UDC 628.513:697.94

FUJI BIOCLEAN ROOM AND BIOHAZARD CONTAINMENT TECHNOLOGY

Madoka Uchida
Yutaka Hishita

1 INTRODUCTION

There are two types of clean room (hereinafter abbreviated to as CR). One of them is mainly to control number of airborne particles, and the other is to control not only the airborne particles but also microorganic particles floating in the air. The latter is called Biological Clean Room (hereinafter abbreviated to as BCR).

The primary purpose of the BCR is to reduce number of microorganisms floating in the air. At present, however, the idea and building technique of the BCR are same as those of the CR for general industries, and based on the dust and germ eliminating functions of High Efficiency Particulate Air Filter (HEPA filter), high volume sterilized air circulating method is used.

The HEPA filter which is used exclusively as a sterilizing equipment for the BCR has been proved to be highly effective in collecting microorganisms floating in the air, through a number of experiments, and the performance has been insured with the actually operating systems. *Table 1* and 2 indicate results of germ filtering efficiency measurement on HEPA filters conducted by Furuhashi and others, and *Table 3* indicate number of microorganisms floating in a class 10,000 (JIS Class 5) CR and data for difference in number of germs between inside and outside of a class

Table 1 Germ collection efficiency of HEPA filter

Type of air filter	Number of experiments	Germ filtering efficiency (%) Mean ± S.E.	Air zole passing velocity (m/s)
DOP–99.97 filter (Flanders)	20	99.9999±0.0000	0.025
DOP–99.97 filter (Cambridge)	19	99.9994±0.0007	0.025
DOP–99.97 filter (Oshitari)	20	99.9964±0.0024	0.025
DOP–95 filter (Oshitari)	17	99.989 ±0.0024	0.025
DOP–75 filter (Oshitari)	20	99.88 ±0.0179	0.05
NBS–95 filter (Flanders)	20	99.85 ±0.0157	0.09

Table 2 Germ filtering efficiency of HEPA filter

Method of test / Filter	Filtering efficiencies of various test methods					
	DOP	NBS Dust in the air	AFI Dust in the air	NBS Artificial dust	AFI Weight method	Germ
Viscous type, oil impregnated tectile, collision type filter	0~2	5~12	3~10	50~60	65~75	10~60
Viscous, oil impregnated metal fiber collision type filter	0~2	5~12	3~8	55~60	70~75	10~60
Dry, medium efficiency filter or electric dust collector available in the commercial market	45~55	80~85	60~70	98~99	NA	90~95
Dry high efficiency filter or electric dust collector available in the commercial market	65~75	90~95	78~88	99+	NA	95~99
Dry hospital type filter	95~98	99+	99+	NA	NA	99+
HEPA filter	99.97	NA	NA	NA	NA	99.99+

NA: Not applicable to that powder.

Table 3 Example of test results on microorganisms floating in air cleaning equipment

Air cleaning equipment	Test method	Result (Number of colonies)	
		Within air cleaner	Control
Class 3 clean bench installed in an experiment room	Dropped germ Petri dish method, 10 minute open	0	26
	E. Coli spray drop Petri dish method, 10 minute open	0	∞
Class 3 clean booth installed in a work room	Milipore filter adsorbing method, (60 *l*)	0	5
Class 5 CR	Slit sampler method, (60 *l*)	(within CR) *l*	6

100 (JIS Class 3) Clean bench.

As described above, number of microorganisms floating in a BCR can be reduced to a practically sufficient level. The BCR must be constructed with the building interior finishing, cleaning, operation management, equipment maintenance and other individual element systematically. This paper introduces the present status of the BCR and the technical accomplishments.

