Aseptic Production, Gowning Systems, and Airborne Contaminants

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DIGITAL STOCK

Using a modified dispersal chamber, the authors have studied the protective efficacy of cleanroom clothing systems. Study results show that the state of a cleanroom clothing system—new or much used—influences the protection efficacy of the system. Suitable combinations of cleanroom underwear and cleanroom garments also improve the protection of the clean environment against airborne contaminants from people.

Because the production of sterile products for global sale requires manufacturing conditions that comply with increasingly strict national and international requirements of cleanliness and documentation, sterile drugs today are manufactured in classified cleanrooms.

In the International Organization for Standards' (ISO) standard 14644-1, "Clean rooms and Associated Controlled Environments," a cleanroom is defined as,

> "A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room and in which other relevant parameters, *e.g.*, temperature, humidity, and pressure, are controlled as necessary." (1)

As technical solutions have improved, current good manufacturing practice (CGMP) requirements regarding manufacturing conditions (*e.g.*, the total number and viable number of airborne particulates) for sterile drugs have become more stringent. The various international GMP requirements, however, have not yet been fully harmonized. For pharmaceutical cleanrooms, the most commonly used guidelines and requirements are:

- The US Food and Drug Administration's CGMPs, which apply to products sold in the United States;
- The European Union's GMP Annex 1, which applies to products sold in the European Union (EU).

Annex 1 of the *European Commission (EC) Guide to Good Manufacturing Practice* provides supplementary guidance regarding the application of the principles and guidance of GMPs to sterile products (2). The guidance includes recommendations for standards of environmental cleanliness for cleanrooms.

Table I lists, as of January 2005, the maximum permitted or recommended number of viable particles in cleanrooms according to EU Annex I, FDA GMPs, and the US Pharmacopeia (*USP 24–NF 19*) (2, 4, 5). Neither ISO 14644-1 nor 14698-1 defines the number of viable airborne particles permitted in a classified cleanroom or associated controlled environment (1, 3).

To avoid contamination originating from the process, all materials (ingredients and products) transferred into clean aseptic areas must be sterile-filtered, autoclaved, or cleaned and sterilized in some other way. In addition, personnel must enter the cleanroom through appropriate changing rooms.

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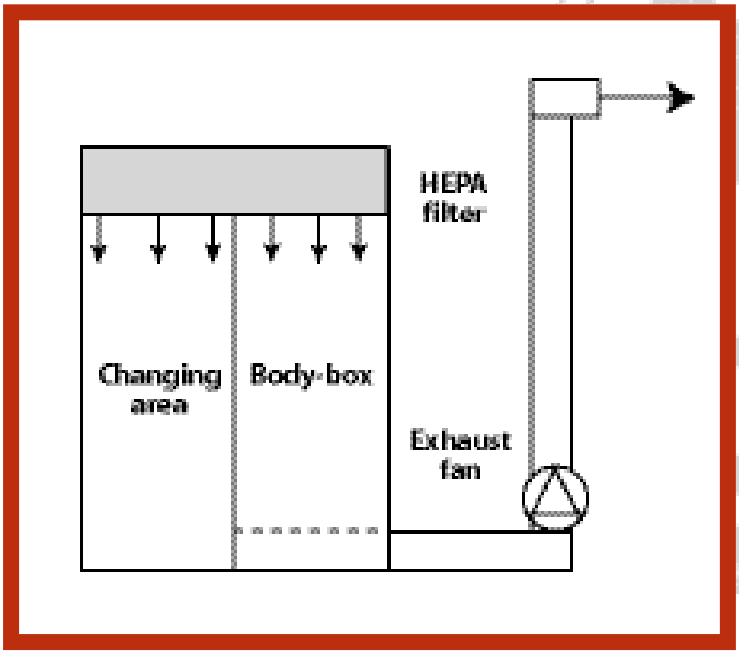

Figure 1: The arrangement of a modified dispersal chamber (body box) used to study the protective efficacy of clothing systems.

Because supply air is filtered through HEPA-filters, the main sources of airborne particles in cleanrooms are people and operating machinery. People are the principal source of viable airborne particles. Thus, to protect the air quality, operators should wear specially designed cleanroom clothing.

