

Digital Twin for biological safety cabinet to reproduce performance and field certification test specified by NSF/ANSI 49 - 2019

Ryota Muta^{1*}, and Kazuhide Ito¹

¹ Faculty of Engineering Sciences, Kyushu University, Fukuoka, Japan

Abstract. The airflow balance test to confirm the physical performance of biological safety cabinets (BSC) is standardized by JIS K 3800 in accordance with NSF/ANSI 49 in Japan. In the test, *Bacillus atrophaeus* spores are used as an evaluation index, which poses a few limitations, such as their dependence on the environmental conditions to be tested. The aim of this study is to develop BSC digital twins and discuss reasonable alternatives for performance evaluation tests while maintaining the accuracy of the bacterial test performance. Herein, we provide the outline of a digital twin model for BSCs and the results of a numerical analysis, where a passive scalar is used as an alternative pollutant in the evaluation index.

1 Introduction

A biological safety cabinet (BSC) is a biohazard countermeasure device that is designed to perform the following three functions by controlling the airflow in the cabinet: (A) Personal protection, (B) Product protection, and (C) Prevention of cross-contamination between samples inside the cabinet. BSCs are classified into three types in terms of their structure and ventilation system, and NSF/ANSI 49 - 2019 applies to Class II (laminar flow) BSCs designed to minimize hazards inherent in tasks involving agents assigned to biosafety levels 1, 2, 3, or 4. In Class II, the air entering the cabinet is either recirculated into the cabinet or exhausted through a HEPA filter. This standard defines a performance test for confirming the basic requirements of BSCs. In Japan, JIS K 3800:2021 is standardized such that it is consistent with NSF/ANSI 49. [1] In this standard, a bacterial test using *Bacillus atrophaeus* spores is specified; however, this bacterial test is costly and time consuming, as it requires the incubation of the culture medium for more than 24 h.

Hence, this study is performed to develop a BSC digital twin and discuss reasonable alternatives for performance evaluation tests while maintaining the accuracy of the bacterial test. First, the flow field in a BSC is visualized via computational fluid dynamics (CFD) simulation. Subsequently, the bacterial test is reproduced by analyzing the passive scalar, i.e., the *Bacillus atrophaeus* spores. In addition, the effect of the differences in the blowout and inlet velocities of the BSC (offset conditions) on contaminant transport is investigated.

2 Materials/Methods

In this study, we reproduced an analysis model used for conducting a performance test based on the airflow balance test (JIS K 3800) in an ideal laboratory environment, as shown in Figure 1. In this standard test, a cylinder ($\phi 0.063 \text{ m} \times 0.58 \text{ m}$) was placed at the center of the BSC to account for the effect of the arms of a worker/operator using a BSC. To analyze the effect of the cylinder, an analysis model without a cylinder was also reproduced. The height of the BSC aperture was set to 0.25 m.

The JIS airflow test comprises three tests: (A) Personal protection, (B) Product protection, and (C) Prevention of cross-contamination between samples inside the cabinet as mentioned before. The contaminant generation positions for each test are shown in Figure 2. The contaminant generation surface was set to $\phi = 0.014 \text{ m}$. A hypothetical contaminant with the same physical properties as air was assumed to be the contaminant, and a passive scalar was generated from the abovementioned positions. The contaminant concentration distributions in and around the BSC were analyzed by solving the scalar transport equation, as shown in Eq. 1, after a steady-state flow field analysis was performed via CFD simulation. Here, the scalar transport equation with ensemble averaging operations is presented, assuming that the flow field is analyzed using the Reynolds-averaged Navier–Stokes model.

$$\frac{\partial \bar{\phi}}{\partial t} + \frac{\partial \bar{U}_j \bar{\phi}}{\partial x_j} = \frac{\partial}{\partial x_j} \left(\left(D_\phi + \frac{\nu_t}{\sigma_t} \right) \frac{\partial \bar{\phi}}{\partial x_j} \right) + S_\phi \quad (1)$$

* Corresponding author: muta.ryota.048@m.kyushu-u.ac.jp

Here, the effective diffusivity constant D_ϕ represents the molecular diffusivity, σ_t the turbulent Schmidt number (1.0), and ν_t the turbulent viscosity.

