

Performance of materials used for biological personal protective equipment against blood splash penetration

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Abstract: For occupational safety, healthcare workers must select and wear appropriate personal protective equipment (PPE), protective clothing, and masks as countermeasures against exposure to infectious body fluids and blood splash. It is important for healthcare workers to ensure the protective performance of each PPE against penetration of pathogens. The International Standards Organization (ISO) 22609 test evaluates the effectiveness of medical facemasks to protect against penetration of splashed synthetic blood. However, in this method, the protective performance is determined only visually, without quantification of leaked liquid volume. Therefore, in this study, we modified the ISO 22609 test method to quantify the volume of leaked liquid and obtain a more accurate assessment of the protection performance. We tested non-woven and woven materials used for masks or protective clothing, and the performance of each material was classified using this new method. We found that the quantity of leaked synthetic blood was dependent on the structural characteristics of each material. These findings will allow healthcare workers to select the most appropriate PPE for a given situation or task.

Key words: Personal protective equipment, Personal protective clothing, Nonwoven, Woven, Penetration, Blood splash, Hazardous biological agents

Introduction

Accidental occupational infections occur in laboratories, hospitals, and animal handling facilities as well as in some industries, pharmaceutical and food production, and agriculture¹.

In the case of accidental infection, the most common routes of pathogen entry are aerosol inhalation, percutaneous inoculation through needles or broken glass, animal bites or scratches, direct contact with contaminated surfaces, and accidental ingestion through a pipette^{2, 3}.

The most common hospital-acquired infections are those associated with surgery; in the gastrointestinal tract, bloodstream, or urinary tract via a catheter; and ventilator-associated pneumonia^{4, 5}. Ebola virus, Middle East respiratory syndrome coronavirus, hepatitis B virus (HBV), and norovirus are pathogens that have been linked to occupational infection^{6–9}.

To mitigate the risks of accidental infection, healthcare workers (HCWs) must wear appropriate personal protective equipment (PPE) in environments where they are exposed to pathogens. However, HCWs should be aware of the performance and suitability of different types of PPE in specific situations. For example, during an Ebola virus outbreak in western African in 2014, secondary accidental infections occurred in hospitals outside the affected

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Table 1. Tested fabrics

Type	Woven					
Sample No.	1	2	3	4	5	
Materials	Polyester 50% Cotton 50%	Cotton 100%	Polyester 65% Cotton 35%	Polyester 65% Cotton 35%	Polyestr 100%	
	Plain	Twill (Katsuragi) No. 14	Twill (Katsuragi)	Weather	High density	
Finishing	Non-repellent	Absorption	Repellent 5	Repellent	Repellent	
Weight (g/m ²)	211	290	267	170	159	
Use	Mask, Inner	Mask, Gown, Drape	Gown, Drape	Gown	Gown	
Protective performance classification by the pressurized cell test (JIS T 8060)	<Class 1	<Class 1	Class 1	<Class 1	Class 1	

Type	Nonwoven					
Sample No.	6	7	8	9	10	11
Materials	PP 100%	PET 45% Wood pulp 55%	PP 100%	PP and PE	PE 100%	PE 100%
	Spunbond	Spunlace	SMS	SMS	Flushspun	Flushspun
Finishing	—	Repellent	—	—	—	—
Weight (g/m ²)	70	70.4	56	51.45	41.0	41.0
Use	Gown, Protective clothing	Mask, Gown, Drape	Protective clothing	Protective clothing, Gown	Protective clothing	Protective clothing
Protective performance classification by the pressurized cell test (JIS T 8060)	<Class 1	Class 1	Class 1	Class 1	Class 3	Class 2

countries^{10, 11}). In response to this crisis, the World Health Organization and U.S. Center for Disease Control and Prevention published guidelines for HCWs treating Ebola patients^{12, 13}) that included wearing PPE covering the entire body—i.e., masks, personal protective clothing (PPC), head covers, gloves, goggles, and boots. The guidelines also stipulate the selection and use of PPE with high performance in terms of protection from sprayed liquids, such as contaminated body fluids.