For every operator in a critical (grade A or B) area, clean and sterile protective garments, including sterile gloves and masks, must be provided for each working session. Cleanroom clothing must be of high quality, clean, and sterile (sterilized or adequately sanitized). Separate laundry facilities for such clothing are desirable.

Table I: Maximum permitted or recommended airborne viable particles (colony-forming units/m^3) in pharmaceutical cleanrooms.

EU GMP Annex 1		FDA 2004 (4)		*USP 24–NF 19* (5)	
Grade	**Maximum particle count (cfu/m^3)**	**Area**	**Maximum particle count (cfu/m^3)**	**US customary class**	**Maximum particle count (cfu/m^3)**
A	<1	Critical area	1	100	<3
		Background to critical area, US Customary Class 1000	7		
B	10	Background to critical area, US Customary Class 10,000	10	10,000	20
C	100	Controlled area, US Customary Class 100,000	100	100,000	100
D	200				

People

People disperse fragments from the skin. The airborne dispersion of such particles varies from person to person and from time to time. According to Akers, during aseptic production, most cases of microbiological product contamination are caused by airborne contamination (6). Within a cleanroom, people are the main source of such microbiological airborne contaminants.

According to the requirements for current good manufacturing practices (CGMPs), all personnel entering an aseptic production area must be qualified with regard to gowning techniques. People within the aseptic production and the critical areas are frequently monitored by a combination of sampling methods. People with skin problems, who are referred to as *shedders*, should not work in an aseptic cleanroom.

Cleanroom clothing

The primary purpose of cleanroom clothing is to protect products and the processing environment from airborne contamination. Clothing should cover a person as completely as possible to prevent significant numbers of contaminants from being dispersed into a cleanroom. The properties of the fabrics used for cleanroom clothing can be assessed by measuring air permeability, particle retention, and pore size. The fabric itself should disperse only a minimal number of particles and be resistant to wear and tear. The effectiveness of cleanroom clothing will deteriorate, however, because of factors such as aging, wear, washing, drying, and sterilizing.

Today's aseptic cleanroom clothing consists of a combination of disposable or reusable coveralls, hoods, long boots, and accessories such as gloves, face masks, and goggles. All items must be of appropriate high quality, clean, and sterile (sterilized or adequately sanitized). For reusable parts, the washing process as well as the sterilization process or the sanitization processes must be validated.

Testing the material properties for aseptic clothing is mainly performed by the material or clothing manufacturer. Most tests are conducted on new material and study particle generation, particle filtration, and resistance to wear.

At KTH (the Royal Institute of Technology) in Sweden, a modified dispersal chamber has been used to study the protective efficacy of cleanroom clothing (see Figure 1). Comparative studies have been performed in the dispersal chamber on selected clothing systems of both new systems and systems washed and sterilized 50 times. Detailed results have been reported by Ljungqvist and Reinmüller (7, 8).

The tests performed. The two reusable cleanroom clothing systems studied were two sets of the same model made by a single manufacturer. The materials consisted of continuous filament polyester fabric with an electrostatic

Table II: The effect of using special cleanroom underwear on the filtration efficacy of particles and colony-forming units (cfu)*.

	Aerobic cfu/s		
	Mean value	Confidence interval	
Part (number of test subjects)		Lower	Upper
Part 1 (n = 5)	0.51	0.24	0.78
Part 2 (n = 5)	0.24	0.10	0.39

*The 95% confidence intervals (t-distribution) for source strengths of airborne aerobic cfu/s from test subjects dressed in new cleanroom clothing systems. Parts 1 and 2 comprised all 10 test subjects. The five test subjects in part 1 wore a cleanroom undershirt and short underpants; the five test subjects in part 2 wore a cleanroom undershirt and long-legged cleanroom underpants.

Table III: Performance of cleanroom clothing systems after various numbers of washing and sterilization cycles.*

		Particles/s and colony-forming unit (cfu)/s		
Clothing	Contaminant	After 1 wash and sterilization cycle	After 25 wash and sterilization cycles	After 50 wash and sterilization cycles
High-quality cleanroom clothing	Particles ≥0.5 µm	585	3950	2860
	Particles ≥5 µm	9	70	36
	Colony-forming units	0.38	0.49	1.14

*Comparison of data (mean values) of the source strength (generated particles and colony-forming units [cfu] per second). People were dressed in various clothing systems washed and sterilized once, 25 times, or 50 times, respectively.

discharge (ESD) stripe. The weave, weight (g/m^2), and surface treatment, however, varied. A disposable system also was tested for comparison. Several types of underwear were tested in combination with the cleanroom coveralls.

