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Doctoral Dissertation

Microbiological contamination risk management in aseptic room environment by three dimensional air flow analysis and microbiological risk characteristics

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July 2008

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無菌室環境における微生物汚染リスクの制御と微生物汚染リスクの特長について

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July 2008

Contents

1. Introduction 1

1.1. Preface 1

1.2. Environmental air conditions in aseptic rooms 2

1.3. Air flow pattern in aseptic rooms 5

1.4. Visualization of air flow patterns in aseptic rooms 6

1.5. Three dimensional air flow mapping (3D-AFM) and local mean age of air (LMAA) calculation using computer simulation 7

1.6. LMAA by Computational fluid dynamics (CFD) 7

1.7. Risk-based categorization of aseptic rooms 9

1.8. Risk assessment tools for aseptic rooms 12

1.9. Scope of this dissertation. 16

1.10. References 22

2. Establishment of Critical Contamination Risk Locations ("Hot Spots") in Environmental Monitoring by three-dimensional airflow mapping (3D-AFM) and Particulate Evaluation 26

2.1. Introduction 26

2.2. Materials and Methods 27

2.2.1. Facilities 27

2.2.2. 3D-AFM measurements 28

2.2.3. Environmental measurements 29

2.3. Results 34

2.3.1. Three-dimensional airflow analysis 34

2.3.2. Environmental monitoring 41

2.4. Discussion 48

2.5. Conclusion 50

2.6. References 50

3. Monitoring minimization of Grade B environments based on risk assessment using 3D-AFM and computer simulation 53

3.1. Introduction 53

3.2. Materials and methods 55

3.2.1. Facility 55

3.2.2. Three-dimensional airflow measurement 56

3.2.3. Environmental monitoring 57

3.3. Results 62

3.3.1. Experimental results of three-dimensional airflow 62

3.3.2. Average air velocity in each Area 67

3.3.3. Airflow analysis using computer simulation 68

3.3.4. Local mean age of air (LMAA) 69

3.3.5. Environmental monitoring results 69

3.4. Discussion 73

3.4.1. Cleanliness and contamination risk level of Grade B rooms 73

3.4.2. Microbial air monitoring and particle monitoring efficiency in Grade B rooms 74

3.4.3. Grade B gowning rooms 76

3.4.4. Adequate monitoring program in a Grade B environment 76

3.4.5. Small stagnant spot 77

3.4.6. Hotspot localization using three-dimensional airflow measurements and computer simulation 78

3.5. Conclusion 79

3.6. Glossary 80

3.7. References 81

4. Comparative results of air flow characteristics mapped by CFD simulation and actual measurements of 3D-AFM and particle concentrations 83

4.1. Introduction 83

4.2. Materials and Methods 85

4.2.1. Facilities 85

4.2.2. Airborne particle measurement 85

4.2.3. Measurement and analysis of a 3D-AFM 86

4.2.4. Three-dimensional computer simulation of airflow 86

4.3. Results 87

4.3.1. Airborne particulate measurement results 87

4.3.2. Three-dimensional simulation of airflow 87

4.3.3. SVE-3 as LMAA and air velocity simulation 90

4.4. Discussion 92

4.4.1. CFD simulations of reliability 92

4.4.2. Relationship between air velocity and SVE-3 94

4.4.3. Air velocity distribution, age distribution and local air retention area relationship 94

4.5. Conclusion 95

4.6. References 96

5. Proposal for a New Categorization of Aseptic Processing Facilities based on Risk Assessment Scores 98

5.1. Introduction 98

5.2. Materials and Methods 99

5.2.1. Facilities 99

5.2.2. Media fill run and microbiological monitoring 100

5.2.3. Risk assessment methods 101

5.3. Results 104

5.3.1. Summary of media fill run and microbiological monitoring 104

5.3.2. Risk assessment Results 106

5.4. Discussion 107

5.4.1. Category definitions 109

5.4.2. Comparison of calculated risk scores 110

5.4.3. Proposed frequency of media fill runs 112

5.5. Conclusion 114

5.6. References 116

6. Risk management and Deviation handling 118

7. General Conclusion 122

8. Acknowledgments 124

9. Publication list 126

1. Introduction

1.1. Preface

Current medical products manufacturing must assure the safety of the patient through quality assurance technology. Among medical products, sterile injections without terminal sterilization, namely aseptically prepared injections, are considered risky products in manufacturing. Based on GMP (good manufacturing practice), regulatory authorities consider aseptic processing of pharmaceutical products a high-risk manufacturing activity (*1–3*).

One of the major risks in aseptic product manufacturing is microbial contamination in clean rooms during manufacturing. With the exception of a failure of the as sterilization process, the most probable source of contamination is airborne particles, such adhering microbes, which might be in contact with products through air before sealing during open container processing. Given the presence of microbes with invisible particulate contaminants in the surrounding aseptic processing environment, I believe that safe production is assured using a visualized and comprehensive contamination risk assessment method which can scientifically predict the potential risks of contamination. The risk assessment of aseptic rooms, however, has historically been empirical. Random or speculated risk points are selected as sampling points. Those monitoring points have been considered reasonable because in aseptic rooms, there is generally no scientific information to identigy critical high risk locations as control point.

Here, we propose a new method for determining environmental monitoring points based on scientific and clear data. In subsections 1.2-1.8, we examine the current base information of aseptic room air conditions to be achieved, characteristics of air in aseptic rooms, computer simulations for air flow characterization, and risk assessment of aseptic processing.

In section 1.9, we discuss the possible methods to improve quality assurance of aseptic environmental air based on the newly introduced concept of air characterization in aseptic rooms which composes the majority of this dissertation.

1.2. Environmental air conditions in aseptic rooms

The studies reported in this thesis focus particularly on Grade B rooms, which are aseptic rooms. The Grade B environment surrounds Grade A, the critical core production area. In the United States Grade B is defined as Class 1000–10,000 whereas, Grade A is Class 100, which indicates that the volume limit for a 0.5 micron particle concentration is 100 counts per cubic feet. For the manufacture of sterile medicinal products, four grades are present in the EU GMP Annex 1 and Japanese Pharmacopeia General information section 29. Definitions of Grade are below.

Grade A: The local zone for high risk operations, e.g. filling, stopper bowls, open

ampoules and vials, aseptic connection process. Normally Grade A conditions are provided by a laminar air flow work station. At the working position for open clean room applications, laminar air flow systems should provide homogeneous air speed ranging between 0.36 – 0.54 m/s (guidance value).

Grade B: Background environment for the grade A zone. Used for aseptic preparation and filling.

Grade C and D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

Table 1.1. summarizes clean area air classifications and the recommended action levels of microbiological quality by the U.S.A. authority FDA and European Union.

Table 1.1. Air Classifications *a*

Clean Area NASA Class (0.5 μm particles/ft^3)	ISO Class ***b***	**EU GMP Annex 1 Grade**	> 0.5 μm particles/m^3	Microbial Active Air Action Levels ***c*** (cfu/m^3)	Microbial Settling Plates Action Levels ***c,d*** (ϕ 90 mm; cfu/4 hours)
100	5	**A**	3,520	1 ***e***	1 ***e***
1000	6	**-**	35,200	7	3
10,000	7	**B**	352,000	10	5
100,000	8	**C**	3,520,000	100	50

a- All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.

b- ISO 14644-1 designations provide uniform particle concentration values for clean rooms in multiple industries. An ISO 5 particle concentration is equal to Class 100 and approximately equals EU Grade A.

c- Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological action levels due to the nature of the operation or method of analysis.

d- The additional use of settling plates is optional.

e- Samples from Class 100 (ISO 5) environments should normally yield no

microbiological contaminants.

1.3. Air flow pattern in aseptic rooms

In Good Manufacturing Practice (GMP) guidance documents, environments surrounding the critical area, namely Class 1000–10,000 and Grade B, are usually treated as uniform spaces (*4-6*). Historically, a turbulent clean room is treated as ideally homogeneous in particle concentration, whereas a turbulent airflow pattern is treated as well-mixed. These areas, however, are neither uniform nor homogeneous (*7*). In other words, classification of Class 10,000, Class 1000, and Grade B is not an adequate zoning definition for environments surrounding the critical area.

In general, the existence of air streams and heterogeneous concentrations of airborne particles in a clean room are recognized during the performance qualification of the heating, venting and air conditioning (HVAC) system, as well as by airflow visualization (smoke study), which includes the monitoring and evaluation of additional physical parameters, including as air velocity (*8,9*).

However, understanding of the overall relationships between these test criteria remains unclear in the overall context of clean room performance. A significant reason for this difficulty is the absence of comprehensive organized information, such as in a visualized air flow mapping of an aseptic room.

1.4. Visualization of air flow patterns in aseptic rooms

Several significant air monitoring points are found when the entire airflow within a room is visualized. We propose to evaluate the following air zones in an overall turbulent aseptic room (Figure 1-1) using three-dimensional airflow mapping (3D-AFM). Actual measurements of 3D-AFM reveal different character zones within the aseptic room.

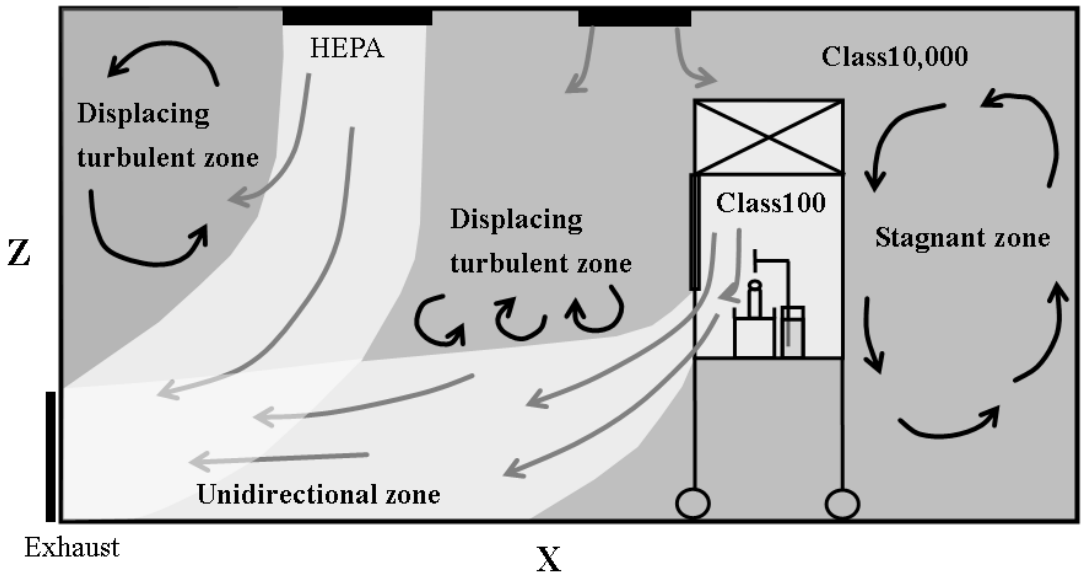

Figure 1-1. Types of zones in a clean room

a) Unidirectional zone

i) A part of air mass flowing in the same direction from the Grade A (Class 100) booth

b) Turbulent zone

i) Displacing turbulent zone: portion of the turbulent zone contacting the unidirectional zone

ii) Stagnant zone: independent air zone consisting of circulated airflow

c) Main stream: large stream extending from the higher air-supplying zone to the higher exhausting zone.

Airflow is visualized using actual air measurements and 3D-AFM, which facilitates the prediction of the location of contamination occurrence and the point where contamination is efficiently removed by airflow.

1.5. Three dimensional air flow mapping (3D-AFM) and local mean age of air (LMAA) calculation using computer simulation

3D-AFM is performed by actual air velocity measurements, which require a subtratntial amount of human resources and time. Computer simulation are therefore used to replace manual measurement and optimize the environmental monitoring point. Computer calculation of local mean age of air facilitates the determination of air characteristics due to the general speculation that LMAA is possibly comparable to the concentration of contaminant. Additionally, LMAA mapping gives a visually clear understanding of high risk locations.

1.6. LMAA by Computational fluid dynamics (CFD)

3D-AFM is an analytical method which provides a more useful overview of clean room performance. In general, CFD-based computer simulation models are efficient in designing clean rooms. The model requires the air velocity and temperature at air supply points in the clean room. The simulation provides useful metrics of the Scale for Ventilation Efficiency 1-6 (SVE-1-6) (*10*); for example, the dimensionless age of SVE-3 yields a meaningful air-age map to evaluate the average risk of contaminant presence. Further, SVE3 suggests air supply design optimization for the clean room (*11-14*), and is used to estimate the age of air. SVE-3 is defined as follows:

$$SVE3 = \frac{C_X'(X)}{C_S} \qquad \text{Equation 1}$$

$$C_S = \frac{q}{Q} \qquad \text{Equation 2}$$

where SVE-3 is the scale for ventilation efficiency 3 at position X; $C_X'(X)$ (kg/m^3) is the contaminant concentration in the case of uniform generation throughout a room; q (kg/s) is the contaminant generation rate; Q (m^3/s) is the airflow rate; and C_S (kg/m^3) is the perfect mixing concentration. SVE-3 corresponds to the age of supply air itself.

To find hot spots in a clean room, SVE-3 mapping using the CFD model is a suitable approach [“recommended”?] as it is visually comprehensive. LMAA indicates the time the air at the measuring point spent floating in the room after air

supply from a HEPA filter, and is considered an indicator of air pollution. When LMAA is short, the measured point shows relatively fresh air supply. In the case of an aseptic room, this low LMAA [check] represents a low risk air point in fresh air, similar to air after moving through a HEPA filter, where the air contains no particulates and microbial contaminants.

SVE-3 is calculated from the local averaged age of air and divided by the number of air changes per hour, and the resulting value is non-dimensional [please check].

The LMAA and the air particulates in a clean room are speculated to be in some relationship. However in case of aseptic room, in very low particle concentration condition, one pessimistic result was reported (*15*).

1.7. Risk-based categorization of aseptic rooms

Recent improvements in aseptic production facilities, through the use of technologies such as the Restricted Access Barrier System (RABS), the Blow Fill Seal (BFS) and the isolator, have drastically lowered the risk level of microbial contamination. Currently, the target Sterility Assurance Level (SAL) of 10^{-6} is expressed as [or "represents"?] either the lethal biological population, used as an indicator during terminal sterilization, or as the probability of the presence of microbial contamination units during aseptic processing. In the latter case, traditional media fill runs cannot yield an assurance level of 10^{-6} due to limitations of media fill batch sizes in the region of $10^3/10^4$, which only mathematically

assures a SAL of approximately 10^{-3}. However, based on accumulated empirical results from past media fill runs (*16*) and on calculated simulation results (*17*), current advanced aseptic technology potentially achieves the theoretical 10^{-6} SAL in aseptic processing.

Contamination risk levels have considerably decreased with the use of modern advanced technology. The FDA has indicated the possibility of reducing the frequency and batch size of the media fill test when advanced technology is introduced in a facility (*18*). Similarly, the USP has proposed reducing monitoring frequency when an isolator system is used. In addition, the USP has suggested accepting non-microbial qualification tests for extremely clean, ISO Class 5, Grade A air (*19*). Current industrial targets for preventing microbial contamination may be achieved by a flexible approach to validation. Further, manufacturing costs may potentially be reduced to a more reasonable level, while ensuring reliable sterile products based on risk management appropriate to the facilities used.

Achieving a SAL value under 10^{-6} is expected in cases where an isolator is completely separated from the operator. When an isolator is used, a Grade B (ISO 7) environment is not required in the surrounding area. With the use of RABS and BFS, however, a Grade B (ISO 7) surrounding environment is required due to direct human access during installation and subsequent periodic human

interventions during processing (*20*). While RABS is capable of achieving a high SAL value of 10^{-6} (*21*), current guidelines require an equivalent set of environmental monitoring methods as for conventional Grade B rooms.

Compared to conventional facility design, RABS use is generally known to significantly lower the risk of contaminant delivery from the surrounding Grade B environment to the Grade A area [check]. Given this situation, simplifying the environmental monitoring program based on risk assessment should be possible. The monitoring of interventions and equipment installed in a Grade A environment should be an adequate and effective method of monitoring direct contamination risks at any point in time during operations. In contrast, the quality of the surrounding Grade B air does not directly affect Grade A operations due to the rapid dilution of contamination risk provided by the air barrier which is created by the Grade A(ISO 5) unidirectional air flow when the RABS door is opened. In Grade B environments, contaminant scattering by operators constitutes a significant risk. Contaminated Grade B air may potentially contaminate the intact surface of the operator's gown and gloves, as well as autoclaved items, and may then be transported into a RABS Grade A environment. These contaminated surfaces are potentially in contact with surfaces in the RABS Grade A environment during setup and periodic interventions. Surfaces which may have been in contact with products in a RABS Grade A environment should be directly monitored. Because the risk of Grade B air contaminants invading a Grade A

environment is usually extremely low due to air and physical hard barriers, the primary potential source of contamination comes from scattered Grade B air contaminants coming into contact with gowns, gloves and autoclaved items. These contaminants are evaluated by measuring the overall accumulated amount of contaminants in the Grade B environment during production activity. If assessment of contamination hotspots can determine the maximum biological contamination level of a room, it should be possible to select one representative hotspot and use it as a daily monitoring point to effectively predict the overall risk potential for the Grade B room. However, particularly in extremely clean Grade B environments where traditional microbial sampling methods are not sufficient to determine contamination risk due to their low sensitivity, identifying contaminants caused by human presence at a specific point may not be a reliable approach. For risk control purposes, extremely clean Grade B environments should be subjected to intensified room qualification criteria and a daily monitoring program targeted towards environments suitable for the use of a RABS barrier system.

1.8. Risk assessment tools for aseptic rooms

Risk assessment tools for the analysis of aseptic processing facilities have undergone rapid development, particularly following the publication in 2002 of the FDA's *Pharmaceutical cGMPs for the 21st Century –A Risk-based Approach* (*22*). This initiative was established to promote the introduction of new and improved production technologies that will contribute to assuring the quality and

safety of products for consumers. The pharmaceutical industry has developed significant flexibility in introducing state-of-the-art technology, which results in high quality, high-performance aseptic production operations. In this streamline industry obtained the tools required to balance quality benefit and resource consumption based on scientific risk assessments during the improvement process.

To assure sterile product manufacturing, aseptic processing facilities, which have an improved low-risk level, should employ a new set of aseptic criteria instead of using traditional media-based microbiological test methods, which are considered insufficient in extremely clean areas. For example, in the revised USP<1116> (*19*), microbiological monitoring in ISO Class 5 is not always necessary. The document states that "if a Class 5 designation-rated hood is used for control of nonviable particulates, microbiological testing is not required." Instead of media-based tests, firms can conduct continuous particle monitoring for extremely clean ISO Class 5 zones when control of particulates is the critical objective. In addition, a chapter introduces a new risk-based criterion based on "contamination incident rates for aseptic processing". Coming from an understanding of the low microorganism detection capability of microbiological methods, as well as the inherent variability in the number of colony-forming units recovered, this concept can also be applied to the media fill run.

In the case of media fill (also known as process simulation) (*23*) testing, although numerous surveys of historical data have appeared, discussion of the actual efficacy or usefulness of this test in a modern, advanced aseptic processing facility has been insufficient. The general criterion given for the quality attribute of sterility is a sterility assurance level (SAL) of 10^{-6}. Tidswell proposed a Quantitative Risk Modeling and Simulation. A Monte Carlo simulation based on empirical microbial test results of the individual processes showed a microbial ingress risk value of 2.39 x 10^{-6} for the model conventional aseptic processing (*24*,*25*). However, aseptic processing discussions regarding acceptance criteria have historically focused on the idea that the process capability of aseptic operations is approximately 10^{-3}, which is actually based on media fill run results rather than true sterility assurance. Therefore, any consideration of SAL related to aseptic processing is substantially different in concept from the notion of sterility as applied to terminal sterilization.

The major function of the media fill run is to determine the risk of human-related microbiological contamination. However, improved aseptic processing facilities such as the isolator, which can effectively and severely limit human access to aseptic environments, may confer a contamination level so low as be undetectable by conventional microbiological analysis. The Restricted Access Barrier System (RABS) and Blow-Fill Seal (BFS), which also minimize human access during core aseptic operations, may also not greatly benefit from media fill runs due to

their extremely low actual risk of microbiological contamination (*26*,*27*). In contrast, conventional aseptic operations, which cannot be carried out without regular direct human intervention, can be effectively evaluated for microbiological contamination risks using traditional media fill runs. In addition, although investigators such as Sutton (*26*), Sanderson(*27*) and Nagarkar (*28*) mention the poor quantitative reliability of traditional microbial monitoring methods in clean environments, useful information may still be obtained from microbial contamination risks in conventional aseptic rooms.

The risk assessment approach can prove to be a reasonable tool to distinguish whether a facility should be monitored by traditional media-based tests or by an alternative method. By comparing risk assessment scores with experimental media based test data, we evaluated the possibility of defining the limits of usefulness of traditional media based testing and identifying a category of lower risk in aseptic processing where traditional media based testing lacks efficiency. The results reported in this study can serve as a first step towards establishing more flexible risk- and science based validation and monitoring requirements for advanced aseptic processing.

A number of published risk assessment tools which provide scientific numerical scores simplify theoretical risk assessment.. However, very few assessment tools able to simply estimate categorization of aseptic facilities are accessible for

individual sites. Examples of simple risk assessment methods include the modified FMEA assessment tool proposed by Whyte-Eaton (*31*) and a convenient risk assessment tool for aseptic processing suggested by Akers and Agalloco (*32*,*33*). These tools are nonetheless considered reliable for simultaneous comparison of facilities by an individual or group.

1.9. Scope of this dissertation.

In view of the current regulatory focus on a science and the risk-based approach to pharmaceutical manufacturing and process control (*8*,*34*), author has developed an improved method to evaluate the contamination risk in aseptic environment. This method is a combination of 3D-AFM and intensive environmental monitoring (IEM) including airborne particle monitoring, airborne microbial monitoring and microbial surface monitoring. Furthermore a LMAA mapping by computer simulation can assist the 3D-AFM, and air qualification.

In section 2, a primal investigation study to make a proof of concept of this 3D-AFM method is reported. A small aseptic Room1 shown in Figure1-2 for clinical trial injection production was investigated by the 3D-AFM method. The comparison of repeated actually measured 3D-AFM and IEM was attempted. As a result 3D-AFM is considered a useful and applicable method.

