Emergency measures	Emergency measures ①Health care for workers in emergencies ②Operation protocol for disinfection ③Operation protocol for contact in emergencies	Yes	Yes
Education and training	Education and training ① Characteristics of microorganisms ② Entry process for controlled areas ③ Operation protocol in controlled areas ④ Waste and disinfection ⑤ Emergency measures	Yes	Yes

2.4. Biosafety guideline for laboratory facilities

2.4.1. Biosafety guideline for BSL1 laboratory facilities

Special facilities are not required by either the Japanese or the WHO guidelines.

2.4.2. Biosafety guideline for BSL2 laboratory facilities

Item	Requirement	Japan	WHO
Facility	Manufacturing facilities are separated from other facilities. Restriction of entry to the manufacturing facilities.	No	No
	Prevention of microorganism leakage.	No	No
	Double-door entry.	No	No
Air	Air supply through an HEPA filter linked to the exhaust system (negative-pressure control).	No	No
	Air of high work rooms is exhausted through an HEPA filter (circulation is available).	No	No
Manufacturing	Operations with microorganism aerosols are performed in a safety cabinet (class IIA or better type) or equivalent system, and the air is exhausted directly outside through an HEPA filter (circulation is available).	Yes	Yes

2.4.3. Biosafety guideline for BSL3 laboratory facilities

Item	Requirement	Japan	WHO
Facility	The manufacturing facilities are separated from other facilities. Restriction of entry to the manufacturing facilities.	Yes	Yes
	Prevention of microorganism leakage.	Yes	Yes
	Double-door entry.	Yes	Yes
Air	Air supply through an HEPA filter linked to the exhaust system (negative pressure control).	Yes	Yes
	Air of a high-risk work rooms are exhausted through an HEPA filter.	Yes	Yes
Manufacturing	Operations with microorganism aerosols are performed in a safety cabinet (class IIB or better type) or equivalent system, and the air is exhausted directly outside through an HEPA filter.	Yes	Yes

3. Biosafety of influenza vaccine production

In accordance with the revision of the Pharmaceutical Affairs Law (Ministerial ordinance on manufacture facilities, manufacturing processes, and quality control of pharmaceuticals, 1999), we established biosafety countermeasures for influenza vaccine manufacturing facilities. The policy suggests that current influenza vaccine can be manufactured by BSL2 manufacturing facilities, but that BSL3 manufacturing facilities should be used for new type influenza vaccines (using highly virulent viruses, such as the H5 type).

The reasons are as follows:

1) Infection of workers should be prevented.
2) Infection of the community through workers should be prevented.
3) Virus release into the field should be prevented.

However, the vaccine must be manufactured by GMP. These points should be considered, and biosafety countermeasures vaccine production for new types of influenza should be discussed.

3.1. Manufacturing facilities

3.1.1. The manufacturing facilities for influenza vaccine should be separated from other facilities (Figure 1).

3.1.2. Protocol of display of entrance restriction and permission of entrance should be managed for entrance restriction to the manufacture facilities. In addition, physical entrance restriction by security doors (card type, fingerprint type) might be an effective means of entrance restriction (Figure 2).

3.1.3. Information should be displayed in controlled areas where viruses are used on a biohazard label (BSL level, name of an administrator, a phone number for emergencies, etc.) (Figure 3)

3.1.4. Wall, floor, and ceiling surfaces should be smooth, and there should be not cracks or dust for airtight containment. Materials should be resistant to chemical agents and antiseptics.

3.1.5. An anteroom with a different air pressure should be provided before the work room. There should be double doors, and the doors should not open at the same time (Figure 4).

3.1.6. The workers and materials should be moved one-way to prevent cross contamination (Figure 4).

3.1.7. An air curtain or double doors should be set up in entrance of eggs to prevent contamination by insects.

3.1.8. The refrigerator and incubator for eggs should be designed to prevent virus release outside.

3.1.9. A shower or antiseptic agent spray machine should be installed at the entrance to the work room for use in accident.

3.1.10. A shower or antiseptic agent spray machine should be installed in work room for use in accidents.

3.1.11. A foot control for the sink faucets and hand washer or automatic system should be installed to prevent of cross contamination (Figure 5).

3.1.12. The seed virus stockroom should be locked, and the key stored by a responsible person. The biohazard label should be displayed on the door.

3.1.13. The work space should be large enough to prevent accidents.

3.1.14. A backflow prevention device should be installed in the drainage system (Figure 6).

3.2. Air conditioning system (Figure 7)

3.2.1. Independent air conditioner.

The air conditioner should be independent from other work areas.

3.2.2. Negative pressure control.

The controlled area where viruses are used should be a relatively negative-pressure habitat to maintain the airtightness of the establishment. In addition, the negative pressure habitat that one step is already low should be demanded from the working area where aerosol occurs more. Difference in pressure is also needed between loculus of 10-15 Pa degrees to maintain negative pressure.

3.2.3. Monitoring of the work area.

Air flow between rooms having different air pressures should monitored with a differential manometer (preferably with an alarm system).

3.2.4. Control of exhaust to outside the work area.

An HEPA filter should be inserted in the air supply and exhaust system for controlled areas where viruses are used. The air of at least high-risk work rooms (inoculation room, harvest room) should be exhausted directly outside through an HEPA filter.

3.2.5. Countermeasures for unexpected air conditioner situations.

The air conditioners in controlled areas should be capable of disinfecting with gas, such as formalin. If an air conditioner accidentally stops, no virus should leak outside the room (for example, air supply and the configuration that a damper of exhaust system closes promptly or air supply and exhaust system with HEPA filter configuration of double).

3.2.6. Emergency measures

An emergency electrical power source for continuous air conditioner operation in blackout emergencies should be secured.

3.3. Prevention of virus leakage during vaccine production

3.3.1. Prevention of virus leakage during inoculation and harvesting (Figure 8).

1) Virus inoculation of eggs and virus harvesting from eggs should be performed in a safety cabinet (class IIB or higher type) or equivalent system, and the air should be exhausted directly outside through an

HEPA filter.