(A) The personal protection test is performed to evaluate whether workers are appropriately prevented from exposure to contaminants used inside the BSC. Therefore, contaminants are generated from the inside of the BSC toward the outside. An analysis that reproduces test (A) is used to evaluate whether contaminants leaked from inside the BSC. (B) The product protection test is performed to evaluate whether the contamination of samples in the BSC from outside the BSC is prevented effectively. Therefore, contaminants are generated from the outside toward the inside of the BSC. An analysis that reproduces test (B) is used to evaluate whether contaminants entering the BSC reach the workbench in the BSC. (C) The prevention of cross-contamination between samples inside the cabinet is tested to evaluate whether contamination between multiple samples inside the BSC is prevented effectively. An analysis that reproduces test (C) is used to evaluate whether contaminants spread toward the center of the workbench. Therefore, contaminants are generated near the walls of the BSC. Table 1 lists the cases used in this study. For each airflow test, the respective blowout and inlet velocities were set based on JIS K 3800. The actual operating conditions of the BSC were a blowout velocity of 0.40 m/s and an inlet velocity of 0.55 m/s (Cases 1-1, 2-1, 3-1, 4-1, and 5-1). Offset conditions were set for conditions in which the BSC did not function as intended (Cases 1-2, 1-3, 2-2, 2-3, 3-2, and 4-2). Table 2 lists the numerical and boundary conditions for the CFD simulations performed.

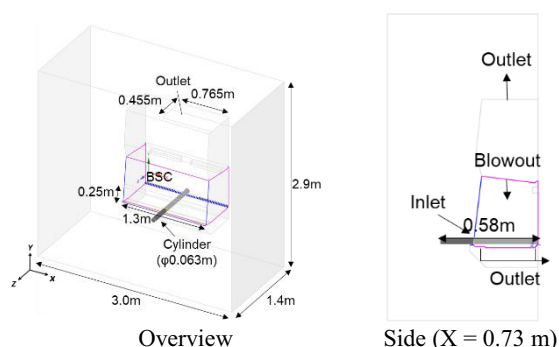


Fig. 1. Analysis model (with cylinder)

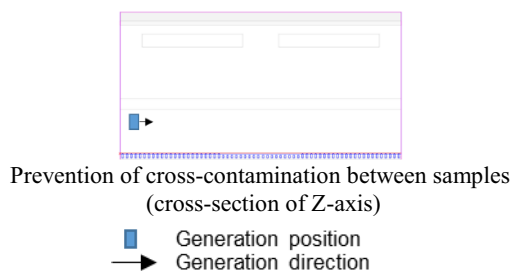
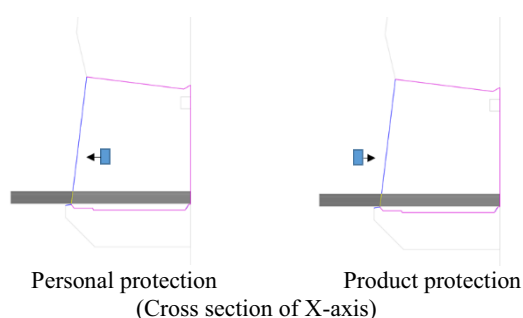


Fig. 2. Contaminant generation position

Table 1. Analysis cases

Airflow test	Case	Cylinder	Blowout velocity (m/s)	Inlet velocity (m/s)
A	1-1	-	0.40	0.55
	1-2		0.45	0.50
	1-3		0.35	0.50
	2-1	○	0.40	0.55
	2-2		0.45	0.50
	2-3		0.35	0.50
B	3-1	-	0.40	0.55
	3-2		0.35	0.60
	4-1	○	0.40	0.55
	4-2		0.35	0.60
C	5-1	-	0.40	0.55