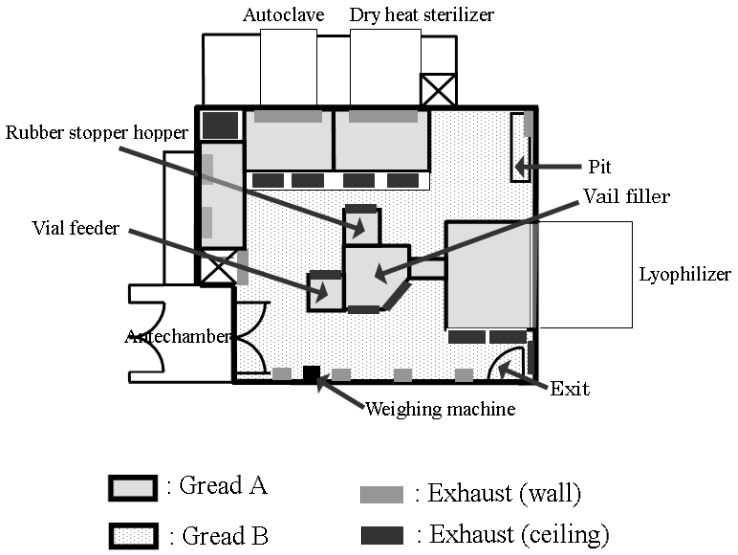

Figure1-2. Layout of aseptic Room1 for clinical trial material production

Author has improved this 3D-AFM method as a convenient risk assessment tool of aseptic environment.

In section 3, an expanded scale study is presented. The3D-AFM method is applied for a large size aseptic Room2 in Figure1-3 which is using for commercial injection drug production. In this study variety of intensive microbial and particle monitoring were conducted. The relation of actual contaminants and air flow characteristics translated from 3D-AFM is discussed. LMAA-3D-AFM associates the translation of actual 3D-AFM to a practically meaningful risk level.

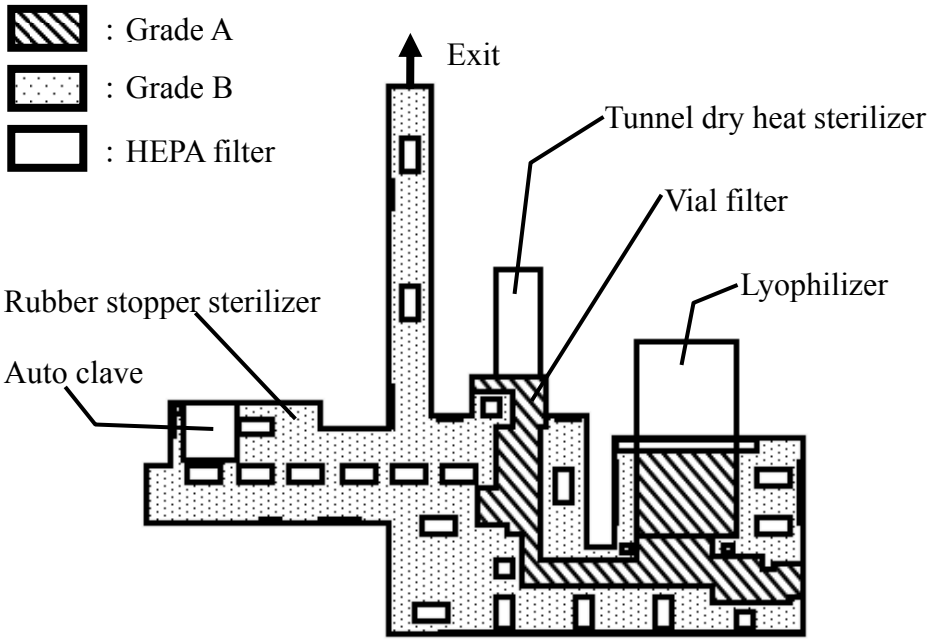

Figure 1-3. Layout of Aseptic Room2. Takaoka Plant №1 suite.

In section 4, mapping of LMAA and accuracy of computer simulation are determined by using another's result of 3D-AFM which study data obtained from another large aseptic Room3 in Figure1-4 for commercial production.

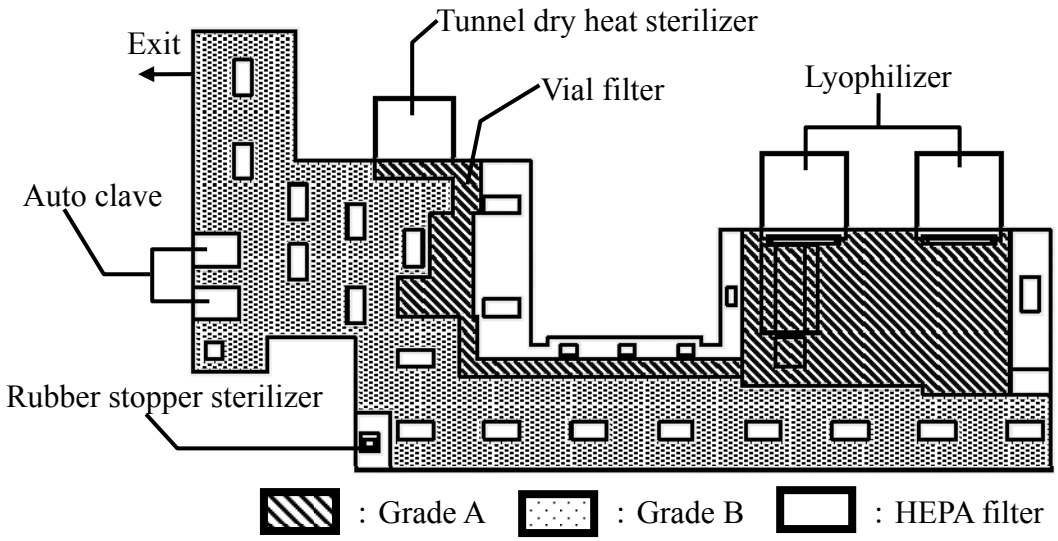

Figure 1-4. Layout of Aseptic Room3. Takaoka Plant №3 suite.

Prediction of contamination risk by a scientific risk assessment tool is able to mitigate the risk of contamination from environmental air. However clean room performance and manufacturing technology are very widely variable. Author conducted the 3D-AFM case study for three different aseptic rooms. Author determined repeatedly the predictability of actual 3D-AFM by LMAA simulation in case studies of section 3 and 4.

In section 5, author focused the difference of risk level of contaminants in an aseptic room. Three case studies in section 2 to 4 used highly ventilated aseptic rooms those have 60 to 80 times per hour air change ratio. In those kind of high performance aseptic room always gives zero base results for microbiological monitoring. As the concept of Grade A of aseptic room 2 and 3 in Figure 2 and 3

are Restricted Access Barrier System (RABS), the risk level of product contamination in Room 2 and 3 should be further lower than the contamination risk level in Room 1, even the contaminants clearance capability in Grade B is same among Room 1 to 3. However the microbiological environmental monitoring can not differentiate the product contamination risk level of those rooms 1 to 3. Microbiological tests are not detectable enough to clarify the product contamination level depending on the capability of product protection from contaminants in a facility.

In view of the current regulatory focus on a science and the risk-based approach to pharmaceutical manufacturing and process control (*4*,*5*), aseptic production facility can be classified to 4 categories based on their aseptic processing technology. Categories are proposed in section 5.

In section 5, author discussed the practical risk assessment of aseptic processing facilities using two published risk assessment tools. As a result a conventional facility having acceptable aseptic processing lines gave relatively high risk scores. The facility showing a rather high risk score level demonstrated the usefulness of conventional microbiological test methods. Those facilities should be risk assessed by author's three dimensional air analytical method.

In section 6 author discuss the 3D-AFM and LMAA method is also useful for

investigations in case of out trend and out of specification of asepsis. A good application of the method is for the purpose of an investigation in case of out of trend in environmental monitoring results. For the reliable justification the three dimensional air analytical method should be employed.

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2. Establishment of Critical Contamination Risk Locations ("Hot Spots") in Environmental Monitoring by three-dimensional airflow mapping (3D-AFM) and Particulate Evaluation

2.1. Introduction

This section 2 shows the results of one case study in a small aseptic room utilizing the three dimensional air flow analytical method. The particle distribution map of a Grade B environment based upon extensive analysis was found to correspond to room airflow, as visualized by air vector mapping. The actual annual environmental monitoring data, which include airborne particles and microbes, as well as other microbial monitoring data, are also presented with respect to their relationship to the airflow pattern.

When considering the risk of microbial contamination originating in the surrounding environment entering the critical area, operators and objects in clean room (such as machines and materials) must be evaluated for risk. Aseptic processing experts appear to agree that contamination of human origin presents the greatest risk to product. We believe that in order to obtain a better performance model of the entire aseptic processing area, improved methodology is necessary.

We propose an improved method that uses a combination of 3D-AFM and

intensive particle analysis within the aseptic processing area.

2.2. Materials and Methods

2.2.1. Facilities

The aseptic room (Figure 2-1) has a floor area of 56 m^2 and a height of 2.4 m. An air exchange rate of 68 air changes/h was used in this research study. The air change ratio is calculated by dividing the supplied air volume per hour by the volume of the room. The supplied air blows from the ceiling HEPA filters, and it does not include the air change in the clean booth. If the air supplied by the Grade A clean booth were considered, the air exchange rate would be 170 air changes/h. A clean booth for an ampoule-filling machine had been installed near a pit pitch just before June 15, 2001, but it has since been removed.

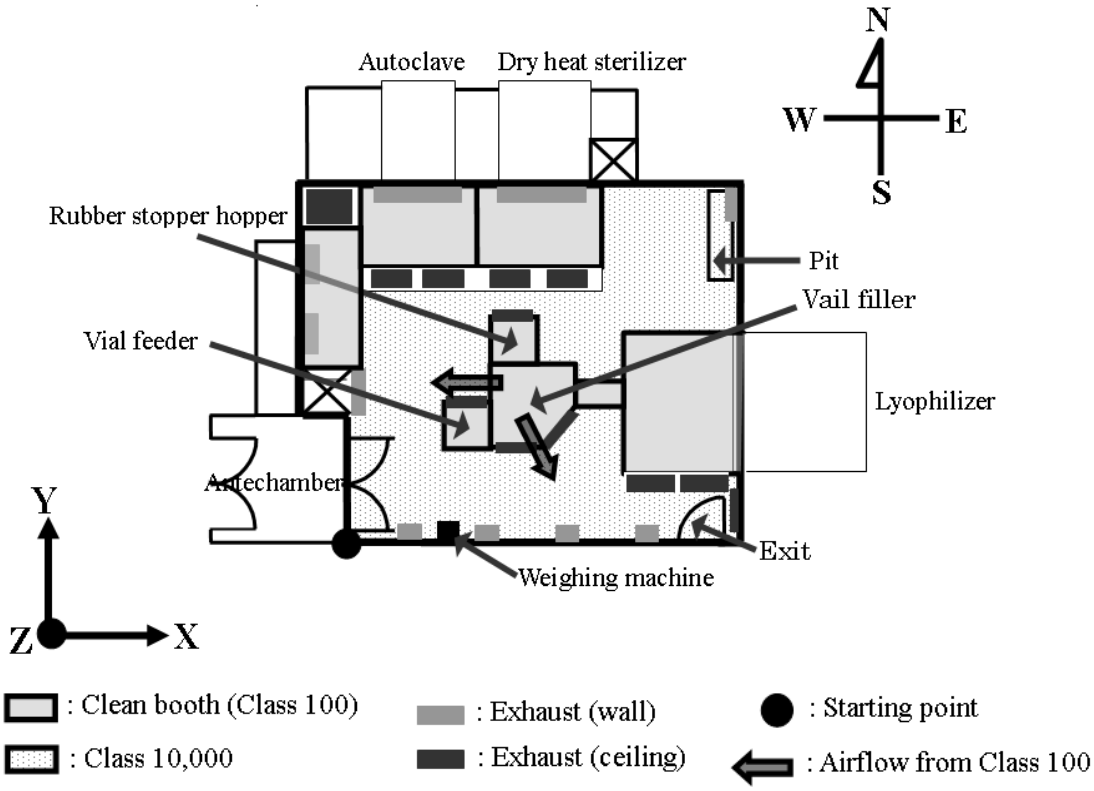

Figure 2-1. Aseptic Room Layout

2.2.2. 3D-AFM measurements

a) Measuring equipment. Three-dimensional air velocities were measured with an ultrasonic anemometer for clean rooms (model WA-590) and software WASP-007 (KAIJO, Tokyo, JAPAN) for the three-dimensional anemometer measurement program.

b) Measuring points. The starting point was marked in the Southwest corner on the floor of the aseptic room (Figure 2-1). From the starting point, the East direction was designated as the X-axis and the North direction was

designated as the Y-axis. On the XY axial plane, 50 x 50 cm grids were created. In addition, the vertical direction from the starting point was designated the Z-axis. Measuring points were set at heights of 50, 100, and 200 cm in the direction of the Z-axis above all cross points of grids on the floor of the aseptic room.

c) Measuring method. Three-dimensional air velocity was measured for 15 sec/point. The sensors were set parallel to the Z-axis in an upwards position. Measurements of three-dimensional air velocities were performed in the at-rest condition; no personnel were present in the clean room during the study except for the analyst.

d) Frequency of measurement. Measurements were taken twice with different equipment layouts. The first was in May 2000 when the clean booth for an ampoule-filling machine was installed near the pit within the aseptic room. The second measurement was done in January 2002 after the ampoule-filling machine was removed.

2.2.3. Environmental measurements

a) Measuring equipment and method

i)Airborne particle. One foot3 was measured three times consecutively using HIACROYCO 243A air sampler (Grants Pass, OR), and an average of the three measurements was adopted as a measured value. The particle diameter used in the study was 0.5 μm for particle counts evaluation.

ii)Active microbial air sampling. At each monitoring point, 1000 L (1 m^3) of air sample with agar strip was taken with the air sampler RCS Plus (Biotest, Frankfurt, GERMANY).

iii)Settling plate. A 9-cm diameter petri dish of agar was opened for an hour at the monitoring point.

iv)Microbial surface monitoring. A 25-cm2 agar contact plate (Clean Petan, EIKEN KIZAI, Tokyo, JAPAN) was used for surface monitoring.

v)Airborne particle continuous monitoring. In order to observe the steady state levels of airborne particle in the aseptic room, 0.3-μm and 0.5-μm airborne particles were analyzed continuously for 60 min in the Grade B zone.

b) Measuring points and the times of measurement.

i)Intensive monitoring in Grade B zone

- Airborne particles. 0.5-μm airborne particles were measured at 50 and 100 cm heights at 38 places in Grade B zone (Figure 2-2). Measurements were carried out three times during clean conditions, immediately after cleaning, and again just after a maintenance break to evaluate the clean room under less ideal conditions. This monitoring was conducted after the removal of the ampoule-filling machine at Region A.
- Active microbial air sampling. Airborne microbes were measured once at a 100-cm height at 18 places (Figure 2-2) after the periodical

monitoring operation

- Settling plates. Settling microbes were measured 3 times at 18 places (Figure 2-2) on the floor after the periodical monitoring operation.
- Microbial surface monitoring. Surface microbial contamination was measured three times at 40 places (Figure 2-3) after the periodical monitoring operation

ii) Annual environmental monitoring. Monitoring for 0.5-μm airborne particles at a 100-cm height, active microbial air monitoring at a 100 cm height, settling plates at a 100-cm height, and surface sampling were carried out every Friday with the machinery at rest and then with it in operation. These analyses were conducted for one full year from January 2001 through December 2001. Sampling points were at two locations near the pit and near the weighing machine of the Grade B zone (Figure 2-2). Surface sampling was conducted on the wall near the pit (sampling point 13) and on the weighing machine table (sampling point 32).

iii) Airborne particle continuous monitoring. Airborne particle continuous monitoring for 60 min was measured once after operation at 50-cm and 100-cm heights at two places, sampling point 27 and 30 in Figure 2-2.

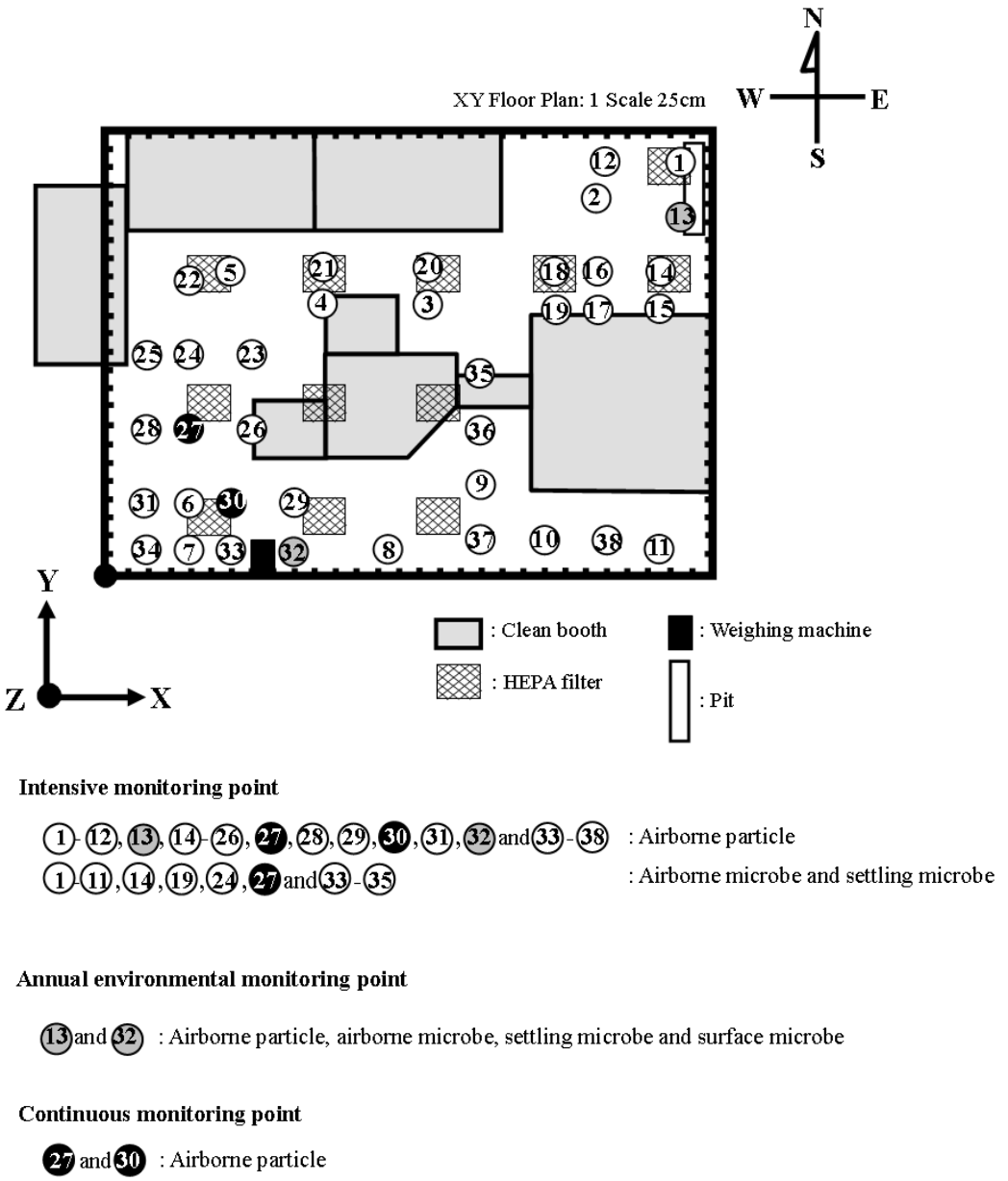

Figure 2-2. Measuring points of intensive monitoring (airborne particle, airborne microbe, and settling microbe), annul environmental monitoring, and airborne particle continuous monitoring

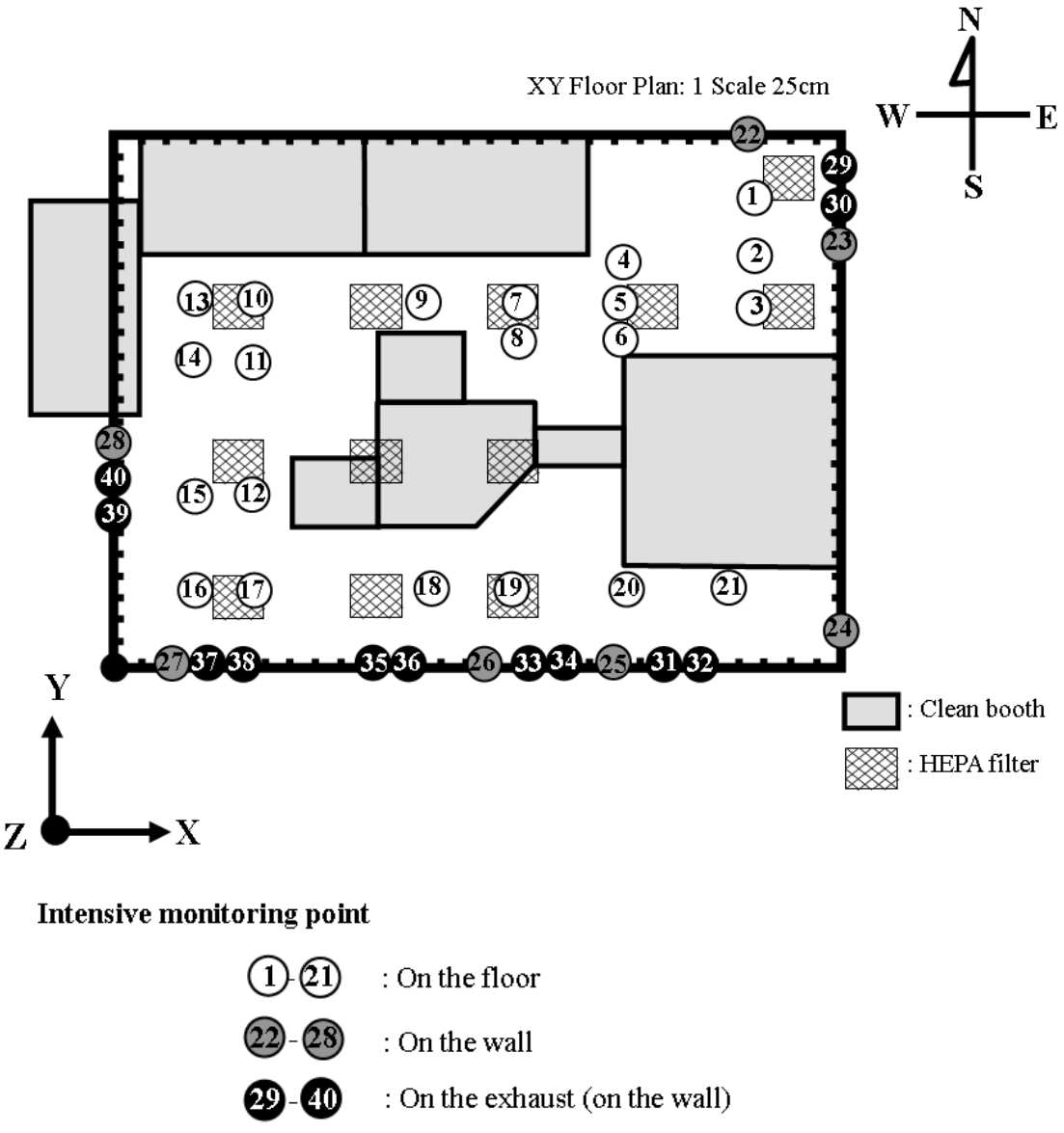

Figure 2-3. Measuring points of intensive surface monitoring

2.3. Results

2.3.1. Three-dimensional airflow analysis

The results of two separate measurements are shown in Figures 2-4 and 2-5. The first measurement is shown in Figure 2-4 and the second is in Figure 2-5. These are angled views from the top, X-Y-Z dimensions. The arrow in the map indicates the direction of airflow; its velocity is shown by the length of the arrow. At each measurement height of 50, 100 and 200 cm, the observed parameters were mapped. In Figure 2-5, there are three kinds of significant regions, designated A, B and C. From the figures a main stream is found in both maps. It flows from the Northeast corner in the map to the West side, and then flows to South and finally to the East side. This main stream's direction is basically the same in both studies. A small difference appears above Region B. In the case of Figure 2-5, this flow appears at the 200-cm height, whereas it appears at the 100-cm height in Figure 2-4. In this section, the results of the second measurement are discussed further in order to develop a clearer understanding of the analysis in consideration of the intensive monitoring results presented in Table 2-1 and Figures 2-6 and 2-7.

