GUIDE ASPEC

ISOLATORS: QUALIFICATIONS AND MAINTENANCE

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ISOLATORS: QUALIFICATIONS AND MAINTENANCE

CLEANROOMS, CONTROLLED ENVIRONMENTS & CONTAINMENT LABORATORIES

ACKNOWLEDGEMENTS

This guide is an updated version of a french document first published in 2002.
It is the result of work by a group of multidisciplinary isolator technology experts.

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PREFACE

Isolator technology has become widely established in the last decade in the production of injectable medicines in both the pharmaceutical industry and in hospitals. The quality of the production process is dependent on the quality of the qualification and maintenance of the production equipment.

Following the success of the first edition of this guide on isolator qualification, the multidisciplinary working group wished to **update the information on qualification and to expand the original work to include isolator maintenance**.
This new edition adopts a pragmatic approach incorporating the experience of specialists in the fields of hospital and industrial pharmacy, equipment suppliers, and service providers. The guide continues to be a training **aid for isolator users**. This publication incorporates a summary section on qualifications and maintenance in addition to practical guides for the implementation of qualification tests and maintenance operations.
This second expanded edition should be quickly adopted by isolation technology professionals as the new reference document.

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For the prevention and study
of contamination

ASPEC © March 2017
64 rue nationale
75013 PARIS
ISBN 978-2-910218-21-8
EAN 9782910218218

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INTRODUCTION

Isolators are commonly found in various applications in the pharmaceutical industry, hospital pharmacy (**FIGURE 0.1**), the cosmetic industry, the food industry and in research and development. Isolators are used for aseptic production activities (weighing, filling, formulation, bulking, etc.), for microbiological tests (sterility testing, for example, **FIGURE 0.2**), and for the protection of personnel.

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FIGURE 0.1 – Manufacture of cytotoxic drugs in a Hospital Pharmacy

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FIGURE 0.2 – Isotest® Isolator for sterility testing

In the hospital and industrial pharmacy, the aseptic filling of pouches or vials with liquid products is carried out in positive pressure isolators (**FIGURE 0.3**). Applications, such as the handling of toxic non-sterile powders, fine chemistry applications, pharmaceutical radionuclide production, nuclear medicine unit activities, and Bio-hazardous activities, require negative pressure isolators.

Isolator technology is now also being used in cell therapy, in the production of Advanced Therapy Medicinal Products (ATMP), in microbiological containment areas (**FIGURE 0.4**) and in controlled atmosphere and containment areas.
It is the responsibility of users to choose between positive and negative pressure isolators, depending on their own risk assessment.
This guide focuses on isolators used for aseptic drug filling line operations, therefore for the most part, with positive pressure isolators.
However the approach described in this guide can also be applied to positive pressure isolators used for non-aseptic processes, and for negative pressure isolators.

This publication tackles the qualification testing and the maintenance of isolators.

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FIGURE 0.3 – Industrial filling line for pharmaceutical vials

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FIGURE 0.4 – Example of an isolator in a biological risks containment area

It is the responsibility of users to manage their own isolator technology procedures, to keep themselves up to date, and to monitor the qualification and maintenance operations of their installation over time in order to guarantee its performance. An isolator is a complex system (interfacing with transfer systems and production equipment), requiring the skill of its user to ensure the integrity of the contained environment.

In conclusion, an isolator:

- Is a barrier, interfacing with other chambers or equipment, enabling the protection of products, operators and the environment.
- Requires skilled, trained personnel (users, test and maintenance personnel...)
- Is a technology that continues to develop and which now allows for ongoing control of environmental parameters (particles, the microbiological status of the air and surfaces...)

The isolator is not just an item of air treatment equipment but a complete system interacting with its environment. The environmental conditions of the cleanroom in which the isolator is housed (temperature, relative humidity, air change rate) can affect the efficacy and aeration time of the isolator's bio-decontamination cycle.

In order to comply with GMP and Good Practice Guide in hospitals (French BPP) requirements an isolator used for preparation of injectable medicines should be in at least a grade D environment.

The alarms, sensors and recording system of the isolator can be linked to those of the room housing the isolator.

1

GENERAL INTRODUCTION TO ISOLATORS

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1 DEFINITION OF AN ISOLATOR

An isolator is an item of equipment which uses a leak-tight physical barrier, as described in the ISO standard 10648-2, to bring about a separation:

- Between two environments, external and internal, separated and controlled
- Between a process and personnel

A summary of isolator definitions taken from standards, guidelines and regulatory recommendations can be found in **PARAGRAPH 1 OF CHAPTER 16**. Chapter 16 also standard lists leak rates for isolators. Depending on use, personnel/the environment or the product must be protected, or personnel/the environment and the product must be protected. Isolators are therefore either at positive or negative pressure.

- The type of leak rate of an isolator can be described as follows : Isolators with permanent leak-tightness ('closed' isolator, with leak-tight transfer systems)
- Isolators with sequential leak-tightness ('open' isolator, involving entrance of components and exit of products)

Leakage rate is an important element in the performance of the isolator:

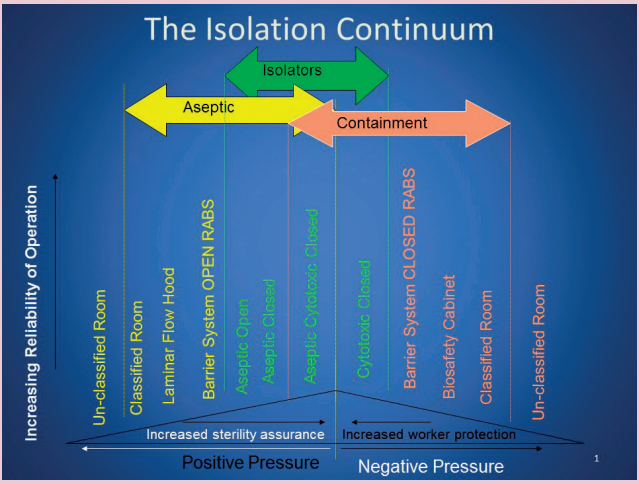
- Under positive pressure, it influences the level of operator exposure to the sterilizing agent in the room in which the isolator is located.
- Isolators under negative pressure have the predominant function of protecting personnel.

Leakage rates can affect the quality of bio-decontamination at the site of the leak during the bio-decontamination process.

The leak rates generally used for isolator are based on the ISO 10648-2 standard:

- in the order of 0.5% V/h for a sterility test isolator, an isolator used for the preparation of cytotoxic drugs, a filling lineisolator, at a test pressure of generally 1.5 to 3 times the nominal working pressure. The test is carried out at ambient temperature
- ≥ 2.5 % V/h for a filling line isolator, at test pressure of generally 1.5 to 3 times the nominal working pressure. The test is carried out at ambient temperature.

The use of air treatment equipment and rooms in accordance with need (aseptic or containment) is shown in **FIGURE 1.1**.



PDA, TR 34

FIGURE 1.1 – Positioning of the isolator in relation to air treatment facilities

The type of isolator air circulation system may vary: Unidirectional flow ('laminar') or non-unidirectional flow ('turbulent')(depending on the intended objective for non-viable and viable particulate concentrations, at rest and/or in activity, see **FIGURE 1.6**).

The performance objectives for the quality of the air in an isolator for pharmaceutical applications are given in the Good Manufacturing Practices guide (GMP), Annex N° 1: Manufacture of sterile medications, and summarized in **TABLE 1.1 AND 1.2**.

TABLE 1.1: Microbiological monitoring of controlled atmosphere areas 'in activity', in accordance with GMP, Annex 1

RECOMMENDED MICROBIOLOGICAL CONTAMINATION LIMITS[1]				
CLASS	AIR SAMPLE CFU / m³	PETRI DISHES DIAMETER: 90 mm CFU/ 4 HOURS [2]	CONTACT AGAR PLATES DIAMETER: 55 mm CFU/ PLAQUE	GLOVE PRINTS (5 FINGERS) CFU / GLOVE
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

[1] *Values indicated are average values*

[2] *Petri dishes can be exposed for less than 4 hours*

CFU = 'Colony Forming Unit`

TABLE 0.2 Particle classification of controlled atmosphere areas, according to GMP, Annex 1

		AT REST		IN ACTIVITY	
CLASS	EQUIVALENT CLASS IN ACCORDANCE WITH ISO 14644-1	MAXIMUM NUMBER OF PARTICLES PER m³ , EQUAL TO, OR LARGER THAN,			
		0,5 µm	5 µm	0,5 µm	5 µm
A	ISO 4.8 at rest and in activity	3 520	20	3520	20
B	ISO 5 at rest and ISO 7 in activity	3 520	29	352 000	2 900
C	ISO 7 at rest and ISO 8 in activity	352 000	2 900	3 520 000	29 000
D	ISO 8 at rest	3 520 000	29 000	Not defined	Not defined

➲In the case of a pharmaceutical grade A filling line isolator at rest and in activity, air flow is unidirectional flow (average air velocity 0.45 m/s ± 20 %).
➲In the case of aseptic preparation in a closed system, or the use of an isolator for sterility testing, where the objective to be attained is a class A at rest both 'unidirectional flow' and 'turbulent flow' technologies can be used.

2 DESCRIPTION OF AN ISOLATOR

An isolator incorporates different elements:

- A soft-walled or rigid chamber (enclosure)
- A ventilation system composed of sub-assemblies which deliver, extract and sometimes recirculate air.

The ventilation system may be part of the isolator or located separately in a technical area.

- An integrated or stand alone sterilizing agent generator.
- Gloves, sleeves and or/ half suit
- Peripheral elements for interfacing with other equipment or processes, which typically include three broad categories:

– Connection systems (**FIGURES 1.2 TO 1.4**):

e.g. : Leaktight Double port system (DPTE®) commonly known as rapid transfer ports ('RTP': 'Rapid Transfer Port): mobile RTP or RTP fixed to the structure of isolator.

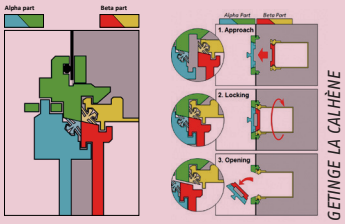


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FIGURE 1.2 The DPTE rapid transfer system

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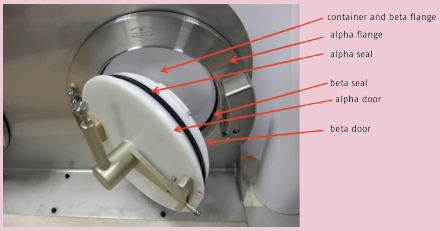
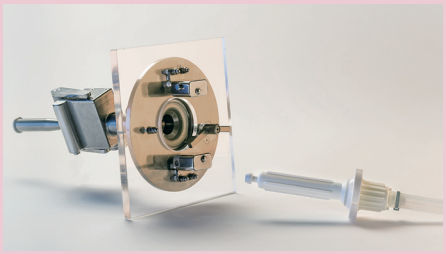


CAPE EUROPE

FIGURE 1.3 – Examples of other rapid transfer systems



SARTORIUS

FIGURE 1.4 – Liquid transfer system in a SART® isolator

– All pass-throughs in the isolator wall such as ports for taking samples and cable glands.
– Evacuation systems:
• Removal of liquids, aspiration of debris (aspirator, vacuum pump etc.);
• 'Mouse hole' for the continuous exit of products.
• Closed system bags, canisters or tubing; to remove products or waste in a sterile sealed container (**FIGURE 1.5**).