2 PRESENT STATUS OF BCR

2.1 Field of pharmaceutical industries

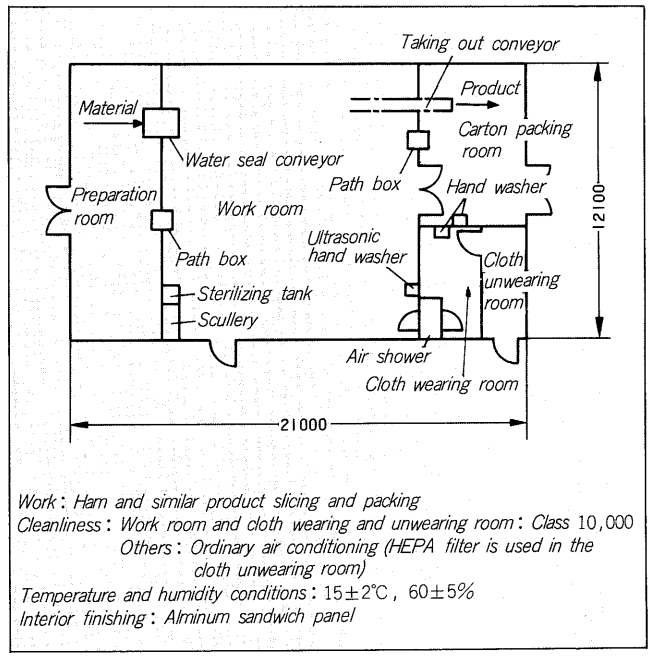
The BCR in Japan populalized through the Standards for Manufacturing and Quality Control of Medical Product -GMP- executed since 1974. In this period, about 1000 BCRs were installed in Japan. As the results of installations and operations of many number of BCRs, level of BCR managing technique was enhanced and effect of the BCR was checked under various conditions. As the results, the effect of air cleaning in improving quality of products was recognized, and the status of BCR has been concreted.

The purposes of installing CRs in the medical product manufacturing industries are two. One of them is to prevent cross contaminations among different and to achieve this purpose, individual handling places are segregated and localized, and air treatment systems are separated. The other is to protect workers from medicaments. To do so, generation of particles at the manufacturing line must be suppressed, and in the event any particles are dispersed, the concentration must be reduced immediately. The methods to accomplish the above described purposes have been, through various trials, digested into two types; One separates a manufacturing equipment into each small chamber, and the other installs that a laminar flow type air cleaning equipment covers on a manufacturing equipment.

Table 4 Effect of introduction of air cleaning countermeasures

Object	Effect	Contents of the object
Medical products, ampoule filling process	Foreign matter mixing rate	Liquid injection process is covered with compact clean booth
Milk bottling process	4 times increase in validity period	A vertical laminar type clean booth is installed immediately above
Ham slicing and packing process	Maintaining flavor and 3 times increase in validity period	A self standing type clean booth is installed
Fish cake cooling and sealing process	2 to 3 times increase of validity period	Same as above
Pastry cooling and sealing process	3 times increase of validity period	A tunnel type clean booth is installed.

Fig. 1 Ham and sausage slicing and packing factory



Work: *Ham and similar product slicing and packing*
Cleanliness: *Work room and cloth wearing and unwearing room*: *Class* 10,000
Others: *Ordinary air conditioning (HEPA filter is used in the cloth unwearing room)*
Temperature and humidity conditions: 15±2°C, 60±5%
Interior finishing: *Alminum sandwich panel*

2.2 Food industries

On the other hand, even before the GMP was executed for medical product manufacturing, it has been tried to keep food manufacturing factories free from germ and dust. As the result effect of remarkable extension of product validity period has been recognized. First, biological clean system was applied practically to milk bottling process, and next, method of BCR was actively employed in the fishery food factories and pastry making factories. It may be especially noted that the BCR is an essential equipment in a sliced food packaging factory during recent years. (Refer to *Table 4*.)

The purposes of sterilizing at food plants are;

(1) Prevention of contamination with pathogenic bacteria-Suppressing adhesion of various harmful microorganisms to foods and preventing food poisoning
(2) Prevention of quality reduction due to germs and bacteria-Preventing quality reductions such as odor, changed taste and discoloring due to decompositions
(3) Extension of storing period-Extending validity period by sterilizing treatment instead of the conventional heating treatment or use of preserving agent.