2) The hole in the egg made to inoculate viruses should be closed with a sealant after inoculation.

3) There should be the function of disinfection system of egg after inoculation.

4) The inoculation and harvest devices should be disinfected.

5) There should be an alarm for emergencies.

3.3.2. Prevention of virus leakage in aerosols.

Operations with virus aerosols should be operated in a safety cabinet (class IIB or higher type) or an equivalent system, and the air should be exhausted directly outside through an HEPA filter.

3.3.3. Airtight devices

1) Before inactivation the virus container should airtight and not made of any weak materials.

2) The manufacturing machines (e.g. centrifugal machine) should be used airtight system for prevention of virus leakage.

3.4. Management of solid waste

3.4.1. Wastes containing virus should be incinerated in a controlled area after appropriate chemical or heat disinfection (Figure 9).

3.4.2. Egg disposal after virus harvesting.

There are two methods (1 or 2) of egg disposal after virus harvesting (Figure 10).

1) Direct transport of the egg to an incinerator by an in-line system.

2) Transport of the eggs to a sterilizer by an in-line system followed by transport to the incinerator after sterilization.

3.5. Management of fluid waste (water)

Waste fluid containing viruses and waste fluid that had come into contact with viruses are drained outside the facility after adequate heat sterilization (Figure 11).

3.6. Sterilization and disinfection

The process of sterilization and disinfection should be validated of enough inactivation of the virus.

3.7. Work clothes

3.7.1. Protective clothing

1) A face mask and air flow pump should be attached to the protective clothing to prevent infection (Figure 12).
2) When high-risk manufacturing processes are performed, the protective clothing should be airtight with positive pressure inside.

3.7.2. Management of protective clothing

The protective clothing should be carried out after appropriate disinfection to outside of controlled area.

3.7.3. Protective clothing for emergency

For occupation of decontamination when contamination happened by fatigues accidents in emergency, air bomb possesses airtight fatigues belonging to it (Figure 13).

3.8. Prevention of workers infection

3.8.1. Health care of workers

Regular physical checkup (2 times per year) should be performed done, and health care should be done oneself everyday. An effective antiviral drug should always be available for emergencies.

3.8.2. Worker education

Regular biosafety training conducted according to the safety regulations.

4. Summary

"Biosafety control measures for pharmaceutical manufacture" have discussed using an influenza manufacturing facility as an example.

Extensive containment measures are necessary, because influenza viruses are highly infectious, and a large quantity of chicken eggs are used in vaccine manufacture.

The biosafety measures discussed here are based on current technology and regulations.
Additional control measures will be needed as progress is made in related sciences and technologies.

Reference

1) Regulations for Manufacturing Control and Quality Control of Drugs and Quasi-drugs (MHO Ordinance No. 16, March 12, 1999).
2) Regulations for Buildings and Facilities for Pharmacies, etc. (MHO Ordinance No. 57, April 30, 1999).
3) Biosafety Guidance for Manufacturing Plants of Biological Products, etc. (PMSB/IGD Notification No. 14, February 14, 2000)
4) WHO: Biosafety Guidelines for Personnel Engaged in the Production of Vaccines and Biological Products for Medical. WHO/CDS/BVI/Mai, 1995).
5) WHO: Laboratory Biosafety Manual (Second edition, 1993).
6) Good Manufacturing Practices for Biological Products, WHO Tech. Rep. Ser., No. 822, 1992.
7) Safety Control Code for Pathogenic Microorganisms (NIID, April 1999).
8) Biosafety Guidelines for Pathogenic Microorganisms (Japanese Society for Bacteriology, 26 August, 2000).
9) Report on New Types of Influenza Virus, Clinical Virology 25(5): 353, 1997.