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FIGURE 1.5 – Example of an isolator with a tubing outlet and a sealed container waste outlet

– Utilities such as chilled water (e.g. air treatment system) or compressed air (e.g. controlling the operation of valves) and electricity.

3 DIAGRAM OF AN ISOLATOR

FIGURE 1.6 Schematic of the different elements likely to be found on an isolator.

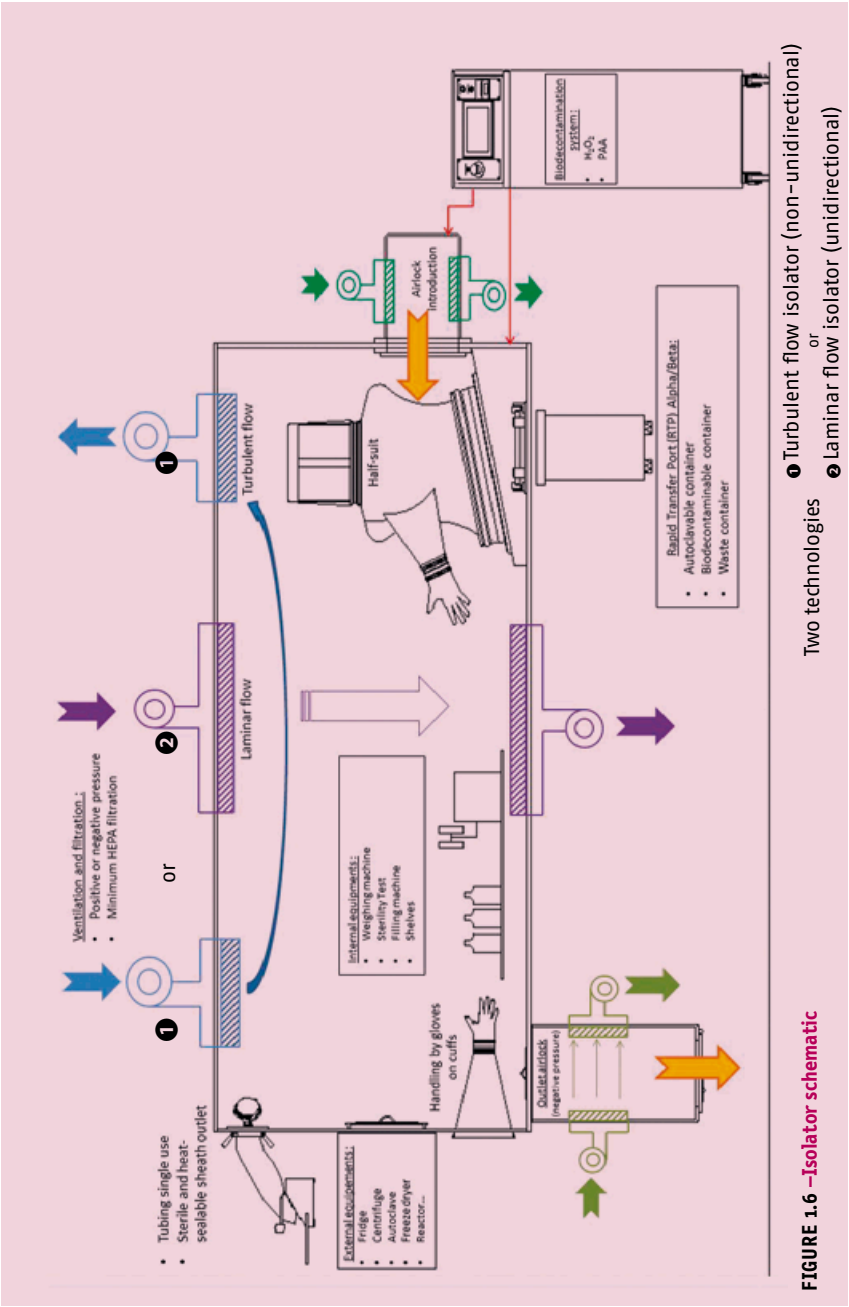

FIGURE 1.6 –Isolator schematic

2

USER SAFETY

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1 RISK ASSESSMENT

In order to reduce risk to employees it is a regulatory requirement that those in charge of facilities conduct an assessment of professional risks and implement a prevention policy using technical and operational measures.

In the event of an intervention by an external company, a prevention plan must be written (*see the example of a **prevention plan** at the end of this chapter p. 23 to 27*). The prevention plan has to be reviewed at least once a year.

The risk assessment and prevention plan are produced jointly by all involved.

In general, risk assessment involves an overview of the overall activity, including products, the processes and equipment in which they are used. The provision of training and information to users is a fourth factor which must be taken into account.

Hazards are an inherent feature of the product (**FIGURE 2.1**).

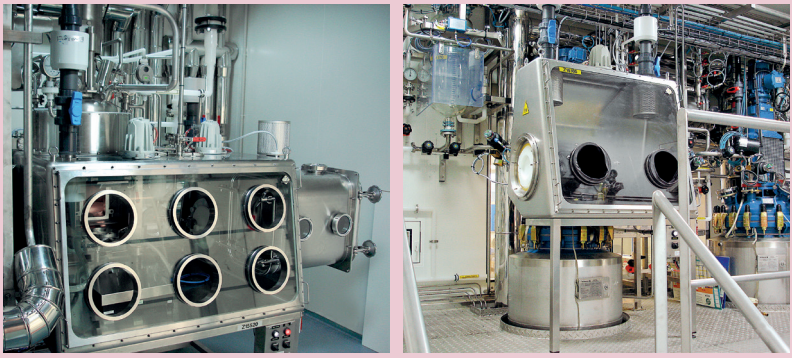

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FIGURE 2.1 – Examples of isolators for fine chemical applications

Conversely, exposure is multifactorial. It is defined by product quantity, the frequency of its use, its physico-chemical properties, the method and equipment used (temperature, agitation, etc.). Risk is a function of the toxicity of the product and the likelihood of exposure.

Example: Changing the flexible canopy of an isolator in which cytotoxic medications in solid form are handled.

The canopy is changed once a week and the operation takes 2 hours. The product is a powder; the operation includes the used of compressed air as a pre-cleaning step and the use of wipes and aqueous cleaning liquids. Replacing the canopy is carried out manually. Exposure to the product in 'vapour' phase is low because of its physical state and the temperatures used. Conversely, exposure to solid aerosols is not negligible as the granules are fine and the operation involves suspension of this powder.

To summarise, risk assessment shows that this operation carries a low to moderate risk, which in practice is that of possible exposure to solid aerosols by inhalation and exposure by skin contact which may occur during wipe cleaning.

Prevention policy: The use of a localised sensor is difficult in this case, so the use of protective respiratory equipment with particle filtration is essential. Substitution of the operation involving compressed air by an aspiration operation is likely to reduce suspension of the powder. Wearing a protective oversuit and suitable gloves limits cutaneous exposure.

2 PREVENTION

A proper risk assessment will clearly determine the resources to be put in place to ensure prevention.

Where biological agents are used, the facility is obliged to decontaminate all equipment before any maintenance tasks are carried out. This decontamination must be the subject of a document communicated to the maintenance personnel (🇫🇷 Following the French decree of 16 July 2007, establishing the technical preventive measures, in particular the containment measures, to be implemented in research, teaching, analytical, anatomical and cell pathology laboratories, post-mortem rooms and industrial and agricultural facilities, where the workforce is likely to be exposed to pathogenic biological agents). In addition to resources for collective protection, it may be necessary provide personal protective equipment (PPE).

Suitable containment systems must be considered for operations upstream and downstream of manipulations carried out using isolation technology in hospital Pharmacies (Preparation of cytotoxic products, Advanced Therapy Medicinal Products*). Wearing gloves for activities judged to carry risk is highly recommended (in connection with contamination of the external surfaces of vials). Dependent on risk assessment, additional PPE may be required.

**ATMP: French Decree of 4 February 2013 delineating the content of initial authorization applications, for authorization renewal or modification for advanced therapy medicinal products prepared with accuracy and the facilities or organizations which prepare these products.*

In terms of the safety of maintenance personnel, the operations which must receive particular attention are those which involve a containment breach: for example, changing a port, a half suit (see **FIGURE 2.2**), etc.

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FIGURE 2.2 – Half-suit isolator

🇫🇷 **French INRS Bibliography:**

- ED 6106 Les appareils de (protection respiratoire – choix et utilisation, 2011
http://www.inrs.fr/accueil/produits/mediatheque/doc/publications.html?refINRS=ED%206106
- ED 118 Gants de protection pour les métiers de la santé, 2004
http://www.inrs.fr/accueil/produits/mediatheque/doc/publications.html?refINRS=ED%20118
- ED 112 Des gants contre les risques chimiques
- ED 6170 Lavez-vous les mains pour vous protéger et protéger les autres, 2013
http://www.inrs.fr/accueil/produits/mediatheque/doc/publications.html?refINRS=ED%206170

Example of an informed prevention plan, derived from INRS recommendations:

Prevention plan

French regulation : art. R. 4511-1 à R.4514-10 du Code du travail

USER COMPANY (CUSTOMER)	OUTSIDE COMPANY (SUPPLIER)
Company name : LAB ABC	Company name: PHARM CONSEIL
Represented by : M DURANT	Represented by: M DUPONT
Contact information :	Contact information:
BAT 20 – Office n°4 – Site of Lyon	Street X – LYON
Phone number : XX XX XX XX XX	Phone number : XX XX XX XX XX

Location of intervention: Builiding 90, level 0, Room C55		
Previous visit	~~Yes~~ / No	Dates :
Intervention(s) CHSCT or Occupational Health and Safety Committee	~~Yes~~ / No	
Description of the intervention:		
Validation of sterilization cycles by H_2O_2 of sterility test isolator		
Maximum number of employees	2	
Date and duration of prevention plan – Intervention schedules		
from 21th of october to 31th of december 2014 – 8h to 18h00		
Specials observations		
No work at night or weekends		

Signature of stakeholders (user company and outside company to take into account of this prevention plan)
Mr.Durant signature
Mr.Dupont signature

Aid organization
Qualifications required by Employees
Means made available

post number :	People to be notified :	How ?
1 65 67	Production manager (in case of spreading of product, fire...)	Phone or cellphone
2 65 45	Nurse (in case of body accident)	Phone or cellphone
3 65 82	Principal	Phone
Exemple : 15 (in France)	First Aid	Cellphone

First aid organization **Materials (location and instructions for use), skills, emergency access, evacuation, etc.):**
See the «Welcome to Lab ABC» brochure with the position of the emergency exits and the assembly points

Training, qualifications, authorizations, qualifications and medical skills required for the intervention:
* Access in ZAC SOP XYZ
* No known allergy to antibiotics

Material means made available to the outside company:
(place, room, products and user company materials)
N/A

Others observations :
N/A

N/A : not applicable

Risks analyzes

In a first time, identify families of risks involved in the joint ventures of two companies. For these last ones, Detail each risk by indicating their nature, location, frequency of supervision. Risks will be listed in desending order of importance. To help you in this analysis, you will find some benchmarks under each risk family.