There are various tests for evaluating PPE performance. The International Organization for Standardization (ISO) 22609 test measures the protection performance of medical face masks with respect to penetration of splashed synthetic blood (SB)¹⁴). In this test, the protective performance is determined by visual inspection. However, a more accurate mode of evaluation based on the quantification of leakage liquid volume is desired, given that our previous study found a positive correlation between volume of leaked SB and the number of microbes that penetrated PPC¹⁵).

In this study, we modified the ISO 22609 test method, using absorption paper to measure leaked liquid volume through woven and non-woven materials used for PPC

or masks, to evaluate their protection performance more accurately.

Subjects and Methods

Test material fabrics

Eleven fabrics used in commercially available PPC or masks at hospitals were tested in this study (Table 1). These fabrics were previously tested for penetration resistance to SB according to the pressurized cell test (JIS T 8060, Fig. 1)¹⁶) and grouped into specific classes according to the response to applied pressure¹⁷). In JIS T 8060, the loaded pressure level is divided into six stages, and the pressure is increased step-by-step at 5-min intervals. Higher-class fabrics were more resistant to pressure—i.e., of those that were woven, samples 1, 2, and 4 were in class <1, whereas samples 3 and 5 were in class 1; and of those that were non-woven, sample 6 was in class <1; samples 7, 8, and 9 were in class 1; sample 11 was in class 2; and sample 10 was in class 3¹⁸). Samples were cut into squares measuring 13 × 13 cm for testing.

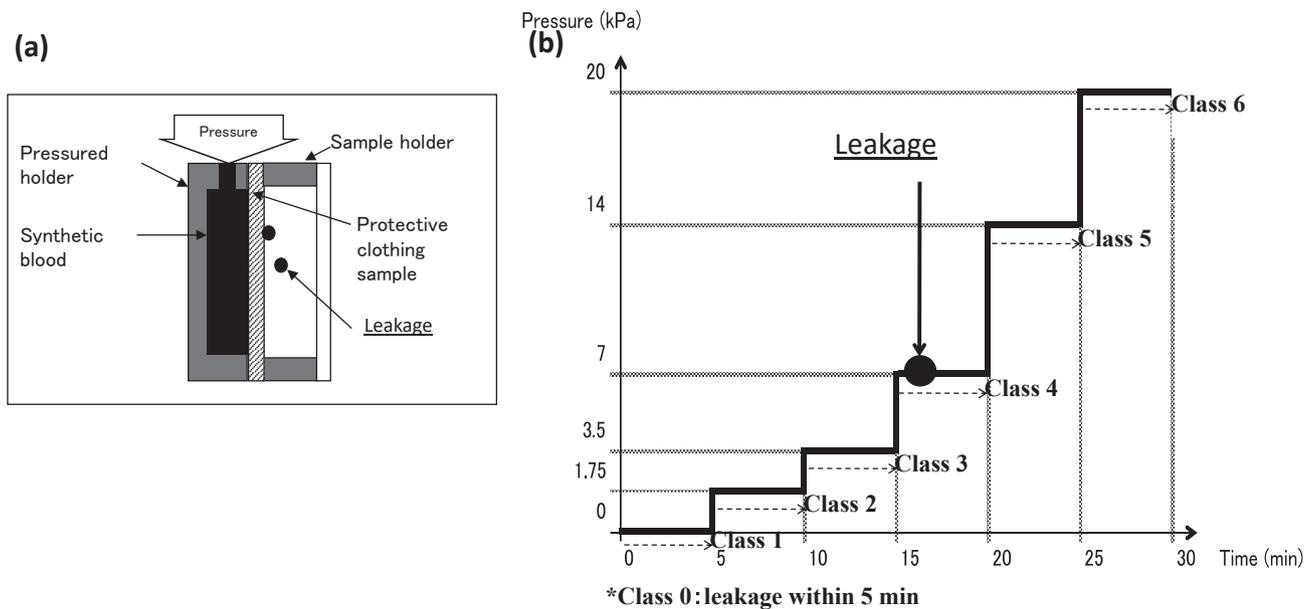


Fig. 1. Pressure protocol according to JIS T 8060.

The diagram of test devices (a) and time course graph of applied pressure (b) based on JIS T 8060 are shown.