The sterilizing treatment using BCR can be classified into two major methods. One method packs products treated by heating under a sterilizing method (long life milk, etc.), and the other packs products which cannot be heated to sterilized due to the nature of the products under a sterilizing method and distributed with the products kept in a low temperature (sliced ham pack, etc.)

Inspite of limitations of food decomposition preventing agent, the primary flavor and taste of the food can be maintained and improvement of food safety can be realized by the sterilizing treatment mentioned above. With these methods, merchandises can be easily developed by using a wider distributing system, and the economical effect has

been extremely increased.

As an actual example, *Fig. 1* shows BCR of a ham slicing and packing plant.

2.3 Field of medical treatment

To the field of medical treatment which is the source of idea of the BCR, large volume air circulating type BCR has been introduced also. At present, majority of the BCRs applied to the field of medical treatment are those applied to operating rooms and rooms attached to the operating rooms, and it is said that the BCR is effective in preventing contagion or infection when replacement an artificial internal organs to human body. Further, in a medical treatment room of a certain type, the BCR is believed to be effective as a method to prevent infections.

For the effects of the BCR in the medical treatment field, not so many quantitative evaluations have been reported because the objectives are humanbeings and factors to be extracted are complicated. However, as of 1982, number of BCRs installed and operated in Japan exceeded 300, and it seems this fact indicates recognitions of the effect. Further, it should be noted that the idea for the BCR in the medical treatment field has been widely employed as a new method to the overall hospital air conditioning systems.

2.4 Countermeasures for biohazard

As for handlings of microorganism and gene which have been noticed as a driving force of technical innovation, the researching is very active and becoming wider. Consequently, it is anticipated that, depending on how they are handled, potentials of serious hazard given to the researchers and surrounding environment may increase. For this reason, the handling methods which were conventionally managed by the researchers by themselves were examined, the necessity of legalization under the common standards was evoked and the legalization was executed (USA, 1976). Following this movement, also in Japan, the standard for handling etiological microorgamisms the hazard level of which is high was established as an intra regulation (plan) of the National Institute of Health (JAPAN). Thus, the technical standard for handling equipment was initially was stipulated.

Thereafter, the notice No. 42 [Recombination DNA Experiment Guidance at Research Agent, etc. such as University] dated March 31, 1979 was issued from the Ministry of Education for handling recombination DNA. The revision was made (Notice No. 131, August 31, 1982) thereafter up to the present.

The purpose of the technical standard for equipment in the place where etiological microorganisms and genes are handled is to prevent (physical containment) accidental dispersion of microorganism from the specified handling place. Technically, a biohazard cabinet [also called biological safety cabinet (Bio, S.C.) is used as a primary containment equipment, and further, the experiment room itself is used as a secondary containment facility.

Fig. 2 Biological safety cabinet (Fuji Electric made)