Presence of elements containing asbestos on or near the place of intervention:	
YES ☑ NO ☐	User company will hand over the asbestos diagnosis to the outside company

Internal traffic risks :	YES ☑ NO ☐
Documents submitted to outside company :	
* traffic ways	
* parking places	
* Contractors access: Parking area for unloading vehicles, special rules for driving within the site compound * Access to the place of work or requirement for access: in the case the work is to be carried out in an area with different levels or in an upper level In the both cases : '– Condition of the floor, obstructions caused by low lying objects which may need to be stepped over, or which present a risk of snagging or potential injury '– Type of shoes used, adherence before, during and after works '– Conformity of stairs, stairs rails, doors (to rooms or lifts), automatically closing and vibility either side **Factors increasing risks:** lifting and manual handing, poor lighting, poor ventilation or enclosed spaces, poor knowledge of working area etc.	

Risks related to fall in height :	YES ☐ NO ☑
In its access and realization: – Work is carried out in an area or requires the operator uses a ladder or any type of mobile platform either to access to the work place or in order to carry out works – Work is carried out at ground level but in proximity of a sudden drop – Work is carried out in an area where object may fall on the operator Factors affecting level of risk or surveillance required : height, load carrying, lighting, space, restrictions, lack of ventilation or confined space, operator physical condition (state of health, sensitive to height etc)	

Risks related to chemicals products:	YES ☑ NO ☐
1–Skin contact or risk of inhalation in case of exposure to hydrogen peroxide H_2O_2 (35% w / w) gas and liquid	
2–Risk of exposure to an antibiotic	
* Products brought to site by the contractor: Toxicity by inhalation or skin contact (+ fire or explosion risk). Estimate probability and level of monitoring. * Product in use on either in the production process or its utilities: Toxicity by inhalation or skin contact (+ fire or explosion risk). Estimate probability ans level of monitoring. * Compatibility of storage during use and disposal of the products listed above (including for example wipes used for spillage) acid product + bleach + emission of chlorine. *Risk related to the combining of products with separately do not present a risk but do if combined with another product. **Critical factors:** Temperature and proximity to a permanent or occasional source of heat, inadequate ventilation, poor knowledge of product routing, inappropriate containers (weight, means of dispensing) missing or poorly labelled. Product transfer between product container and point of use (container, clothes, etc).	

Electric risks	YES ☐ NO ☑
Risk's elements : Condition of installation (earthing, differential trips, state of connectors, cables etc). Environmental conditions : Humidity or potential contact with humidity (hands,...), cleanliness of the equipement Potential risk factors : Working alone, poor knowledge of risks	

Risks related to manual handling:	YES ☐ NO ☑
Risk's elements: type of movements, repetitive movements, amount of effort required. Example: lifting of leads: weight, lift points movements. Critical factors : Site infrastructure (stairs, bins,etc.), work environment, internal traffic	

Biological risks :	YES ☐ NO ☑
Overall at least risks to which the site employees are exposed to. Medical research, risk of infection related to blood, tissue, containers and equipment in contact with substances. **Special risks:** legionaries disease, mites (asthma, allergies)	

Fire / Explosion Hazards:	YES ☐ NO ☑
Components : Combustibles, oxidizing agents, source of heat or energy * Energy source: naked flame, sparks, friction (gives rise to static, fermentation, shocks, cigarettes) * Conbustibles: all type of combustible materials, wood, paper, chemical products, store or spilled, dust * Oxidizing agents: the air mostly but occasionnally products stocked on site by the client	

Risks related to co-activity (user company / outside company)	YES ☐ NO ☑
Identify in this section if works to be carried out by the contractor increase exisiting risks or create new risks for the client and vice versa. Do not omit movement of construction equipment or vehicules.	

Risks related to encountered nuisances (noise, temperature, confined space, lighting, radiation, dust, etc.)	YES ☐ NO ☑
In general likely to increase risk either concurrently or between each other. The essential element in appreciating the risk is the type of work (physical or intellectual: increase or not of the effects of risk) exposure time and exposure levels are important. Ability to overcome risks related to the ambient environment is an important element of the analysis. Example: hot or cold room etc. When the ambient conditions are likely to vary in an unpredictable manner consider the extremes. Example: weather: wind temperature, rain, snow, ice, exposure to sunlight etc. * Humidity, temperature * Confined space or work in a poorly ventilated or polluted area the analysis is based on the type of activity, level of pollution, pollution of air by the work carried out (chemical or biological, risk of asphyxiation. Lighting: Analysis of the lack of or excess of lighting, the position of light switch or switches, appropriateness for the type of work to be carried out (example separate lighting where risk of electrocution is likely) evaluate the incidence of natural or artificial lighting on the task to be carried out. * Radiations: Work near of electromagnetic signal generators (radio, infrared, UV, micro-wave etc.) Example: proximity to a mobile telephone aerial, a radioactive source (medical device) welding X ray	

Other risks not mentioned elsewhere: (Aggression, isolated worker, etc.)	YES ☐ NO ☑
Part of identified risks in user company and in its activities (Cf: risk analysis document compliant with article R) Separate the risks identified to the client's site with those associated with contractor's activities.	

SOURCE : INRS

PREVENTION MEASURES (permanent or non-permanent)
Starting with the most critical, define the collective preventative measures (organization, equipment etc.) and individual protection. Treat the remaining risks using training or information (instructions) to reduce risk or level of risk or increase the likelyhood of avoiding the risk. (See supporting document for risk evaluation compliant with article R).
Measures (to be defined by risk):
Skin contact or risk of inhalation when accidentally exposed to H_2O_2 gas and/or liquid
Wear PPE when handling liquid H2O2
Wear a Drager PAC III to detect any leak of H_2O_2 vapours
Orange warning beacon on isolater during H_2O_2 sterilisation cycle
Further Instructions (or rest of precautions):

MEANS IN PLACE FOR MONITORING THE PREVENTION PLAN, REACTUALIZATION AND ITS EFFECTIVE APPLICATION ON THE GROUND
(Agent / user company liaison book or agent / external company report, hazardous situation report, etc.)

3

STERILIZATION (BIO-DECONTAMINATION) AND STERILISING AGENTS

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1 STERILIZATION (BIO-DECONTAMINATION)

Prerequisites for sterilization or bio-decontamination using a chemical agent: **Bio-decontamination and sterilization using chemical agents require clean surfaces, which have been cleaned and dried prior to sterilization.**

➲ Regulatory situation:

Note on the European Pharmacopoeia:

Isolator surface sterilization is not covered by the European Pharmacopoeia: 'Gas sterilization is commonly used for medical devices, isolators, rooms, etc. The use of gases in this context is not covered by the European Pharmacopoeia' (Chapter 5.1.2., Biological indicators for sterilization EP 8.0).

Note on the American Pharmacopoeia:

Chapter 1229.11 of the American Pharmacopoeia USP uses the term 'vapor phase sterilization'. Chapter 800 of the USP gives the terminology 'sterilization' (**SEE CHAPTER 16, PARAGRAPH 2**).

➲ Clarification provided by the EDITORIAL COMMITTEE:

In the case of isolators used for aseptic filling of injectable medicines the acceptable sterility assurance level should be adjusted, dependent on the process involved, to either: 10^4 or 10^6 ;

- for isolators in which the product is not subject to terminal sterilization, and is exposed to the environment (for example, filling operations, preparation of cytotoxic medications), a level of 10^6 is required for critical surfaces (in direct or indirect contact with the product).
- for isolators which operate with closed vials in which the product is not exposed, a level of 10^4 may be sufficient (example: crimping capped vials in an isolator).

The most commonly encountered level of 10^6, is consistent with a sterilization objective. Levels of 10^4 up to 10^6 correspond to a bio-decontamination objective, subject to the knowledge of the initial level of contamination and an existing formalised risk analysis.

For the manufacture of injectable products and for critical surfaces (e.g.: stopper hoppers, sterility testing, parenteral nutrition), a 6 log reduction of spores is imperative.

In hospital pharmacy, the performance objective is also 6 log reduction of spores.

Explanatory note on the history of isolators and their associated terminology:
Isolators were used for the containment of rodents and invalids long before their use in pharmaceutical applications. The expression used at the time was 'isolator sterilization'. When isolators were first used in pharmacy, users wanted to distinguish between **(FIGURE 3.1)**:
– A complete sterilization process which sterilizes the medication in its primary packaging (e.g.: by autoclaving) and which, it terms of envisaged objectives, allows reference to a Sterility Assurance Level (SAL). The sterility assurance level corresponds to a probability of contamination, and signifies that not more than one in a million products that have undergone complete sterilization is likely to be contaminated. In this context sterility assurance level is expressed as a negative power of 10, 10^{-6}.
– A surface sterilization process which does not include the medication as in autoclave sterilization. In this case the sterility assurance level is refered to as a Spore Log Reduction (SLR) using a strain resistant to the sterilizing agent. The log reduction of spores for an isolator is expressed with a positive exponent and signifies that a quantity of spores has been destroyed by the surface sterilization process (10^6 for hospital pharmacy, and for injectable products and critical surfaces in the pharmaceutical industry).

In English the terms sterilisation and bio-decontamination are used to distinguish between complete sterilisation and surface sterilisation. These terms have been translated into French by 'stérilisation' and 'biodécontamination'.
In the past, the term 'stérilisation de surface' has been used in place of 'bio-décontamination'.