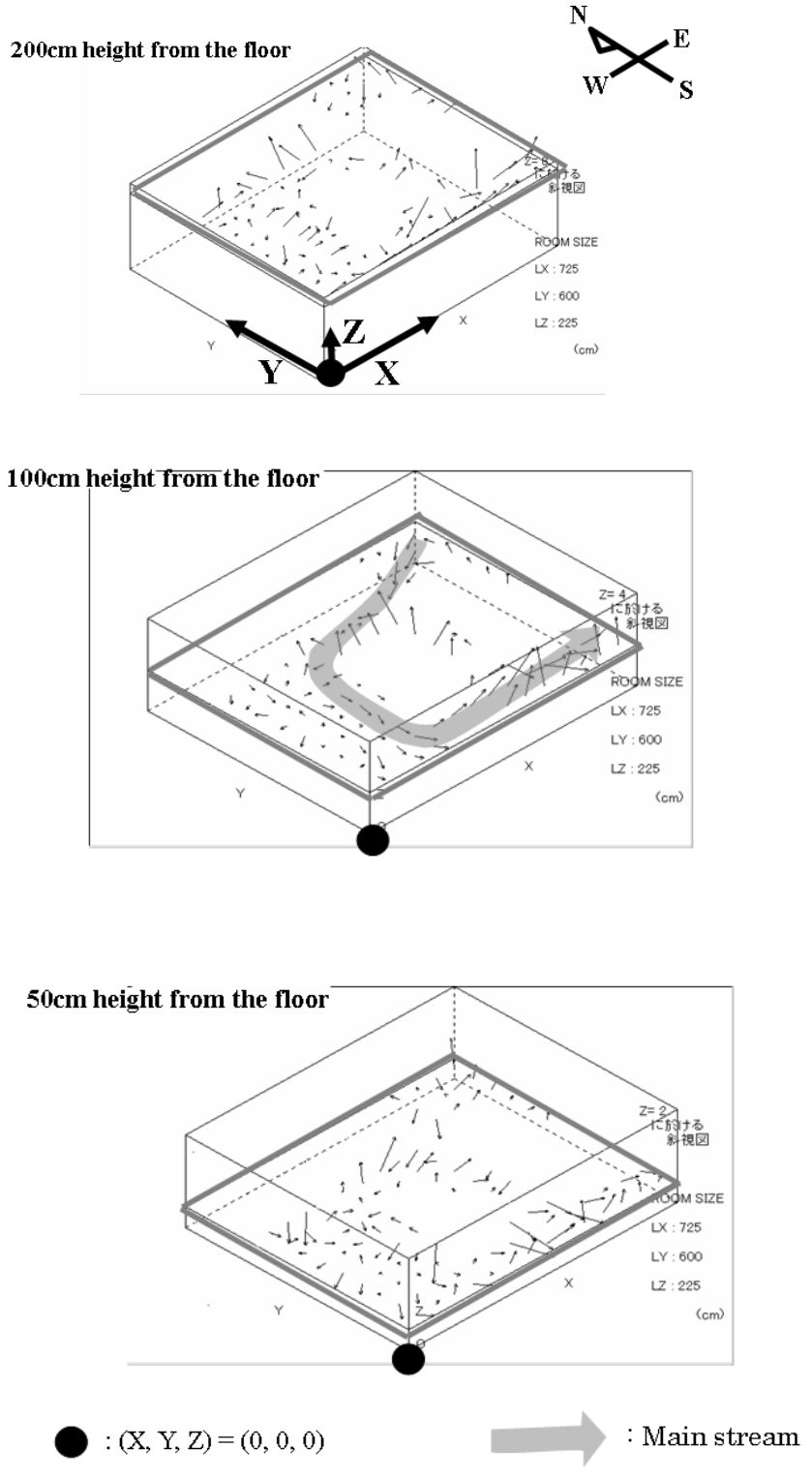

Figure 2-4. The first measurement of three dimensional air velocities

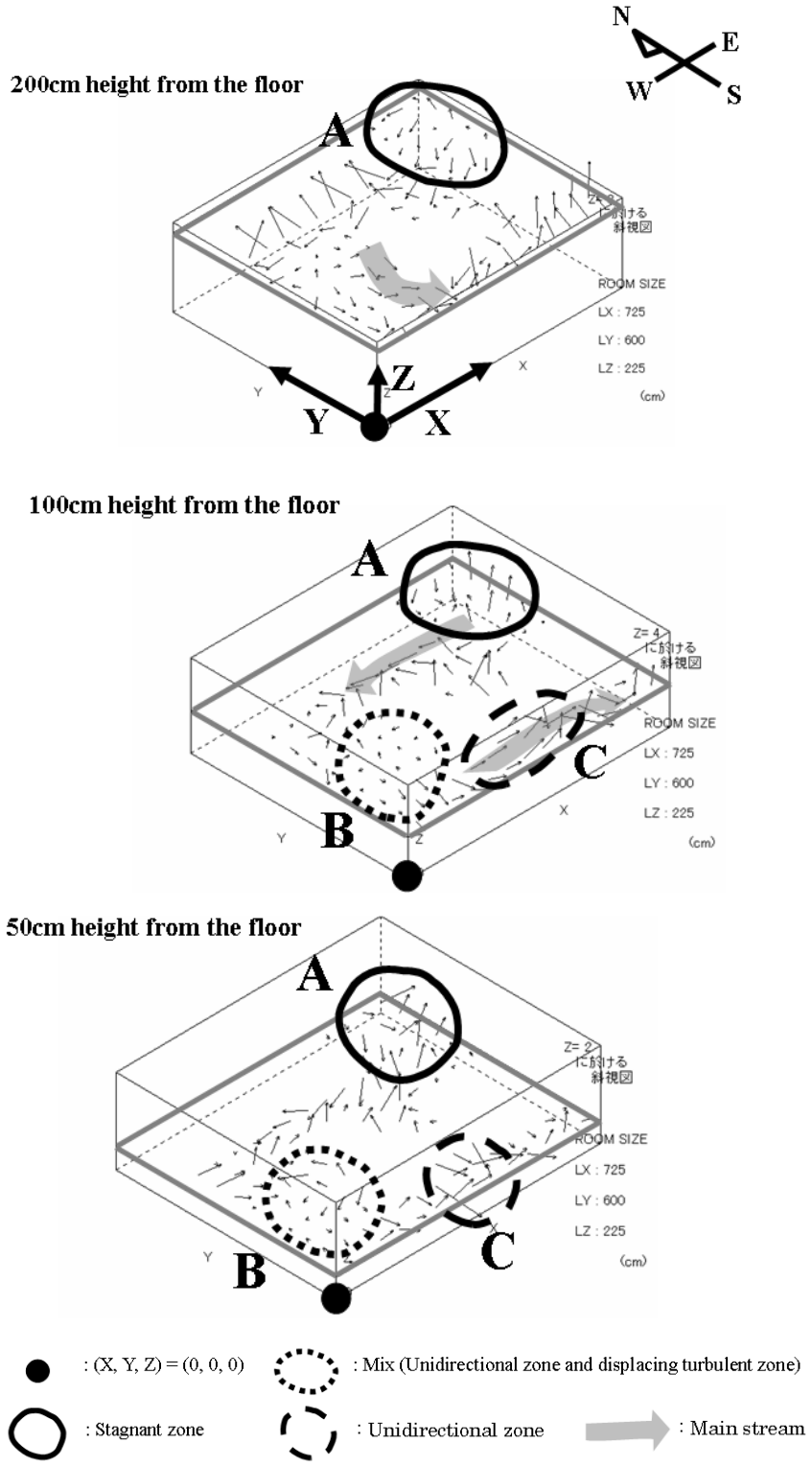

Figure 2-5. The second measurement of three dimensional air velocities

Table 2-1. Airborne microbe, settling microbe and surface microbe monitoring

Sampling point (Reference: Figure 3)	Airborne microbe cfu / m^3 (n=1)	Settling microbe cfu / plate AVE. (n=3)
1 - 11	0	0
14	0	0
19	0	0
24	0	0
27	0	0
33 - 35	0	0

Sampling point (Reference: Figure 4)	Surface microbe cfu / plate AVE. (n=3)
1 - 21 (On the floor)	0
22 - 28 (On the wall)	0
29 - 40 (On the exhaust)	0

Before cleaning

Figure 2-6. Airborne particle counts before cleaning

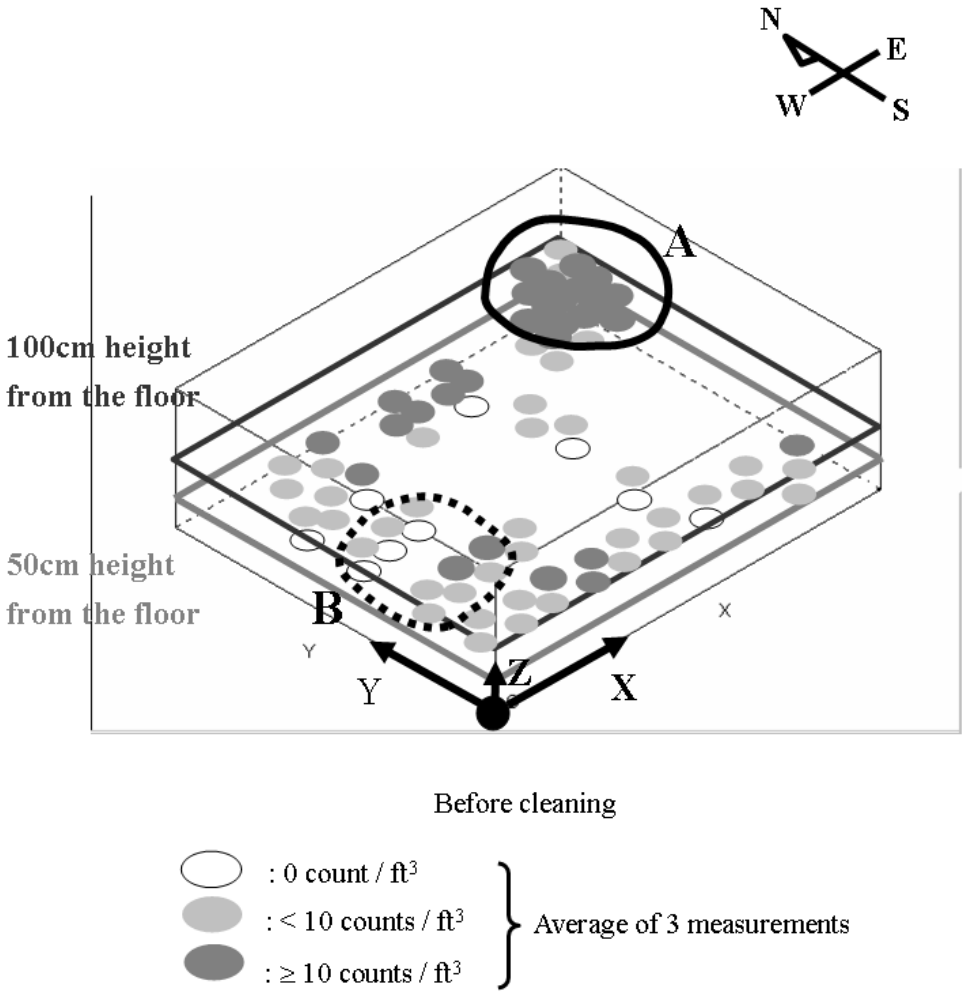

Figure 2-7. The distributed particle concentrations before cleaning

Region A can be considered a stagnant zone with a circulating airflow pattern from 50-cm to 200-cm heights. This airflow in Figure 2-5 can be seen as counter-clockwise from the 50-cm level along the East side wall. The rising arrows around the wall go up at the 100-cm level, and then to the 200-cm level. Flows fall down to 50 cm in the West part of Region A. These arrows show a typical stagnant zone in a turbulent aseptic room animated in Figure 1-1 in page 8.

Region B is a double-layered air zone. It is observed in the horizontal airflow maps of Region B, at 50-cm and 100-cm heights, shown in Figures 2-8-1 and 2-8-2. Several groups of arrows flowing to the same direction appear in these two figures. They are represented by the large gray arrows. In Region B at the 50-cm height, arrows can be seen pointing in the direction of the West-side wall exhaust. This group of arrows indicates an unidirectional flow within (2, 7) to (10, 7) in (X, Z) dimension. In the same Region B at 100-cm height, the arrows are seen to point randomly.

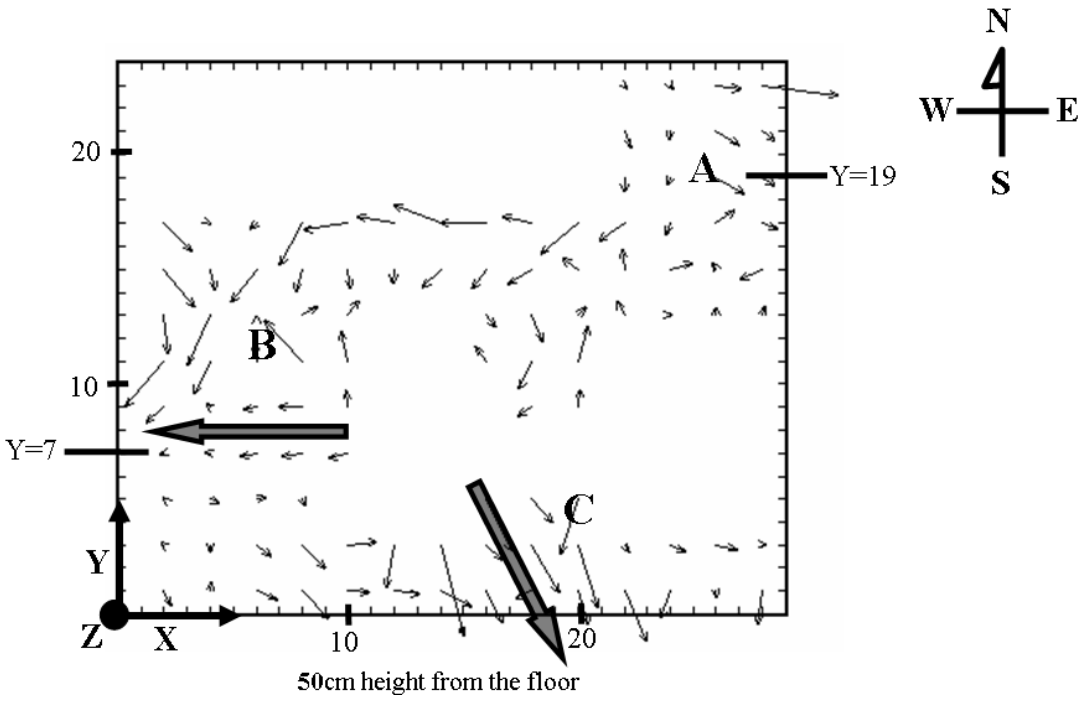

Figure 2-8-1. Airflow pattern of the top view at 50cm height from the floor

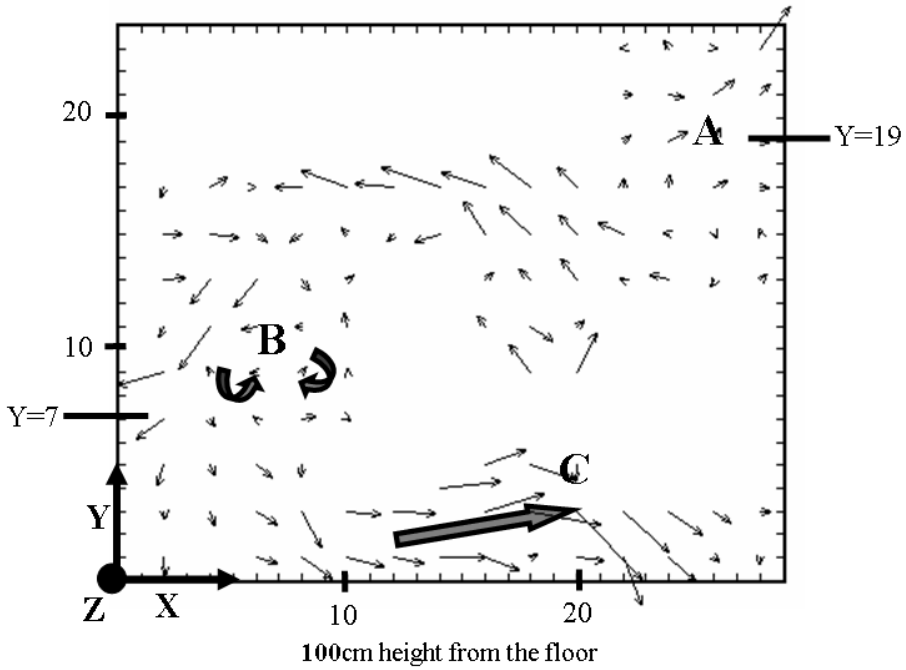

Figure 2-8-2. Airflow pattern of the top view at 100cm height from the floor

For a better understanding of both Region A and B, Figure 2-9, shows side views of the clean room in the X-Z dimensions. The upper part of Figure 2-9, sectioned by Y = 19, shows Region A. It is easy to visualize the circulation within (22, 1) to (29, 9) in (X, Z) dimension. This independent, circulative-type air zone is named the stagnant zone. The lower part of the figure, sectioned by Y = 7, shows Region B where the unidirectional flow within (2, 2) to (10, 2) v in (X, Z) dimension and displacing turbulent flow within (2, 4) to (10, 4) in (X, Z) dimension are clear.

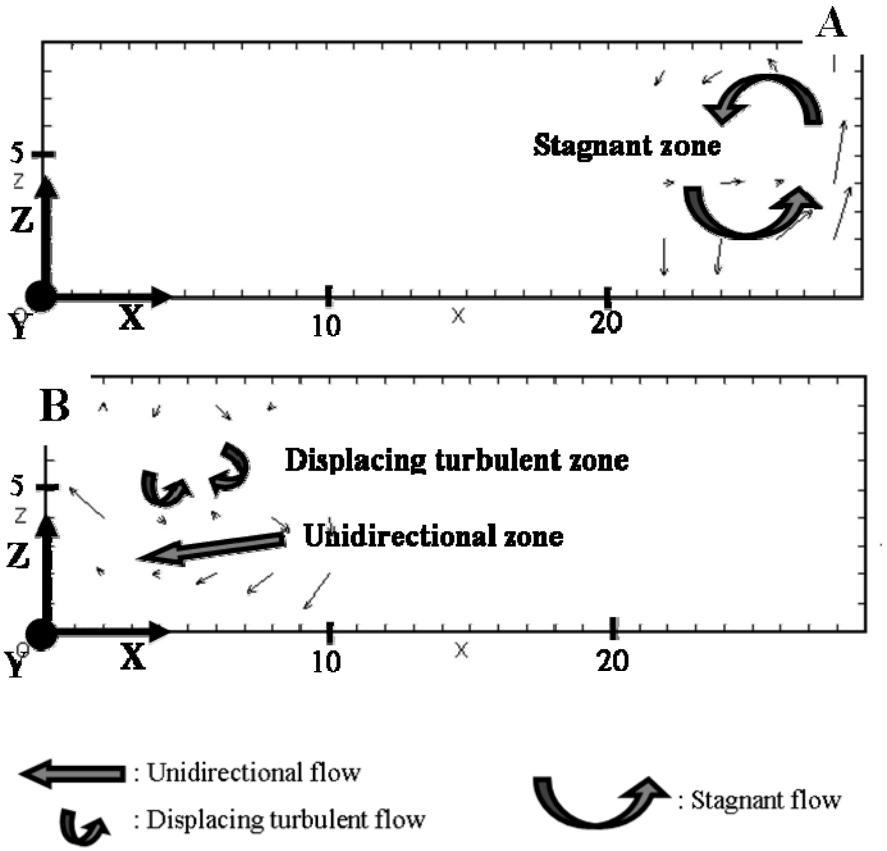

Figure 2-9. Airflow patterns of lateral views

In Figure 2-8, it can be seen that in Region C several parallel arrows within (14, 0) to (23, 3) in (X, Z) dimension also form a unidirectional flow. The origin of this unidirectional flow at the 50-cm height is the clean booth of the filling machine. At the 100-cm height in Region C within (10, 0) to (21, 6) in (X, Z) dimension the main stream moves laterally with a strong parallel unidirectional flow.

2.3.2. Environmental monitoring

a) Intensive airborne particle monitoring in Grade B zone under challenging

conditions. The results of airborne particle monitoring before cleaning at 50-cm and 100-cm heights are shown in Figures 2-6 and 2-7. Figure 2-6 is a horizontal map and Figure 2-7 is in the X-Y-Z dimensions, with an angled view from the top. Ovals show the measured points. The particle counts are averages of three measurements of 0.5-μm airborne particles. A white color means zero count, pale gray means less than 10 counts, and gray means more than 10 counts. The distributed particle concentrations can be seen in a bird's-eye view in Figure 2-7. Region A shows a significantly high level of particle concentration and Region B shows a lower concentration of particles. At each state, before cleaning and after cleaning, airborne particles were measured. However, airborne particles after cleaning were present at a very low concentration. The very low observed counts in this study make it difficult to clearly assess particle distribution. This clean room is basically a highly cleared, sustainable aseptic room. Higher particulate counts were expected in the tests done prior to cleaning. It can be seen in Figure 2-6 that the 50-cm height is "cleaner" than the 100-cm height. The higher-count region and the lower-count region are very significant in the map of the room prior to cleaning. Region A, the stagnant zone, shows higher counts compared to other regions. Region B, the unidirectional and displacing turbulent zone, shows lower counts.