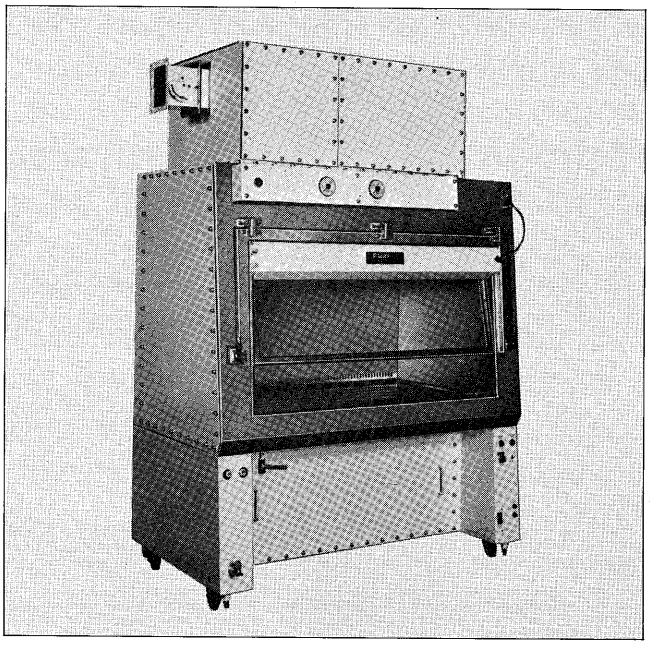


Fig. 2 shows a typical model of Bio. S.C used as the primary containment equipment. The developed above mentioned Bio, S.C. in 1980, and Fuji Bio. S.C. are presently used in various facilities in overall Japan for practical operations Further, Fuji Electric is accumulating actual accomplishments in design and construction of biohazard safety equipment, and is enhancing the completeness of total systems.

Fuji Electric's Bio, S.C developing process will be reported in a separate paper, however, the performance completely satisfies the most authoritative N.S.F. No. 49 (USA).

It should be noted that the above described handling standards are for somewhat the values handled in the scale of an experiment room. It is ruled, for large volume handlings for industrial purposes, that the contents shall be examined by official agents whenever the plan is made and the safety shall be confirmed.

Recently, it was reported that the use of recombination DNA large scale equipment wax authorized by the examination committee, and it has given such an impression as that the hazardous has been relieved all at once. It should be noted, however, that the present restriction relief is not aiming at relief of the handling method or basic containment effect of the equipment but relief of level of hazard for handling class only.

As long as biochemical is one of the cores for technical innovations, for biohazard countermeasures, an enhanced reliability of large scale containment equipment will be the most important subject as industrial handlings are becoming more active and wider in the future.

Table 5 Results of biological inspections on Fuji Bio. S.C.

Tested item	Judgement standard (N.S.F.)	Measuring equipment and measuring position	Measurement result					Judgement	Remarks
			1st time	2nd time	3rd time	4th time	5th time		
Personnel protection test (tested in 5 times)		Upper right	3	4	4	2	1	Good	Result of the measurement at the position where the objective dish can be thoroughly caught by sprayed air zole indicated positive for all in one time to five times.
		Upper left	4	0	1	0	0		
		Lower right	4	4	3	5	2		
		Lower left	4	4	3	5	2		
	Within 20 colonies at a time (Total value of 16 Petri dishes)	impingers sampler	2	5	0	1	1	Good	
Product protection test (tested in 5 times)	Shall be positive (300 colonies or more)	Dish	Positive	Positive	Positive	Positive	Positive	Good	
	Within 5 colonies at time (total of all Petri dishes)	Petri dish on work table (65 dishes/time)	4	3	2	1	2	Good	
	Shall be positive (300 colonies or more)	Dish	3	8	9	21	1	Good	
Cross-contamination preventing test, Tested in 3 times	Colony shall not be detected in the center of or behind Petri dish located in 14 inches from the side wall. (In this case, Center of or behind L3 row or R3 row)	L5 row	Many	Many					
		L4 row	0	1					
		L3 row	0	0	Many				
		R5 row			1				
		R4 row			1				
		R3 row			0				

Table 6 digests the purposes and industrial fields which use the above described BCRs and biohazard containment equipment.

3 TECHNICAL FEATURES OF BCR SYSTEM

For applications of the BCR, there are two types. One of them is of a dry type used in the medical treatment field and powder state of pharmaceutical process, and the other is of a wet type which uses a large volume of water in the food treatment process, liquid state of pharmaceutical process and houses accommodating animals for experiments. However, the BCR is featured in the periodical disinfecting operations against the building interior. For this reason, when building a BCR system, resistance against the disinfecting agent must be taken into considerations.