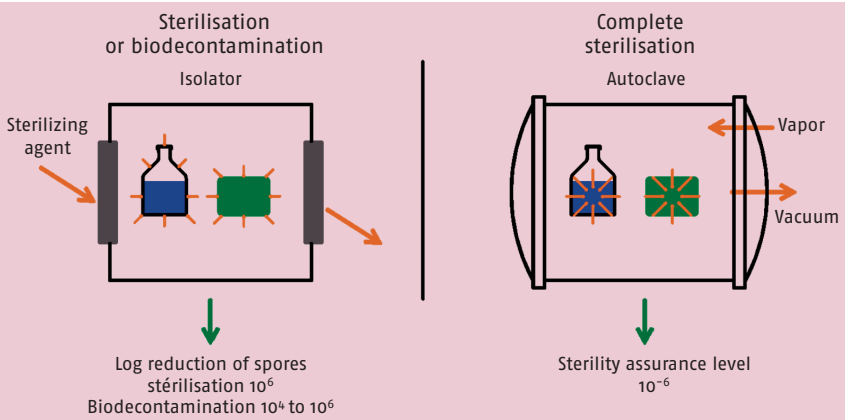

FIGURE 3.1 –Surface sterilization and bio-decontamination in an isolator versus complete sterilization in an autoclave

2 STERILIZING AGENTS

2.1 DEFINITION

A **sterilizing agent** is *a physical or chemical entity or combination of entities which possesses a microbiocidal activity sufficient to obtain sterility under the defined conditions (EN ISO 14 937).*

2.2 NATURE OF STERILIZING AGENTS

The manufacturer's instructions for the pair 'Product – Generator' must be followed.
Two types of sterilizing agents are commonly used:
➲**Solutions containing a mixture of peracetic acid (from 1 to 5%) and hydrogen peroxide (from 8 to 22%), stabilized at ambient temperature, referred to in the remainder of this document as PAA;**

Solutions of hydrogen peroxide (from 30 to 60 %, possibly in lower concentrations in nebulizes) referred to in the remainder of this document as H_2O_2.

Solutions have also been developed that use other sterilizing agents (NO_2...).

2.3 DESCRIPTION OF STERILIZING AGENTS

As a minimum, the supplier must provide the following information:

- The name of the product
- The name and address of the supplier (manufacturer, distributor)
- Composition (w/w concentration of the sterilizing agent and associated tolerance: e.g. : 35 % ± 3 %
- Batch number
- Use-by date (conditions of storage)
- Packaging
- Manufacturer's safety datasheet (indicating the Occupational Exposure Limit (OEL) and the Exposure Limit Value (ELV).
- Certificate of analysis of the oxidizing agent in compliance with regulations (Reach, CLP) and with the manufacturer's recommendations
- Stabiliser content (phosphates, tin,...):

NB: Depending on the origin of the bio-decontamination chemical agent, as well as the nature and concentration of stabilizers, these substances may leave visible residues (in the order of 0.02 % to 0.06 %) in the generator tank or inside the capillary tubing. The risk of accumulating residues is then the reduced efficacy of the sterilization or the bio-decontamination cycle.

- Sterility of the sterilizing agent
- Storage conditions

In addition, it is desirable to obtain the following:

- The technical file including the validation of product efficacy
- Compatibility with the materials in use (equipment, media, consumables)
- Conditions and precautions for use (product/equipment link)
- Use-by date after opening
- Studies dealing with materials permeability:

Little information is available on the permeability of materials to chemical bio-decontamination agents. If this is the case at the end of the performance qualification phase (PQ), once the sterilization cycle and the load have been determined, the user must conduct studies to assess the risks from the interaction of the sterilizing agent with different components of the process, namely:

• Risk of product degradation, (e.g. oxidation generating toxicity to the patient)
• Risk of creating false negatives during environmental monitoring by inactivation of the agar plates used for microbiological tests
• Risk of creating false negatives during sterility testing (bacteriostatic and fungistatic activity affecting test samples).

The xylenol orange method by UV spectrophotometry is which has a sensitivity to 0.25 ppm, can be used to look for traces in the product.

2.4 CERTIFICATE OF ANALYSIS

The certificate of analysis must incorporate the following elements:

- Name of the product
- Batch number
- Name of the supplier
- Analysis: method and tolerance limits
- Analysis results:
  - Concentration,
  - pH
  - Stability over 24h
  - Residues after evaporation
- Use-by date
- Date of manufacture

3 OPERATIONAL PRINCIPLE OF STERILIZING AGENT GENERATORS

The vaporization method uses the sterilization agent in 'vapour' phase, at low temperature, after heating, in order to obtain optimal circulation. There are two evaporation techniques (see Table 3.1):

TABLE 3.1: Techniques for the emission of the sterilizing agent by evaporation

OPEN CIRCUIT	CLOSED CIRCUIT
* heating and distribution using compressed air This can also be done using a ventilator.	* heating and distribution by a ventilator and by recirculation

FIGURE 3.2 summarizes the practicable routes during emission of a sterilizing agent in the isolator

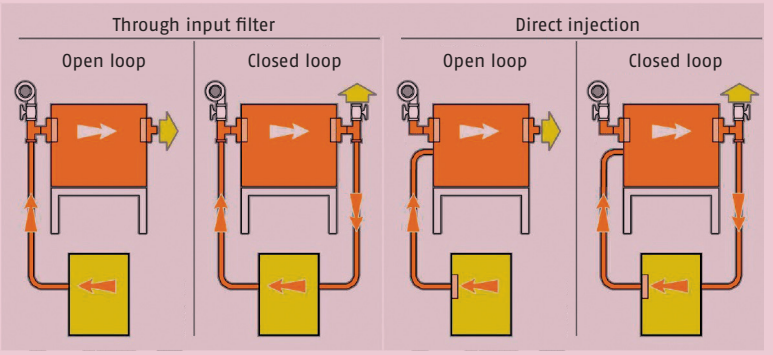

FIGURE 3.2 – Practicable routes for the emission of the sterilizing agent

The **nebulization method** is also sometimes used in isolators. In these cases, the sterilizer controls the quantity of sterilizing agent emitted and the duration of contact time. The sterilizing agent is connected to a fixed nozzle on the isolator wall. Circulation of the sterilizing agent is effected by nebulization of the sterilizing agent under pressure, without passing through the isolator's HEPA filter.

TABLE 3.2 summarizes the principal advantages and disadvantages of the various techniques for generating sterilizing agents.

TABLE 3.2: Advantages/Disadvantages according to the mode of generating the chemical bio-decontaminant agent

MODE OF GENERATION	ADVANTAGES	DISADVANTAGES
In-line evaporation passing through the HEPA filter(s)	Bio-decontamination of the whole system, including the the filters	Longer cycle duration (by a minimum factor of 3)
In-line evaporation without passing through the HEPA filter(s)	Shorter cycle duration	The surface upstream of the filter is not sterilized or bio-decontaminated
Evaporation with recirculation passing through the HEPA filter(s)	Bio-decontamination of the whole system, including the filters	Longer cycle duration (by a minimum factor of 3)
Evaporation with recirculation without passing through the HEPA	Shorter cycle duration	The surface upstream of the filter is not sterilized or bio-decontaminated
Nebulization	Shorter cycle duration	Dependent on the technologies used, there is a risk of wetting the filters and a longer aeration time[1]

[1] *the Dryfog process does not carry this type of disadvantage, delivering a direct injection in static mode which does not pass through the filters.*

Installations also exist where the sterilizing agent is either passed through the inlet filter of injected directly into the chamber so that following initial bio-decontamination of the isolator including the filters, the isolator can subsequently be bio-decontaminated more rapidly. Comment: Whatever the generation system, the extraction ducts must be compatible with PAA which is corrosive (e.g. : PVC). Conversely, galvanized steel ducts are prohibited.

It should be noted that the 'proper distribution' of the sterilizing agent in an isolator is directly related to its design (**FIGURE 3.3**).

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FIGURE 3.3 – Examples of methods of generating the bio-decontaminant agent in an isolator (nozzle, ventilator)

4

BIOLOGICAL INDICATORS (BI)

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2.2 Choice, sensitivity testing and population counts 36
2.3 Investigations 39
3 CERTIFICATE OF ANALYSIS FOR THE INOCULATED CARRIERS 40
4 CONTROLS 40

1 REFERENCE INFORMATION

The biological indicators (BI) most commonly encountered are listed in **TABLES 4.1** and **4.2**.

TABLE 4.1 : Biological indicators commonly used in Europe

PARAMETER	STERILISATION BY H_2O_2 OR APA
Microorganism	**H_2O_2 and APA:** *Geobacillus stearothermophilus* ATCC 7953 and ATCC12980[1] **PAA:** *Bacillus atrophaeus* (formerly Bacillus subtilis) ATCC 9372
Carrier	Inert from a physico-chemical point of view[2]
Population	≥ 1.10^6
Characteristics (indications to be displayed on the label and/or in the certificate of analysis)	- Strain used: Name of the control microorganism, Strain reference number - Population viable under the following conditions (specify the culture medium, the conditions of incubation and methods of recovery) -Specify waste disposal method and the non-pathogenic nature of the strain - D value in accordance with the supplier's test conditions[3] -Storage conditions - Expiry date Waste disposal

[1] in Europe, ATCC 7953 is most often used

[2] It must be sufficiently smooth not to protect the spores. It must not degrade the sterilizing agent nor have a biocidal effect.

[3] The user must, as a minimum, for the same lot of biological indicators, perform a numeration and identification to verify that the BIs have not been affected by transport.

TABLE 4.2 : Biological indicators commonly used in the United States

PARAMETER	STERILISATION BY H_2O_2
Microorganisms (spores)	*Geobacillus stearothermophilus:* ATCC12980 *Clostridium sporogenes:* ATCC11437 (little used)
Carrier	Inert from a physico-chemical point of view[1]
Population	≥ 1.10^6
Survival time	[Log (pop) – 2] x D-value
Kill time	[Log (pop) +4] x D-value
Characteristics (indications to be displayed on the label and/or the certificate of analysis)	- Strain used: (name, reference number, collection) - Population viable under the following conditions (specify the culture medium and conditions of incubation) - D value in accordance with the supplier's test conditions - potentially available: survival time and kill time - Storage conditions - Expiry date - Waste disposal

1 It must be sufficiently smooth not to protect the spores. It must not degrade the sterilizing agent nor have a biocidal effect.

The user must, as a minimum, for the same lot of biological indicators, perform a numeration and identification to verify that the BIs have not been affected by transport (USP Recommendation).

A determination of the D value under conditions validated by the user is advised.

See **CHAPTER 6 PARAGRAPH 5**

2. CHARACTERISTICS

A biological indicator is characterized by the following elements:

- The name of the strain
- The ATCC number or equivalent
- Population and quality of the initial suspension
- Lot number
- Bacterial count (identity) per manufacturer's lot
- Type of carrier
- Number of viable spores per carrier
- D value per manufacturer's lot with specification of operational conditions
- Storage conditions
- Transport conditions
- Disposal conditions

2.1 TYPES OF CARRIER

The carrier must be inert with respect to the sterilizing agent and its method of use.
The following types of carrier are commercially available:

- Paper
- Stainless steel
- Plastic (for example PVC, EPDM, Hypalon)
- Glass

NB: **Paper is historically the most commonly employed carrier for PAA in hospital pharmacy. Stainless steel is most commonly used carrier for H_2O_2.**

TABLE 4.3: Physico-chemical compatibility of the carrier with the sterilizing agent

CARRIER TYPE	COMPATIBILITY WITH	
	H_2O_2	PAA
Paper	-	+
Glass	+	+
Stainless steel	+	+
Tyvek®	+	+
Polystyrène (for example, polyflex®)	+	+

- : The sterilizing agent is incompatible with the material

+ : The sterilizing agent is compatible with the material

2.2 CHOICE, SENSITIVITY TESTING AND STRAIN COUNTS

Choice

The choice of test strains is dependent on the sterilizing agent:

❶ Hydrogen peroxide

Strains ***Geobacillus stearothermophilus*** (ATCC 7953 and ATCC 12 980)

Following a study conducted by an independent laboratory, the two strains are equivalent (*Technical Bulletin : Comparative Biochemical Characteristics of Bacillus stearothermophilus Strains ATCC 7953 and ATCC 12980*).

