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〈報　告〉

Examination of the Optimal Environment of Unilateral Laminar-Flow Clean Benches for Sterile Preparation Based on Wind Velocity Measurements

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Abstract

A clean bench is necessary for maintaining an environment compliant with Class 5 of the International Organization for Standardization 14644-1 (ISO Class 5), ensuring airflow through the workspace with a mean wind velocity of 0.3-0.6 m/s as delineated by Japanese Industrial Standards (JIS) B9922. This study emphasizes wind velocity as a crucial factor influencing ventilation rates and measures wind velocity across various vertical and horizontal planes with respect to the work surface in three different models of clean benches. The findings indicate that among the models tested, the median wind velocity within 15-30 cm from the sash met JIS standards. However, the median wind velocity showed wide variation on the planes at 0-20 cm height from the work surface, while the variation was narrow on planes covered by the sash at 20-50 cm height from the work surface, yielding a constant wind velocity. These observations led to the identification of the optimal preparation area across the models as being "at least 20 cm above the work surface and within a 15-30 cm depth from the sash, with the sash opening maintained at or below 20 cm. This designated area minimizes the risk of particulate and microbial contamination of the prepared drug products, offering a standardized guideline for clean bench operation to ensure product and environmental safety.

Key words: clean bench, airflow, visualization experiment, environmental cleanliness

Introduction

Preparing injection drug mixtures involves the risk of thrombogenesis and sepsis in patients receiving the drugs due to contamination by particulates and microorganisms during preparation. Therefore, there should be maximum caution during preparation to prevent contamination. For this reason, the preparer must maintain sterile techniques and an appropriate preparation environment[1~4)]. The environment used for sterile preparation must comply with ISO Class 5 of airborne particulate-based classifications (1-8) defined by the International Organization for Standardization 14644-1 (ISO 14644-1; Cleanrooms and associated controlled environments-Part 1: Classification of air cleanliness by particle concentration)[5, 6)].

The authors demonstrated that the performance of a clean bench, compliant with ISO Class 5 standards, is significantly influenced by the installation environment, as evidenced by the levels of airborne particulates and free-living microorganisms[7)]. A noteworthy finding of this study is that there is a positive correlation (R= 0.60) between the number of airborne particulates produced during preparation and the number of microorganisms, underscoring the importance of minimizing particulate generation to reduce the risk of microbial contamination in pharmaceutical formulations.

This observation is in contrast with the study of Asano et al.,[8)] which reported that there is no significant correlation between airborne particulate counts and microbial presence in the air. They suggested that

maintaining the ventilation rate close to its maximum capacity (=1) could effectively reduce particulate levels and, consequently, the potential for microbial contamination. This discrepancy highlights the complexity of controlling environmental factors within clean benches and underscores the need for tailored strategies to optimize cleanliness and minimize contamination risks in pharmaceutical product preparation.

Clean benches used for sterile preparation in medical settings are equipped with a built-in air blower and HEPA or ULPA filter. These provide airflow at a constant wind velocity to remove airborne particulates and microorganisms from the workspace and maintain the ISO Class 5 standard for cleanliness. In addition, the mean wind velocity required to maintain ISO Class 5 cleanliness inside a clean bench is 0.3-0.6 m/s, with a tolerance margin of ± 20%, by Japanese Industrial Standards (JIS) B9922[9]. The air passed through the HEPA filter must have an even linear flow toward the work surface and maintain a wind velocity within the specified range. After contact with the work surface, the airflow needs to be vented outside the clean bench through the opening of the front glass (hereinafter "sash"). For wind velocity measurements, the JIS standard B9922 states that "Measurements shall be taken on a plane parallel to the air-blowing surface of the filter or distributing plate and 10 cm downstream from the air-blowing surface, and the mean velocity is determined by averaging measurement values." However, when the maximum distance is assumed to be 35 cm based on the average forearm length in the Japanese population[10], the actual area for injection drug mixture preparations likely differs from the plane specified by the JIS standard B9922, even when considering for range of motion.

In this study, attention was directed toward understanding the airflow dynamics within clean benches, selecting air velocity-a critical determinant of ventilation rate-as the primary indicator. This approach facilitated the identification of an optimal preparation area shared across different models, which reliably upholds ISO Class 5 cleanliness standards during periods of nonoperation, when aseptic procedures are not underway.