Figure 1 Classification of production areas

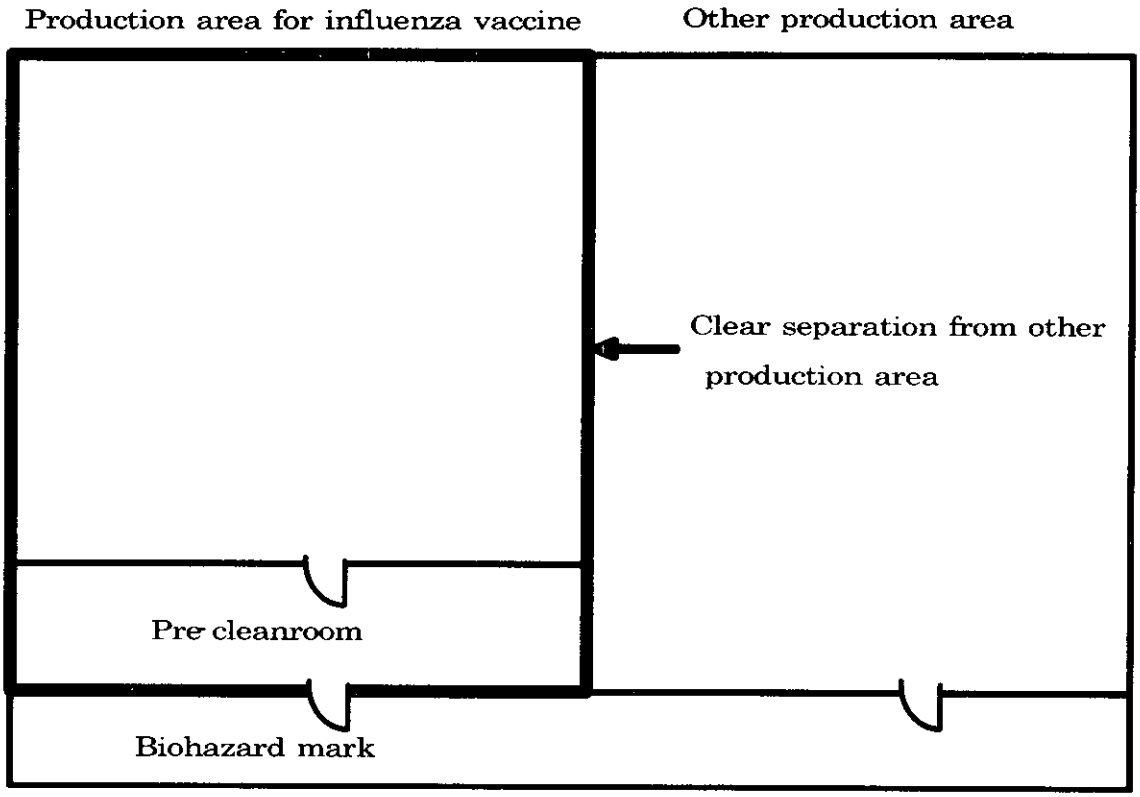


Figure 2 A security door(a card type)