The washing process and sterilizing cycle used in the study consisted of a washing process at a temperature of 73 ± 2 °C for 10 minutes, rinsing steps with filtered deionized water, and dry-tumbling with HEPA-filtered warm air. The sterilization cycle was carried out in a steam autoclave at 121 °C for at least 20 minutes.

The containment tests in the dispersal chamber evaluated the protection efficacy of the clothing systems by measuring the concentration of total airborne particulates and viable particulates (as aerobic colony-forming units, or cfu) in the exhaust air duct. The total number of airborne particles was determined using a discrete particle counter (Hiac/Royco model 245, Pacific Scientific Instruments/Hach Ultra Analytics, Grants Pass, OR, www.pacsciinst.com). Viable particles were collected with a slit-sampler (Impactor FH3/FH5, Markus Klotz GmbH, Bad Liebenzen, Germany, www.fa-klotz.de) and in some cases also with a sieve-sampler (Andersen 6-Stage Sampler, Anderson Samplers Inc., Wendell, GA). All instruments were operated according to the manufacturers' instructions. Microbiological growth medium for all tests was standard medium tryptic soy agar (TSA) in 9-cm Petri dishes. The TSA plates were incubated for at least 3 days at 32 °C followed by at least 2 days at room temperature.

Microbiological results. No significant performance difference was seen between the disposable clothing and the two sets of reusable clothing that were only washed and sterilized once. The variation among the test subjects was greater than the variation between the systems. The use of special cleanroom underwear, however, enhanced the filtration efficacy of both particles and colony-forming units (see Table II). There were five test subjects in each group: the test subjects in part 1 wore a cleanroom undershirt and short underpants and the subjects in part 2 wore a cleanroom undershirt and long-legged cleanroom underpants.

Table III summarizes the results of testing three sets of cleanroom clothing: new clothing (washed and sterilized only once), clothing washed and sterilized 25 times, and clothing washed and sterilized 50 times. More particles were generated after 25 washing and sterilization cycles than after 50. This result suggests that the particles may have been generated from the material itself and not by the activity of the test subjects. The number of colony-forming units generated, however, increased as the number of washing and sterilization cycles increased.

Discussion

The study results show that the source strength—number of particles including the number of colony-forming units generated from people—increases with the number of washing and sterilization cycles even when high-quality cleanroom clothing is used. Some estimates are given in the following example.

Example. In a 90-m^3 aseptic filling room with 20 air changes per hour, the only microbiological contamination source is the operators. The current colony-forming unit limit for grade B areas (under EU GMPs) and for the background to critical areas (under FDA rules) is 10 cfu/m^3. Theoretically, how many operators can be allowed in an aseptic filling room if the limit of 10 cfu/m^3 cannot be exceeded during operating conditions?

This can be determined by calculating the cfu concentration in the filling room by taking the source strength for one person (from Table III) and dividing it by the total air volume flow. The theoretical maximum number of people allowed in the aseptic filling room will be the limit value divided by the calculated concentration for one person.

Table IV shows the source strengths (cfu/s) for high-quality cleanroom clothing and the estimated maximum number of operators allowed in an aseptic filling room with a maximum level of 10 cfu/m^3.

Calculations based on the reported results and the dilution effect of a ventilation system with at least 20 air changes per

Table IV: Source strengths (cfu/s) for high-quality cleanroom clothing and the estimated maximum number of operators allowed in an aseptic filling room with a maximum level of 10 cfu/m³.

Number of washing and sterilizing cycles	Source strength (cfu/s)	Concentration (cfu/m³)	Maximum number of operators
1	0.38	0.76	13
25	0.49	0.98	10
50	1.14	2.28	3

hour and HEPA-filters show that the limit of 10 cfu/m³ can be maintained with three people present in a commonly sized cleanroom for aseptic production (9).

The results of the study show that the state of a cleanroom clothing system—new or much used—is important because it influences the protection efficacy of the system. Suitable combinations of cleanroom underwear with the cleanroom garments also improve the protection of the clean environment against airborne contaminants from people. So far, no other test method than measurements in a dispersal chamber have been reported to give information about cleanroom clothing's protection efficacy during use.

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