Materials and Methods

1. Measurement Environment

There were three different one-sided laminar-flow clean bench models (①PVC1915BNG1, HITACHI Co., Ltd., Tokyo, Japan; ②MCV-161BNU-PJ, Panasonic Healthcare Co., Ltd., Tokyo, Japan; ③MCV-161BNF-PJ, SANYO Electric Co., Ltd., Japan) installed in a normal air-conditioned room. The mean particulate count was 300,000/ft^3 at a room temperature of 23℃ ± 5℃ and humidity of 50% ± 20%. A rectangular configuration was chosen for the clean bench to guarantee uniformity in the work surface area. The models analyzed in this study were as follows: ①MCV-161BNU-PJ (2015) by Panasonic Healthcare Co., Ltd., Tokyo, Japan; ②MCV-161BNF-PJ by SANYO Electric Co. Ltd., Tokyo, Japan; and ③PVC1915BNG1 by HITACHI Co., Ltd., Tokyo, Japan. Model ①is the successor of model ②, with model ③differing in specifications from the other two.

Wind velocity was measured after the airflow in the clean bench had stabilized following a warm-up period of at least 5 min. In addition, only a wind-velocity meter was installed inside the clean benches, and no other items were brought into the clean benches. Calibration of the clean bench was conducted by ensuring that the filter alerts were functioning normally and by performing a preliminary air velocity test. This test verified that both filter leakage and air velocity were in compliance with the JIS B9922.

2. Measurement of Wind Velocity

Wind velocity was measured using a wind-velocity/air-volume meter (Climomaster; 6541-21, Kanomax Japan Inc.). The sash opening was fixed at 20 cm, and the measurement points were set at 55 intersection points of the vertical planes (10, 20, 30, 40, and 50 cm from the sash toward the back of the clean bench) and the horizontal planes (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 cm height from the work surface). At each measurement point, wind velocity was measured once per second, over a continuous period of 20 s.

3. Comparison of Wind Velocity Among Models and Examination of the Optimal Preparation Area

3.1) Comparison Among Models

Among the models tested, we compared wind velocity measured at the plane with (1) the same height from the work surface and (2) the same distance from the sash. The optimal preparation area common to the models was examined.

3.2) Measurement Standard

The JIS standard B9922, which stipulates the cleanliness of clean benches, was used as the measurement standard. The specified values for wind velocity are as