Figure 2-10 shows the image of the side view of Region A. The small stream of unidirectional clean air from Class 100 cannot effectively displace the stagnant circulating air in Region A. Therefore, the airborne particle concentration in this zone is higher than the other zone. Figure 2-11 shows the image of the side view of Region B. Around the floor, unidirectional air from the Class 100 clean booth spills over the 50-cm height. As a result of this, the airborne particles are not detected in the unidirectional zone. The contact-displacing turbulent zone is cleared immediately by the efficient surface interaction with the unidirectional zone. The airborne particles in this turbulent zone of Region B show lower counts.

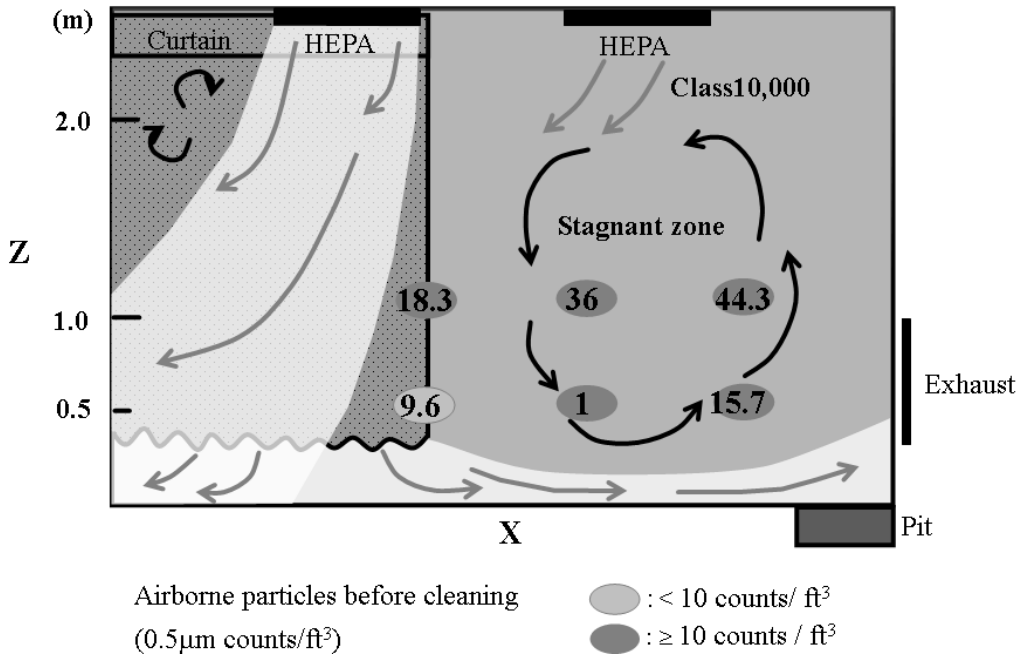

Figure 2-10. The image of the side view of Region A

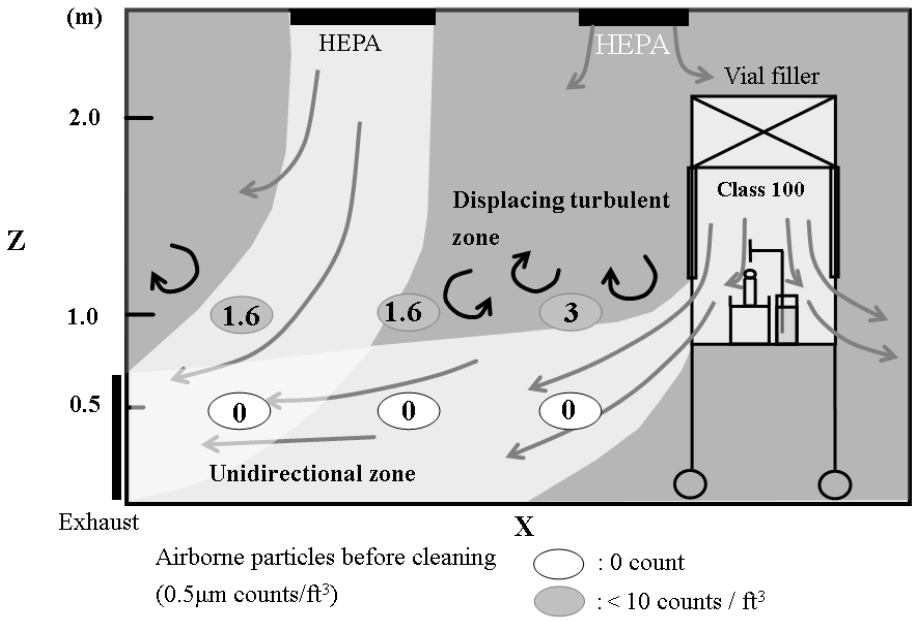

Figure 2-11. The image of the side view of Region B

b) Airborne particle continuous monitoring. Figure 2-12 shows results of continuous airborne particle monitoring. Sampling point 27 is in the unidirectional zone. Sampling point 30 is in a turbulent zone. These two locations show consistent patterns of particle concentration during test period. The unidirectional zone (point 27) has a lower particle concentration than the turbulent zone does (point 30). Therefore, we can conclude that when operators are working within this clean room, they disperse only a very low level of particles. In this case, the distribution pattern of particles depends on the airflow pattern, which can be expected to be consistent in the absence of changes to the HVAC system and

machine layout.

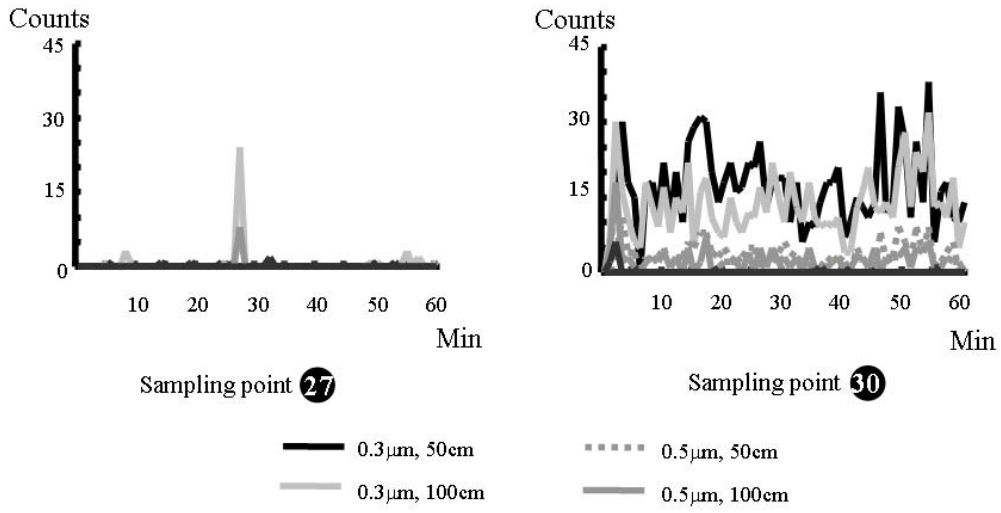

Figure 2-12. Airborne particle continuous monitoring

c) Intensive airborne microbe analysis, settling microbe and surface microbe monitoring in Grade B zone. The results of intensive microbial monitoring show the difficulty of detecting microorganisms in this clean room. Table 2-1 shows the results of microbial monitoring using the active air sampler and settling plates. Counts of airborne microbe from 18 samples (sampling point 18 places×1 time of measurement) and counts of settling microbe from 54 samples (sampling point 18 places×3 times of measurement) were all zero, even though they were monitored intensively after operation. This highlights the relative inefficiency of microbiological air monitoring for the purpose of finding “hot spots”. The results of intensive surface monitoring are given in Table 2-1, and all counts observed in this test from

a total of 120 samples (40 sampling points×3 times of measurement) were also zero. This means that viable microorganisms were not deposited at detectable levels on these surfaces. The few microbes present are considered to be either of human origin or entering on materials entering through the pass box.

d) Annual environmental monitoring. Table 2-2 shows results of annual environmental monitoring. During the period of January 12 to August 6, 2001, a clean booth with an ampoule-filling machine was installed near the pit in the aseptic room. During this period, operators mostly moved around Region A during production. The main stream of this period is shown in Figure 2-4. As a result of this, as seen in Table 2-2, particle counts of sample point 13 and sample point 32, near the weighing machine, are higher than those observed during a non-working condition (see the map in Figure 2-2, Region A). This phenomenon is clearly reflected in Figure 2-10. Emissions during the operation would stay in the stagnant zone a relatively long time instead of rapid passing by due to a high air-change ratio. On the other hand, during the period of June 15 to December 21, 2001, Region A was empty; operators mostly moved around Region B during production, and they rarely moved into Region A. Figure 2-5 show the main stream during this period, and Figure 2-6 shows the particle counts mapping during the same period. The very low counts of particles at sample point 13

in Region A in working conditions for this period means that the main stream flushes particles to the down stream immediately after emission from an operator moving in Region B and near the weighing machine (see Figure 2-2). Therefore, particles dispersed from an operator in Region B could not reach Region A against the main stream. Very low counts of viable microbe in Table 2-2 show the excellent cleanliness of this aseptic room. However, the viable monitoring results do not parallel the particle counts. These test results indicate the inability of microbiological monitoring alone to reflect trends in contamination within the class of environments utilized in the most critical areas within an aseptic processing area. The relatively low sensitivity of microbiological methods limits their usefulness in this regard.

Table 2-2. Annual environmental monitoring

	Sampling point	Average counts / Times of measurement			
		Jan 12 to June 8, 2001		June 15 to Dec 21, 2001	
		Working	Non-working	Working	Non-working
Airborne particle	32	54.9 / 16	3.7 / 15	46.2 / 16	7.3 / 22
	13	54.3 / 19	2.3 / 15	0 / 4	0.5 / 22
Airborne microbe	32	0 / 16	0 / 16	0.1 / 17	0 / 22
	13	0.6 / 18	0 / 16	0 / 4	0 / 22
Settling microbe	32	0 / 15	0 / 15	0 / 11	0 / 1
	13	0 / 17	0 / 17	-	-
Surface microbe	32	0 / 14	0 / 14	0 / 16	0 / 6
	13	0 / 16	0 / 16	-	-

2.4. Discussion

In an aseptic room it is difficult to predict the sampling hot spots based upon the results of microbial monitoring data because viable microorganisms are rarely detected by the sampling (*5–7*). However, using 3D-AFM and intensive challenged particle count monitoring will enable a user to develop an acceptably reliable monitoring plan. This approach also enables one to select the most appropriate sampling locations so that risk can be better assessed. As a result of this analytical approach, less useful sample points could be eliminated. In a highly ventilated clean room, the surrounding Grade A or Class 100 environment approached Class 100 conditions; only in the particle pockets like Region A and around the product weight check location were excursions beyond Class 100 in operation observed. The greatest attention should be paid to the particulate "spikes" emitted by human operators or machines (*1,2,6,7,8*). .The best method for detecting unusual or unexpected spikes is continuous particulate monitoring. Active airborne microbial monitoring at any location may not be as effective as settling plates (*6,9*), see Tables 2-1 and 2-2.

On the floor of this room, settling plates do not detect microbial even in the operating condition. Therefore, keeping monitoring equipment and/or wiping cloths etc on the lower shelf around 50cm from the floor around parallel unidirectional flow does not appear to be risky in terms of microbial

contamination of these items. In the case of this facility, Region A or up-mainstream should not be used for critical preparations or activities during or proceeding manufacturing operations is in Region B. Access to the filling machine from Region B and C would be safe because of the effective clearance in these interface zones as a result of consistent spilled unidirectional flow from Grade A and the resulting highly displacing airflow pattern.

Evolving technologies such as isolators and the blow-fill-seal aim to minimize the possibility of microbial contamination from the surrounding environment to the critical area by reducing the likelihood of human contamination. These technologies may reduce the risk of environmental contamination in aseptic processing compared to the conventional clean room which permits minimal access of qualified operators. The high potential for control of human contamination has made isolators and blow-fill-seal important alternatives to clean rooms. On the other hand, modern conventional clean rooms for aseptic processing can still be a low risk and convenient alternative, especially when the risk analysis approach described in this article is applied.

We believe that the aseptic processing environments defined in regulatory documents of authorities from around the world (*3,4,10*) would be better controlled if the sampling program was designed as a result of careful, in depth analysis of the entire aseptic processing environment. The Grade B surrounding

environment, which serves as an interface zone with the critical Grade A environment, should not affect cleanliness in the critical zone when the Grade A area is widely exposed to this interface zone. For this reason the interface zone should be qualified with respect to the release of viable contamination by aseptic processing operations.

2.5. Conclusion

The use of 3D-AFM enables a user to accurately assess risk and to determine the operating characteristics of zones within an environment. When evaluating the air zone mapping combined with particle count data, it is possible to optimize the working conditions within a turbulent Grade B area. Because of the inability of microbiological sampling methods to detect low levels of contamination, risk can be better assessed by the airflow evaluation techniques described in this study. We believe this method has great value in the analysis of the performance of existing clean rooms and also in the qualification of new clean rooms. Furthermore, we believe this analysis can lead to the elimination of inefficient environmental sample point selection.

2.6. References

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3. Monitoring minimization of Grade B environments based on risk assessment using 3D-AFM and computer simulation

3.1. Introduction

By utilizing the developed 3D-AFM and LMAA approach in section 2, author propose a practical risk-based monitoring approach which minimize the representative number of monitoring points used for microbial contamination risk assessment. Author conducted a case study on an aseptic clean room, newly introduced and specifically designed for the use of a Restricted Access Barrier System (RABS). However, we also found the floor surface air around the exit airway of the RABS EU GMP Annex 1 Grade A, ISO Class 5, room was always remarkably clean, possibly due to the immediate sweep of the piston airflow which prevents dispersed human microbes from falling in a Stokes-type manner on settling plates placed on the floor around the Grade A exit airway. In addition, the airflow is expected clean with a significantly low LMAA.

Based on these observed results, we propose a highly simplified daily monitoring program to assess microbial contamination in Grade B environments. Instead of particle or microbial air monitoring, we recommend the use of microbial surface monitoring at the main air exhaust. To locate hotspots we propose using a combination of computer simulation, actual airflow measurements and intensive environmental monitoring sampling at the environmental qualification stage.

These measures would be sufficient to assure the efficiency of the monitoring program, as well as to minimize the number of surface sampling points used at exhausts in environments surrounding a RABS.

Author conducted intensive microbial monitoring in a Grade B room under various conditions. We repeatedly obtained zero counts using traditional microbial monitoring methods. The results demonstrate the difficulty in finding microbial contamination hotspots for this type of clean environment. Although the presence of a large number of operators and the absence of sterile garment usage may yield occasional positive microbial monitoring results, such results would not clearly correspond to those obtained by particle monitoring. To identify theoretical hotspots, therefore, we then determined the three-dimensional airflow according a previously published and predictable method which used airflow analysis data collected through actual measurement and intensive environmental monitoring (*1*). Computer simulations are also expected to support experimental data (*2*). Machida and Maekawa reported (*3*) that computer simulation can predict locations where contaminants tend to remain in a clean room, defined as "hotspots". This is achieved through computer calculation and mapping of the local mean age of air (LMAA, 9), which calculates the average time particles remain after their entry into a room (*4*). Using this system, results obtained from the analysis of three-dimensional air velocity measurements and computer simulation were comparable. Overlapping of the microbial hotspots, particle

hotspots and stugnant airflow hotspots allowed the determination of distinct microbial contamination hotspots.

Here, based on the results of these experiments and simulations, we propose a reliable and an effective sampling method to determine specific sampling points. This simple and minimal sampling method is suitable for use in extremely clean Grade B environments in which RABS is used, but can also be usefully applied to conventional Grade B environments.

3.2. Materials and methods

3.2.1. Facility

The Grade B aseptic room used in this study has a floor area of 175 m^2 and a height of 3.0 m. The layout is shown in Figure 3-1. For computer calculations, the aseptic room was divided into five areas, Areas 1 to 5. Area 5 is physically segregated from other areas by a wall and door. The RABS is installed in Areas 2, 4 and 5, shown by the shaded area in Figure 3-1. The RABS is equipped with unidirectional air flow Grade A cabinets and HVAC coming directly from the ceiling. The cabinets open at the bottom. The air from the RABS HVAC exhausts from the bottom of the cabinet. The aseptic processing equipment includes a filling machine, an automated loading system and a crimping machine. The inside of the RABS, shown by the shaded area in Areas 2, 4 and 5 of Figure 3-1, is a Grade A area. The air change rate for the Grade B area is 80 times per hour, and

the total air change rate for the aseptic room, which is the arithmetic average of Grade A and B areas, is 180 times per hour. Human interventions are usually restricted, except during setup, unplanned interventions, and after completion of production. Material transportation is carried out in a closed system.

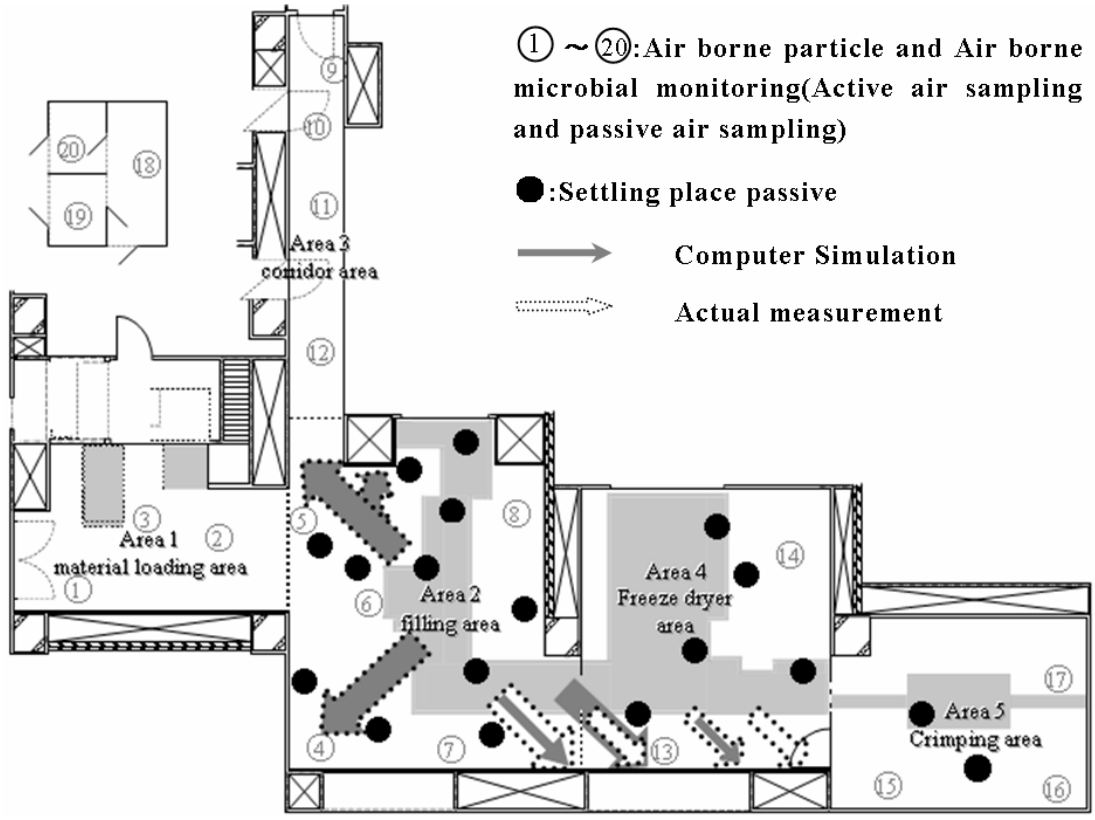

Figure 3-1. Facility layout, Area 1-5 and sampling points

3.2.2. Three-dimensional airflow measurement

a) Measuring equipment

Three-dimensional air velocities were measured with an ultrasonic anemometer model WA-590 used for clean rooms, and the

three-dimensional anemometer measurement software WASP-007 (KAIJO, Tokyo, JAPAN).

b) Measuring method

The three-dimensional air vectors in each area were measured using a 100 x 100 cm grid lattice, with measurement points created on the floor. The vertical measurements were taken at points located 50, 100 and 200 cm above the floor grid. The air vectors at each point were measured for 15 seconds. During measurements, the HVAC systems of both the aseptic room and RABS system were in operation, whereas the production machines were not in operation because during the production the operators would obviously be operational and could affect the measurements quite significantly. 3D-AFM were created for each divided area at points 50, 100 and 200 cm above ground level.

c) Computer simulation analysis of airflow

Based on the theoretical air velocity at the aseptic room's air outlet, simulations were conducted by Asahi Kogyosha Co., (Tokyo, JAPAN). The LMAA in each area was simulated using the computational fluid dynamics (CFD) module from Software Cradle Co., Ltd.

3.2.3. Environmental monitoring

Intensive microbial and particle monitoring was conducted for a period of six months during the qualification stage. Initially the non-sterile challenged conditions were monitored for six weeks. Five to fifteen people conducted machine qualification activities wearing non-sterile garments. Six people simultaneously collected various monitoring samples. The biological active air sampling points are shown in Figure 3-1, from numbers 1 through 20. Passive air monitoring using settling plates on the floor was also performed at points 1 through 20, and additionally at 18 black-spotted places in Figure 3-1. Surface sampling with contact plates was conducted at 152 points, including points 1 to 20 from Figure 3-1, 90 points on the floor and 40 points on perforated panels. Samples were collected on Days 1 to 9 in Table 3-2 from all defined monitoring points after every two to five operations days during which Areas 1 to 5 were all in use. Conditions on Days 1 to 3 had the greatest potential for contamination because of after heavily contaminating operations, whereas those from Days 4 to 9 had only moderate potential.

After a full cleanup of the areas, monitoring under full aseptic conditions was performed during the final three months. Three operators were normally present, and six were present during media fill conditions. The active and passive air sampling were performed which points are depicted by numbers 1 to 20 in Figure 3-1. Forty-three surface sampling points were used, including 24 points on the floor and 13 on different perforated panels.

Table 3-2. Environmental monitoring under challenged assessment conditions

Monitoring Method / Monitored Timing	Airborn microbial monitoring by active air sampling		Airborn microbial monitoring by passive air sampling		Surface microbial monitoring		Airborne particle count/ft³
	Total count/40 point	Incident Ratio	Total count/23 point	Incident Ratio	Total count/152 point	Incident Ratio	0.5 μ<
Day 1.	12	23%	4	17%	1490	91%	132.2
Day 2.	15	15%	0	0%	94	36%	51.6
Day 3.	2	5%	0	0%	243	41%	65.4
Average		14%		6%		56%	
Day 4.	0	0%	0	0%	2	1%	55.5
Day 5.	4	8%	0	0%	35	16%	75.1
Day 6.	0	0%	0	0%	27	11%	72.5
Day 7.	4	8%	0	0%	9	4%	59.9
Day 8.	0	0%	0	0%	1	1%	74.0
Day 9.	3	5%	1	4%	0	0%	32.4
Average		3%		1%		5%	

Day 1: No cleanup, approximately 15 people operating wearing a non-sterile, one piece garment worn on top of normal clothes.