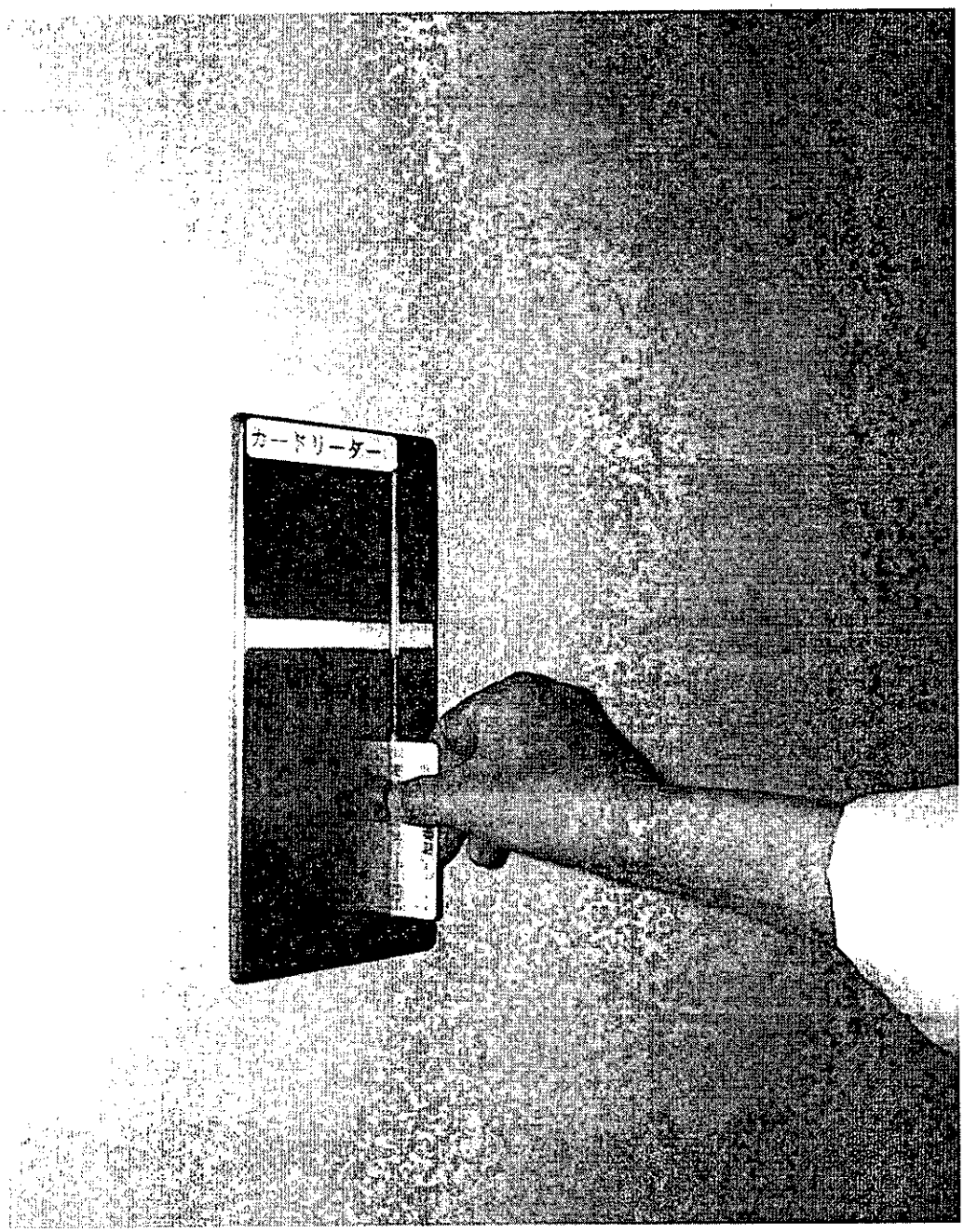


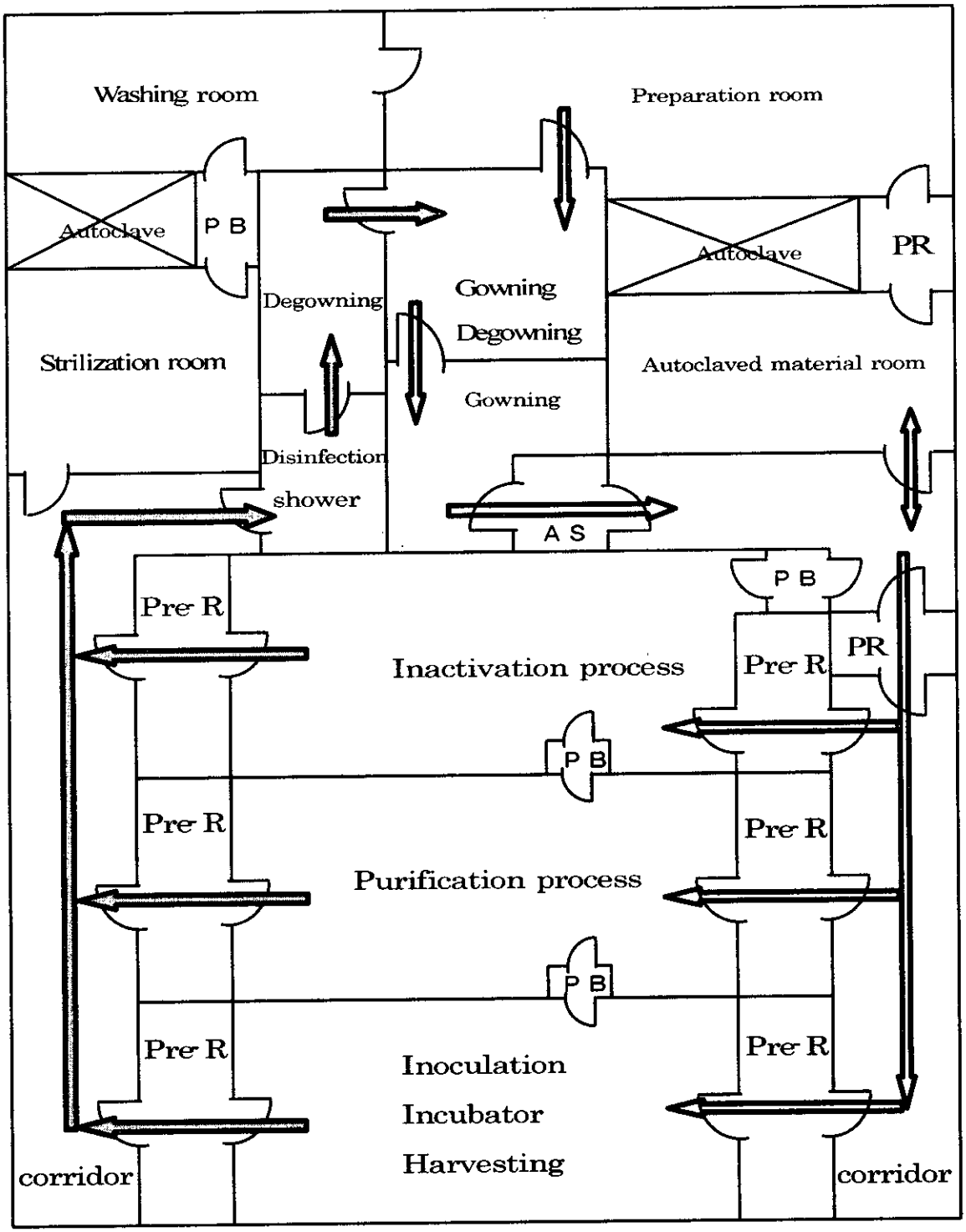
Figure 3　Biohazard mark

BIOHAZARD

Authorized person only

Area name	
Name of microorganisms	
Level	
Responsible person	
An emergency address	

Figure 4-1 Personnel flow



PB(pass box) AS(air shower) PR(pass room)

Figure 4-2 Material flow

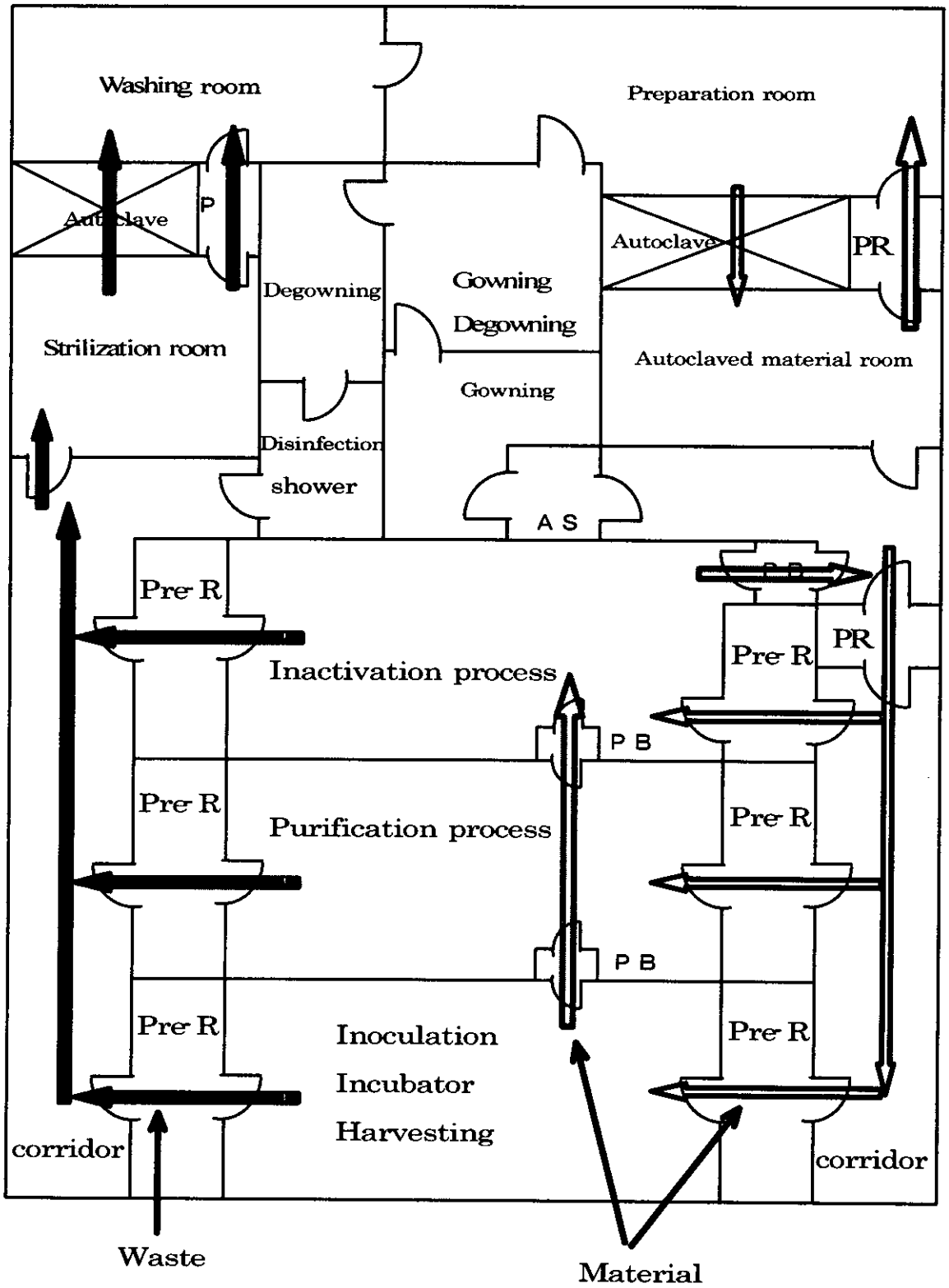
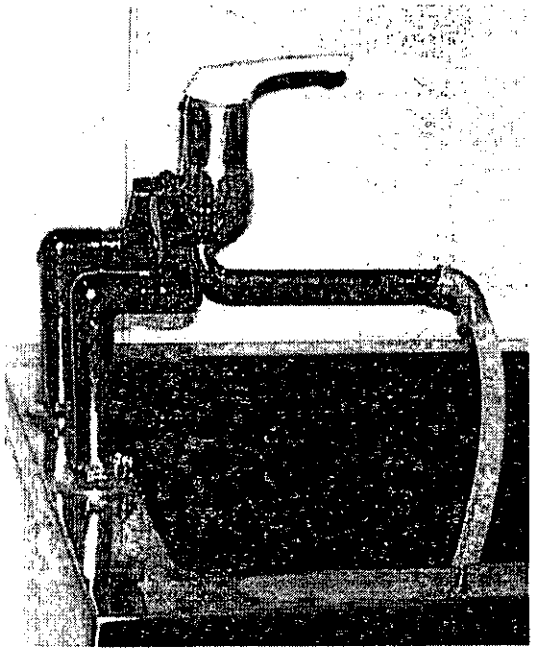