The pressure is increased step-by-step at 5-minute intervals. For example, if it was observed visually that the SB did not leak through a protective clothing sample at 3.5 kPa but did leak at 7 kPa after more than 15 minutes, the sample was classified into Class 3.

Testing apparatus and procedure

The experimental setup of the testing apparatus based on ISO 22609 is shown in Fig. 2a. The apparatus consisted of a testing booth equipped with a splash gun, sample holder, and a splash pressure control unit. We tested woven and non-woven materials used for PPC or masks at impact pressures of 16.0 and 21.3 kPa, which were the same as those for SB in ISO 22609. We used Kimtowel paper (Nippon Paper Crexia Co., Tokyo, Japan) to absorb and easily visualize the leaked liquid. The fabric sample was placed in the sample holder along with a sheet of the absorbent paper with a diameter of 8 cm. The distance between the splash gun and sample holder was 30 cm. A 2-ml volume of SB (Synthetic Blood Reagent Mix: ISO 16603; Johnson, Moen & Co., Rochester, MN, USA) was ejected from the splash gun onto the sample, which was then removed from the holder along with the paper. The back of the sample was checked for leaked SB and the area was measured to determine leakage volume (Fig. 2b). The test was repeated five times for each sample.

Estimation of SB penetration volume

The area of leakage (length and breadth of the ellipse) on the absorption paper was measured using a ruler. The SB penetration volume was estimated from the measured area based on a linear standard curve obtained before the

test by analyzing the correlation between the dispensed volume of SB and detected area, using the following equation: detected area (mm^2) = $3.7844 \times$ dispensed volume of SB (μl); $R^2 = 0.9987$.

Results

Quantification of leaked SB volume for woven samples

We tested and estimated leaked SB volume on absorption paper for five woven samples (Fig. 3). The volume was correlated with splash gun pressure for all samples except 2 and 3, which had twill weave structures (Katsuragi) (Table 1). Samples 2 and 3 had similar leakage volumes at 21.3 and 16.0 kPa. The volume varied by more than 100-fold between samples 1 and 2 and sample 5. Accordingly, the five woven samples were classified into two groups by this test method: samples for which leakage volumes at 21.3 kPa were >100 and $<50 \mu\text{l}$ were grouped as low and high-performance groups, respectively.

Quantification of leaked SB volume for non-woven samples

We tested six non-woven samples and estimated the volume of SB that leaked onto absorption paper for each sample (Fig. 4). Leakage volume was correlated with splash gun pressure for all samples except 10 and 11, which had flashspun fabric structures (Table 1). Samples 10 and 11

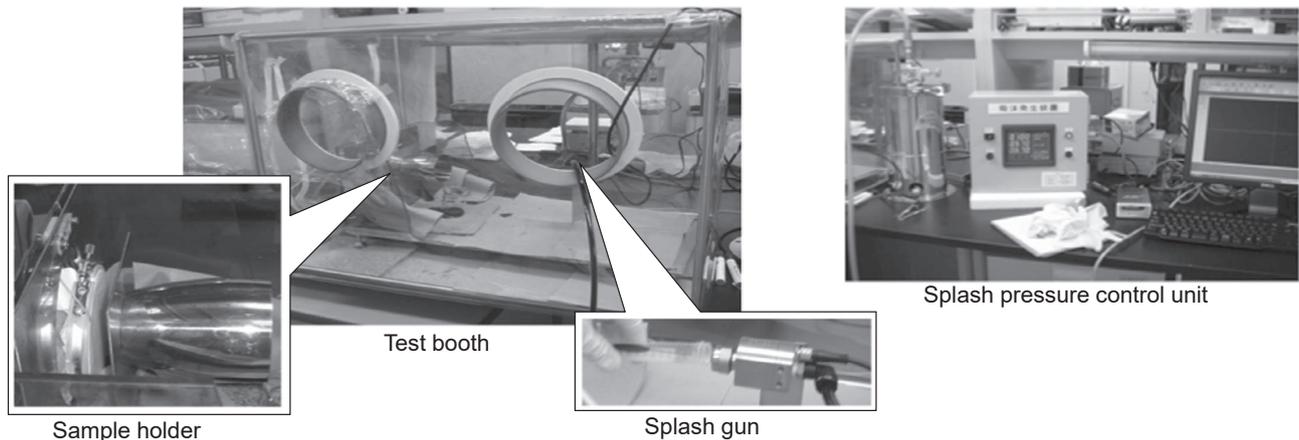


Fig. 2a. Experimental setup of testing apparatus.