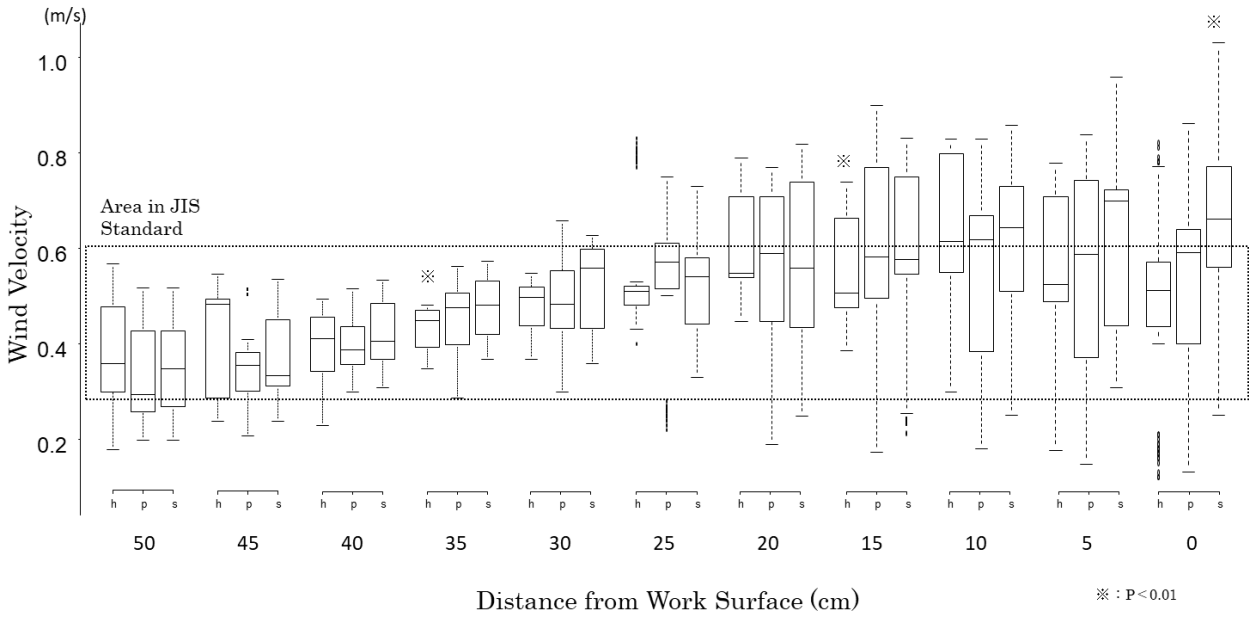

Fig. 1　Relationship between the distance from work surface (height) and wind velocity

Differences in wind velocity among the models were analyzed using the Mann-Whitney U test with Panasonic MCV-161BNU-PJ(p) as the reference (P＜0.01).

h: HITACHI PVC1915BNG1, p: Panasonic MCV-161BNU-PJ, s: SANYO MCV-161BNF-PJ

follows: "(1) The rated wind velocity of the clean bench is in the range of 0.3-0.6 m/s, (2) the mean wind velocity is within ±20% of the rated wind velocity, and (3) the wind velocity at each measurement point is within ±20% of the mean wind velocity." Of these, (1) was adopted in this study.

4. Visualization of Airflow

Using an airflow visualization device (Air Clean Viewer, ACV-201A1, AIRTECH JAPAN, Ltd., Tokyo, Japan), the airflow inside the clean benches was visualized by releasing mist with a particle size of 3 μm. The sash opening was fixed at 20 cm. The airflow visualization device was installed on the air-blowing surface above the clean bench at 10, 20, 30, 40, and 50 cm distance from the sash, and the mist was released in a downward, vertical direction.

5. Statistical Analysis

The Mann-Whitney U test was used to analyze differences in wind velocity among the models (the level of significance was set at a P-value of ＜0.01 for all comparisons). The statistical analysis was performed using software EZR version 1.36 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Results

1. Relationship Between the Distance from Work Surface (Height) and Wind Velocity

Fig. 1 shows the wind velocity at different heights from the work surface in each model (h: HITACHI Co., Ltd. PVC1915BNG1, p: Panasonic Co., Ltd. MCV-161 BNU-PJ, s: SANYO Electric Co., Ltd. MCV-161BNF-PJ). In all models, the median (IQR) wind velocity on the planes at 30-45 cm height was within the standard range, while that on the planes at 5-25 cm height exceeded the standard range at the 75% percentile. In addition, there were significant differences among models on the planes at 0 and 15 cm height (P<0.01) **(Fig. 1)**.

2. Relationship Between the Distance from Sash (Depth) and Wind Velocity

In all models, the median (IQR) wind velocity on the planes at 0-15 cm depth exceeded the standard range at the 75% percentile. In addition, the median (IQR) wind velocity on the planes at 20-30 cm depth was within the standard range, although there were significant differences among models (P＜0.01) **(Fig. 2)**.

3. Examination of the Optimal Preparation Area

In all models, the median (IQR) wind velocity on the planes at 30-45 cm height from the work surface and at 20-30 cm distance from the sash (depth) was within