❷ Peracetic acid

Strains ***Geobacillus stearothermophilus*** (ATCC 7953 and also ATCC 12 980)
Bacillus atropheus (ATCC 9372)
Clostridium sporogenes (ATCC 11437)

For applications using peracetic acid as a sterilizing agent, ***Bacillus atropheus*** (ATCC 9372) is the strain most commonly used, further to resistance studies (Block, Hoet and Thorogood).

Resistance testing (D value)

When the Tyvek® packaging is removed from the biological indicators, these are referred to as inoculated carriers. The terms BI or inoculated carriers are used in accordance with user preference.

It is essential to determine the resistance of the inoculated carrier relative to the method and the sterilizing agent to justify the parameters of a sterilization cycle (USP recommendation).

D value studies consist in determining the time required to reduce an initial population of microorganisms by a factor of ten (a 1 log reduction).

The manufacturer's D value and count are indicators of the quality of a given lot of biological indicators. This information can provide a database for the acceptance of subsequent lots of biological indicators (revalidation).

The D value is highly dependent on the 'isolator/generator' system under consideration (concentration, temperature, etc.). So for the same lot of biological indicators, the D value can change depending on the system used to determine it.

It is therefore recommended that users should determine the D value of a biological indicator lot on their own installation during initial qualification and prior to requalification.

As a general rule, it should be remembered that biological indicators are by definition 'biological' and that in consequence their resistance to the sterilizing agent can vary significantly.

It advised that sterilization cycles should be developed with a safety margin sufficient to take account of the natural variability in the resistance of biological indicators.

The fraction-negative method for determining a D value (USP, <55>) is the easiest of to implement.

According to the EN ISO 14161 standard, *this analysis requires a determination of positive or zero growth, and uses aseptic transfer of inoculated carriers intact into liquid culture medium. Transfer must be carried out without any mechanical, microbiological or thermal effects on the inoculated carriers. Several methods of this type are used and are collectively referred to as fraction negative methods or quantum methods. Positive or zero growth is observed relative to the number of inoculated carriers tested.*

Fraction negative methods are described in Appendix C of standard EN ISO 14161. It should be noted that the BI must be removed from the chamber in the shortest possible time.

Numeration

The European Pharmacopoeia recommends numeration when using a population of microorganisms greater than 5.10^5.

The American Pharmacopoeia recommends a population of microorganisms greater than 1.10^6.

In practice, a bioburden of greater than 10^6 per carrier is used.

If users manufacture inoculated carriers themselves, they will need to take account of standard ISO 11138-1.

Use-by date, transport and storage conditions

The supplier must be able to reference stability studies including transport and storage conditions specified by the manufacturer in order to set the use-by date and the D value. As a consequence, the end-user has a duty to scrupulously respect the transport and storage recommendations determined by the manufacturer.

Conditions of use and incubation

➲ *Use*:

Biological indicators used for validation and surface sterilization can be taken out of their primary packaging. BIs removed from their primary packaging become inoculated carriers (not usable for a requalification in activity).

In some cases, an equivalence may be found between the resistance of the inoculated carriers and the biological indicators including its primary packaging where the primary pack is permeable to the sterilizing agent vapours.

Where cold storage is used, inoculated carriers must be left to reach to ambient temperature and laboratory conditions before opening the outer packaging. This generally takes 1 to 2 hours. To avoid condensation on the inoculated carriers, it is wise to keep them with some desiccant in a sealed bag.

During qualifications of the sterilization cycle, the use of the biological indicators must be carefully controlled. The temperature of the biological indicators at the moment of exposure, must always be the same, preferably this should be the ambient temperature of the isolator. The period intervening between removal of the BI from the storage chamber and their introduction into the isolator must be defined and reproducible.

The positioning of each BI at each locations is important. Each BI must be oriented in the same manner. The primary packaging should not be covered (for example, by adhesive tape). During recovery of BIs, avoid gathering the collected BIs together. Keep them separated.

➲ *Incubation*:

Each BI must be placed individually into culture without being in contact with other BIs. Temperature and incubation time should be defined with reference to the manufacturer's indications.

The European Pharmacopoeia gives the following culture conditions (**TABLE 4.4**)

TABLE 4.4: Culture Conditions (European Pharmacopoeia)

INOCULATED CARRIER	MEDIUM[1]	TEMPERATURE (in °C)	INCUBATION TIME
Bacillus atrophaeus	TSB[2]	30 – 35°C	7 days
Geobacillus stearothermophilus	TSB[2]	55 – 60°C	7 days

[1] *To demonstrate that there is no inhibitory action on the growth of the strain*

[2] *TSB: Medium containing casein hydrolysate and soy (European Pharmacopoeia – 2.6.12)*

It is recommended that the inoculated carriers should be incubated without delay.
- Take account of the particular conditions linked to the sterilizing agent during the strain regrowth step (traces of sterilizing agent which could disrupt microbiological growth)
- Precautions must be taken to ensure transfer of the inoculated carriers into culture medium :
• If possible, transfer should be carried out in an isolator
• Use sterile gloves, and if need be, sterile tweezers
• If need be, use, one set of tweezers per biological indicator and per location
• During transfer operations, avoid contaminating the surfaces involved

NB: Any handling irregularity that occurs during incubation should be noted, for example if the biological indicator is dropped in or outside the chamber during the sterilization cycle or during recovery.

2.3 INVESTIGATIONS

Non-compliant (positive) BIs are handled in accordance with the recommendations of the PDA Technical Report, TR51, 2010, Chapter 8. In the event of a non-compliant BI an investigation should be carried out.
The following approach is to be applied to all non-conformities relative to positive BIs following their incubation as part of a sterilization challenge.

The basis of the investigation lies in checking and documenting the following points:
- Confirm the cycle parameters (acceptance, report and alarm), including the room and the machinery connected to the chamber.
- Receipt of BIs (supplier's certificate, D value and counts)
- Verification of the implementation of the validation protocol (operator training)
- Verification of compliance with the operational parameters of the procedure (sterilization of the isolator, use of sterilizing agent generators, operator training)
- Sterilizing agent certificate (type, expiry date, dilution,...)
- Review of calibrations (treatment of the air in the room, isolator, sterilizing agent generator)
- Review of maintenance data
- Comparison with previous cycles (temperature graph, humidity measurements, sterilizing agent concentration and pressure).
- Analysis of temperature data (surfaces, chamber and isolator)
- Previous history of non-conformities in the same location position involved (recurrence).

Investigation assessment
– After the investigation has been conducted and if no cause has been identified, the most likely cause for the positive will be an unusually resistant indicator.
– The investigation must be thoroughly documented before proceeding to the step of running a confirmation test.
– In the event that the investigation concludes that there is a problem with an unusually resistant indicator, a confirmation test may be conducted:
• with one or several BIs in the position affected
• with the same cycle parameters and the same acceptance criteria

3 CERTIFICATE OF ANALYSIS OF INOCULATED CARRIERS

The certificate of analysis should include:
• The BI Reference
• The address of the supplier
• The type and conditions of sterilization
• Description of the BI (carrier, strain)
• Lot number
• Use-by date
• Count
• D value with reference to the manufacturer's sterilization procedure
• Storage and transport conditions
• Date and signature

On receipt, a numeration will be performed either internally, or by a subcontracting laboratory.

4 CONTROLS

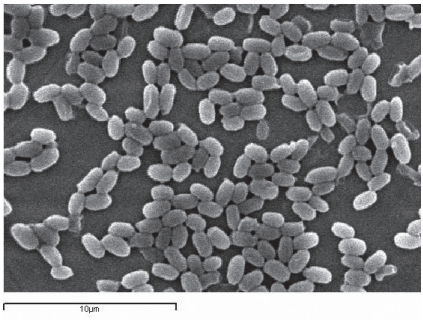
We recommended that at least three controls should be run:
➲**Negative control of the culture medium**. Incubate the control medium in the same conditions as the inoculated carrier. This control is not subjected to the sterilization cycle. It should not result in microbiological growth.

➲**Positive controls:**
One of these is the spore viability control. This control is placed in a leak-tight container impermeable to the sterilizing agent. It must not be placed in contact with the sterilizing agent. The inoculation protocol is the same as that for the inoculated carriers used for validation.
It should result in the growth of spores.

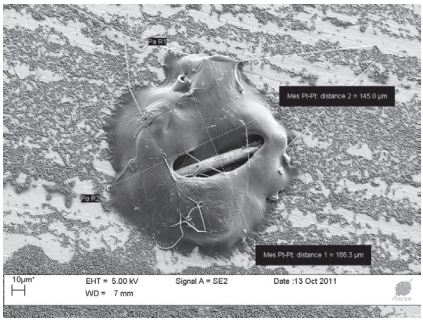
The second positive control is a **control count** conducted outside the isolator which is analysed in the same way.
A fourth control is expected to be run by biological indicator manufacturers. This ensures that the carrier itself (paper, stainless steel...) does not give rise to the growth of bacterial colonies.

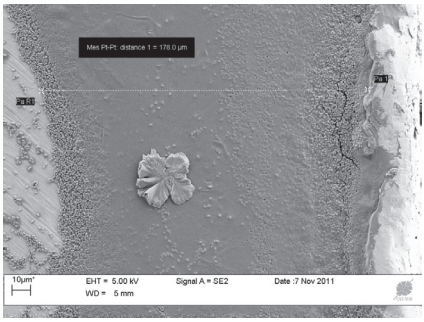
MICROSCOPIC EXAMINATION OF SPORES OF BIOLOGICAL INDICATORS

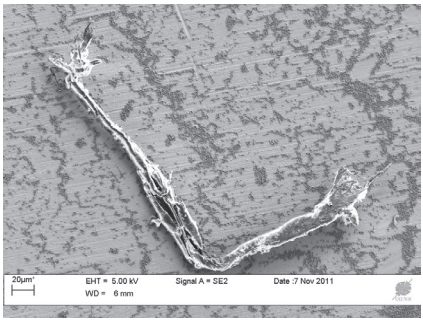
SPORES IDEALLY POSITIONNED (MONOLAYER, FREE OF CONTAMINANT)

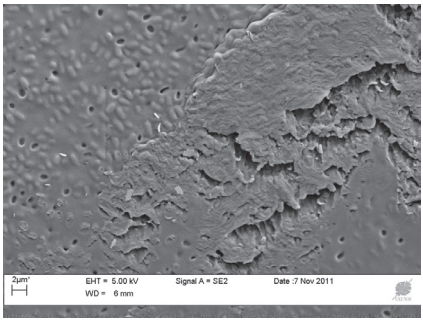


EXAMPLES OF DEFECTS ON BI FOR VHP









5

CHEMICAL INDICATORS, SENSORS AND PROBES

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Qualitative or quantitative sterilizing agent measuring devices can be used for:
- Verifying the correct distribution of the bio-decontaminating agent during the development of bio-decontamination cycle;
- That during routine use the cycle is running correctly (particularly useful in the case of generator models which do not allow access to all cycle parameters);
- Ensuring sufficient aeration in terms of personnel and procedural safety at the end of the bio-decontamination cycle;
- Ensuring that there no process risks: no penetration of the sterilizing agent into the product container, or into the media in test tubes or on media plates.