The testing apparatus consisted of a test booth equipped with a splash gun, sample holder, and a splash pressure control unit. Samples were placed on a sheet of absorption paper in the holder, which had a diameter of 8 cm. The distance between the splash gun and sample holder was 30 cm. A 2-ml volume of SB was ejected by the splash gun at the sample.

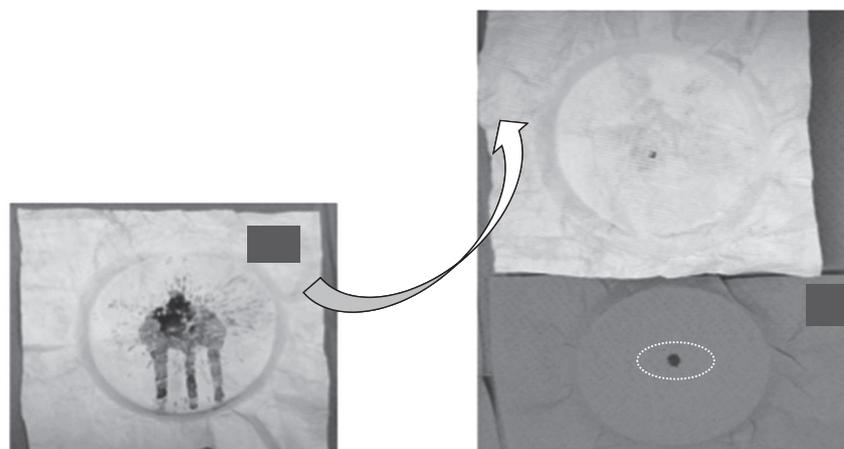


Fig. 2b. Representative image of test sample and absorption paper after the test.

Leaked SB on the absorption paper is encircled by a dotted white line in the bottom right photograph.

had similar leakage volumes at 21.3 and 16.0 kPa. The volume varied by more than 100-fold between sample 6 and samples 10 and 11. Based on these observations, the six non-woven samples were classified into low-, moderate-, and high-performance groups (i.e., samples for which leakage volumes at 21.3 kPa were $>100 \mu\text{l}$, between 100 and $50 \mu\text{l}$, and $<50 \mu\text{l}$, respectively). The above findings indicate that quantitative differences in protection performance among samples were distinguishable by our modified test method.

Comparison of detection sensitivity

The SB detection sensitivity of our test at an impact

pressure of 21.3 kPa was carried out by visual inspection of leaked SB on the back surface of the sample and on the absorption paper. The sample fabric was considered as having failed the splash test if SB leakage was detected (upper two rows of Table 2). For woven sample 3, the fail rate was 5/5 based on the absorption paper and 0/5 by visual inspection; for samples 4 and 5, the rates were 5/5 and 3/5, respectively. For non-woven sample 10, the fail rate was 2/5 based on the absorption paper and 1/5 by visual inspection; for sample 11, the rates were 4/5 and 3/5, respectively. Therefore, leakage could be detected with greater sensitivity using absorption paper than by visual inspection (i.e., the ISO 22609 test).