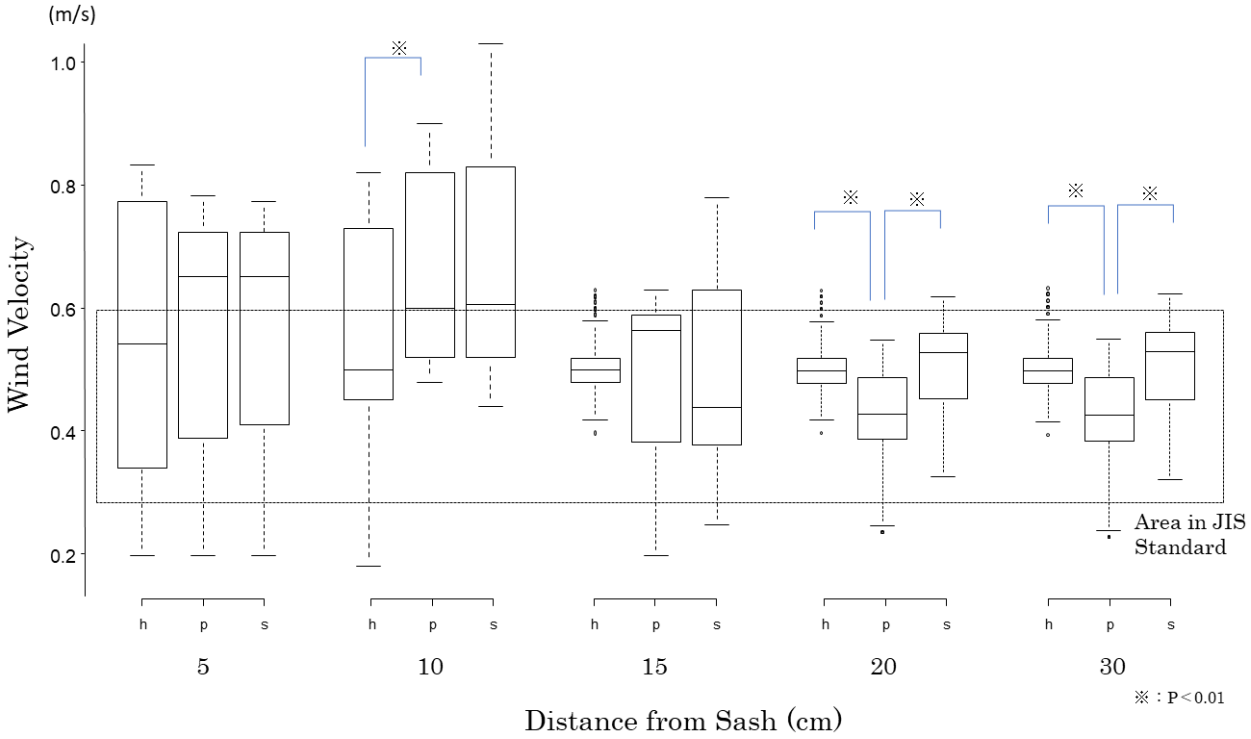

Fig. 2　Relationship between the distance from sash (depth) and wind velocity

Differences in wind velocity among the models were analyzed using the Mann-Whitney U test with Panasonic MCV-161BNU-PJ(p) as the reference (P＜0.01).

h: HITACHI PVC1915BNG1, p: Panasonic MCV-161BNU-PJ, s: SANYO MCV-161BNF-PJ

the standard range.

4. Visualization of Airflow

Fig. 3 shows the center and width of the visualized airflow. In all models, the airflow on the planes at around 40-50 cm height descended vertically toward the work surface, and it drew an arc toward the sash from the plane at approximately 30 cm. Moreover, the airflow that blew from 10 or 20 cm depth was vented outside the clean bench on the planes at 0-20 cm of sash opening (**Fig. 3**).

Discussion

In this study, the IQR of wind velocity on the planes covered by the sash at 25-50 cm from the work surface was approximately within the standard range. However, wind velocity on the planes at 0-20 cm from the work surface tended to have significant variation in all models tested, and the IQR at the 75% percentile exceeded the standard range (**Fig. 1**).

In addition, the IQR on the planes at 15-30 cm from the sash to the inside of the clean bench was approximately within the standard range. However, wind velocity on the planes at 5 and 10 cm from the sash surface tended to have significant variation in all models, and the IQR at the 75% percentile exceeded the standard range (**Fig. 2**).

Ventilation frequency (N) to maintain cleanliness inside the clean bench is expressed as

Ventilation air volume Q (m^3/h)=Ventilation frequency N (times/h)×Ventilated area A (m^2) (1)

In addition, the relationship between wind velocity and air volume is expressed as

Air volume Q' (m^3/h)=Passing wind velocity V (m/h)×Passing area B (m^2) (2)

Because the air volume from the fan filter of a clean bench is constant, we can derive the following:

Ventilation air volume Q (m^3/h)=Air volume Q' (m^3/h).