3.1 Air treating technique

Kinds of microorganism floating in the air which a BCR must eliminate vary toward a wide range form virus to mold, and their sizes of a unit are from 0.01μm to about 50 μm. It is a defined opinion, however, that when they are floating in the air, they are adhered to floating dusts and water drips.

For this reason, the air treating technique of the present BCR uses a large volume filtered air circulating system with HEPA filter. Thus, the air treating equipment may be considered to be the same as ordinary industrial CRs.

Number of microorganisms naturally floating in the air greatly fluctuates depending on the place and season, however, it is generally 10^2 to 10^4 per one m^3 of the air. On the other hand, when number of floating bioparticles is converted to each 1 m^3 air for BCR class 100 to class 100,000, and difference of concentration of floating bioparticles between the space inside the BCR and outside is calculated as a reduction rate, they appear as indicated in *Table 7*. With the values indicated in this table and *Tables 1* and *2* above, when a filter of 95% or higher DOP collecting efficiency is used in the bioparticle filtering system, BCR class 100 can be obtained at the filter blowing outlet. Actually, however, except for a larminar air flow type CR class 100, most of them are turbulent air flow type CRs. *Table 9* shows an example of floating bioparticle calculation of turbulent type CRs. As it is obvious from this example, the present CR air flitering technique is capable of supplying almost completely sterilized air into BCR. Thus, the number of particles generated inside the room decides the number of bioparticles floating in a turblent airflow type BCR as well as in the case of an ordinary industrial CR, and it should be noted that in the most cases, the workers in the BCR are the source of particle generation. Based on the above results, the same air filtering system as an ordinary industrial model shown in *Fig. 3* may be used. Moreover, we

Table 6 Applications of BCR and biohazard containment facilities

Industrial field				Application
BCR	Medical treatment	Surgery	Internal organ transfer/hip joint replacement	Preventions of infection and contagion during operations
		Recovery room		Prevention of infection and contagion after operations
		Special ward	Acute lueemia, injury, asthma	Prevention of infection and contagion/elimination of allergen
		Born baby room	Immature baby/born baby	Same as above
	Pharmaceuticals	Injection medicine manufacturing	Preparation/filling	Prevention of microorganism contamination
		Tablet manufacturing	Mixing, molding, coating	Prevention of quality reduction due to microorganism
		Antibiotic	Cultivation, filling, inspection	Prevention of discomposition due to microorganism
		Medical equipment manfuacturing	Injection needle, blood pack, etc.	Prevention of contamination due to microorganism
	Food	Meat treatment	Ham, sausage, etc.	Prevention of contamination due to microorganism, prevention of quality drop, extension of valid period
		Milk product	Milk, yogurt, etc	Same as above
		Beverage	Juice	Same as above
		Fish cakes		Same as above
		Brewery	Miso, Sake, Beer, etc.	Same as above
		Daily dishes	Salad	Same as above
		Pastry	Cake, etc.	Same as above
	Others	Aseptic animal		Sterilizing growing room and study room
		Plant	Mashrooms	Prevention of contamination due to caultivating microorganism
		Carnivorous animals		Improvement of productivity
		Electronic industry	Electronic parts	Prevention of reliability reduction due to mold
Biohazard containment equipment	Medical treatment	Special ward	Medical treatment for internationally contageous disease	Prevention of infection, isolation
		Surgery		Same as above
	Medicines	Biopharmaceutical	Including gene control method	Protection of worker, prevention of environmental contamination
		Effect test	Animal test	Improvement of worker test tresult
	Others	Etiological microorganism research		Same as above
		DNA recombination research		Same as above

Table 7 Required reduction rate of floating bioparticles

Number of microorganisms floating in the air (microorganisms/m^3)	BCR class	Floating bioparticle value (particles/m^3)	Required attenuation rate
	100	3.5	96.5~99.965
$10^2 \sim 10^4$	10,000	17.6	82.4~99.824
	100,000	88.4	11.6~99.116

also report that there is such a discussion as that HEPA filter may not be used as the final filter when the purpose of a BCR is limited to control floating bioparticles only.