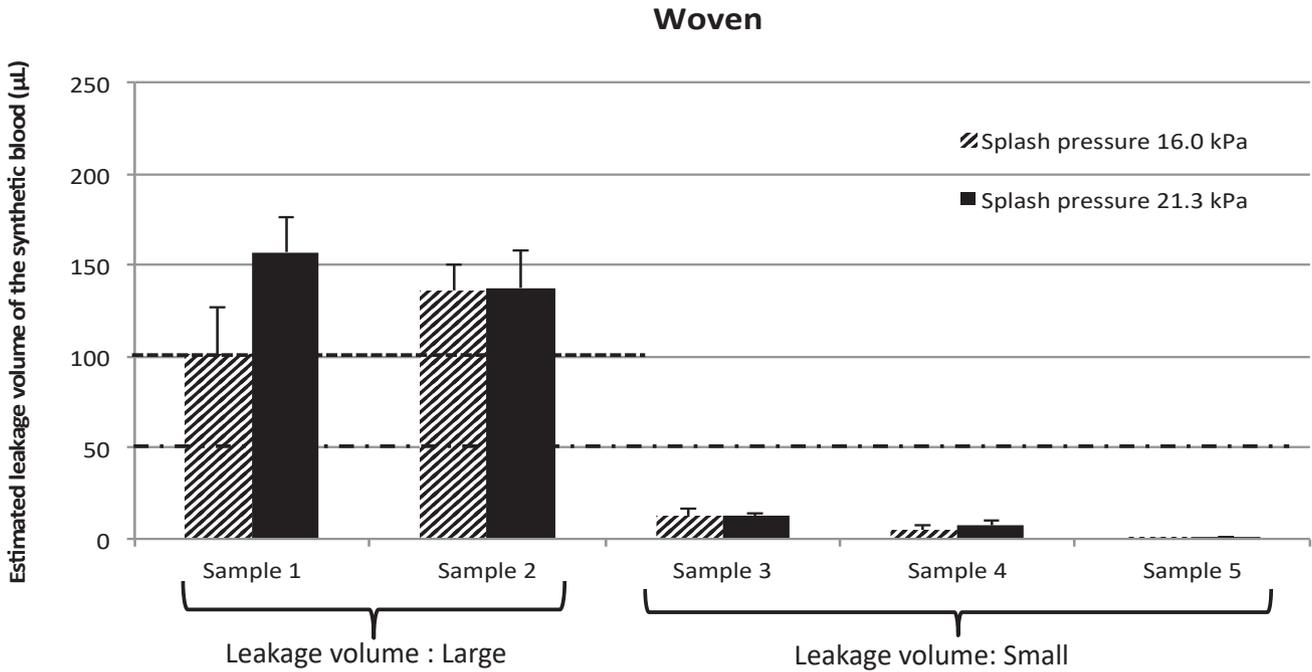


Fig. 3. Quantification of leaked volume of SB on woven fabric.

The volume of SB penetrating five woven fabric samples was quantified. Values represent the mean of five experiments, and error bars denote standard deviation. The five samples were classified into two groups based on leaked volume at 21.3 kPa (>100 µl and <50 µl in large and small leakage groups, respectively).