This suggests that passing wind velocity (V) can be derived from the following:

Passing wind velocity V (m/h)=

Ventilated area A (m^2)/Passing area B (m^2)/Ventilation frequency N (times/h) (3)

The air velocity V (m/s) required for ventilation across a plane D (m) at a specified ventilation frequency N (times/h) can be mathematically expressed

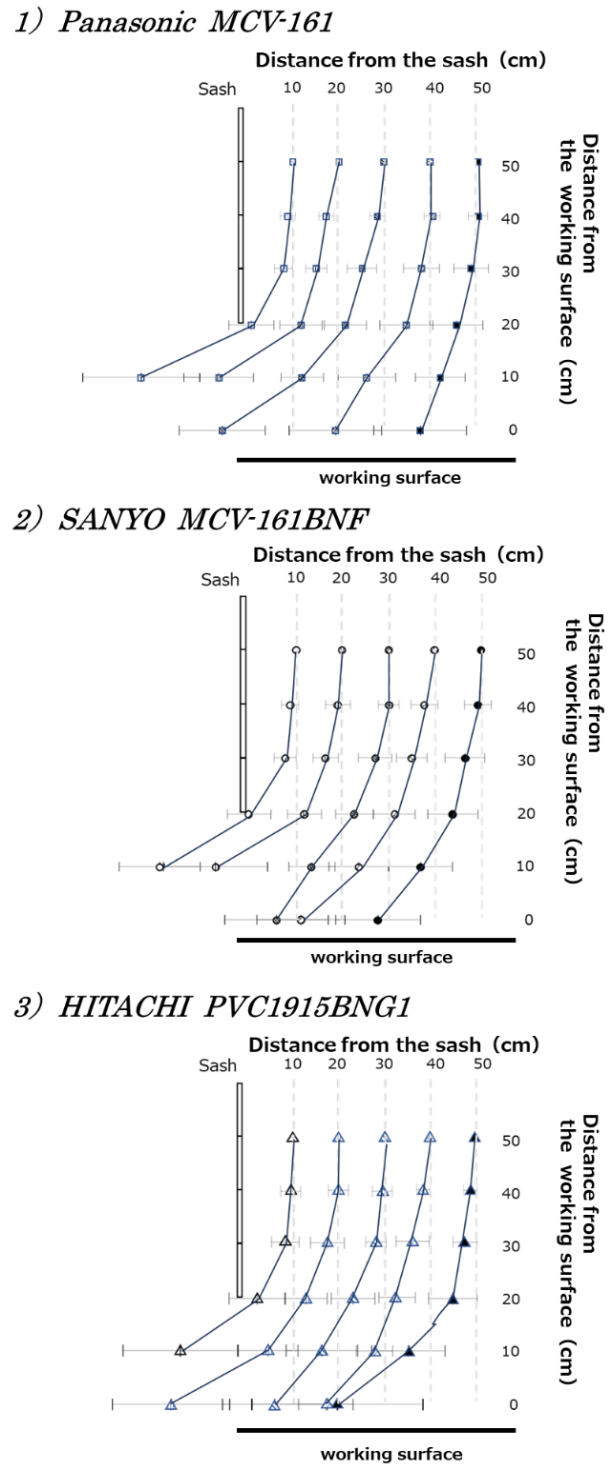

Fig. 3　Visualization of airflow
The line graph shows the spread and center point of the mist at each height of the mist ejected vertically downward from the top of the clean bench.