Table 2. Numerical boundary conditions of CFD simulation

Turbulence model	SST k- ω model
Mesh	Without cylinder case 3,918,843 (Polyhedral mesh)
	With cylinder case 3,765,538 (Polyhedral mesh)
Inflow Boundary	Blowout (Pressure inlet)
Outflow Boundary	Outflow Cases 1-1, 2-1, 3-1, 4-1, and 5-1 0.45 m ³ /s
	Case 1-2, 2-2, 0.47 m ³ /s
Contaminant	Cases 1-3 and 2-3 0.40 m ³ /s
	Cases 3-2 and 4-2 0.43 m ³ /s
Contaminant	Passive scalar C ₀ = 1.0 [-]
	Spraying velocity for scalar 1 m/s (ϕ 0.014 m)

3 Results

3.1 Flow field distribution

Figure 3 shows the flow field distribution results for each case. The results for the (A) personal protection and (B) product protection tests are shown by the central cross-section of the X-axis ($X = 0.73$ m), whereas that for the (C) prevention of cross-contamination between samples inside the cabinet result is shown by a cross-section of the X-axis near the contaminant generation position ($Z = 0.49$ m).

In cases with appropriately controlled wind speeds, such as Cases 1-1 and 3-1, a laminar flow field was formed

from the upper blowout surface of the BSC to the lower workbench. The airflow impinging on the workbench flowed toward the exhaust outlet near the opening surface and the rear of the BSC. In addition, the airflow entering from the opening surface flowed toward the exhaust outlet near the opening surface. However, in the case where the BSC environment was assumed to be hazardous based on the appropriate controlled wind speed, the flow field in the BSC was slightly disturbed. In particular, when the blowout velocity was lower and the inlet velocity was higher, as in Cases 1-3 and 3-2, the flow field distribution near the opening sash of the BSC was non-uniform.

In all cases, the flow fields near the workbench and opening surface were non-uniformly distributed when the cylinder was placed in the center of the BSC.

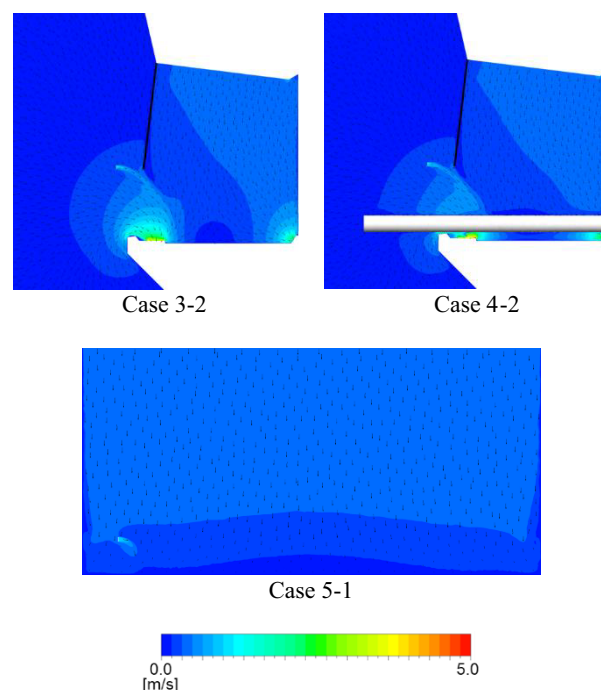
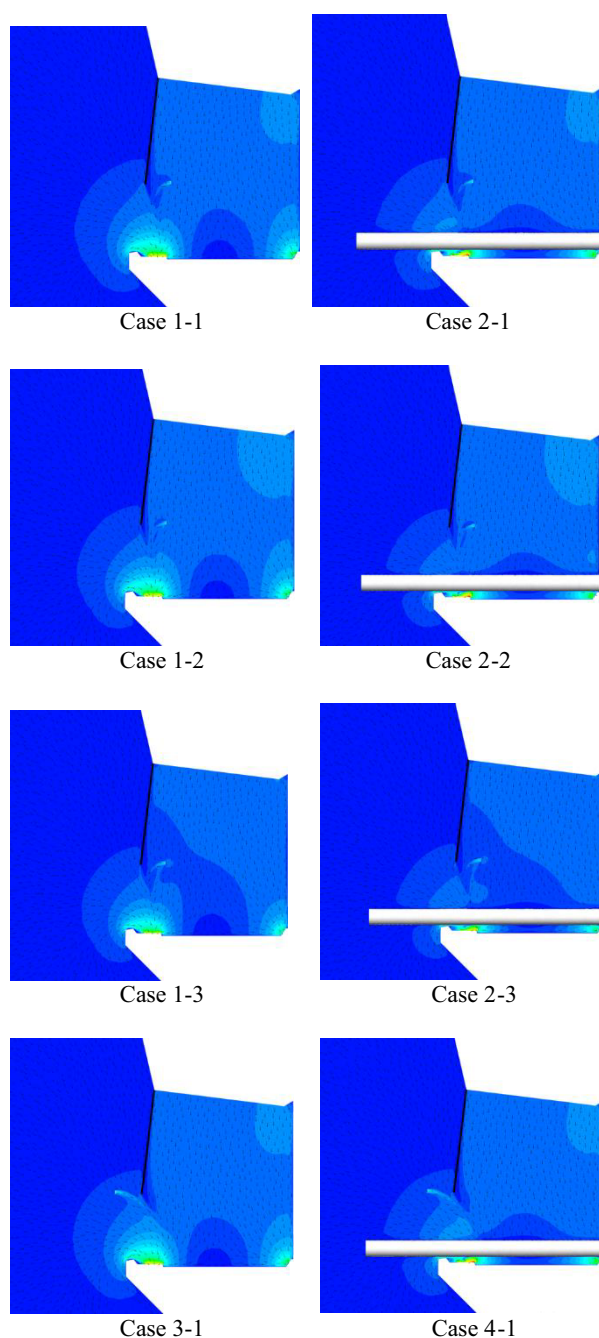