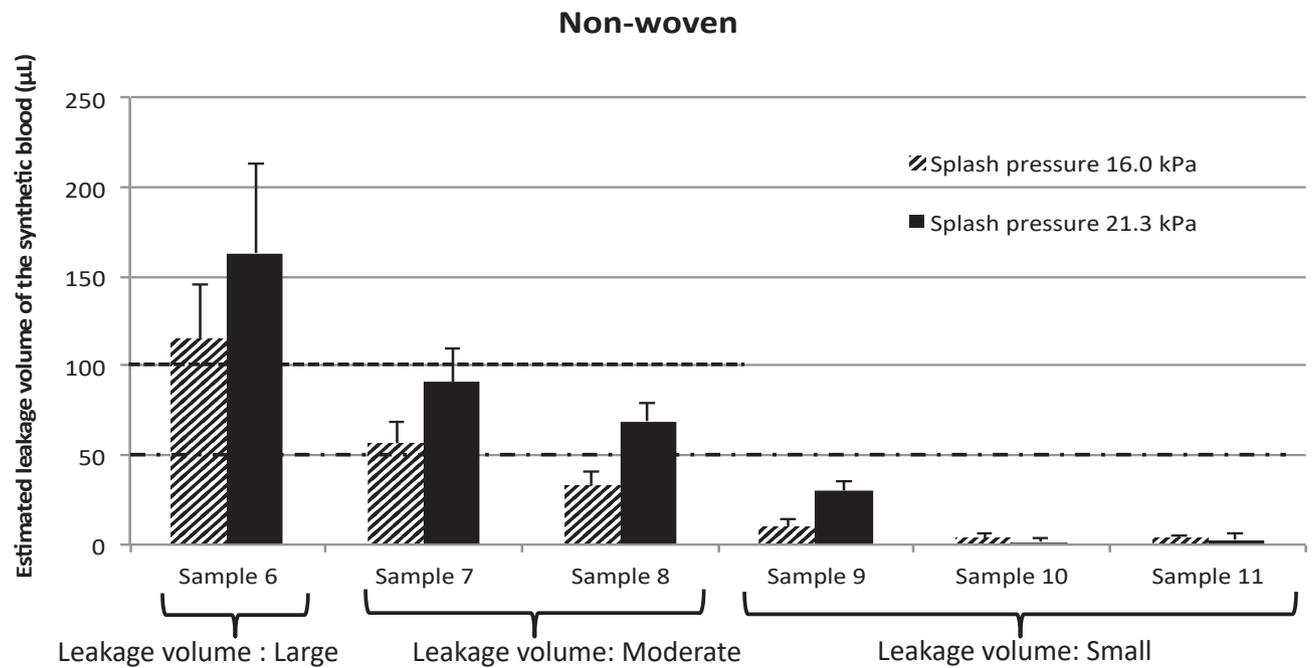


Fig. 4. Quantification of leaked volume of SB on non-woven fabric.

The volume of SB penetrating six non-woven fabric samples was quantified. Values represent the mean of five experiments, and error bars denote standard deviation. The five samples were classified into three groups based on leaked volume at 21.3 kPa (>100 µl, between 100 and 50 µl, and <50 µl in large, moderate, and small leakage groups, respectively).

Table 2. Summary of results

Type	Woven				
Sample No.	1	2	3	4	5
Fail ratio in splash test (by visual inspection) (21.3 kPa)	5/5	5/5	0/5	3/5	3/5
Fail ratio in splash test (using absorption paper) (21.3 kPa)	5/5	5/5	5/5	5/5	5/5
Leakage volume by the quantitative splash test	Large	Large	Small	Small	Small
Protective performance classification by the pressurized cell test (JIS T 8060)	<Class 1	<Class 1	Class 1	<Class 1	Class 1

Type	Nonwoven					
Sample No.	6	7	8	9	10	11
Fail ratio in splash test (by visual inspection) (21.3 kPa)	5/5	5/5	5/5	5/5	1/5	3/5
Fail ratio in splash test (using absorption paper) (21.3 kPa)	5/5	5/5	5/5	5/5	2/5	4/5
Leakage volume by the quantitative splash test	Large	Middle	Middle	Small	Small	Small
Protective performance classification by the pressurized cell test (JIS T 8060)	<Class 1	Class 1	Class 1	Class 1	Class 3	Class 2

Discussion

In this study, we evaluated the protection performance of woven and non-woven materials used in commercially available PPC or masks at hospitals to protect against leakage of splashed blood, using a modified version of the ISO 22609 test. The results are summarized in Table 2. We found that the volume of leaked liquid was dependent on the structural characteristics of each material; samples 3, 4, 5, 10, and 11 had low leakage volumes. Detection sensitivity was improved by using absorption paper rather than by relying on simple visual inspection. In this study, an SB volume as low as $0.05 \mu\text{l}$ that penetrated sample 5 was detected using absorption paper (Fig. 3). Our previous study demonstrated a positive correlation between leaked SB volume and number of penetrated microbes¹⁵). Therefore, quantifying the volume of leaked liquid is a more effective approach for evaluating the protection performance of materials used to manufacture PPC and masks against pathogens than the current method based on visual observation. For instance, HBV DNA concentration in the whole blood of infected patients was found to

be 7.5×10^5 – 4.3×10^8 copies/ml,¹⁹) HBV-infected patient blood contains 37.5 – 2.15×10^4 copies HBV DNA/0.05 μl . Thus, HCWs are at high risk of HBV infection, given that a previous study reported that the minimum amount required for transmission of HBV is approximately 30 copies in the case of chimpanzees²⁰).