There are two distinct types of device based on result obtained, accuracy and cost:
- Chemical indicators (qualitative information)
- Low and high level detectors and measuring probes

1 CHEMICAL INDICATORS

1.1 FOR H_2O_2

Chemical indicators for H2O2 are short-term indicators that are used for initial validation. They provide **qualitative information**.
At least two types of chemical indicators are available:
- **Chemical indicators, for example PCC051® and PCC060®** (PCC060® more recent and less sensitive, is tending to replace PCC051®), manufactured by STERIS
- **Chemical indicators, for example HPV-CI®**, manufactured by BIOQUELL: this type of chemical indicator allows for fast and direct reading of the log reduction, following action of the bio-decontaminating agent. The colour change of this indicator is permanent. They are supplied non-sterile.

© BIOQUELL

FIGURE 5.1 – HPV-CI® Chemical indicator

During validation, chemical indicators are generally distributed through the volume of the chamber. Their distribution and number are determined on the basis of the temperatures and air flow in the isolator.
A Petri dish or contact plate containing phenol red can also serve as a short-term chemical indicator control for the sterilizing agent (pH control).

1.2 FOR PAA

No specific indicator is commercially available for vapour phase PAA. Although designed to test liquid media, the MERCK MERCKOQUANT® (MQuantTM) indicator (no 1.10084.0001), a semi quantitative indicator, allows detection of peracetic acid in vapour phase (**FIGURE 5.2**). The measuring range for PAA is from 5 to 50 mg/l.

© MERCK MILLIPORE

FIGURE 5.2. MERCKOQUANT® (MQuant™) Indicator no. 1.10084.0001

The chemical indicator PCC060, initially intended for use with H_2O_2, is also suitable for use with H_2O_2-PAA mixtures such as SOPROPER®.
The same applies for the HPV-CI (BIOQUELL) indicator which is also suitable for the detection of PAA.
Users must qualify their own test method when using chemical indicators as their response depends on:

- The sterilization cycle: the quantity of sterilizing agent, cycle duration
- The type of sterilizing agent generator and its output
- The volume of the chamber
- The quality of the vector gas and its control

Before beginning trials with the sterilizing agent, measurements of air speed and generator output followed by smoke tests (using a smoke generator compatible with the bio-decontamination process) are generally very useful. Conditions in the isolator can be optimized by conducting this type of test, even before using the bio-decontaminating agent.

Examples of points in an isolator that are critical for the positioning of BIs and CIs are provided in **TABLE 5.1** and visualized in **FIGURE 5.3**.

TABLE 5.1 Examples of critical points in isolator

EMPLACEMENT	DESCRIPTION DE L'EMPLACEMENT	TYPE	POSE BI	RETRAIT BI
01	On the floor near rear and left panels	D Gaz		
02	On the rear panel left top	HT		
03	On the ceiling beam center	HT		
04	Rear panel right top corner	HT		
05	On the floor near rear and right panels	BT		
06	On the floor near front and right panels	CP		
07	On the front panel, top right corner	D Gaz		
08	On the machine center	D Gaz		
09	On the floor near front and right panels	CP		
10	On the left panels, between the glove ports	CP		
11	Rear panel in the middle	HT		
12	Right panel in the glove on the wrist	CP		
13	On the right panel into the RTP port	CP		
14	On the front panel, into the sleeve, glove number 3	CP		
15	On the front panel, center on the window, 20 cm above the gloves	CP		
16	On the front panel, on the upper edge of the machine	CP		
17	On the rear panel, center on rear face of the machine at 50 cm above the floor	CP		
18	On the floor, center at 30 cm from the right panel	CP		

HT : Hot Spot as per temperature mapping study
BT : Cold Spot as per temperature mapping study
D (gaz) : Worst case location in term of gaz distribution as per chemical mapping study
CP : Critical for process as per process analysis (filling point, RTP port, conveyor and so on...)

The diagram showing the positioning of chemical indicators and thermocouples can also be supported by photos.

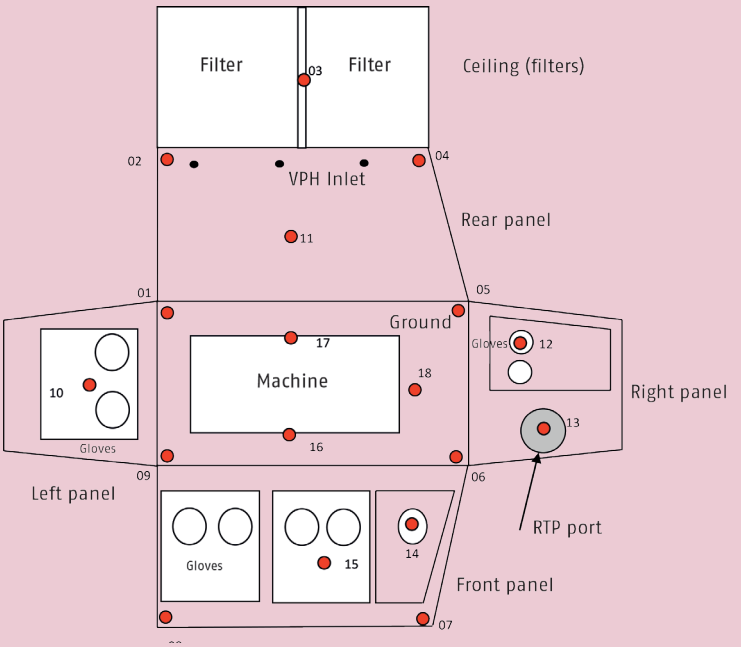

FIGURE 5.3 – Example of located (BIs and CI) in an isolator

2 INDICATORS USED FOR AMBIENT ENVIRONMENT

These indicators are used to check residual levels of sterilizing agent in the isolator or in the surrounding environment.

2.1 FOR H_2O_2

The following indicators in order of increasing accuracy are available for gaseous hydrogen peroxide:

➲A MERCK chemical indicator strip MQuantTM no. 1.10 011.0001®. This means of detection allows a semi-quantitative analysis.

➲A DRÄGER system that takes samples by aspiration with a manual pump connected to a detection tube (**FIGURE 5.4**):

- DRÄGER tube for hydrogen peroxide 0.1/a (Ref. 81 01 041)®
- Range 0.1 to 3 ppm
- Number of pump strokes : 20
- Colour change from white to brown

(Ambient conditions: 10 to 25°C and CH20 from 3 to 10 mg/l)

FIGURE 5.4 – DRÄGER detector tube for hydrogen peroxide 0.1/a (Ref. 81 01 041)®

2.2 FOR PAA

NB : Taking account of the concentrations to be detected, the tube that is commercially available for the detection of acetic acid is not suitable: DRÄGER tube Acetic acid 5/a (ref. 67 22 101), concentrations from 5 to 80 ppm. The detection threshold is not adapted to a quantitative assessment and only allows qualitative assessment.

There is a chemical indicator offered by MERCK-MILLIPORE, **Reflectoquant® n° 1.16975.0001** (**FIGURE 5.5**) which enables a reaction to occur between peracetic acid and an aromatic amine, producing a blue colour which is detected by reflectometry. The measurement range is from 1 to 22.5 mg/l (315 ppm to 6950 ppm).

© MERCK MILLIPORE

FIGURE 5.5 – Chemical indicator, Reflectoquant® no. 1.16975.0001

The MERCK-MILLIPORE chemical indicator strip **MQuantTM n° 1.10 011.0001®**, also enables analysis of PAA.

Tests for the detection of PAA using the DRÄGER tube for hydrogen peroxide 0.1/a (Ref. 81 01 041)® from 0.1 to 3 ppm are conclusive.

NB: Measurements higher than 3 ppm with detection tubes do not exist except for a portable pocket Dräger appliance XAM5100 Ref: 8322750 with a reference sensor 6809170 (**FIGURE 5.7**).

3 DETECTORS AND PROBES

Detectors and probes enable a quantitative analysis to be made. Their use to record the concentration of a sterilizing agent during the surface sterilization cycle (bio-decontamination is desirable when possible.

NB : In the current state of technology, measurement of H_2O_2 concentration is influenced by temperature, humidity and probe calibration and is of limited precision.

3.1 FOR H_2O_2

Surface sterilization of an isolator with H_2O_2 is generally performed using concentrations in the order of 0.3 to 2.0 mg/l (250 to 1400 ppm), the exact concentration being dependent on the surface temperatures of the chamber during sterilization and the humidity level in the isolator when injection is begun.

Detectors commercially available for the quantification of H_2O_2 :

➲ **DRÄGER Polytron 7000®, fixed station measuring system (remote sensor and transmitter/ display unit).** This device can be equipped with a low concentration probe (0 to 50 ppm of H_2O_2) or high concentration probe (50 to 7000 ppm, preset at 4000 ppm of H_2O_2). The device has a measurement variability of 30% (if a surrogate gas is used for re-calibration) with 10% reproducibility when used in the same conditions between calibrations.
NB: the low concentration probe connected to the electronic module can also be used as an indicator of ambient environment.
It is recommended that the probe should be calibrated annually and changed periodically.
Adjustments should be avoided, that is adjustments should be made as infrequently as possible as the calibration reference point introduces substantial variability (see above).
On site calibrations are performed using a surrogate gas such as SO_2. A deviation in the response of the probe is observed in comparison with that observed when hydrogen peroxide is used. The surrogate gas method is less precise than the method of calibration with H_2O_2.

© MSD Riom

FIGURE 5.6 – Polytron Probe 7000® (Dräger) installed on the isolator

NB: Another sensor model is made by ATI, F12 series (distributor: Equipements Scientifiques-France). This is a fixed transmitter for toxic gases equipped with a H_2O_2 concentration probe:
- Electrochemical sensor
- Measurement range:
  - low concentration probe 0-10 ppm; 0-20 ppm; 0-50 ppm; 0-100 ppm
  - high concentration probe 0-1000 ppm; 0-2000 ppm
- Repeatability: ± 1 %
- Output: 4-20 mA; Modbus; RS-232 or RS-485

➲ For more complex installations (isolator production lines, bio-decontamination tunnels), a **spectrophotometer equipped with a near infrared probe** for quantification of H_2O_2 and H_2O **(Guided Wave)** is commercially available.
The spectrophotometer has a self-calibration function and has the following characteristics:
- Measurement range: 0.1 mg/l to 10.0 mg/l (70 ppm to 20 000 ppm)
- Resolution: 0.1 mg/l
- Accuracy of this type of appliance: ± 5 %

DRÄGER portable H_2O_2 detector, model X-am 5100® (sensor ref. 68 09 170) is used **to check for residual concentration** at the end of aeration :

- Measurement range: 0 to 20 ppm
- Detection threshold:0.5 ppm
- Resolution: 0.1 ppm
- Response time: 60 s

It is shown in **FIGURE 5.7**

© DRAEGER

FIGURE 5.7 – Portable H_2O_2 detector, model X-am 5100®

NB: these measuring appliances require probe calibration by the manufacturer.