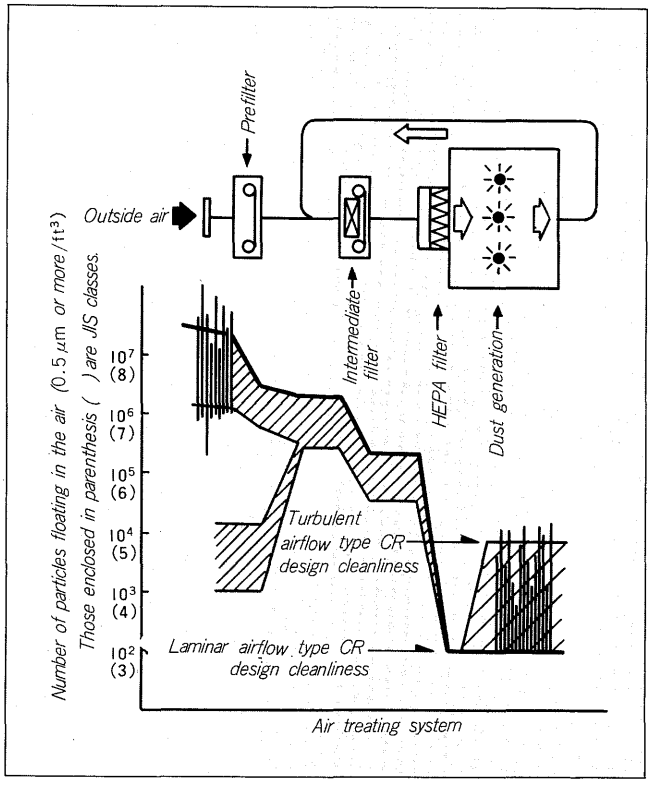
Features of the air treating system of the recent BCR are to limit the place where germ-free atmosphere is required and where the effect of the treatment is large, and to obtain a locally high cleanliness by using clean booth, etc. Especially, in the fields of medical product manufacturing and food industries, those air cleaning suited to the shapes of the manufacturing machines and equipment are popular. *Table 9* shows a list of air cleaning devices used in BCR systems.

As for temperature and humidity conditions of the BCR, in many cases, a low temperature is required in food industries. In this case, the design is made to separate circulated air system from the temperature control system. With an ordinary industrial use air conditioner, a sufficient functions can be obtained for BCR. However, the number of life years is limited to a considerably short period of time. This is mainly due to the situation that mixture of sterilizing agent cannot be avoided. As for the improving countermeasures, it is, first of all, important to use steriliz-

Table 8 Example of estimating calculation for number of bioparticles floating in BCR

Air filtering system	Taken in outside air $Q m^3/h$ — Filter — Bioparticle passing rate P_f — Exhaust $Q m^3/h$ — Number of generated germs (pieces/hour) — G
Calculation	C : Number of bioparticles floating in the room (particles/m^3) C_i: Number of bioparticles floating in the taken in air (particles/m^3) Equation $C = C_i \times P_f + \frac{G}{Q}$ where, C_i : 10^4 (from Table 7) P_f : 0.001 (When collection rate is 99.9%) G : 5000 pieces/minute (assumed to be 1/1000 of particle generation)* Q : 5000m^3/h (Room capacity: 100m^3, Number of ventilations: 50 times/hour) *Masakazu Tuzuki : Air cleaning for air conditioning, p. 397, Soft Science $C = 10^4 \times 10^{-3} + \frac{5 \times 10^3 \times 60}{5 \times 10^3}$ $= 10 + 60$ $= 70$ (pieces/m^3)
Examinations	This value is equivalent to class 100,000 in Table 7. It should be noted that the number of bioparticles floating in the room is decided by the right side second term G/Q almost finally.