The protection performance of each sample material against SB splashes was not correlated with that of each material against SB impact pressure (Table 2 and Fig. 1). This implies that protection performance is dependent on multiple factors—i.e., mainly material structure, but also load condition (splash impact and continuous pressure). Therefore, it is necessary to test materials under various conditions to determine their protective capacity. It is important for HCWs to select suitable PPC and masks certified by testing. Identifying differences in protection performance by various test methods can facilitate PPE selection based on risk assessment so that accidental exposure to infectious agents can be avoided. However, there are few methods currently available for testing the performance of protective materials against hazardous biological agents (Table 3). These tests typically measure the

Table 3. ISO or JIS for PPE

ISO No. or JIS No.	Title
ISO 6530:2005 JIS T 8033:2008(MOD)	Protective clothing—Protection against liquid chemicals—Test method for resistance of materials to penetration by liquids
ISO 13982-1:2004 JIS T 8124-1:2010(MOD)	Protective clothing for use against solid particulates—Part 1: Performance requirements for chemical protective clothing providing protection to the full body against airborne solid particulates (Type 5 clothing)
ISO 13982-1 Amd1:2010	Protective clothing for use against solid particulates—Part 1: Performance requirements for chemical protective clothing providing protection to the full body against airborne solid particulates (type 5 clothing) Amendment 1
ISO 13982-2:2004 JIS T 8124-2:2008(MOD)	Protective clothing for use against solid particulates—Part 2: Test method of determination of inward leakage of aerosols of fine particles into suits
ISO 16602:2007 JIS T 8115:2015(MOD)	Protective clothing for protection against chemicals—Classification, labelling and performance requirements
ISO 16602:2007/Amd1:2012	Protective clothing for protection against chemicals—Classification, labelling and performance requirements—AMENDMENT 1
ISO 17491-1:2012 JIS T 8032-1:2015(MOD)	Protective clothing—Test methods for clothing providing protection against chemicals—Part 1: Determination of resistance to outward leakage of gases (internal pressure test)
ISO 17491-2:2012 JIS T 8032-2:2015(MOD)	Protective clothing—Test methods for clothing providing protection against chemicals—Part 2: Determination of resistance to inward leakage of aerosols and gases (inward leakage test)
ISO 17491-3:2008 JIS T 8032-3:2015(MOD)	Protective clothing—Test method for clothing providing protection against chemicals—Part 3: Determination of resistance to penetration by a jet of liquid (jet test)
ISO 17491-4:2008 JIS T 8032-4:2015(MOD)	Protective clothing—Test method for clothing providing protection against chemicals—Part 4: Determination of resistance to penetration by a spray of liquid (spray test)
ISO 17491-5:2013 JIS T 8032-5:2015(MOD)	Protective clothing—Test methods for clothing providing protection against chemicals—Part 5: Determination of resistance to penetration by a spray of liquid (Manikin spray test)
ISO 16603:2004 JIS T 8060:2015(MOD)	Clothing for protection against contact with blood and body fluids—Determination of the resistance of protective clothing materials to penetration by blood and body fluids—Test method using synthetic blood
ISO 16604:2004 JIS T 8061:2015(MOD)	Clothing for protection against contact with blood and body fluids—Determination of the resistance of protective clothing materials to penetration by blood-borne pathogens—Test method using Phi-X174 bacteriophage
JIS T 8122:2015	Protective clothing for protection against hazardous biological agents
ISO 22609:2004 JIS T 8062:2010(MOD)	Clothing for protection against infectious agents—Medical facemasks—Test method for resistance against penetration by synthetic blood (fixed volume, horizontally projected)
ISO 22610:2006	Surgical drapes, gowns, and clean air suits, used as medical devices, for patients, clinical staff and equipment—Test method to determine the resistance to wet bacterial penetration
ISO 22612:2005	Clothing for protection against infectious agents—Test method for resistance to dry microbial penetration

penetration of a liquid/particle or the permeation of molecules through PPE materials or membranes²¹). For handling hazardous biological agents such as microorganisms, PPE that protects against microparticles with a size of ~20 nm is required. Therefore, materials with high protective performance against penetration or permeation of liquid or small particles are equally suitable for protection against hazardous biological agents.

In conclusion, the results of this study provide a basis for evaluating and selecting materials for PPC or masks based on their capacity for protection against splashed blood, which can be quantitatively analyzed using our modified method. The performance information of PPE can help HCWs select PPE suited to biological hazards based on the risk of infection.

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