Figure 5 Tap/Washer

Elbow type

肘式蛇口



Automatic hand washer

自動手洗い器

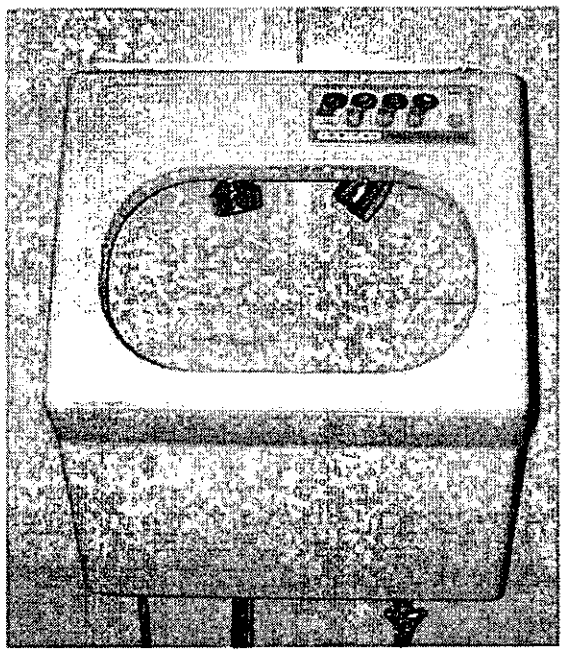
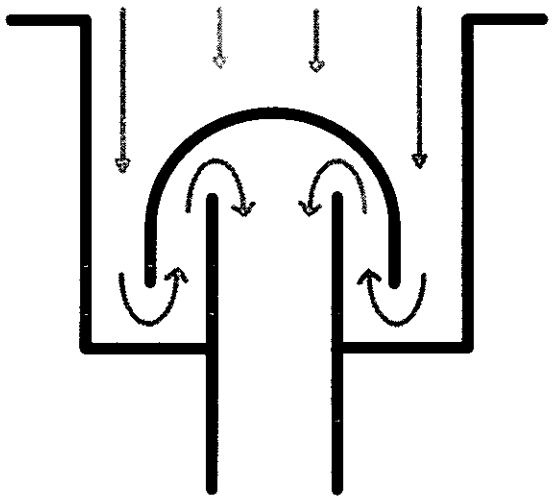
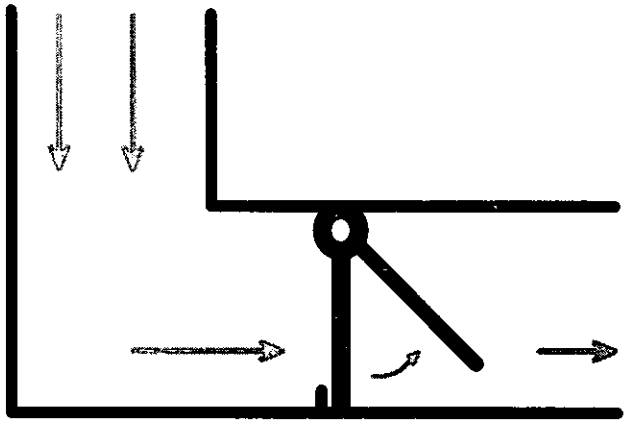