Fig. 3 CR air treatment system diagram



ing agent having a low corrosiveness. Further, technically, a damper is installed on the duct system, the machine interior is finished with resin lining, or maintenance is conducted more frequently. In a place where particles are generated considerably at medical product industries, local dust collection must be performed thoroughly, and particles in the returned air must be eliminated before the air reaches the air conditioner.

For the HEPA filter to be used, those of a plastic separator should be selected, and also for the filter gasket, suitability of the gasket with the used chemicals must be confirmed.

3.2 Accessorial equipment

For equipment attached to BCRs, sterilizing or germ eliminating functions must be added to those for industries use.

The largest source of generating dusts and germs is a worker. To reduce the potential of this contamination source, there is no method other than executions of sterilizing and germ elimination by the workers by themselves in accordance with the predetermined procedure. Archtecturally, the equipment should be so arranged that the workers can be managed easily.

First of all, sterilizing and hadn washing facilities should be made available so that workers sterilize their hands and fingers. A large volume of water used to wash their hands must be thoroughly sterilized by means of an ultraviolet ray or membrane filter. The hand washer valve should be of a type operated by a foot or knee. Further, for drving hands after washing, an air towel is recommended. On the other hand, in a special pharmaceutical industrial field, overall worker body sterilizing showers must be installed.

Fig. 4 Ampoule booth

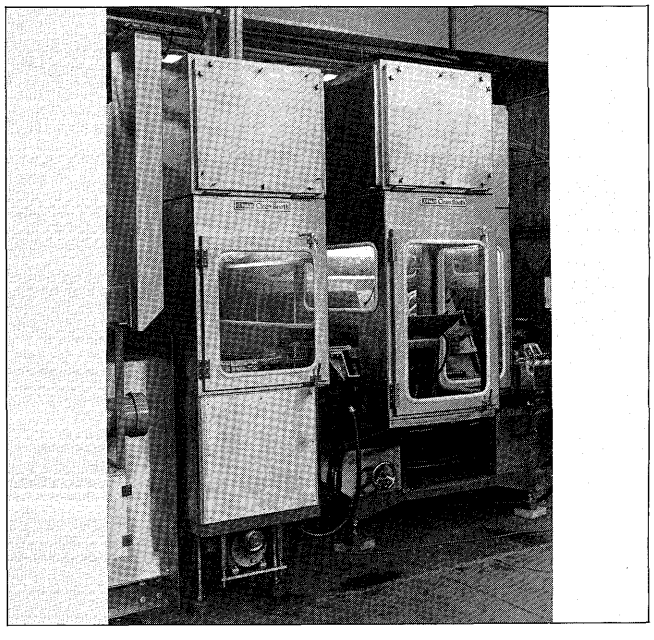


Table 9 Examples of air cleaning equipment for food and pharmaceutical industries

Type of equipment	Features	Cleanliness	Application
Prefabricated CR	•A clean unit installed. •Diameter of duct for air conditioner can be reduced. •The interior finishing is aluminum sandwich panel	Classes 1000 through 100,000. However, the class immediately below the clean unit is 100.	(Suited to modify the already existing building) •Ampoule filling •Bottling •Sliced food pack
Clean booth	•Can be moved freely to any desired place. •Installed in such a manner as that the manufacturing equipment is covered.	Class 100	•Ampoule filing •Bottling •Sliced food packing •Cooling •Storing •Raw material check
Compact clean booth	•The main portions are covered with vertical larminar flow	Class 100	•Tablet molder •Powdery medicine measuring/ packing machine
Clean chamber	•Clean air is blown into the tablet molder cover, and air exhausted from the cover is filtered and recirculated.	Air supply: Class 100 to 10,000	•Ampoule filling •Small bottling
Clean hood	•Installed for powder mixer •Dispersed powder is sucked and recirculated after filtering	Class 1000 through 10,000	•Powder packing packing machine •Mixing
Clean bench	•Workers can work by opposing	Class 100	•For bioexperiments
Biohazard safety cabinet (Bio, S.C.)	•Worker protection •Product protection •Prevention of cross contamination	Air supply: Class 100	•DNA recomposing experiments •Harmful microorganism handling work •Harmful chemical handling work
Air shower, path box	•Air shower for people and objects •Those for automatic transporting trays are also available	Jet air: Class 100	•CR entrance/exit •Commonly used with air lock room •Convenyor opening

Items delivered into the BCR must be sterilized as much as possible before entering into the BCR, and when the items cannot be sterilized, they are passed through air shower to eliminate dust.