There is a similar device the PortaSens portable detector for quantification of residual H_2O_2 concentrations, manufactured by ATI (Équipements Scientifiques-France).

For detecting traces of the chemical agent H2O2 in the air (pharmaceutical preparations...), an appliance is available made by PICARRO, model G2114®, Cavity Ring Down Spectroscopy (CRDS):

- Measurement range: 0 to 100 ppm
- Detection threshold: 10 ppb
- Temperature range: 10 to 35°C
- Response time: < 2 min

3.2 FOR PAA

The presence of peracetic acid can only be detected **qualitatively** not quantitatively with the acetic acid detection tube, Ref: 67 22 101.

The dose-ranging studies of acetic acid/peracetic acid in air are french documents:

- Fiche METROPOL 068, INRS, H_2O_2 et APA
- INRS Note documentaire: ND 2274, Evaluation des expositions a l'acide peracetique lors d'operations de désinfection

Other methods:

Monitoring the humidity level during the cycle is an indirect method of following the concentration of PAA in an isolator as it develops. There are data-recording hygrometry probes which are resistant to PAA.

6

STAGES OF QUALIFICATIONS

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3 INSTALLATION QUALIFICATION (IQ) 53

4 OPERATIONNAL QUALIFICATION (OQ) 54

5 DEVELOPMENT CYCLE (DC) 68

6 PERFORMANCE QUALIFICATION (PQ) 76

1 ISOLATOR QUALIFICATION FLOW CHART

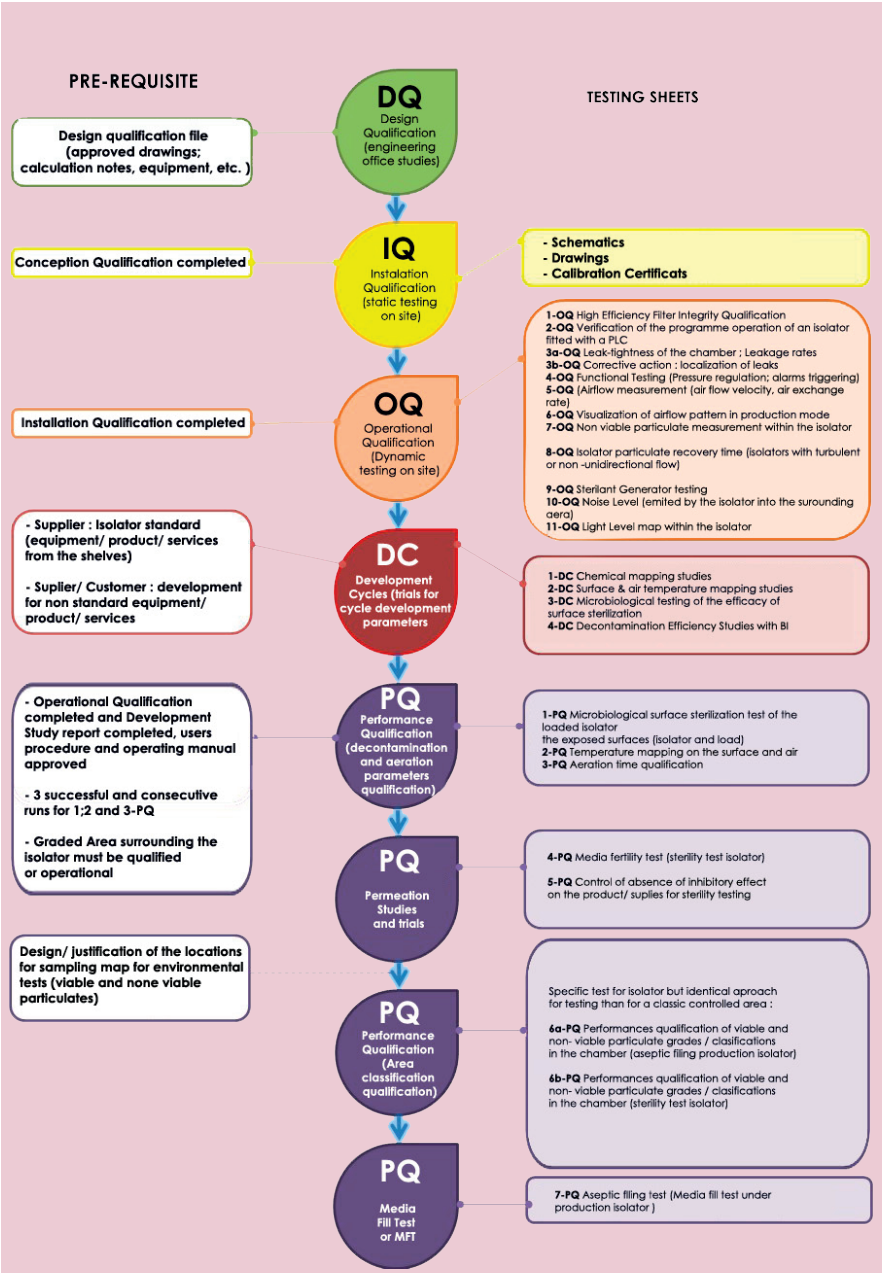

FIGURE 6.1 – Qualification of a project for the preparation of injectable medicines

Note 1: The order shown in which the tests are carried out at each step of the project can be modified according to individual practices, in other words the test planned for step n maybe executed at step n –1. An example of this is the leak test which is generally carried out during OQ can be performed at the end of IQ,

Note 2: The above flow diagram has been designed for a production isolator project and can be simplified to suit less complex projects.

Note 3: Some qualification tests are also applicable to negative pressure isolators.
The more important parameters for negative isolators (Pharmacy radionuclides/ Containment Level 3 and 4 laboratories/Production of cytotoxic medications) are:
- Protection of the operator
- The positive or negative pressure test corresponding to the use of the isolator
- Leak rate test
- According to the french Good Preparation Practices Guide (2007) in hospital pharmacy, the classification of the surrounding room with a negative pressure isolator has to be better than that the classification of one housing a positive pressure isolator.

Note 4: Qualification work must be carried out jointly by the user and the isolator supplier, with the involvement of a multidisciplinary team.
Note 5: In the management of the project, there may be tests at the manufacturer's factory (Factory Acceptance Test or FAT) and in situ (Site Acceptance Test or SAT) as well as commissioning operations which may correspond to contractual milestones.
Cycle development testing (CD) and in-house final testing should be the responsibility of the manufacturer.

2 DESIGN QUALIFICATION (DQ)

This first fundamental step of the qualification phases consists of verifying the users' requirements, the User requirement specification, and the documents transmitted by the manufacturer comply with GMP recommendations and good engineering practice.
The sequence of operations for an isolator technology design project are as follows :
➲**The user**, represented by a multidisciplinary team describes his needs, which are detailed in the User Requirements Specification (URS). This will include:
- The applicable standards
- A description of the process

The load to be sterilized by the chemical agent
- Inflow of materials: raw materials, items and components
- Outflow of materials: products, waste
- The utilities required (air, gas: compressed air... and fluids)
- The required language for project documents
- Computer interfaces (data acquisition and recording, logging of alarms, etc.).

If the isolator is housed in an existing building, the detailed plans of the utilities in this building will be attached to the file sent to the supplier.

➲The manufacturer responds by providing the functional specifications of the equipment.
➲The user approves the functional specifications.

➲The manufacturer proposes a design for the isolator using tools such as a digital model produced with CAD and/or a physical model that has undergone ergonomic trials (see **FIGURE 6.2**). A good 'fit' must be ensured, in particular with regard to the ergonomics, between the isolator, the room, and the application.

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FIGURE 6.2 – Isolator mock up and finished unit

During design studies,
➲the Process and safety alarms are defined taking into account the performance of the measurement sensors to be used on the equipment (isolator, bio-decontaminant generator).
➲Define type and position of pass throughs (monitoring probes).
➲Take into account Maintenance operations and access to the isolator

The prerequisite for the design stage are:
➲The load must be defined.
➲And it is recommended that the layout and the functional specifications of the isolator are verified, particularly with respect to safety (independent extraction for surface sterilization, compliant air treatment, etc.).

3 INSTALLATION QUALIFICATION (IQ)

Installation qualification starts on completion of design and is carried out on site. **The isolator is turned off**. This phase incorporates the following:
➲A document review (supplied with the equipment)
- Description of the system
- Equipment list, parts list(as an example, filters, control instrumentation)
- List of drawings:
  - Assembly drawings
  - Installation drawings
  - Piping and instrumentation diagram (PID)
  - Wiring diagrams, automation flow diagrams, etc.
- List of test procedures (on installation and as routine)
- User manuals and operating procedures
- Preventive maintenance manual (recommendations by the supplier for the maintenance of the installation)
- Material compatibility with the sterilizing agent
- Summary list of materials with drawing cross references (material conformity certificates, mill sheets conform to EN 10 204 standards etc.)
- Reference listing of component parts including part numbers and technical datasheets
- Verification of operator interface screens

➲Static verification (compliance with the requirements specification)

- Presence and identification of major components
- Visual inspection of each component
- Dimensional check
- Materials check (material certificates, roughness)
- Welding check (Welding Procedure Qualification (QMOS), Description of Welding Procedure (DMOS))
- Inspection of ancillary equipment
- Certificates of compliance for each item of equipment
- Calibration certificates for all measurement probes
- Filter manufacturer certificates
- Visual check of connections including those of peripherals
- Check of electrical cabling against wiring
- Mechanical and ergonomic check of ports, half suits, sleeves, fluid connections, filter access
- Verification of connections

At the end of installation qualification, a report is issued and signed by client and the supplier for the tests performed by the supplier.

Installation qualification is generally a phase documentary review and compliance (diagrams, calibration certificates, materials certificates, etc.) whilst operational qualification focuses on dynamic testing.

4 OPERATIONAL QUALIFICATION (OQ)

Operational qualification begins after completion of installation qualification (or if necessary and with the customer/users authorisation whilst IQ still being run but only in the case where there are no 'blocking' non-conformities).

OQ can be conducted internally or sub-contracted to the equipment supplier or to an independent body. At the end of operational qualification, a report is issued.

It is important to clarify that all measuring equipment used must be accompanied by a certificate of calibration, or as a minimum a valid verification certificate.

In the following pages operational testing is described in the form of test sheets arranged in order of execution.

The order in which tests are carried out must be relevant to the intended operation of the isolator for example test all motorised valves before testing pressure regulation.