Figure 6 Backflow prevention devices for waste water

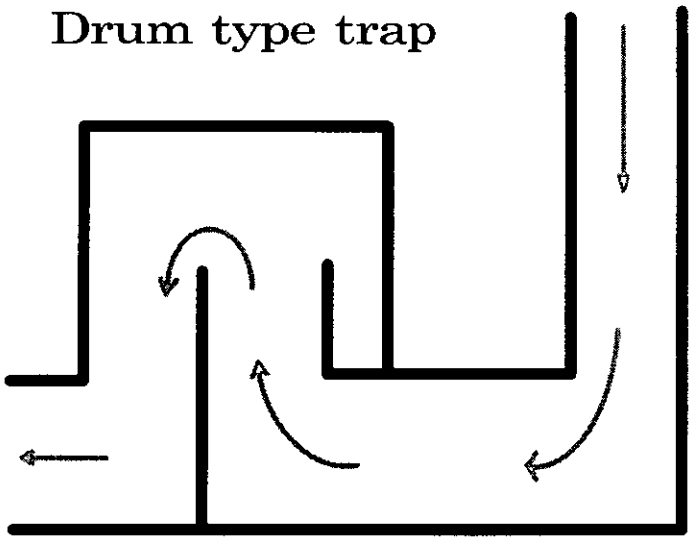
bowl type trap



Check valve



Drum type trap



S type trap

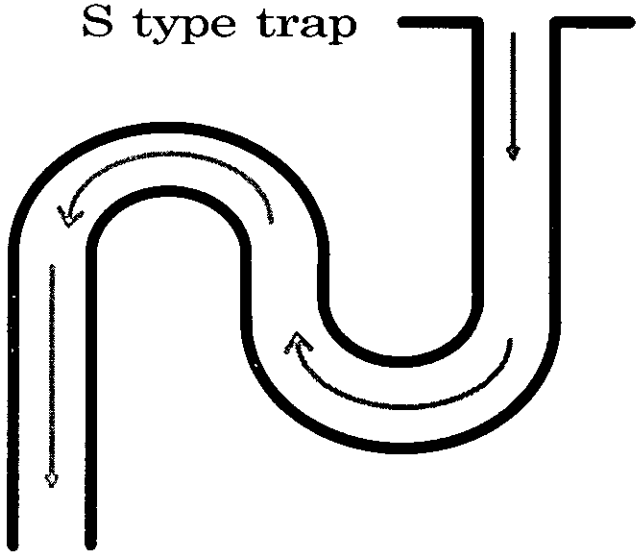
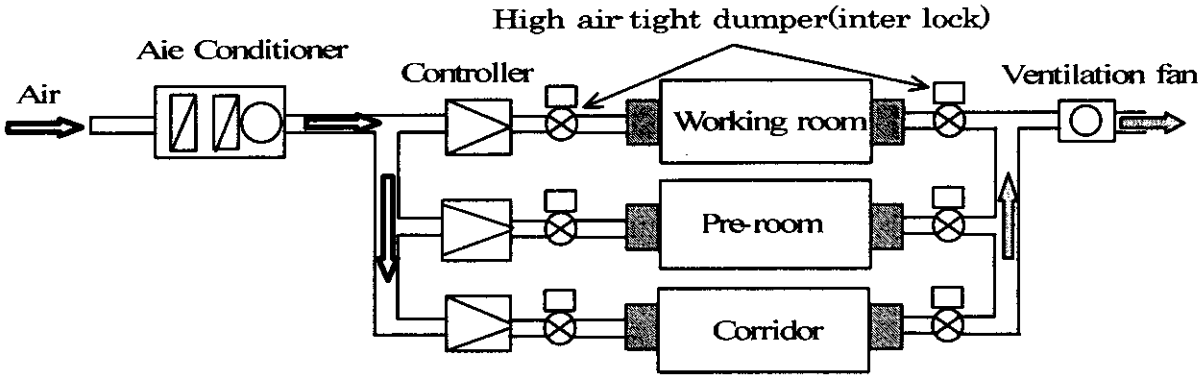