Fig. 3. Flow field in and around the BSC
(Cases 1-1 to 4-2: $X = 0.73$ m; Case 5-1: $Z = 0.49$ m)

3.2 Contaminant concentration distribution

Figure 4 shows the contaminant concentration distribution results for each case. The results for the (A) personal protection and (B) product protection tests are shown by the central cross-section of the X-axis ($X = 0.73$ m), whereas that for the (C) prevention of cross-contamination between samples inside the cabinet is shown by the cross-section of the X-axis near the contaminant generation position ($X = 0.19$ m) and BSC workbench surface.

The results for the (A) personal protection test indicate that the contaminants sprayed from the inside of the BSC toward the outside were exhausted by the exhaust outlet near the opening surface in all the cases. However, in Cases 1-3 and 2-3, where both the blowout and inlet velocities were lower, the contaminants propagated toward the outside near the opening surface. The blowout velocity inside the BSC was intended to transport the contaminants toward the exhaust outlet, whereas the inlet velocity was intended to prevent contaminants from leaking outside the BSC. In the cases involving a cylinder (Cases 2-1 to 2-3), contaminants impinging on the cylinder were transported along the cylinder to the exhaust outlet. No leakage to the outside of the BSC was observed along the top of the cylinder. Similarly, the results of the (B) product protection test indicate that contaminants from outside the BSC were exhausted by the exhaust outlet near the opening surface in all the cases. No contaminants were shown to enter the workbench surface of the BSC, with or without a cylinder. The results of the test for (C) prevention of cross-contamination between samples inside the cabinet test indicate that contaminants were exhausted to the exhaust outlet at the rear of the BSC.