Day 2 and Day 3: After a brief cleanup, 6 to 15 people operating similarly to Day 1.

Days 4-9: After a full clean-up and sanitization, six people operating wearing non-sterile clean garments.

Microbial surface monitoring

For surface monitoring, a 25-cm^2 soybean casein digest medium contact plate (Clean Petan, EIKEN KIZAI, Tokyo, JAPAN) was used. The floor, wall and perforated panel surface were sampled at 152 measuring points.

Airborne microorganisms monitoring by passive air sampling (settling plate)

A 9-cm diameter petri dish containing soybean casein digest medium was opened for one hour at monitoring points. Settling plates were set on the floor.

Airborne microorganism monitoring by active air sampling (centrifuge air sampler)

One cubic meter of air was sampled with an soybean casein digest medium strip using an RCS PLUS air sampler (Biotest, Frankfurt, GERMANY). Active airborne microorganism sampling was performed during the first six weeks at 50 and 100 cm above the floor surface.

Airborne particles

One cubic feet of air was consecutively sampled three times using the HIAC ROYCO 243A air sampler (Grants Pass, OR), and the average of the three measurements was used as the final measured value. For particle count evaluation, all particles greater than or equal to 0.5 μm particle diameter were evaluated. Sampling points are shown in Figures 3-1 and 3-2, from numbers 1 through 20.

Airborne particles were measured at 50, 100 and 200 cm above the floor surface.

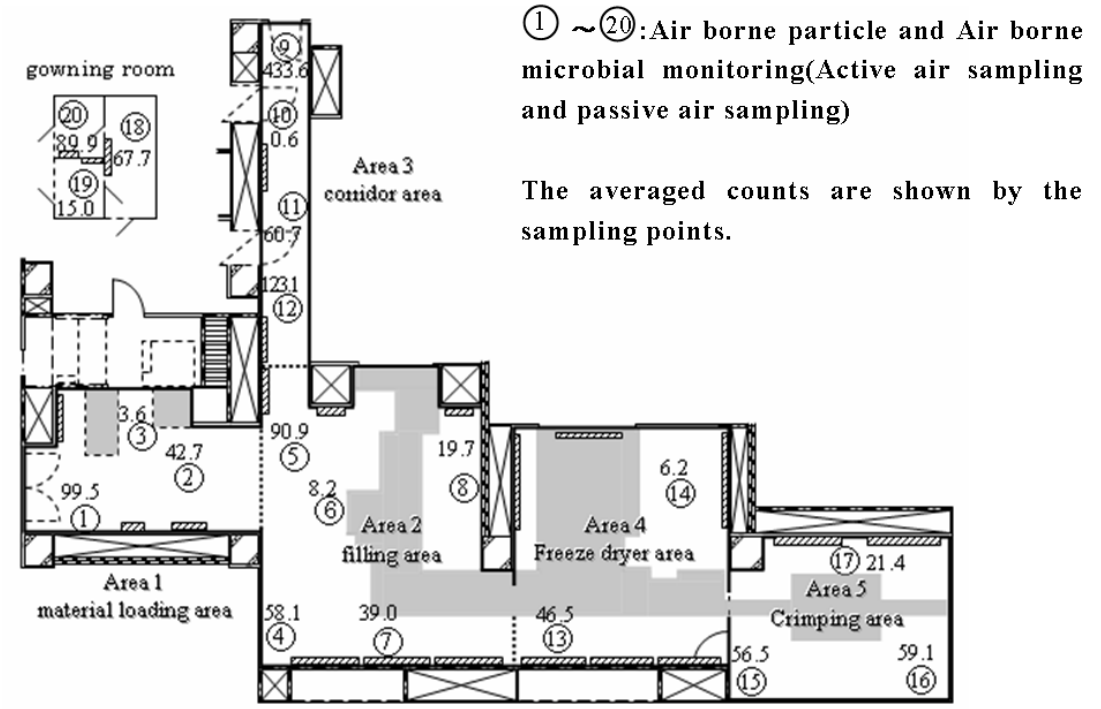

Figure 3-2. Average of 0.5μm airborne particle counts at the height of 50, 100 and 200 cm for 9 days

3.3. Results

3.3.1. Experimental results of three-dimensional airflow

The 3D-AFM of Area 2, which contained the installed RABS filling cabinet, is shown in Figure 3-3-1. Area 3, consisting of a corridor connecting the gowning room to the aseptic operational area, is shown in Figure 3-3-2. Because airflow characteristics are significant in these two areas, they were selected as representative of all five areas.

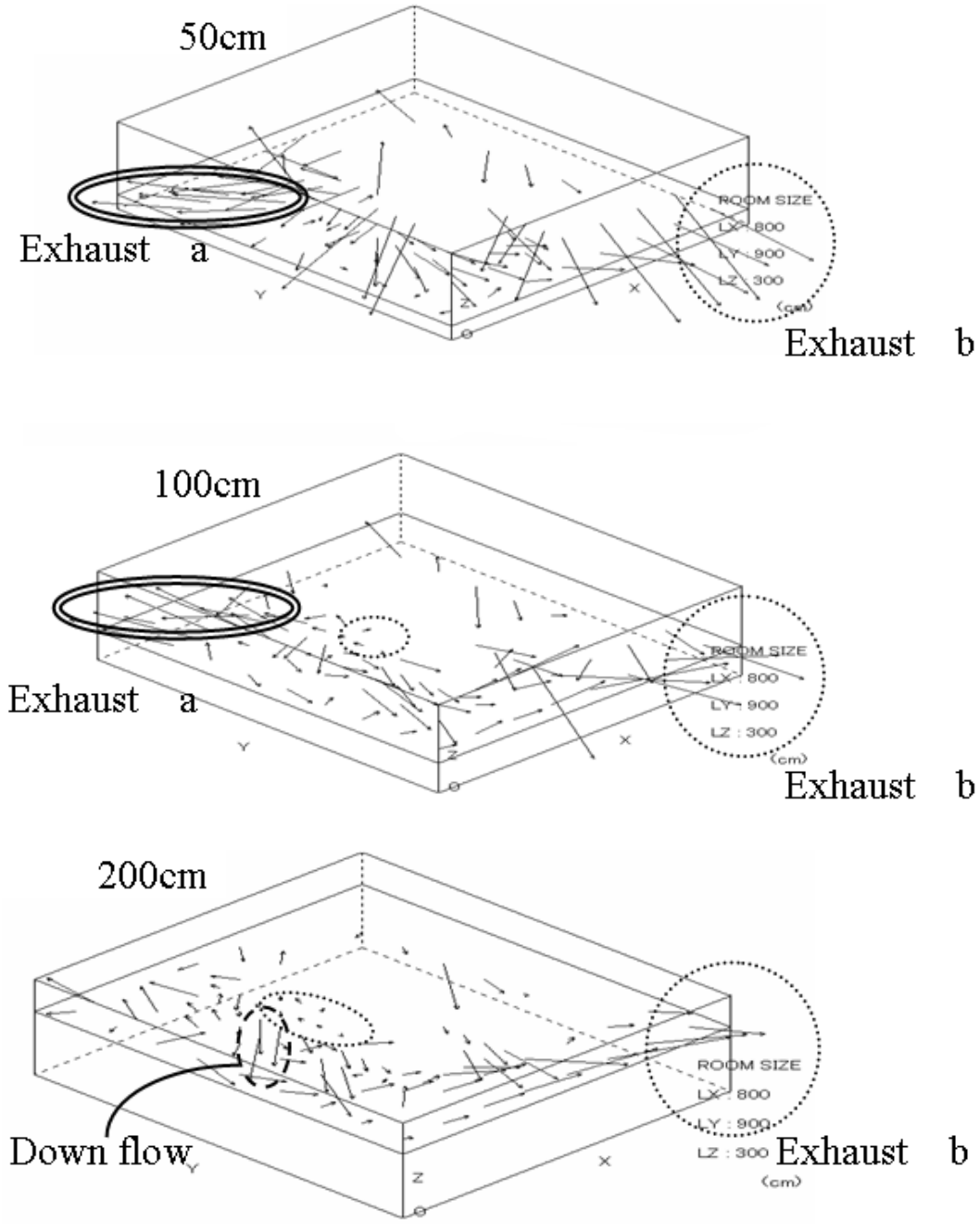

Figure 3-3-1. Airflow vector diagram of the Area 2 Filling area at 50, 100 and 200 cm above ground

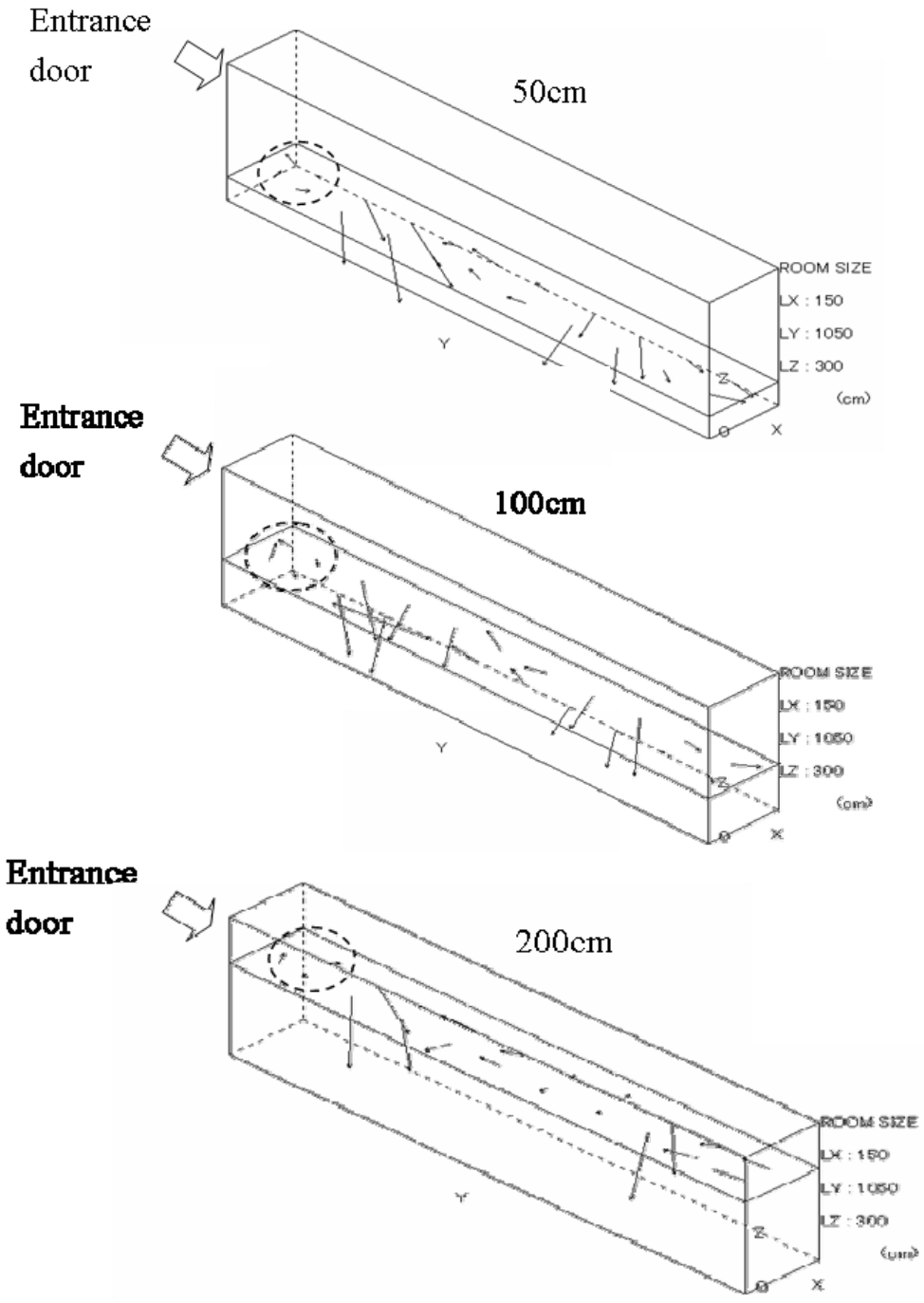

Figure 3-3-2. Airflow vector diagram of the Area 3 Filling area at 50, 100 and 200 cm above ground

The major air streams flowing to Exhaust **a,** double-circled in Figure 3-3-1 at 50 cm and 100 cm above ground, appear on the line showing the RABS Grade A exit airway. The empty white central area in Figure 3-3-1 represents the RABS space. The air streams, namely piston airflows on the floor surface, are represented by arrows labeled Flow X, Y and Z on the computer simulation map in Figure 3-4-2. No piston air streams to Exhaust **a** are found at 100 cm in Figure 3-4-1, although they are clear at 50 and 100 cm in Figure 3-3-1 and at 50 cm in Figure 3-4-2. Exhaust **b,** represented by a dotted circle in Figure 3-3-1, also collects the piston airflow at 50 cm. Exhaust **b** also collects strong circulating air streams from the left corner of the room at 100 cm and 200 cm. No clear streams to Exhaust **a** and **b** at 100 cm are observed in Figure 3-4-1. Piston airflow can also be seen in Area 4.

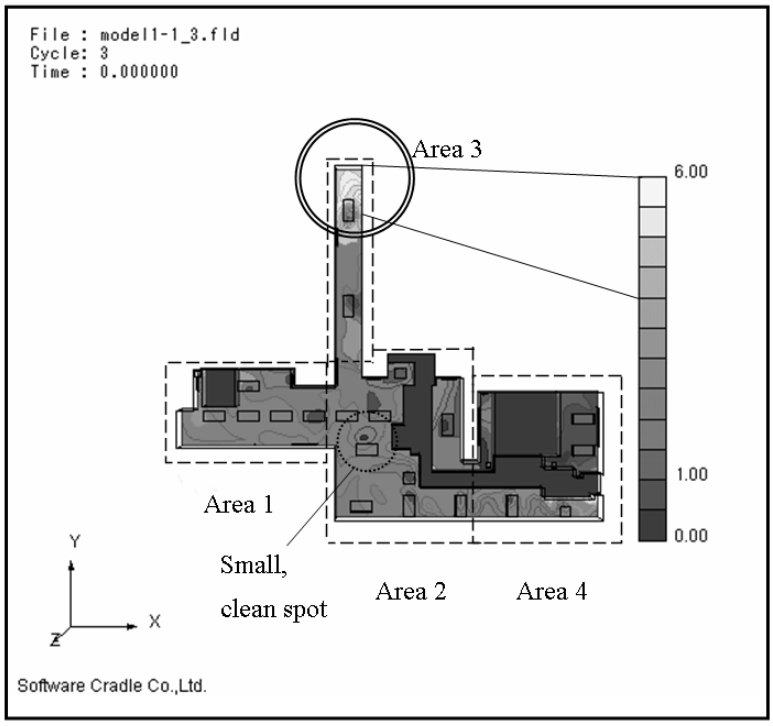

Figure 3-4-1. Average age of air in the aseptic room at 100 cm above ground.

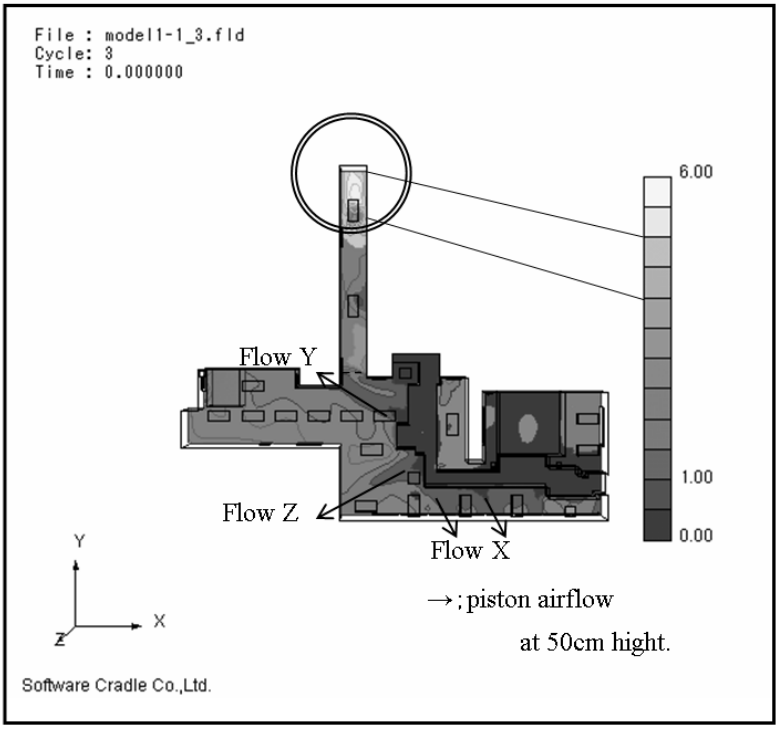

Figure 3-4-2. Average age of air in the aseptic room at 50 cm above ground

Piston airflows observed in both the actual measurement results and computer simulation results are summarized by arrows in Figure 3-1. The two results are in agreement for Exhaust **a**, as well as in one run at the corner of sampling point number 4 in Figure 3-1. The other arrows also generally agree in direction but not in relative strength. In Figure 3-3-1, a weak airflow spot was observed in the center of Area 2 at 100 cm and 200 cm, circled by a dotted line. Other areas also have several weak turbulent airflow spots.

From actual measurements, Area 1 showed a uniform air velocity distribution with no stagnant zone, which is representative of a turbulent air mixing zone. This was also observed from the computer simulation results, shown in Figures 3-4-1 and 3-4-2. In Area 3, a significant stagnant zone consisting of a type of dead leg air circulation was observed in front of the entrance door. This is represented by dotted circles in Figure 3-4.

3.3.2. Average air velocity in each Area

The average air velocity in each area is summarized in Table 3-1. In Areas 2 and 4, the air velocity tended to be higher at lower elevations, where piston air streams flow. However, no correlation between air velocity and elevation from the ground was found in Areas 1, 3 and 5, with only normal mixing turbulent zones considered to be present. In Areas 2 and 4, piston air flows from the RABS Grade A exit airway to the exhausts. These were observed in both the actual

three-dimensional airflow map in Figure 3-3 and the computer simulation map in Figure 3-4, where arrows cover zones with a considerably low LMAA value. A correlation between the piston airflows and the lower average particle concentrations at 50 cm in Areas 2 and 4 is also observed.

Table 3-1. Average air velocity for each measured point in each divided area

Computer simulation

Area		Area 1	Area 2	Area 3	Area 4	Area 5
Average air velocity (cm/s) ***Average particle counts (counts/ft3)***	*50 cm	27.0 ***65.8***	61.1 ***32.7***	31.6 ***178.5***	109.0 ***7.9***	42.6 ***50.7***
	*100 cm	26.8 ***51.5***	44.3 ***40.3***	32.5 ***151.4***	51.4 ***37.2***	37.6 ***48.5***
	*200 cm	27.2 ***28.5***	34.5 ***56.5***	24.1 ***131.0***	5.9 ***34.1***	35.3 ***37.9***

Areas 2 and 4 show significantly rapid air velocity at 50 cm. These areas take into account the piston airflows on the floor surface. The particle concentrations at 50 cm were lowest in Areas 2 and 4, whereas higher elevation points gave smaller particle counts in other areas.

*: Height from the floor

3.3.3. Airflow analysis using computer simulation

The air velocity distribution, LMAA and airflow vectors for Areas 1 to 4 drawn by computer simulation are shown in Figures 3-4-1 and 3-4-2. The simulated results generally agree with the experimental airflow data.

3.3.4. Local mean age of air (LMAA)

A LMAA map is a clear way of mathematically representing air cleanliness in a room. Lower LMAA values denote faster air changes. The end of the corridor in Area 3 shows a significantly high LMAA value, depicted by a double circle in Figures 3-4-1 and 3-4-2. This zone corresponds with the stagnant zone shown in Figure 3-3-2. Areas 2 and 4 show a lower LMAA value at 50 cm than at 100cm. There is overlap between the low LMAA zones at 50 cm and the zone where piston airflows are represented as strong unidirectional vectors in Figure 3-3-1. The piston airflow zone at 50 cm generally corresponds to the significantly low LMAA zone in Figure 3-4-2.

A small spot with a significantly low LMAA value at 100 cm is marked as the "small, clean" spot in Figure 3-4-1. This corresponds to sampling point 6 in Figure 3-2, with a particle count of 8.2 particles $\geq 0.5\,\mu$ per cubic foot. A ceiling HEPA is located over this spot and the effects of its down-flow are observed at 200 cm in Figure 3-3-1. Low particle counts can be determined through the use of LMAA.

3.3.5. Environmental monitoring results

The conventional microbiological monitoring method is not sufficiently sensitive for the increasing cleanliness levels found in today's clean rooms. To measure the

level of contamination in a room, the incident ratio is used, which is a percentage of the number of times microbes are detected over the total number of times monitoring is conducted. Table 3-2 shows the monitoring results from a room under challenged conditions, with Day 1 as the day with the most contamination detected. Incident ratios for the major part of surface-borne microbes and for airborne microbes were 91% and 23%, respectively. Under these conditions, no microbes were detected by passive air monitoring of the floor. An approximately 1/5 to 1/10 reduction in the average incident ratios from each microbial monitoring method was observed after Day 4. However, no significant difference was found between particle counts from Days 2 and 3 and those from Days 4 through 9. In addition, no correlation was found between particle counts and the microbiological cleanliness of the room. Almost no passive airborne microbes were detected on the settling plates placed on the floor. No significant correlation was found between airborne microbe counts and surface microbe counts. This might be due to the various types of daily operations taking place in the room.

Table 3-3 shows the monitoring results from the room under full aseptic conditions after a complete cleanup. Airborne microbes were rarely detected in Areas 1 through 5. After cleanup, considerably different cleanliness levels were observed in the gowning room and in Areas 1-5. A substantial number of microbes was detected in the gowning room by passive air sampling and surface monitoring, whereas no microbes were detected by active air sampling. In contrast, only a few

surface microbes were detected from the floor and perforated panels of the aseptic processing room, and no surface microbes were detected on the operational touch panels and walls of machines. No microbes were detected in air samples from Areas 1-5.