Figure 7-1 Air conditioner

An example of air conditioner for high hazard rooms



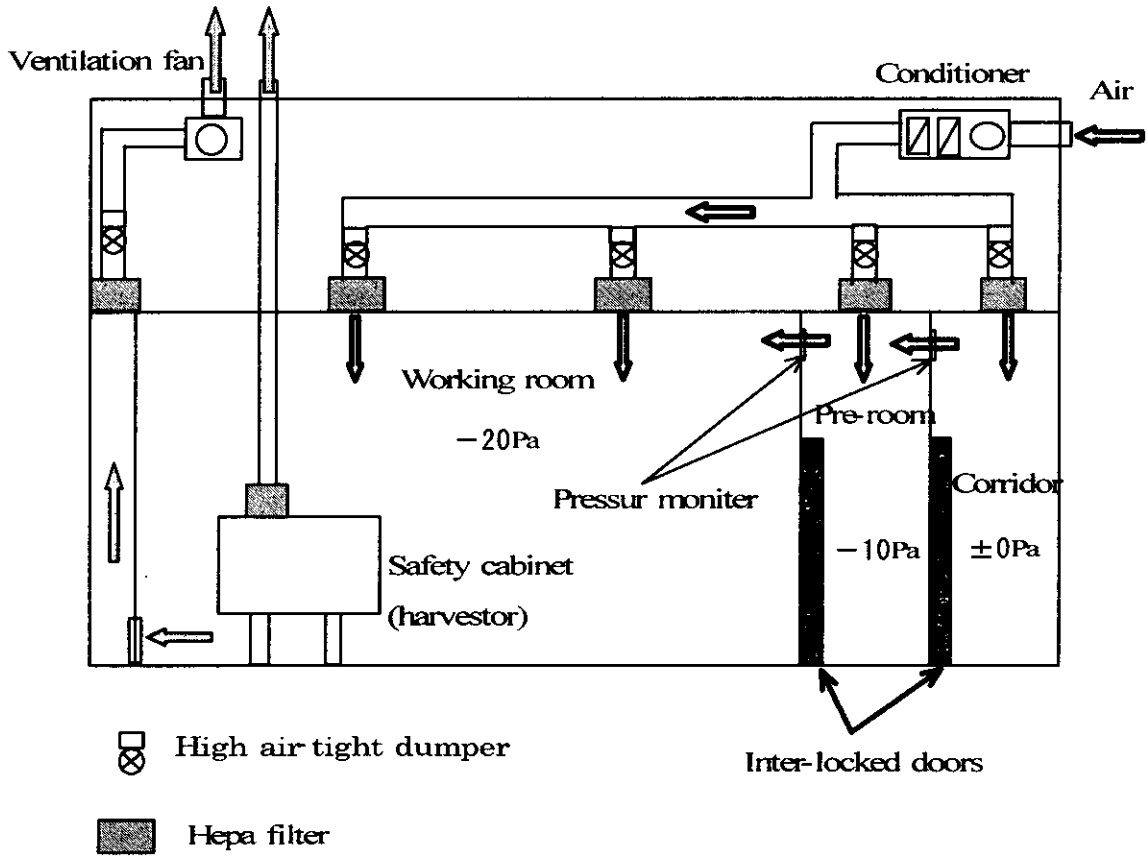
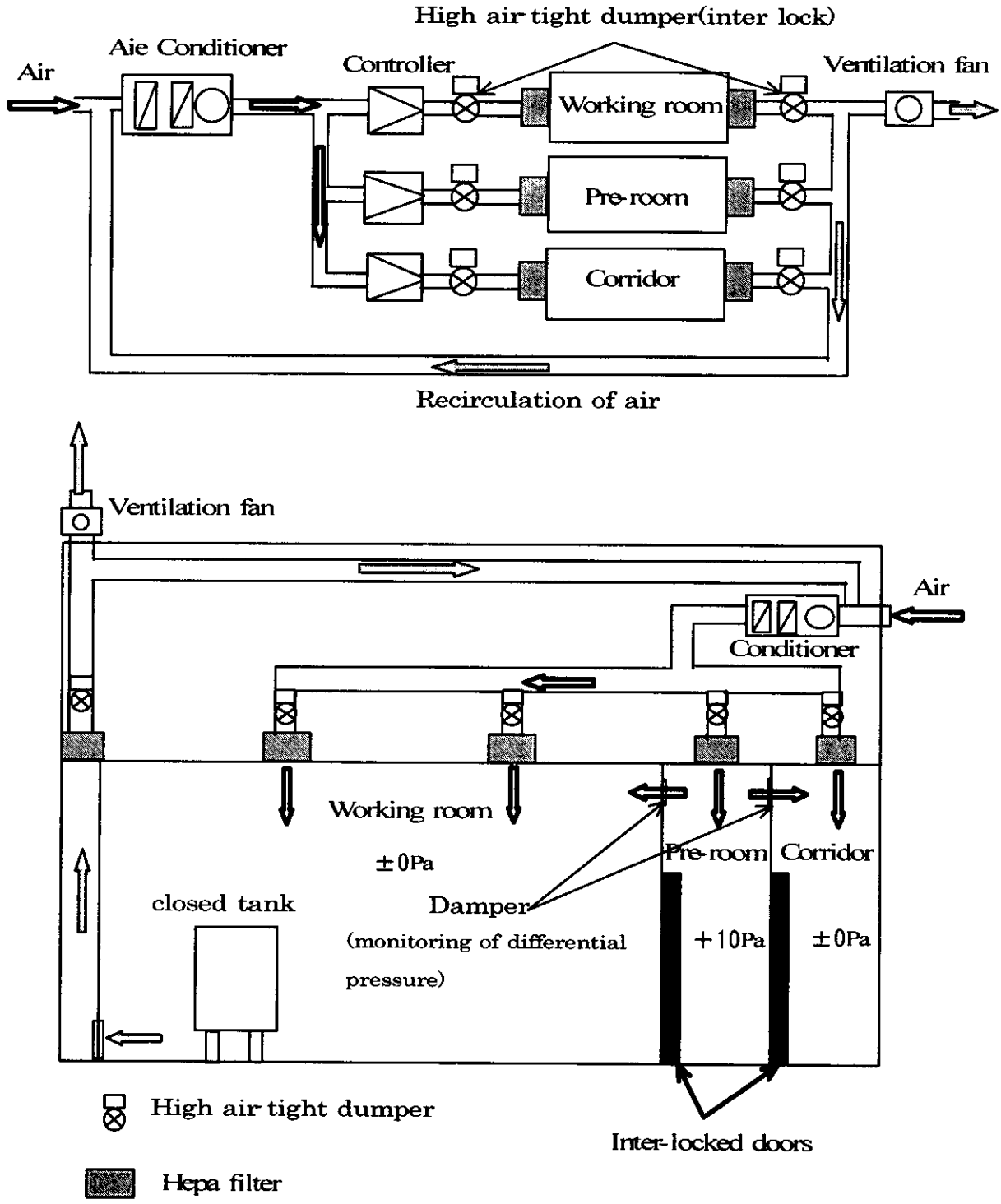
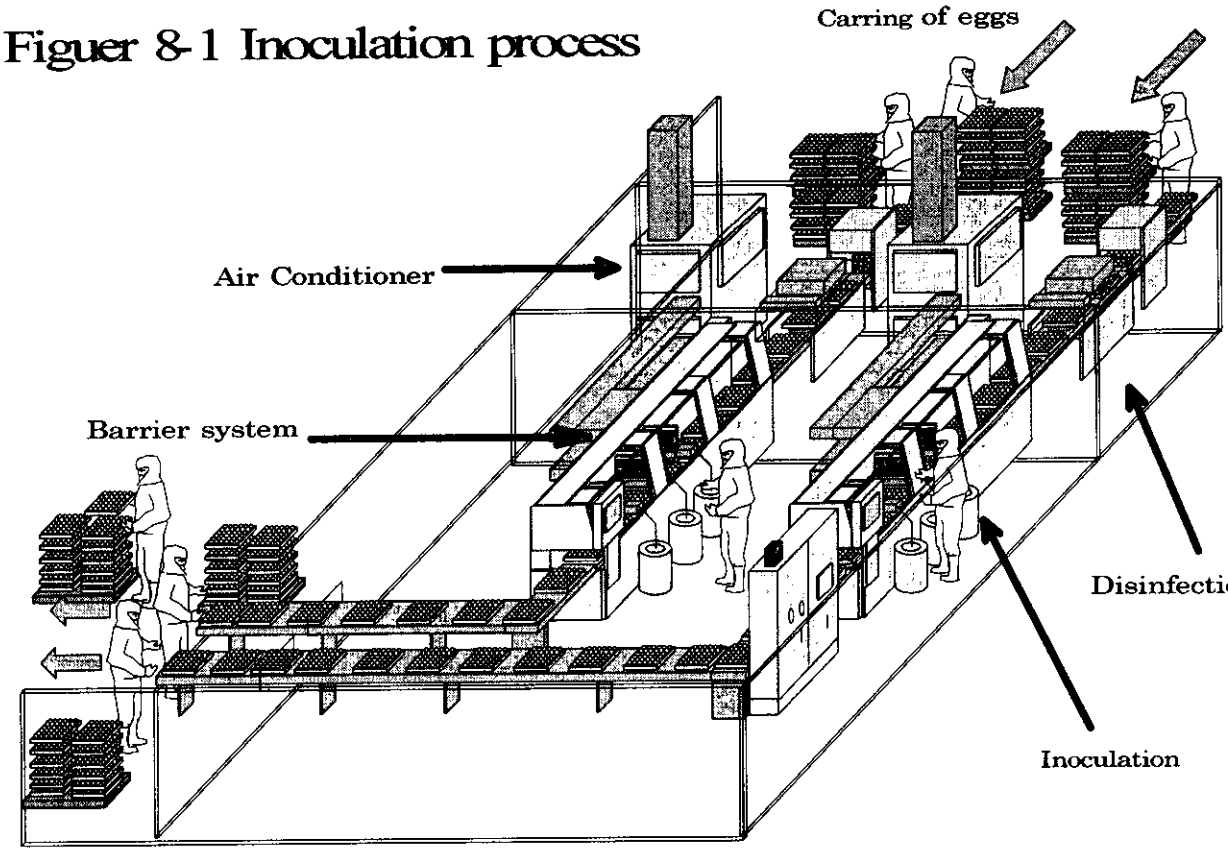