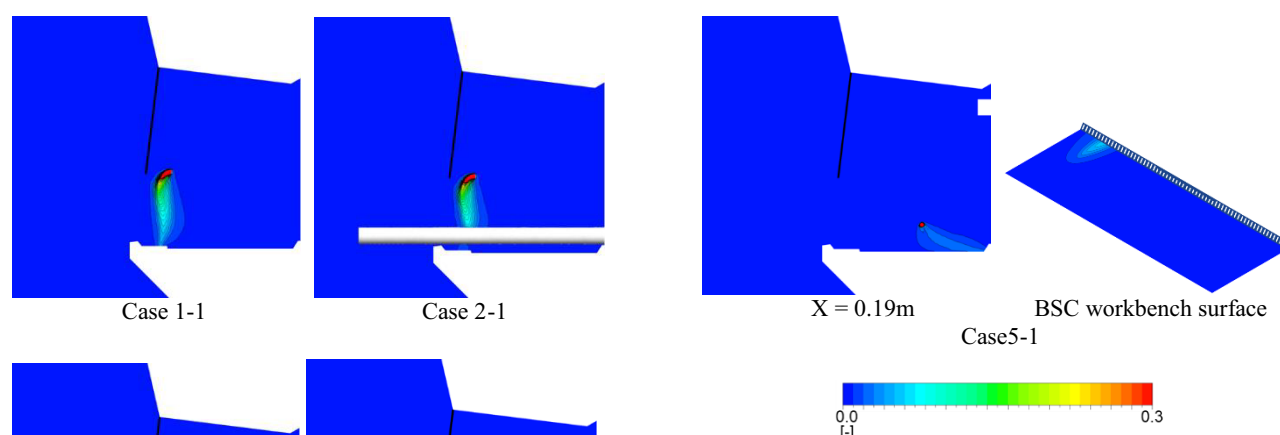


Fig. 4. Concentration distribution in and around the BSC
 (Cases 1-1 to 4-2: $X = 0.73$ m; Case 5-1: $X = 0.19$ m)

4 Discussion and Conclusion

In this study, the bacterial test standardized by JIS K 3800 was reproduced by analyzing the passive scalar, i.e., the *Bacillus atrophaeus* spores.

The results of the (A) personal protection test show no leakage of contaminants outside the BSC in any case. However, the contaminants spread near the opening surface when the blowout velocity became lower (Cases 1-3 and 2-3). This implies that the BSC should be tested under low blowout velocities to evaluate its containment performance. In the actual performance test by authors, some results indicated the leakage of *Bacillus atrophaeus* spores from the BSC. However, the reproducibility of the test results was low when multiple tests were conducted. In other words, some results did not indicate leakage outside the BSC. We assumed that these leaks were due to the background concentration in the actual test environment. Therefore, most of the *Bacillus atrophaeus* spores would have exhausted at the exhaust outlet near the opening surface, as indicated by the current analysis results. The results of the (B) product protection test results show that contaminants entering from outside the BSC did not spread to the workbench and were exhausted through the exhaust outlet in all cases. Under the conditions of low blowout velocity and high inlet velocity, the contaminants entered the BSC through a slightly wider region (Cases 3-2 and 4-2). In the actual performance test, no *Bacillus atrophaeus* spores were observed on the workbench. The results of the test for (C) prevention of cross-contamination between samples inside the cabinet test show that contaminants sprayed from the side of the BSC were exhausted to the exhaust outlet before spreading onto the workbench. In the actual performance test, two cases involving *Bacillus atrophaeus* spores were confirmed: one in which *Bacillus atrophaeus* spores were detected around the exhaust outlet near the opening surface, and the other around the exhaust outlet near the rear of the BSC, as in the current analysis. However, one must confirm that the *Bacillus atrophaeus* spores do not enter the workbench at least 0.36 m away from the side wall in test (C). In both the actual test and the current analysis, no

contaminants were indicated in the workbench more than 0.36 m away from the side wall.

The results above, which are consistent with those of the airflow balance test, indicate the flow field and contaminant distribution required to maintain the three functions of the BSC.

In future studies, we may consider changing the passive scalar of the CFD analysis to particulate matter using Lagrange method. In addition, actual measurements using tracer gas and particles should be conducted to verify the accuracy of the CFD analysis results. By comparing the analysis results based on a passive scalar (Eulerian method) and particulate matter (Lagrangian method), as well as by comparing the results of the actual test with those involving *Bacillus atrophaeus* spores, a reasonable alternative for performance evaluation tests can be proposed. Furthermore, we have analyzed the relationship between fume hood performance and the laboratory environment, and we will also analyze the same for BSC performance. [2,3]

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