3.3 Archtecture

To improve effect of the sterilization is a feature of the BCR smoothness of the interior surface is important. Archtecturally, paint finished construction having a less joint is recommended. Especially, the floor should be painted unless it is for medical treatment facility.

When BCRs are observed from the phase of archtechtural structures, medical products and foods must be manufactured in compliance with the Pharmaceutical Law and Food Sanitation Law in addition to the Archtectural Standards. Out of these, in a food treating factory which is mostly in a highly moistened working atmosphere, it is important to prevent contaminations with microorganisms caused by water condensed on the interior wall surface. Same problems occur in medical product manufacturing process also, but there is an example which solved the problem by providing the room with a circumferential corridor and by raising temperature on the interior wall surface. Especially, when building a BCR in a cold area, a sufficient consideration is required for the room layout in addition to the heat insulating design.

Next, for furnishings, aluminum air tight window frames are used. The air tight performance should be Class 1.0 or higher, and one side drawing type should be selected. Further, those having a firm crescent must be selected. For daily used doors also, those of aluminum air tight type should be selected, and for a door of large size, those steel doors finished with paint are recommended. For door knob bar type is recommended. For the doors inside the CR, swing type, automatic opening closing type, those with packings and air-tight type are used in response to the opening and closing frequencies. When selecting a door, using ease is often sacrificed as being particular about the barrier function. In a BCR system, it is necessary to know the limit of barrier function of a door and window and to design the system so that the function is maintained by air stream flowing from the room to the outside. Further, one of the BCR function reducing factors is a broken wear for CR worn by a worker, which causes contaminating substances. To prevent this breakdown, edges of furnishing must be thoroughly chamfered.

Out of various BCRs, in a facility where a large volume of water is used, the floor must be finished in a non-slippery surface for the working safety.

Further, when employing a floor draining construction, static differential pressure between inside and outside of the CR should be taken into consideration to decide the trap construction. As for a hing of water sealing, it should be about ten times as great as the maximum static differential pressure. When volume of used water is extremely small, a screw type air-tight cover is used.

4 POSTSCRIPT

Troubles due to microorganism are expanding from injuries to living things to overall industrial products. Especially, as functions of industrial products are multiplied and treating dimensions are becoming finer, troubles due to microorgaisms will invite more attentions as well as the adverse effects of microparticles. Further, to enhance levels of safety and quality of medical products and foods, the importance of BCR and biohazard containment facilities will increase more and more.

On the other hands, the biochemical technologies which actively use microorganisms and DNA have unlimited possibilities as a supporting and driving force of the technical innovations, and it is considered that the utilizations will be proceeded more widely and in larger scale. Under the circumstances like this, it is inclined to invite insufficiency of correspondence at the management phase, and it is anticipated that the effect of a facility may not be displayed.

For this reason, it is necessary to enhance such a trend as to proceed more effective, economical and practical technique based on the technologies for the BCR and biohazard containment facilities accumulated in the past.

As the treated products will be varied, the qualities will be increased more and more and preciseness will be increased, small scale systems will be used more widely.

As the purposes of the BCR and biohazard containment facility are to prevent troubles due to microorganisms and more serious nuisance, we have to know that the potential of the hazard exists regardless of the facility scale.

For this reason, further higher technical integrations will be needed in a small scale facility like described above rather than those of a large scale.