Table 3-3. Detection ratio of microorganisms from environmental monitoring

	Aseptic Grade B Room			Grade B gowning room		
Validation stage	Airborn microbial monitoring by active air sampling	Airborn microbial monitoring by passive air sampling	Surface microbial monitoring	Airborn microbial monitoring by active air sampling	Airborn microbial monitoring by passive air sampling	Surface microbial monitoring
Pre-Media Fill	0.0% (0/17)	0.0% (0/5)	0.0% (0/60)	0.0% (0/3)	0.0% (0/3)	50% (3/6)
PQ	0.0% (0/39)	0.0% (0/39)	0.0% (0/216)	0.0% (0/27)	3.7% (1/27)	25.9% (7/27)
Media Fill	0.0% (0/43)	0.0% (0/43)	1.6% (4*/260)	0.0% (0/30)	3.3% (1/30)	20.0% (6/30)
Stability sample production	0.0% (0/125)	0.0% (0/117)	0/0% (0/646)	0.0% (0/81)	3.7% (3/81)	25.4% (16/63)

Incident ratio: % of detection times/sampling times

* Two microbes came from the floor's shallow pit for cleaning wipers, another from "Exhaust **a**" in Figure 3-3-1, and the other from a perforate panel in Area 1.

Figure 3-2 shows a map of the average particle concentrations in Areas 1-5. The arrows indicate the piston flow as determined using the 3D-AFM and computer simulation maps. No significant correlation was found between piston flow and average particle concentrations. However, sampling point 9 at the end of corridor,

shown as the double-circled zone in Figure 3-4 as well as the dotted-circle zone in Figure 3-3-2, shows a significant count of 434 particles.

Figure 3-5 is a comparison of the incident ratio of active air sampling near the perforated panels and the incident ratio of surface microbes on the perforated panel in Table 3-2 for Days 4 through 9. Microbes were mainly detected on specific exhaust perforated panels and at the end of the corridor. In Areas 2 and 4 the perforated panel surface sampling generally showed a higher incident ratio than the active air sampling under challenged conditions.

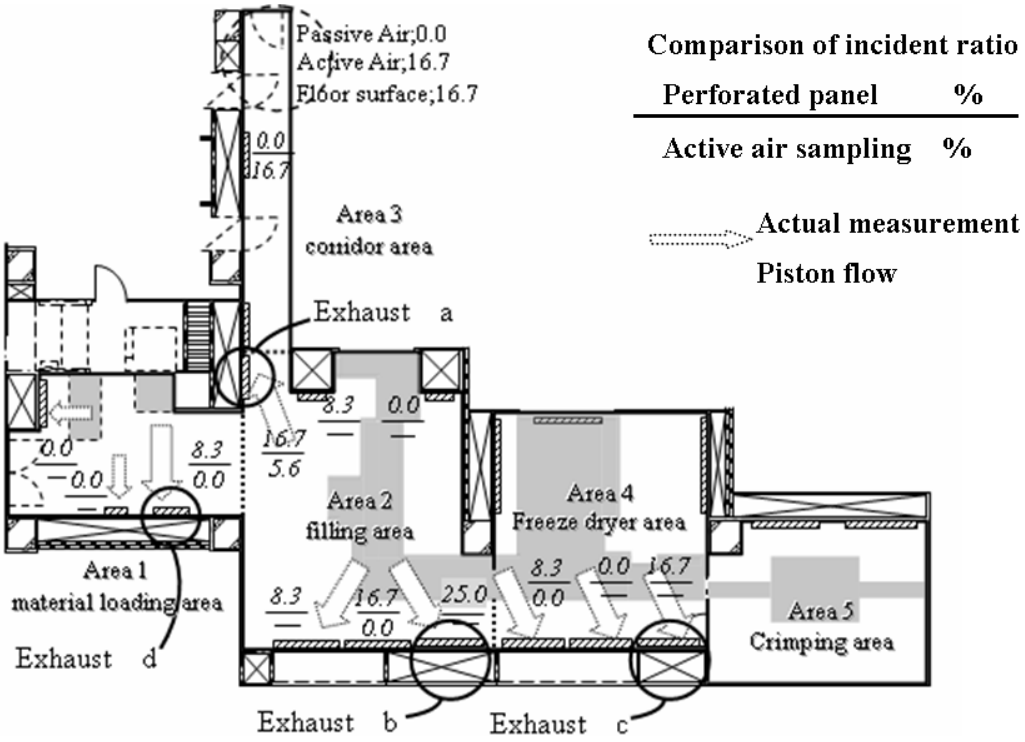

Figure 3-5. Comparison of the incident ratios from air sampling and surface sampling during Days 4-9

3.4. Discussion

3.4.1. Cleanliness and contamination risk level of Grade B rooms

The total environmental monitoring data shown in Table 3-3 reveal that microbes were rarely detected after a complete room cleanup. These results show that extremely clean conditions can be achieved in an entire aseptic room through the use of a high performance HVAC system designed for Grade B rooms. The microbial contamination risk from our Grade B room to the aseptic products in the RABS Grade A environment is therefore expected to be extremely low. Based on the risk calculation method proposed by Sandle (*5*), which gives one point for every one microbe detected in a Grade B room using a microbial monitoring method and five points for every one microbe detected in a Grade A room, the critical threshold value for the overall monitoring result during a batch production day is suggested to be 25 points. As shown in the footnote of Table 3-3, the maximum microbial counts per sample, detected from the floor in one day, were 2 CFU, thereby giving a maximum value of two points, much lower than the proposed threshold value of 25 points.

The USP Forum <1116>Microbiological Evaluation of Clean Rooms and other controlled Environments (*6*) recommends a contamination incident rate for Grade B environments of no more than 3% for airborne microbes and surface microbes, independently of sampling point sensitivity to contaminants. Low contamination counts were observed at predictable hotspots, shown in Table 3-3. When

evaluating the incident ratio trends of this Grade B environment, the 1.6% incident ratio found during a media fill run under the most contaminated conditions is considered a suitably low value.

To assess the risk level of a room using the proposals from both Sandle and the USP Forum <1116>, monitoring of a selected number of hotspots on perforated panels using a microbial surface monitoring method is sufficient. The other monitoring points in the room are considered significantly clean and below the risk level threshold.

3.4.2. Microbial air monitoring and particle monitoring efficiency in Grade B rooms

Results from Tables 3-2 and 3-3 show that after room cleanup, microbial air monitoring was inefficient in assessing the contamination level of the extremely clean Grade B room. In contrast, surface sampling at specific perforated panels from the exhaust seemed efficient. Results from Table 3-2 also show that particle monitoring could not clearly distinguish the room's change in microbial concentration conditions. For example, even though microbial counts were higher on Days 2-3 than Days 4-9, particle counts were similar.

The average incident ratios during Days 1-3, shown in Table 3-2, indicate that without room cleanup and clean garments, each monitoring method is sufficiently

efficient in detecting microbial contamination risks. For example, during this period, the incident ratios from active air sampling and surface sampling were 14% and 56%, respectively. After Day 4, ratios subsequently dropped to 3% and 5%. Based on these incident ratio results, surface sampling seems to be an effective method of detecting microbial contamination risks in Grade B environments. However, comparing the efficiency of sampling methods by analyzing the total average ratio under different sampling conditions is difficult. To clarify the differences between monitoring methods, their local incident ratios were compared. Figure 3-5 shows the local incident ratios from perforated panel surface monitoring and from active air monitoring, where the higher local incident ratio values for surface monitoring at Exhausts a, b, c and d ranged between 8.3-25.0% in Areas 1, 2 and 4. In comparison, with the exception of the corridor in Figure 3-5, active air sampling showed lower incident ratio values ranging between 0.0-5.6%. These results demonstrate the superior efficiency of perforated panel surface sampling over active air sampling in Areas 1, 2 and 4. Collecting contaminants using an air sampler during the monitoring of piston airflow seemed inefficient due to the air's low LMAA. In contrast, the exhaust collecting the major air streams provides reliable overall recorded surface contamination data, and appears better suited as a hotspot sampling point. In corridor Area 3, where a circulating stagnant zone is present instead of piston airflow, the incident ratios from active air sampling and perforate panel sampling were 16.7% and 0.0%, respectively. In this case, active air sampling of the stagnant zone appears superior

to surface sampling.

3.4.3. Grade B gowning rooms

Based on the high incident ratio from the gowning room's passive air sampling results shown in Table 3-3, the use of settling plate monitoring from the floor is possible in some cases. However, Andon mentions that "passive air sampling is inferior to the active air sampling for its low recovery level. There may be no advantage in performing two parallel samplings for the detection" (*7*). In the case of the gowning room, although surface sampling on perforated panels was the most efficient sampling method, air monitoring using a settling plate on the floor was more efficient than active air sampling, due to the gowning room's clearly designed down-flow in narrow rooms, the absence of piston air flow and the high risk of scattering particles from human operators.

3.4.4. Adequate monitoring program in a Grade B environment

From the above discussions about the incident ratios in Tables 3-2 and 3 and Figure 3-5, we suggest an adequate daily monitoring program for this Grade B processing room environment. For Area 3, active air sampling and floor surface sampling in the area depicted by a dotted circle in Figure 3-5 would represent an adequate monitoring program. For Areas 1, 2 and 4, only one to four surface samplings of perforated panels at Exhausts **a**, **b**, **c** and **d** would be sufficient to efficiently detect the contamination risk of a Grade B room. As seen in Figure 3-5,

monitoring results at the Exhaust **b** surface showing an incident ratio of 25% indicates, as seen in Table 3-2, a whole room contamination level of approximately 5%. This is close to the action level where, for the same conditions and as shown in Table 3-2, a 3% incident ratio is expected for airborne microbial monitoring by active air sampling, which is also the incident ratio recommended by the USP Forum <1116> for Grade B environments. Surface monitoring at Exhaust **b** is apparently able to provide the earliest action level alarm warning of problems in this Grade B environment.

3.4.5. Small stagnant spot

According to Figure 3-3-1, a stagnant spot of weak airflow is present in the center of the clean room. However, due to the presence of piston airflow and down-flow, this is not considered a hotspot. This small stagnant spot, indicated by the low LMAA area inside the dotted circle in Figure 3-4-1, is located near particle sampling point 6 in Figure 3-2. The 0.5 μm particle counts at 50 cm and 200 cm above ground were 0-3 and 2-47 particles, respectively, with an average count of 8.2 particles for all elevations, which is considerably clean (Figure 3-2, point 6). Wada and Ogawa showed that the correlation between the contaminant dilution time of stagnant spots similar to point 6 and air velocity of the piston airflow is an important parameter for calculating room air change ratios (*8*). This suggests that local small areas of air stagnation can be quickly diluted in this highly ventilated room, with the exception of the segregated large stagnant zone in Area 3.

3.4.6. Hotspot localization using three-dimensional airflow measurements and computer simulation

The computer simulation depicted in Figure 3-4 predicts the presence of piston airflow and clean air zones with low LMAA values at 50 cm above ground for Areas 1, 2, 4 and 5, as well as the stagnant zone with a high LMAA value for Area 3. Actual measurements of the three-dimensional airflow accurately located the critical exhausts, shown by a group of strong unidirectional arrows in Figure 3-3-1 and 3-4-2, as well as the local weak stagnant zone. In addition, a small down-flow from the ceiling HEPA can also be located at 200 cm on the map in Figure 3-3-1. However, a detailed map of the border and corner air zones could not be mapped due to a limited time schedule and consequent small number of actual measurement points.

When understanding of a room's airflow is limited, computer simulations can assist and cover details such as room borders and corners. Although the present simulation could not accurately predict the strength of the piston flow shown in Figure 3-1, it could accurately predict the "Small, clean spot" shown in Figure 3-4-1. In cases when the number of actual sampling point measurements must be minimized, it is possible to conduct computer simulations alongside actual measurement to provide a better understanding of a room's air circulation. Furthermore, computer simulations can provide feedback regarding actual

intensive monitoring results, and in addition help provide a better understanding of the assessed conditions of an aseptic room during its environmental qualification stage.

3.5. Conclusion

From a practical point of view, the low sensitivity of microbial air monitoring means it is not an adequate method of determining contamination risk in extremely clean Grade B environments. Further, particle monitoring cannot detect changes in microbial contamination risk in this type of Grade B environment due to its higher detection threshold. In extremely clean Grade B environments used as a surrounding for RABS, microbial monitoring of the surface of critical exhaust perforated panels appears to be the most reliable daily monitoring method during manufacturing operations.

The exact monitoring points can be determined using a combination of 3D-AFM simulations, mapping of the average local air age, actual three-dimensional airflow measurements and intensive environmental monitoring during the environmental qualification stage. Selection of the optimal monitoring program based on the setting to be assessed, analysis of the sampling points and the incident ratio for each microbial monitoring method can provide useful information about contamination risk.

3.6. Glossary

Hotspot; A hotspot is a location that has a high contamination probability, caused by scattering of human microbes.

Local mean age of air (LMAA); The local mean age of air is calculated as the average time air remains after entry into a room. When a room is perfect turbulent this value is same as the reciprocal number of the number of air changes per hour. When a room is perfect piston airflow that is a half of the reciprocal number the number of air changes per hour.

Piston air flow; Major piston airflow is the unidirectional airflow coming from the RABS cabinet opening, and running along the floor surface and occasionally directly reaching the air exhaust. Perfect piston air flow is laminar air flow.

3.7. References

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room. *Process Validation for Production for Sterile Product* (in Japanese), Morikawa, K., Ed.; Kodansha: Tokyo, **2006,** 429-437.

4. Comparative results of air flow characteristics mapped by CFD simulation and actual measurements of 3D-AFM and particle concentrations

4.1. Introduction

In this section distribution maps of local age of air and air flow velocity produced by CFD simulation were compared with actual measurements of particle concentrations at eighteen monitoring points as well as 3D-AFM in 1-m grids in an aseptic pharmaceutical manufacturing room. As a result, the air flow patterns assessed by both approaches of simulation with the standard κ-ε model and actual air vector measurements were comparable under conditions of high air change time's aseptic room. In this case study particle concentration and LMAA showed no clear relationship due to very low counts of particles. However, CFD simulation displayed its advantages in giving comprehensive air flow pattern and recovery efficiency at local points in aseptic room.

If actual contamination risk can be predicted by LMAA, it can be a beneficial approach which dose not requires heavy actual measurements. Machida conducted the trial which aimed to discover a hot spot in a clean room. It was shown that LMAA correlates with distribution of airborne particulate concentration in the room where an air change rate is low and where particle concentration is high. However in case of LMAA simulation on Room1 Figure 1-2, actual measurement

3D-AFM did not match to calculated LMAA. In order to examine whether critical location presumption by LMAA by CFD simulation which show the metrics of local air age and air particulate concentration in aseptic room of high air change rate is possible. When a more exact simulation is performed, using aseptic room without air leak from a door, the measurement result of the actually measured 3D-AFM and the result of measured air particulate would be comparable. and it would make clear to verify whether presumption of the hot spot by CFD is possible.

In case of section 2, Grade B of Room 1 Figure 1-2, did not give clear result of correlation between the LMAA and air particulate concentration. The reason was not found out although it was considered that there might be some deficiency of used parameters, even those given as measured value for calculation. The real condition may more complicate on account of air intake and exhaust through invisible gap of seals and door slits in the room. After the LMAA simulation author conducted actual 3D-AFM which carried out as independent research. Based on the 3D-AFM measurement by author, there was a partial deficiency of air flow velocity in CFD simulation LMAA mapping. The simulated LMAA result was inaccurate around the doors. Author thought that this may have influenced correlation with LMAA and air particulate concentration.

Author conducted one more case study to determine the accuracy and the

usefulness of LMAA by CFD. Another facility which is a newly introduced qualified aseptic room was employed for this study. In this study LMAA is expressed as SVE-3.

4.2. Materials and Methods

4.2.1. Facilities

The 3D-AFM was performed in the aseptic room in which the filling machine which manufactures a freeze-drying vial, a capping machine, a freeze-drying machine, and automatic carrying-in equipment were installed. This aseptic room is 240m2 of floor area, and the zone of Grade A is established in the interior of a room of Grade B. An air change rate is 80 times per hour in Grade B. Grade A is 270 times per hour. Temperature was controlled at 21±3 degrees. Humidity was controlled in 40-70%.

4.2.2. Airborne particle measurement

The used particle counter is HIAC/ROYCO243A (Royco). Figure4-1 shows measuring point ① - ⑱. Sampling time was one-minute for 1 ft^3. To check off the tube flushing it took 1 minute after sampling. More than 0.5 μ airborne particulate was counted. In July 2004 measurements run three times a day and collect the average value of three days. Also measurements performed at ⑦ ⑫ ⑮ in April 2005 to March 2006.

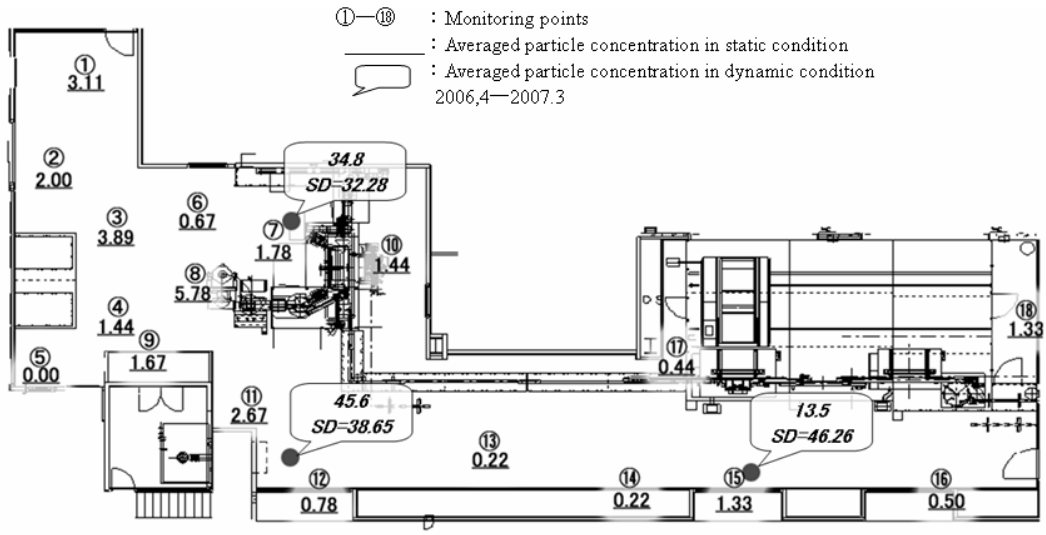

Figure. 4-1 Air born particle concentration at each monitoring points in aseptic manufacturing room (0.5μm)

4.2.3. Measurement and analysis of a 3D-AFM

Measurement is done on grid which is 1 m square and 1 m in height measurements in the aseptic room based from the corner. The measurement equipment of three-dimensional ultrasonic anemometer (WA-590, KAIJO) vector is using to measure airflow and using the software WASP-007 (KAIJO Companies) for analysis and visualization.

4.2.4. Three-dimensional computer simulation of airflow

For three-dimensional computer simulation of air flow velocity, three-dimensional heat fluid analysis system STREAM for Windows Ver.4 and standard κ-ε / CFD models (SOFUTOUEAKUREIDORU) was employed. The LMAA distribution in

Grade A, B in aseptic room was simulated in condition of the overall ventilation rate is 270 times per hour.

4.3. Results

4.3.1. Airborne particulate measurement results

Figure4-1 shows measured concentration of airborne particles at ① - ⑱ of more than 0.5 μ m. In static condition the averaged particle concentration was less than 10 ft^{-3} at all points and the value is low enough. in as low. At point ⑦ ⑫ ⑮the average value of 13.5 to 45.6 units ft^{-3} were obtained during the period and a standard deviation of 32.8 to 46.2 per ft^3 seems in large variation.

4.3.2. Three-dimensional simulation of airflow

Figure4-2 shows air velocity distribution of the simulation results. Grade B shows 0.2 m s^{-1} to 1.82 m s^{-1} of air velocity. The averaged air velocity around ③ ⑥ was below 0.20 m s^{-1} , this is low value. The air velocity distribution around exhaust, ⑯ ⑰ ⑱, was high of 0.4 - 1.0 m s^{-1} .

Figure4-3 to Figure4-5 are visualized simulation air velocity and measured 3D-AFM.. These simulated distribution and actual 3D-AFM are comparable. The combined map of airflow pattern and LMAA works well as a comprehensive presentation.