as V=D×N/60. Considering the airflow in the clean bench traverses through the HEPA filter and is directed toward the work surface to ensure a consistent airflow, the necessary ventilation frequency N to maintain ISO Class 5 cleanliness is estimated at approximately 300 times/h. Consequently, for any location within the clean bench, if (N=300), the air velocity at the measurement point set by JIS B9922, 10 cm below the air outlet (D=0.1), calculates to 0.1×300/60=0.5 m/s, aligning with the JIS standard range of 0.3-0.6 m/s. However, **Fig. 1** shows that the velocity tends to increase as it approaches the work surface, resulting in a nonuniform IQR of wind speed across the 0-20 cm height from the work surface for all models tested. Furthermore, **Fig. 2** shows that the IQR of air velocity at 5-10 cm and 15-30 cm distances in front of the clean bench vary, suggesting that the airflow does not maintain laminarity up to the work surface. This variation indicates that the ventilation rate needed to eliminate airborne particulates and microorganisms from the clean bench is not uniform across different areas. **Fig. 3** shows that the airflow within the clean bench demonstrates a tendency to arc toward the sash aperture from approximately 30 cm in height. Notably, at the 0-20 cm sash aperture, the airflow does not directly impact the work surface but is instead expelled outside the clean bench. This observation indicates a reduced airflow density at the 0-20 cm sash aperture necessary for sustaining cleanliness, necessitating an increase in passing air velocity V to preserve ISO Class 5 cleanliness standards. Such a requirement may account for the observed elevation in air velocity at the 0-20 cm sash aperture, as shown in **Fig. 2**, surpassing the standard range. This diminished airflow density potentially contributes to a decreased capacity for particulate removal within this area and significantly influences airflow turbulence during operational activities. In related research conducted by the authors, involving the preparation of high-calorie infusion solutions, it was recommended that “preparation should be conducted at a depth of at least 15 cm from the sash” for aseptic procedures in a one-sided laminar-flow clean bench, to mitigate risks of airborne particulate and microbial contamination[13]. In this study, while the median (IQR) aligns with the JIS standard across all models within the 15-30 cm plane from a horizontal (depth) perspective relative to the sash, a variance in IQR is observed. From a vertical (height) standpoint, the median (IQR) exhibits broad variability on the 0-20 cm sash opening plane, contrasting with a more constrained range on the 20-50 cm plane from the work surface shielded by the sash, where a stable wind velocity is anticipated.

This study has one limitation: its execution in a controlled environment devoid of typical operational conditions, such as the introduction of materials into the clean bench or the execution of formulation tasks. The introduction of objects, such as drugs and syringes, and the involvement of the pharmacist's actions during preparation are expected to generate more turbulence during actual aseptic formulation. However, since most items are positioned within 0-20 cm from the sash opening, their impact on the airflow and cleanliness in the area extending 20-50 cm from the work surface, which is shielded by the sash, is presumed minimal.

Furthermore, this investigation used a rectangular clean bench, characterized by a consistent volume from the air outlet to the work surface. It is noteworthy that some commercially available clean benches feature a design where this space volume expands from the air outlet surface toward the work surface. Despite these variations, the airflow dynamics at the opening and the consequent decrease in ventilation rate at the lower part of the opening (0-20 cm surface) are expected to be similar. Consequently, the findings suggest that the optimal preparation area, applicable across all models, is maintained with the sash positioned at 20 cm or less, ensuring a height of at least 20 cm from the work surface and a depth of 15-30 cm from the sash. Key considerations for maintaining cleanliness are managing airflow and monitoring changes in air velocity. Thus, validating the performance of the clean bench and its proper management emerges as crucial for the quality assurance of aseptic preparations. While controlling airborne particulates generated during formulation is vital for minimizing microbial contamination risks in pharmaceutical preparations, practical challenges such as the preparer's technique and handling of the preparations play a significant role. However, the study posits that contamination risks can be mitigated through the instantaneous removal of airborne particulates[8, 11].

The methodologies used in this study for air velocity measurement and airflow visualization offer straightforward and effective means to assess clean bench performance. These methods are considered applicable for use in formulation facilities, marking the initial demonstration that clean benches furnish a conducive environment for the formulation of injectable drug products.

Conflicts of Interest: All authors declare no conflicts of interest.

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風速測定を用いた片側層流クリーンベンチにおける無菌調製最適環境の検討

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要　　旨

クリーンベンチは作業空間に，日本工業規格（Japanese Industrial Standards；JIS）B9922 に規定される平均風速 0.3～0.6 m/s の気流を送ることで，International Organization for Standardization 14644-1 の Class 5（ISO Class 5）に準拠した環境を維持している．我々は，換気率に影響を及ぼす因子として風速に着目し，機種の異なる 3 台のクリーベンチを用いて，作業面に対して垂直および水平の風速測定を行った．Sash からの奥行き 15-30 cm 面ではどの機種においても Median（IQR）は JIS 規格に合致している．しかし，作業面からの高さ 0-20 cm 面では Median（IQR）の巾が広いが，Sash が覆っている作業面から 20-50 cm 面では狭く一定の風速が得られていた．以上から各機種間に共通する最適な調製エリアは，「Sash を 20 cm 以下に保ち，作業面から高さ 20 cm 以上かつ Sash から奥行き 15-30 cm」となり，このエリアで調製を行うことで，調製された医薬品への微粒子および微生物混入を防ぐことができると考える．

Key words：クリーンベンチ，気流，可視化実験，清浄度