Figure 7-2 Air conditioner

An example of air conditioner for low hazard rooms



Figuer 8-1 Inoculation process



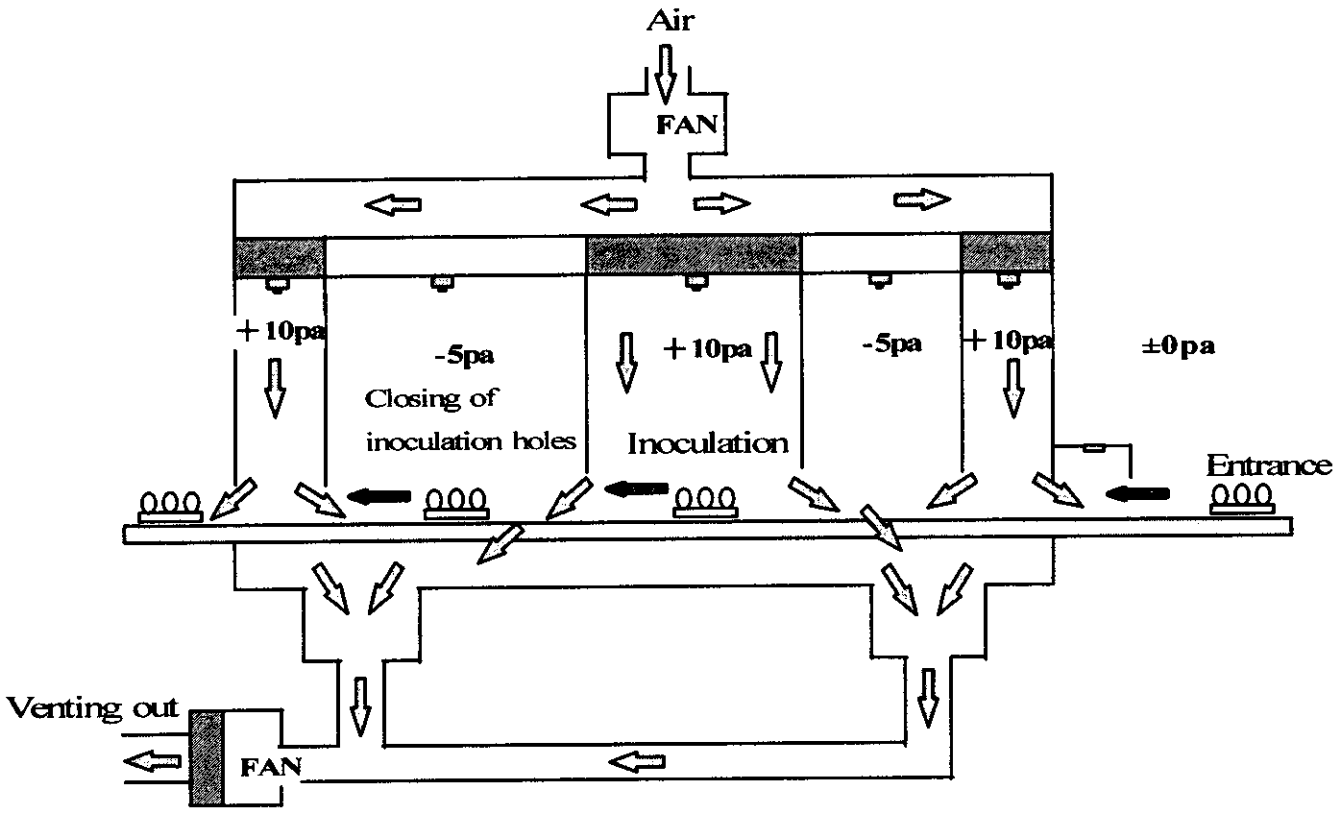


Figure 8-2 Harvest process of virus

Hrvest process of virus