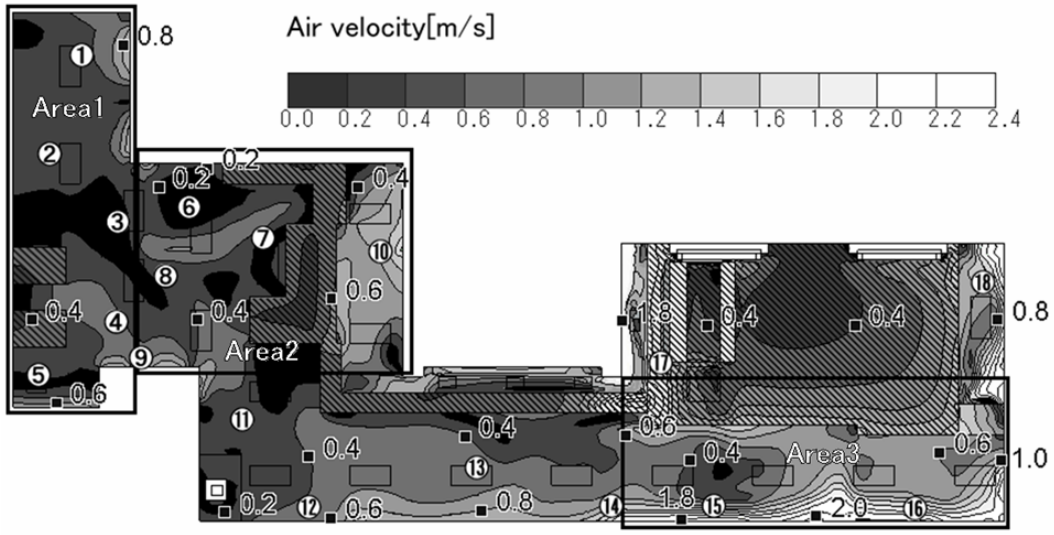

Figure. 4-2 Simulated distribution of air velocity

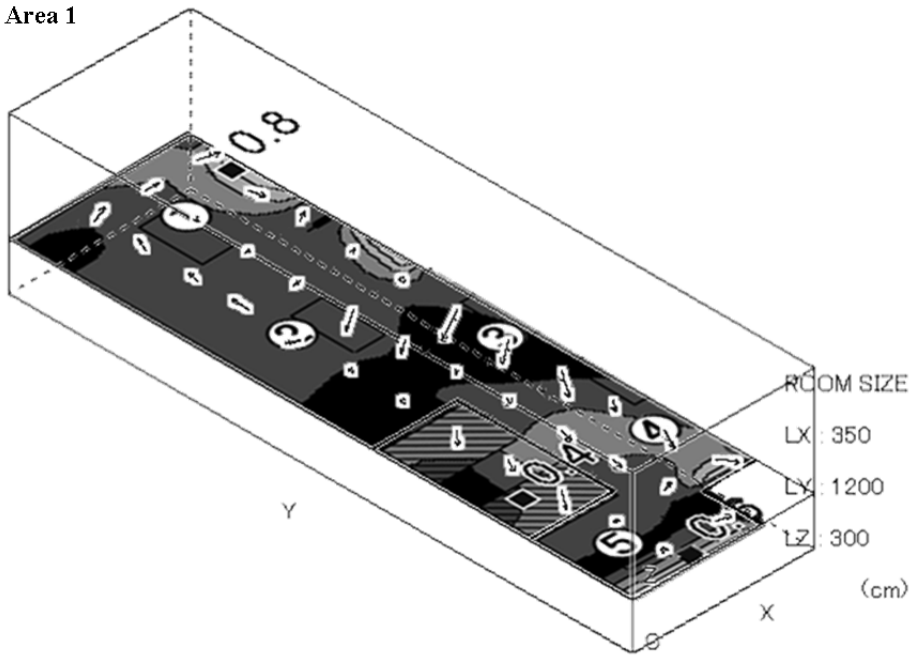

Figure. 4-3 Actual air vectors and simulated distribution of air velocity in Area 1

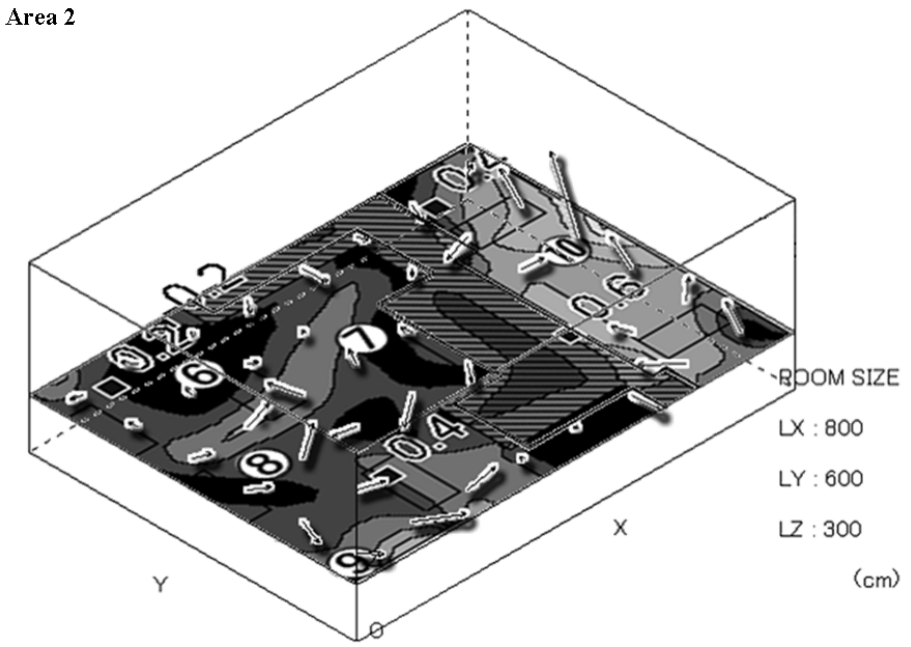

Figure. 4-4 Actual air vectors and simulated distribution of air velocity in Area 2

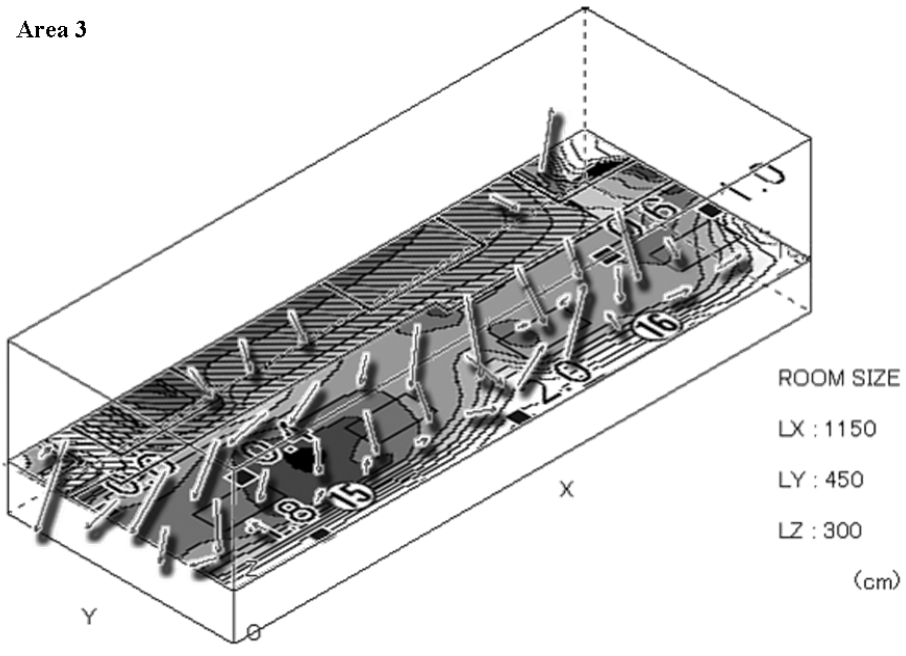

Figure. 4-5 Actual air vectors and simulated distribution of air velocity in Area 3

4.3.3. SVE-3 as LMAA and air velocity simulation

Figure4-6 is the focused partial figure of SVE-3 distribution in Area 2. Figure4-2 and Figure4-4 shows comparability of air velocity from both simulation and actual measurement. This Figure4-6 shows the predictability of the value of SVE-3 from air velocity. Small vectors around ⑥ in Figure4-4 is in white in Figure4-6. The SVE-3 looks near 2.25, very high value which means slow ventilation.

Figure4-7 shows the entire results of the local air age as SVE-3. In the aseptic room SVE-3 distributes from 0.25 to 2.25. The whole room ventilation rate is 270 times. SVE-3 1.0 is therefore calculated at 13.3 s (3600 s/270 times).

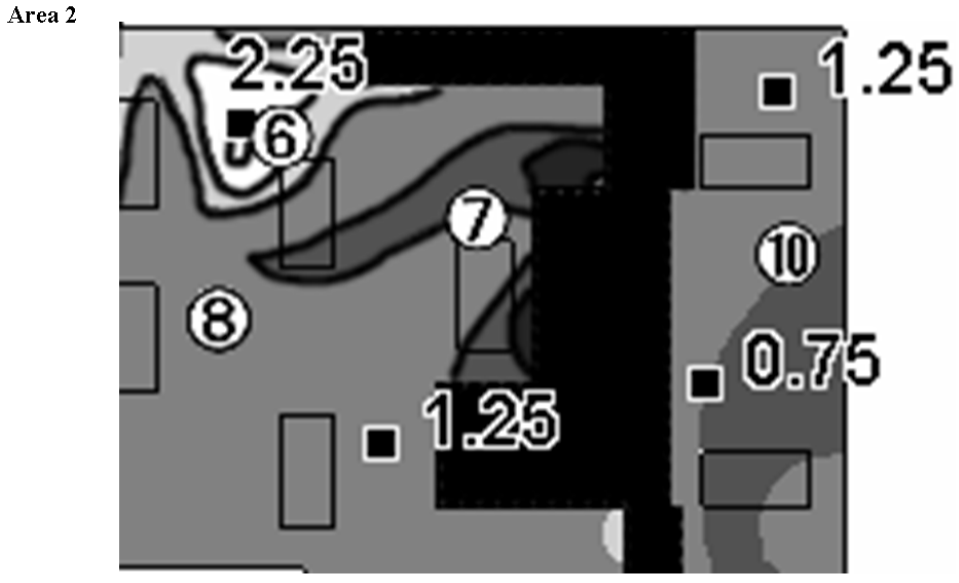

Figure. 4-6 distribution map of local mean age of air simulation (SVE-3)

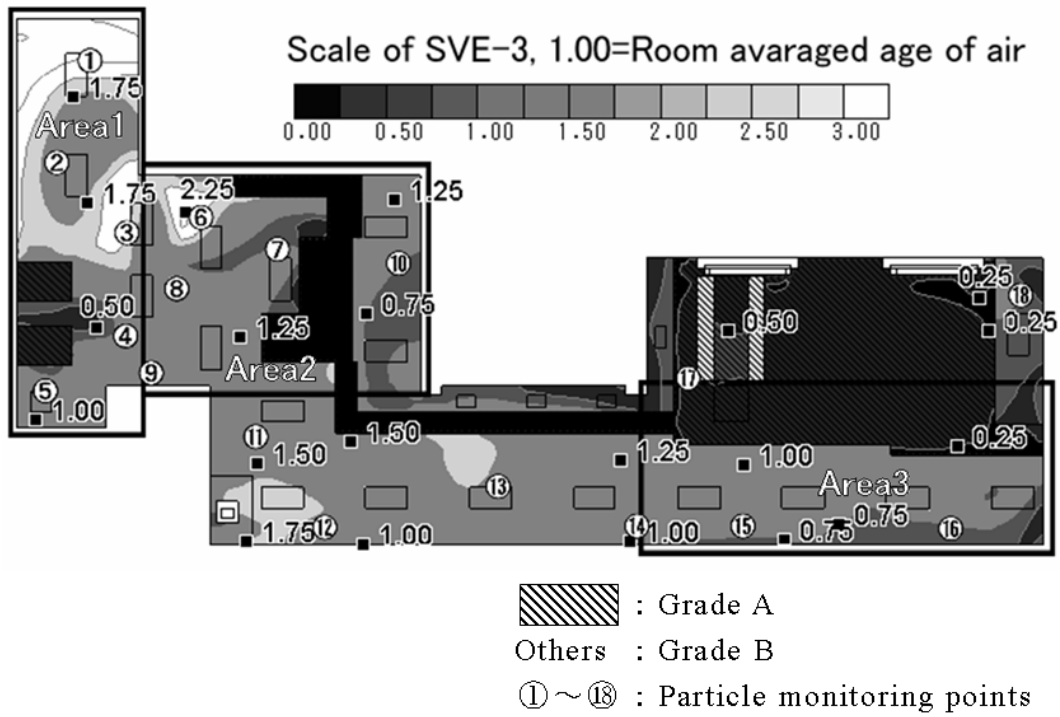

Figure. 4-7 Simulated distribution of local age of air as SVE-3 value

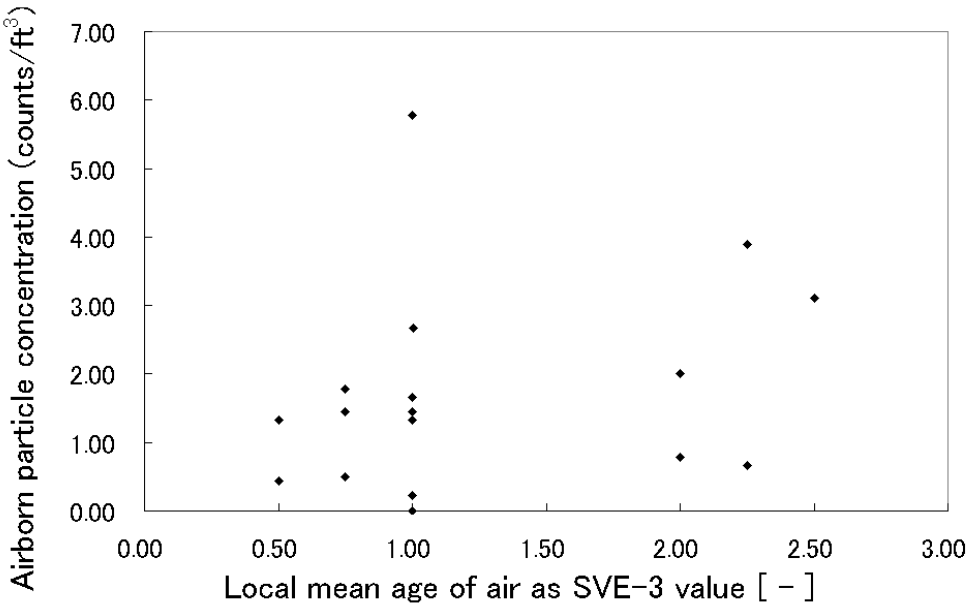

Figure. 4-8 Relationship between local age of air as SVE-3 value and particle concentration at each monitoring points

Figure4-8 shows SVE-3 and the corresponding concentration of airborne particles. In SVE-3 increase the value, higher concentration of airborne particles tend is observed. However even SVE-3 is in higher value in several points the low concentration of airborne particles are observed.Figure4-9 shows the relationship between local air velocity and SVE-3. The local air velocity and SVE-3 is in correlation to the inverse of the air age. In this study case, the coefficient of 0.4 and 0.8 to two groups are observed in the figure.

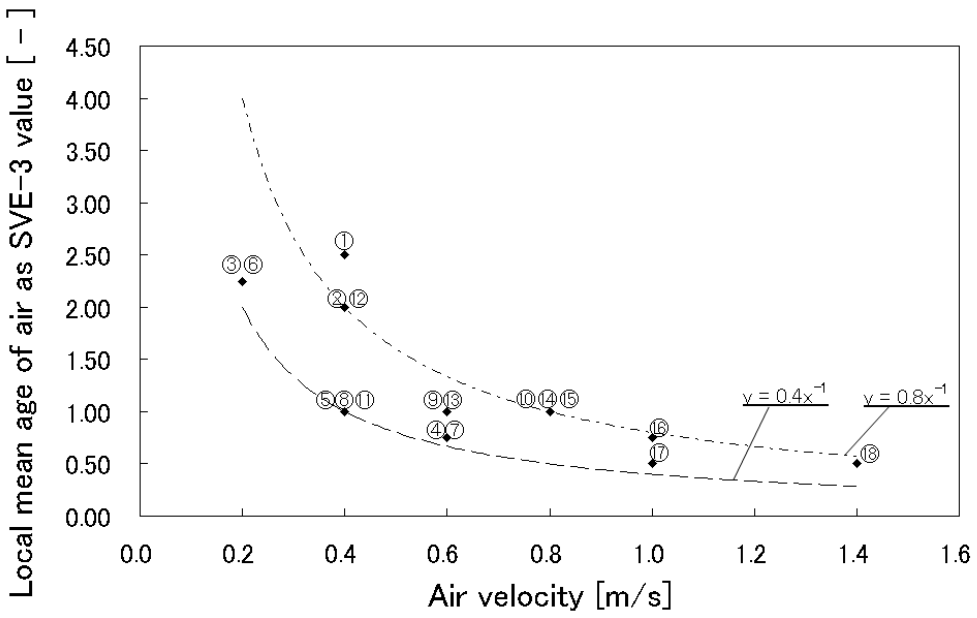

Fig. 4-9 Relationship between simulated local age of air as SVE-3 value and air velocity

4.4. Discussion

4.4.1. CFD simulations of reliability

To simulate the airflow pattern by the κ-ε standard models observed it is relatively consistent. The use of the κ-ε standard model for the analysis of air in the facility is revealed to be appropriate. The κ-ε standard model is assumed that in case of isotropic turbulence the direction is highly regulated to one way. As a result the diffusion of particle is influenced too much. Therefore the RNG κ-ε model which considers the viscosity of air is recommended in some case (*1*,*2*). However, based on this study the κ-ε standard model is still usable for such a high risk location finding purpose. Nishioka indicate this widely used the κ-ε standard model is usefulness (*3*). Measured 3D-AFM and CFD simulation by the κ-ε standard models give good match in Figure4-2 and Figure4-3, 4-4, 4-5 comparison. The overall analysis of the mapped pictures is found useful and sufficient for the purpose of hot spot finding.

a) Relationship between local concentration of particle and SVE-3

This study could not fined clear relationship between local concentration of particle and SVE-3. In Figure4-8 the concentrations of airborne particle are widely distributed from 0.00 to 5.78 on the SVE-3 value is 1.00. In case of SVE-3 value is 2.25, concentrations of airborne particle are also widely distributed in the 0.75-3.89. The results indicate that in Grade A where is usually very low concentration of airborne particles, therefore surrounding environment can be influenced easily from a person or a machine operations, it is difficult to show stable relationship in short

period observation. Ogawa reported (*4*) a study to support this hypothesis.

4.4.2. Relationship between air velocity and SVE-3

Figure4-9 shows that at measuring point ① - ⑱ there are two groups of relationship between simulated local age of air as SVE-3 and air velocity. Data collected at 1.0 m height from floor. The space expects no influence of air supply from HEPA filter on ceiling and of air exhaust upon floor. The area around measurement point ④ ⑤ ⑥ ⑦ ⑧ ⑪, those points give the inverse coefficient of 0.8 in Figure4-9, looks in much complicated area where several strong clean air streams and small displacing zones are mixed. Therefore displacing efficiency is speculated better than that of area around series ① ② ⑩ ⑫ ⑭ ⑮ ⑯ ⑱, those points show the inverse coefficient of 0.4.

4.4.3. Air velocity distribution, age distribution and local air retention area relationship

Slow air vector region where distribution is black colored near ③ ⑥ ⑫ in Figure4-2 meets to white colored region where SVE-3 is high in Figure4-7. However in case of ⑮ which is black in Figure4-2 and black which means low SVE-3 in Figure4-7. Author speculated the reason.

In aseptic room there are three zones those are classified in Figure1-1. In other words, ***a***; unidirectional airflow zone, ***b***; stagnant zone, ***c***; displacing turbulent zone (5). Wada reported ***c*** can appear in case of highly ventilated clean room.

Over 40 times per hour of air change time, the ***c*** zone appears over the ***a*** zone. In this kind of ***c*** / ***a*** structure gives exaggerate recovery time. When air change time is 80 times per hour, the recovery rate is about 1.6 times faster than that of expected (*6*).

Suwa reported (*7*) , the LMAA decreases with air change time. In high air change time case the distribution of LMAA becomes broad. LMAA is a value of time that can be calculated from SVE-3 and air change time (*8*). Thus in case of this study the SVE-3 vale 2.0 outputs 26 s. This value is still very fast compared with the averaged LMAA of 90-60 s in case of 40-60 times air change clean room. LMAA instead of SVE-3 gives clear target time when finding critical control point to be monitored in aseptic room.

4.5. Conclusion

In recent years, sterile room with a high frequency ventilation is considered much safe in terms of microbial contamination. In which high ventilation room the LMAA from SVE-3 is very low value. Based on this section's experimental results, LMAA did not clearly explain the data of the relationship with particle counts in highly ventilated aseptic room. However, the simulation results and the actual 3D-AFM give good matching. This result can be used for prediction of contamination risk. As a primary risk assessment using CFD is useful to explore the possible high risk points.

4.6. References

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rate based on averaged contaminant distribution. Study on evaluation of ventilation effectiveness of occupied space in room. *J. Architect. Plan. Environment. Eng.*. **2000**, 529, 31-37

5. Proposal for a New Categorization of Aseptic Processing Facilities based on Risk Assessment Scores

5.1. Introduction

Even 3D-AFM is useful for the risk assessment of aseptic processing facilities, it was performed using two published risk assessment tools. Calculated risk scores were compared with experimental test results, including environmental monitoring and media fill run results, in three different types of facilities. The two risk assessment tools used gave a generally similar outcome. However, depending on the tool used, variation was observed in the relative scores between the facilities. For the facility yielding the lowest risk scores, the corresponding experimental test results showed no contamination, indicating that these ordinal testing methods are insufficient to evaluate this kind of facility. A conventional facility having acceptable aseptic processing lines gave relatively high risk scores. The facility showing a rather high risk score level demonstrated the usefulness of conventional microbiological test methods.

Considering the significant gaps observed in calculated risk scores and in the ordinal microbiological test results between advanced and conventional facilities, we propose a facility categorization based on risk assessments. The most important risk factor in aseptic processing is human intervention. When human intervention is eliminated from the process by advanced hardware design, the aseptic processing facility can be classified into a new risk category which is

better suited for assuring sterility based on a new set of criteria, rather than currently used microbiological analysis. To fully benefit from advanced technologies, we propose three risk categories for these aseptic facilities.

Here, to clarify the relationship between media fill test results and risk assessment scores among aseptic processing technologies, we evaluated three different types of existing aseptic facility. After comparing results from risk scores, media fill tests and microbiological environmental monitoring data, we propose a new categorization of aseptic processing facilities based on risk assessment scores, accumulated environmental monitoring data and media fill test results.

5.2. Materials and Methods

5.2.1. Facilities

Three typical aseptic processing lines for lyophilized products in current commercial use were chosen to compare risk assessments. The data used were derived from three different plants of the Astellas Pharma group in Japan.

a) Plant A: A lyophilized vial production line equipped with an isolator, an ampoule processing system including an ampoule washer, a tunnel sterilizer and a filling machine. The filling machine equipped in the isolator is located in a Grade C room, while a lyophilizer and automated guided vehicles are located in Grade B rooms. There is no direct human access to the product during the aseptic operations.

b) Plant B: A RABS system equipped with cabinets with HVAC and an automated loading system. The aseptic processing equipment includes a vial washer, a tunnel sterilizer, a filling machine and crimping machines. Aseptic processing machines are all in Grade A cabinets which are located within a surrounding Grade B room. The Grade A cabinet locally opens to the Grade B room. Human interventions are restricted and only allowed before and after production. Material transportation is done in a closed system.

c) Plant C: Conventional barriers are installed around the processing machines in a Grade B room. Regular human intervention is required to supply stoppers and vials. The loading system is also manually operated by using closed carrier containers.

5.2.2. Media fill run and microbiological monitoring

a) Plant A: Media fill run results for 2005 and 2006 were used. Surface monitoring results and active air monitoring results for microbiological contaminants, obtained using a membrane air sampler (SALTORIUS MD-8 air sampler), were compiled from 2005 to 2006. Air sampling volumes were 1 m^3 and 0.5 m^3 for Grade A and Grade B, respectively. The air was monitored once a day during manufacturing.

b) Plant B: Media fill run results for 2002 and 2006 were used. Active air monitoring was done using air samplers (RCS plus air sampler).

Monitoring results were compiled from 2005 to 2006. Air sampling volume was 1 m^3. Air was monitored once or twice daily during manufacturing. Surface sampling was conducted using clean petan plates, with a surface area of 25 cm^2 .

c) Plant C: Media fill run results for 2005 and 2006 were used. Active air monitoring was done using air samplers (MAS-100 air sampler). Monitoring results were compiled from July 2006 to December 2006. Air sampling volumes were 1 m^3 and 0.5 m^3 for Grade A andGrade B, respectively. Air was monitored once a day during manufacturing. Surface sampling was conducted using Rodac plates, with a surface area of 25 cm^2.

5.2.3. Risk assessment methods

a) Whyte-Eaton method(1)

This method, called the "overall deposition model", numerically evaluates risks by using four parameters: amount of microbiological contamination, ease of dispersion or transfer, proximity of source to the critical area, and effectiveness of control method. The actual risk evaluation system is based on a modified FMEA approach. For the purpose of overall risk assessment, time was not taken into account. The calculations were done using the equation below:

Risk of microbiological contamination = A x B x C x D

A = microbiological contamination in/on a source;

B = ease of dispersion and transfer;

C = proximity of source to the critical area;

D = effectiveness of control methods.

When using RABS and an isolator, automated operation registers zero counts, as seen in Table 5-1. Compared to conventional assessment methods, manual operations in RABS show a low score due to the minimization of human access time and risks for human error. In this method, weights of risk score were defined as ranging from one to three.

Table 5-1. Calculated Risk scores by the Whyte-Eaton method for the three model plants.

Aseptic processing for:	Practice	Calculated risk scores		
		Plant A	Plant B	Plant C
Setup	Filter housing and aseptic hose connection	0	0	9
	Aseptic hose breakage and leakage	0	0	4.5
	Aseptic tank set up	0	0	1.5
	Connection to filler	0	0	8
Filling	Environmental monitoring	0	0.25	4.5
	Container tumbling and breakage	0.25	0.25	12
	Needle and conveyer line adjustment	0.25	1	8
	Closure sterilization and feeding	0	0.25	8
	Container feeding	0	0	8
	Container loading	0	0	4.5
Lyophilization	Vial to tray loading	0	0	12
	Transfer to lyophilizer	0	0.25	4.5
	Tray to lyophilizer loading	0	0	12
Total Risk factor score		0.5	2.0	96.5

Total aseptic risk score was calculated by summing individual scores for setup,

filling, and lyophilization.

b) Akers-Agalloco method (2,3)

This method is based on actual risk factors rather than theoretical concepts, critical models or occurrences, and also focuses on the effect of personnel. Significant risk factors to be considered in aseptic processing are summarized below.

A. risk factors in aseptic processing for compounding

B. risk factors in aseptic processing for setup

C. determination of intervention risk (I_R)

D. risk factors in aseptic processing for filling

E. risk factors in aseptic processing for setup and filling

F. risk factors in aseptic processing for lyophilization

From the preset risk contribution list, the exact number of risk contributions can be easily selected. For example in Table 5-2, risks of lyophilization were evaluated for seven factors. One of these factors, thermocouples, has a preset score of 10. Since Plant C uses ten thermocouples, this score is multiplied by 10.

As shown here, when the factor taken into consideration possesses manual microbial contamination risks, the Akers Agalloco method amplifies the risk contributions.

Table 5-2. Calculated risk scores by the Akers-Agallocco method including aseptic compounding risk contribution.

Practice	Calculated risk scores		
	A	B	C
Aseptic compounding risk contribution subtotal	0	0	90
Aseptic setup risk contribution subtotal	3.75	3.75	600
Aseptic filling risk contribution subtotal	90	90	1080
Intervention risk (I_R)	0.00224	0.00292	0.00475
Intervention-adjusted aseptic filling risk	0.2016	0.2628	5.13
Aseptic setup and filling risk contribution subtotal	3.9516	4.0128	605.13
Aseptic setup and filling risk contribution subtotal (with environment factor)	0.0395	4.0128	907.695
Lyophilization risk contribution subtotal	2.4	36	172,800
Total aseptic risk contribution	**2.440**	**40.013**	**173,798**

Aseptic filling risk contribution subtotal x I_R = Intervention-adjusted aseptic filling riskIntervention-adjusted aseptic filling risk + Aseptic setup risk contribution subtotal = Aseptic setup and filling risk contribution subtotalAseptic setup and filling risk contribution subtotal x Environmental technology = Aseptic setup and filling risk contribution subtotal (with environment factor)Aseptic compounding risk contribution subtotal + Aseptic setup and filling risk contribution subtotal (with environment factor) + Lyophilization risk contribution subtotal = Total aseptic risk contribution.

5.3. Results

5.3.1. Summary of media fill run and microbiological monitoring

Media fill run results for each of the three plants are summarized in Table 5-3. All three aseptic processing facilities easily achieved the minimum criteria for passing the media fill tests. The environmental monitoring data are summarized in Table 5-4. The microorganism detection frequency was remarkably low in plants B and C. Microorganisms were occasionally detected for each type of measurement in plant C.

Table 5-3. Media fill run results

Plant	Number of contamination (%)	Number of filled containers	Number of runs	Filled containers/ run
A+A'	0 (0)	65,000	13	5000
B	0 (0)	351,000	13	27000
C+C'	2 (0.003)	60,000	12	5000

A': Lyophilized ampoule production line. The manufacturing environment and facility design concept are the same as Plant A.

C': Lyophilized ampoule production line. The manufacturing environment and facility design concept are the same as Plant C.

Table 5-4. Summary of microbiological monitoring results in each category of aseptic processing facility.

		Category 1 Isolator (two-year results) Plant A	Category 2 Advanced Unmanned System (Two-year results) Plant B	Category 3 Conventional (six-month results) Plant C
Grade A	Active air sampling	0% (0/93)	0% (0/1392)	0.66% (3/458)
	Surface sampling	0% (0/122)	0% (0/712)	1.42% (5/353)
Grade B	Active air sampling	—	0.16% (1/608)	14.00% (164/1171)
	Surface sampling	—	0.20% (4/1978)	1.95% (23/1180)
Body	Body, finger sampling	0% (0/732)	0.11% (1/900)	0.51% (4/783)

5.3.2. Risk assessment Results

a) Whyte-Eaton method (Table 5-1)

Risk assessment is summarized in Table 5-1. Plant C showed a score of approximately 10^2, whereas Plant A and B showed a score of approximately 10^{-1} and 10^0, respectively.

b) Akers-Agalloco method (Table 5-2)

The duration of operations corresponds to the evaluation scores found in

Table 5-2. For example, when using risk contributions of aseptic setup for score estimation, the conventional Plant C requires 60 minutes of manual operation to set up the filling elements in the Grade A room and yields a score of 60 based on the risk contribution score rule. The complexity score for operations requiring manual assembly after autoclaving has a preset listed value of 10. After multiplication of these scores, the aseptic setup risk contribution subtotal score for Plant C was 600. Plants A and B required 5 minutes for setup, 1 minute for simple sterilization after setup, and 0.75 minutes for safe product transfer using a final filter before filling. Multiplication of the corresponding scores (5 x 1 x 0.75), gave a subtotal of 3.75 for both plants.

Sub-risk assessments and overall assessment are summarized in Table 5-2. Plant C showed a score of approximately 10^5 whereas Plant A and B showed a score of approximately $10^{0\sim1}$.

5.4. Discussion

Analysis of Table 5-3 shows that media fill runs (process simulation) were insufficient in plants A and B, since no positive results, or "hits", were observed in any of the tests. In terms of evaluating the human operational contamination risk in aseptic manufacturing, since the media filled vials are not exposed to human operation in isolator based aseptic processing systems. Therefore media fill runs

are meaningless as a validation method. In case of RABS since the media fill was conducted just for intervention activities, no detection indicates that contamination was well controlled, allowing for analytical limitations, within the aseptic operations. No recovered contamination "hits" may indicate that the limited of detection of media fill runs has been reached. Of course, it should be noted that there has never been any evidence presented that product made in facilities which consistently produce zero contamination rates on media fill tests are in any way risky to the end user.

For plant B, 13 runs of 27000 media filled units were used over five years and the results gave no hits. The potential contamination level of plant B may be under $3.7x\ 10^{-5}$. Environmental monitoring results in Grade A of plants A and B also suggest insufficient evaluation in these areas through microbiological methods (Table 5-4). According to the proposal in the revised USP <1116>, ISO Class 5 advanced aseptic processing facilities can be qualified by particulate analysis and other parameters. The results shown in Tables 5-3 and 5-4 support this proposal.

It is important to establish the criteria used to determine which category of facilities requires monitoring of microbiological contaminants using traditional media. We propose the use of risk assessment scores for this purpose, and propose the following aseptic processing categories.

5.4.1. Category definitions

Category 1: Completely closed aseptic processing systems using isolators. The risk score calculated by the Akers-Agalloco and Whyte-Eaton method is approximately $10^{0\sim1}$ and $10^{-1\sim-2}$, respectively. No "contamination hit" is observed in the historical media based tests.

Category 2: RABS, BFS, and other improved facilities that allow only minimum local human access. Physical barriers are provided between personnel and the production line. The risk score calculated by the Akers-Agalloco and Whyte-Eaton method is approximately $10^{1\sim2}$and $10^{0\sim1}$, respectively. No "contamination hit" is observed in the historical media based tests.

Category 3: Conventional aseptic processing facilities. Hardware improvements are not possible in this facility to successfully achieve zero contamination by media-based tests. Historical contamination hits are observed in media fill runs and microbiological monitoring.

Category 3-: Facilities accepted by authority inspections under current GMP. Microbes are however frequently detected through media-based tests. Some facilities falling under Category 3 have high levels of risk as defined by the assessment tools, and therefore are be expected to

produce more “hits” in microbiological tests. When risk scores are sufficiently high, concerns about the acceptability of a facility are beyond the scope of this study.

5.4.2. Comparison of calculated risk scores

Based on the Whyte & Eaton model and Akers-Agalloco method, significant score differences were found among plants. The results of the assessed scores are summarized in Figure 5-1. The Akers-Agalloco method showed a greater gap in assessed risk scores between conventional plants A and C than advanced plants A and B. From the modeled risk assessments, these three types of plants can be classified into two or three categories. Additionally, the Akers-Agalloco method takes into account the time factor for human intervention and other processing. As a result, the score is amplified in cases where frequent manual intervention is required during the process. Therefore, the Akers-Agalloco method may reflect, to a great extent, real operational risks.

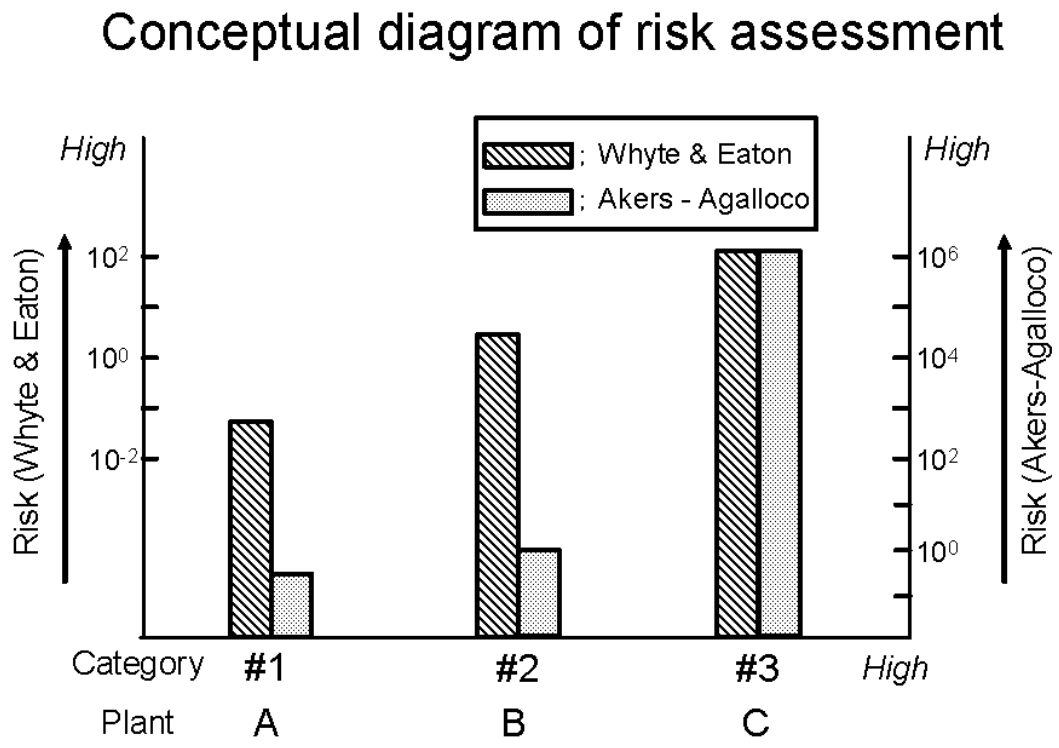

Figure 5-1. Risk score comparison used two risk assessment tools in three existing facilities. A significant gap is observed between Plant C and Plants A, B

Based on the differences in the technologies used in each plant, plants A, B and C can be classified into Categories 1, 2 and 3, respectively. When Category 2 is scored using the Whyte-Eaton method, this category is intermediary between Categories 1 and 3. This method predicts that a significant microbiological difference should exist between Categories 1 and 2.

However, with the Akers-Agalloco method, the risk score in Category 2 is similar

in range to Category 1, and significantly lower than Category 3. These results correspond to the media-based microbiological test results. In particular, the improved facilities in both Categories 1 and 2 gave all-zero detections. The risk assessment gap should be significant between a facility in which contamination is occasionally observed and a facility that consistently produces no “hits”. As mentioned above, with the Akers-Agalloco method, the time factor amplifies the score gap. Some plants falling under Category 2 may be assigned a 10- to 100-fold larger value than plant B if they allow more human access.

5.4.3. Proposed frequency of media fill runs

This categorization shows the flexible application of media-based tests to each category. Although the capability of detecting microbiological contamination risks is limited, the media fill run could be applied to each category depending on need. The requirements are summarized in Table 5-5.

Table 5-5. Proposed frequency of media fill runs.

Category	Frequency of proposed media fill runs		
	Initial validation, investigation for OOS	For automated processes	For processes with human interventions
1	1	—	—
2	3	—	1 / Year
3	3	2 / Year	2 / Year
3-	3	2 / Year	3 / Year (All personnel and processes)

The lowest risk scores for Category 1 can be achieved in appropriately designed isolators similar to those used in plant A. In this case, routine performance of the media fill run would give only zero positives, even with numerous vial runs. In this category, the media fill run should be used for initial validation or in OOS investigations. Media fill run results in Table 5-3 show remarkably low contamination risks in plants A and B. In these facilities, microbiological tests are limited due to low assessment sensitivity. However, media fill tests still seem to be a useful way to evaluate possible unexpected machines defects, although it is clear that the frequency of these tests can be reduced. We therefore propose a modified utilization of the media fill test in cases where advanced aseptic processing technology is based on risk score. The current criteria of machine defect frequency in worldwide use may be the same as the one used in Category 3.

Plant C should be improved by upgrading the procedures leading to reduced human interventions, or by introducing new technology to eliminate them completely. We recognize that Plant C is broadly representative of conventional aseptic processing, and that different risk levels existing in these conventional facilities depend on a number of variable activities rather than easily identified risk factors.

Although the media fill is inaccurate in evaluating the aseptic integrity of a

manufacturing process, the current recognized criteria is zero contamination in 5000 units. Regarding these media fill test criteria, Agalloco and Akers mentioned "no statistically significant difference from zero" (*4*). The scientific reasoning behind this, clearly presented by Kawamura and Abe (*5*), states that "one or two positives in 10,000 fills are enough to assure integrity". This is shown by the consistent zero positive results in our historical data. In such a case, facilities falling under Category 3 can be allowed to manufacture. However, Category 3 should be improved until achieving consistently zero positives. Additional media fill runs may be necessary in Category 3 to increase the probability of detecting contamination in production operations. It should be noted that facilities in Category 3 would make an effort to improve procedures so that risks can be reduced.

New facilities and OOS investigations require overall qualification in critical environments and equipments, as well as in human operations. Category 1 does not have human access contamination risks, but does have mechanical failure risks. This can be evaluated after one media fill run. Categories 2 and 3 may potentially have systematic error risks from human access. Three media fill runs may detect these.

5.5. Conclusion

Our findings suggest the use of risk scores to categorize existing aseptic facilities.

The combined evaluation of a facility's risk scores and its accumulated media based data is appropriate to categorize aseptic processing systems. While we understand that the data presented in this study are necessarily limited. We hope that in the future additional relative risk score comparisons can be accumulated. We believe that an in-depth analysis of these data would enable a firms to determine to what extent they should employ traditional microbiological methods, and help them to recognize that their processes may not obtain benefit from traditional microbiological analysis. In many state-of-the-art aseptic operations, maintenance of engineered process control parameters and continuous particle monitoring will provide increased assurance over traditional microbiological analysis. In addition, media fill tests for all processing categories can be considered a means of discovering hidden risks in overall processing and training during aseptic operations. However, the usage of the media fill run should be flexible and should also depend upon risk scores. Here, results based on the Akers-Agalloco method show significant differences between advanced and conventional technologies. We also show that simple risk assessment is convenient and provides a clear numerical score to justify aseptic facility categorization, which may eventually lead to abandoning inefficient traditional media-based tests in advanced facilities and promote the introduction and improvement of a new set of aseptic risk detecting systems that may include improved microbiological tests.

5.6. References

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6. Risk management and Deviation handling

In the revised "GMP Ministerial Ordinance on Drugs and Quasi-drugs" announced by MHLW in December 2004, "deviation control" was stipulated. In response to this, Manufacturer needs to prepare Standard operational procedure (SOP) to control and handle deviations appropriately and any deviation has to be documented. When critical deviation is occurred, impact assessment on the quality has to be also performed. If the deviation may have quality impact, the deviation has to be notified to Licensed Marketing Approval Holder of the product. Therefore, manufacturing unit or quality unit in manufacturer is required to have sufficient knowledge and ability to execute root cause analysis, impact assessment and corrective action/preventative action (CAPA). In this article, by taking up the following three cases, how to handle deviations such as root cause analysis, impact assessment of quality and CAPA has been discussed.

1)Deviation from the standard operating procedure in granulation process

2)Deviation from the specification in pharmaceutical water

3)Deviation from the humidity limit in stability chamber

In each case, insufficient handling example is first introduced and then desirable way of thinking is shown along with appropriate example. Points to be considered are also discussed for a more appropriate handling.

Table 6-1.Ministerial ordinance, the notice describes the management of deviance

CMP ministerial decree	No. 0338001 section3.3
Article 15 manufacturers, manufacturing and deviation from the procedure (hereinafter referred to simply as "deviant"). Arise if there is a pre-specified, in accordance with such procedure, then are pledged to work to take place.	**15.(2) The first-term "predetermined" and the business officials familiar with the contents of the pre-specified as a business manager with the staff's commitment to Article 6, Section 4, based on the provisions of the proper documents it define1.**
1 Record the contents of deviance. 2 In the event of a serious deviation in the next has work to do. A) Deviation of product quality to assess the impact of the measures required to adopt it. B) To define the measures and evaluation results to create a record, store and the quality department reported that the document. C) Report by the provisions of measures and evaluation results, the quality department to be reviewed.	(1) The first issue of the provisions of Section 1 of the structure of manufacturing equipment and procedures and other manufacturing process management and quality control methods pertaining to the norm for all that will be applied. (2) The second issue of the provisions of Section 1 is the norm all of the major manufacturers have decided that if you deviate conduct of business. (3) Section 1 of the No. 2 and イ ratings necessary measures for an important business for the department to report quality, quality for the department confirmed that.
2 Manufacturers are from the quality department, and procedures based on the number preceding paragraph ハ of the second confirmed record created and kept up with the record number ロ, as well as manufacturing manager of the document that we properly reported .	(6) The second section of the law, administrative or manufacturing engineer is responsible for the Section 1, Clause 5 of the second issue to properly carry out operations that allow them to the manufacturing manager or engineer responsible for reporting to the Of those. (7) And the judge did not deviate from serious after the first test or production lot more about the principle, the subsequent impact concerning the extent of deviation should be evaluated.

Comparison of regulations on management deviance

Table 6-2.Comparison of descriptions in GMPs

Category 1	Category 2	cGMP	EU GMP	WHO GMP	ICH Q7A
					API GMP
Production management	Object	All the deviation	All the deviation (Indirect representation)	Serious deviation	All the deviation
	Correspondence	Record and satisfactory explanation	Deviation to approve the (qualified)	Investigate, Record	Record and the contents revealed a serious departure to investigate the cause of the conclusions to record
	Involvement in the quality control department	Checking and approval	Qualification is desirable for the quality control department	Rating	Resolve serious deviation in the survey confirm that
Quality management	Object	All the deviation (Indirect representation)	None	All the deviation	None
	Correspondence	Record and satisfactory explanation		Recorded in the survey indicate that it is a record	

In this thesis we propose to use a combination of the measurement of 3D-AFM, particulate monitoring, and microbial monitoring to achieve an improved evaluation of the aseptic room. These methods enable the user to locate risk points or hot spots which cause out of specification of deviation during an investigational activity. Furthermore for the purpose of CAPA as well as to optimize personnel and material flow improvement, the 3D-AFM can be useful. A further benefit of this approach is the reduction or elimination of less informative monitoring

locations, resulting in better monitoring efficiency without a loss in data critical to the evaluation of the aseptic environment.

7. General Conclusion

Emerging facility replacement from conventional aseptic clean rooms to isolators or RABS systems may give pressure to the user of existing conventional factories. However this article proposes that the conventional existing facility can be classified in an appropriate risk category of aseptic processing and the suitable improvement of environmental risk mitigation method could be available for it. One of the option is three dimensional air flow analysis that can predict the consistent high risk spots method which is considered to be able to improve the risk contributor of detestability even the sensitivity of microbial test in aseptic room is very low.

CFD simulation of LMAA roughly predict actual measurement of 3D-AFM. The usability and usefulness of 3D-AFM have determined consistently. Combined analysis of LAMM mapping by CFD simulation and 3D-AFM characterizes the air in aseptic room. Characterization of air gives accurate contamination risk assessment to find out control point in aseptic room.

On the other hand modern facility employed new technology like isolator would not require the traditional microbial tests to assure the asepsis. For the balanced quality assurance for the patients and the earth, the risk based and science based approach is very reasonable. The most meaningful application of 3D-AFM method is for risk assessment and finding out monitoring points in Grade B. In

case of Category 2 to 4 The usability was determined. Especially for a conventional aseptic room, the 3D-AFM combined with LMAA and IEM method is useful to improve the risk reduction system.

Before using the 3D-AFM method, users have better to assess the category of the whole aseptic manufacturing technology by utilizing numerical aseptic risk assessment tools those recently developed. In case of the processing line is an isolator system which has complete barrier between the surrounding clean room and the processing line, the evaluation of the surrounding environmental air is not meaningful.

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9. Publication list

Hirohito Katayama, Takashi Higo, Yuji Tokunaga, Yukio Hiyama and Kaoru Morikawa

Establishment of critical contamination risk locations (Hot Spots) in environmental monitoring by means of three-dimensional airflow analysis and particulate evaluation

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Monitoring minimization of Grade B environments based on risk assessment using three-dimensional airflow measurements and computer simulation, *PDA J Pharmaceut. Scie. Technol.* (in press).

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Proposal for a New Categorization of Aseptic Processing Facilities based on Risk Assessment Scores, *PDA J Pharmaceut. Scie. Technol.* (in press).

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Comparative results of air flow characters mapped by CFD simulation and actual measurements of 3D-AFM and particle concentrations.

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Case studies on how to handle deviations “Point to consider for a more appropriate handling” *PDA J. GMP Validat. Japan.* **2006,** 8, 78 – 85.