The performances of the isolator are tested under normal operating conditions but in the absence of any product.

Test QO	High efficiency filter integrity qualification	Sheet No. 1-OQ

SCOPE OF THE TEST

This test verifies the integrity of the inlet and exhaust filters, and if applicable, the recirculation filters. Each high efficiency air filter leaves the factory with a manufacturer's certificate. Transport and assembly can affect its performances. It is necessary to test the integrity of the media, lute, seals and assembly of the filter on the isolator. In hospitals and in the pharmaceutical industry, the test is normally done using the EMERY 3004® aerosol test (or equivalent: DEHS, for example, in accordance with appendix C of standard EN ISO 14644-3), Injection of test aerosol upstream of the filter to be tested and detection of the same aerosol downstream of the filter using a photometer.

The test is carried out at the nominal flow rate of the isolator ventilation system.

REFERENCE DOCUMENTS

- ISO 14644-3 : Test methods
- EN 1822-1
- Manfacturer's filter certificate

EQUIPMENT

Anemometer or equivalent for the prior checking of air speeds/blower output
Aerosol generator (hot and cold generation) (paragraph B.6.2 of standard ISO 14644-3)
EMERY 3004 or equivalent
Photometer (detector)
If hot-generated aerosols are used, an inert carrier gas: nitrogen, for example
Alternative solution: Optical counter method with generation (paragraph B.6.3 of standard ISO 14644-3)

PROTOCOL

For inlet filters, the test aerosol is generated upstream of the filter. In this case, the integrity test is conducted in the direction of air circulation.

For recirculation or exhaust filters, the test aerosol is emitted downstream of the filter being tested.

Filters used for protecting the environment are tested by injecting the test aerosol into the chamber and conducting the detection process outside the isolator.

The upstream concentration of the test aerosol must be between 20 and 100 mg/m3.

The photometer is used to detect possible leaks by simple scanning using a square or rectangular probe (speed in the order of 5 cm/s for a square probe of 3 cm x 3 cm).

The maximal distance between the photometer probe and the filter is 3 cm. The dimensions of the probe, the scanning speed and the scanning distance relative to the filter are specified in standard ISO 14644-3.

ACCEPTANCE CRITERIA

Permeation through the HEPA filter (in general, H14): < 0.01%

OBSERVATIONS

• The inlet air filter and the recirculation or exhaust filters must all be tested at the same time.

• Injection and measurement points should ideally be included in the isolator being design.

• After the integrity test, it is recommended that **a double the bio-decontamination cycle of the isolator and generator system should be carried out**, with the aim of eliminating oily aerosol residues (Emery, PolyAlphaOléfine...) remaining from the integrity test.

• In the case of periodic tests or an operational qualification test on an isolator which has already been in service, in which toxic products are handled, the safety of the technician responsible for conducting this test must be ensured; chemical decontamination is then required.

OQ Test	Verification of the program operation of an isolator fitted with a PLC	Sheet No. 2-OQ

SCOPE OF THE TEST

This test has the objective of verifying the conformity of each phase with the functional analysis used for the validation of the PLC program. The verification of the program functioning can also be used to test alarm functions

Note 1 All items of functional analysis must be executed, run and verified.

Note 2. Verification of the operator interface screens is performed during IQ Prerequisites for this test: Isolator ventilation system tested, functional testing performed, program version verified.

REFERENCE DOCUMENTS

GAMP (Good Automatic Manufacturing Practice) CFR 21 Part 11 ; BPF/LD11
Program validation document and functional analysis supplied by the manufacturer
The software can be graded from 1 to 5, according to its complexity, in accordance with GAMP classification

EQUIPMENT

No test equipment

PROTOCOLE

The program can incorporate several phases, for example:
- 'self-test' phase which can include a leak-tightness test
- a sterilization phase
- an aeration phase
- a 'waiting production' phase
- a production phase
- a production failure phase
- an emergency phase

ACCEPTANCE CRITERIA

- The correct execution of each phase in accordance with the functional analysis
- The handling of alarms for each phase
- The compliance of the operator interface with the operator manual

REMARKS

During testing, all equipment such as sterilization or monitoring devices must be connected.

OQ TEST	Leak-tightness of the chamber (Leakage rates)	Sheet No. 3a-OQ

SCOPE OF THE TEST

This test consists of determining the leakage rate (Lr) of the isolator, by pressure drop or pressure increase. Following the configuration of the isolator , the leakage rate can impact on:

- Operator safety

- The efficacy of the bio-decontamination cycle (leakage of sterilization agent in the network upstream of the chamber)

Lr is the ratio of the leakage output from the chamber under normal conditions of use (pressure and temperature) and the volume of the chamber.

REFERENCE DOCUMENTS

Standard ISO 10 648-2: Containment chambers – Classification according to their leak-tightness and associated testing methods
- For negative pressure chambers: pressure increase method
- For positive pressure chambers: pressure-drop method

Standard ISO 12 807 : Safety of transport of radioactive matter. Tests of package integrity.
NB: These two standards must be adapted to the conditions of use of isolators.

EQUIPMENT

• Precision thermometer to measure the temperature inside and outside the isolator
• Precision micromanometer or precision electronic probe 0.1 Pa

PROTOCOL

Before the test, ensure that the temperature and pressure in the room are stabilized. Limit access to the room.

For isolators under positive pressure (pressure-drop method)
Seal all the isolator openings, after having placed the precision thermometer inside the equipment.
Pressurize the chamber (20 or 50 Pa above the test pressure).
Close the air inlet and allow pressure to fall to test pressure in order to homogenize the pressure in the isolator (for example, 100 Pa for a flexible isolator and 150 Pa for a rigid isolator).
One the test pressure is reached, record the drop in pressure during one minute.
Record the temperature during the test.

ACCEPTANCE CRITERIA

The criteria provided by the manufacturer can be used, for example:

Isolator type	Test pressure	Pa/min	Lr
Flexible isolator	100 Pa	1.6	0.1 %
Rigid isolator	150 Pa	8	0.5 %

Where the leakage rate obtained does not meet expectations, the leaks must be located and repaired (see test sheet 3b OQ)

REMARKS

Application of the method
➲ This method allows for the testing of both flexible and rigid positive pressure isolators..

Advice on running the test
➲ The leak testing of positive pressure isolators using the pressure-drop method enables the risk to operator safety during surface sterilization cycles or manipulation of toxic products to be quantified.
➲ During the test, do not place the isolator next to a heat source.
➲ If there is a doubt regarding the stability of the pressure in the room (reference pressure for the test) record room pressure every second in parallel to that of the isolator.
➲ The influence of a possible variation in temperature on the result can be minimized by conducting the test over a very short period. However there is a limit imposed by the accuracy of the measuring appliances and by the classification of the chamber (low leakage rate for a high leak-tightness classification).
➲ It is acceptable to repeat the phase of pressure increase and stabilization several times before the running the test.
➲ Where the isolator is connected to other equipment (like an autoclave), the leakage rate from the interface of all equipment connected to the isolator must be determined.
➲ During every leak-tightness test, the sleeves and half suits must be installed in the same configuration.

On the subject of leakage rates:
➲ For routine leakage rates, see **CHAPTER 1 PARAGRAPH 1.**
➲ Leak testing can be repeated routinely, prior to bio-decontamination cycles or maintenance operations.
For information, there are several formulas for leakage rates, for example containment chambers in nuclear facilities:

According to standard ISO 12807, the leakage rate is defined by the formula,

LR (in % vol.h^{-1}) = 1/ (ρ *r*T)*(dP/dt)*100
• ρ = 1.19 kg/m3 (air density)
• r = 287 J/kg°K
• T = Temperature in °K
dP/dt = pressure variation over time

TEST OQ	Corrective action: Localization of leaks	Sheet No. 3b-OQ

SCOPE OF THE TEST

This test is conducted when the required leak rate level is not obtained. This test is also used during maintenance.
The objective of this test is to locate possible leaks using a tracer gas (ammonia). The method described below is based on the use of a NH_3 indicator cloth impregnated with bromophenol blue that turns from yellow to blue when in contact with ammonia.

REFERENCE DOCUMENTS

TEST EQUIPMENT

- Ammonia leak detection cloth: 1 m × 1 m. The impregnated cloth is packaged in a pouch. The cloth has an operating life of 2 years, from receipt; e.g. Arpege Industrie (www.arpege-industrie.com) or FISHER (Ref. 11346524)
- Personal protection equipment (mask and gloves)
- 25% ultra-pure ammonia or equipment comprising an ultrasound transducer and an ultrasound detector (for example American equipment SDT170: Measurement range -10 to 120dBµV; Accuracy: ± 0.5 dBµV; Background noise: -5dBµV; Resolution: 0.1 dB µV) – see **FIGURE 6.3**

FIGURE 6.3 SDT70 equipment

TEST PROTOCOL

Attach strips of the test cloth inside the isolator. Introduce an ammonia solution (200 ml for example) into the isolator in a container that has a large evaporation surface (100 cm^2 minimum). All the test indicators inside the isolator should change colour if the correct amount of ammonia has been used. The isolator should be closed during gas diffusion. Once diffused in the isolator open the outlet valve to circulate NH_3 in the piping.

Close all valves and maintain the isolator at a positive pressure of around 100 Pa. Wait for a minimum period of 5 min.

Take a strip of NH_3 detector cloth and apply it to all the external surfaces of the isolator including the connections: doors, sleeve connections, seals and welds. The detection cloth must be held in place at each test point for 30 seconds.

ACCEPTANCE CRITERIA

Absence of colour change in the indicator cloth.

REMARKS

- Gloves must be worn when handling the indicator cloths.
- Each test must be carried out under the same conditions.
- The indicator cloths must be stored away from sunlight.
- It should be noted that H_2O_2 probes are incompatible with the use of NH_3 and must therefore be dismantled before the test.
- The isolator must be well ventilated after the test before opening.

OQ TEST	Functional testing (pressure regulation ; alarm triggering)	Sheet No. 4-OQ

SCOPE OF THE TEST

The objective of the test is to evaluate:

• The response time of the isolator in the event of pressure changes associated with events such as the use of gloves or the half suit, the connection of an additional volume.

• The stability of the pressure in the isolator (hysteresis).

This test can also be used to evaluate the correct functioning of the isolator's automatic control systems, actuators, valves and ventilators: pressure regulation, opening/closure of valves.

NB: this test may also be included in the verifying the functioning of programmes during functional specification testing and (cf. Test sheet 2-OQ).

REFERENCE DOCUMENTS

Functional specification

EQUIPMENT

Calibrated pressure probe connected to a system capable of recording pressure as a function of time.

PROTOCOL

Record the pressure of the isolator.

ACCEPTANCE CRITERIA

This test verifies that pressure regulation is stable when the isolator is at rest, and allows a level for alarm activation to be set that is compatible with the use of the isolator.

REMARKS

• Pressure must be stable in the room where the isolator is housed.
• Dependent on applications, this test can be performed in parallel with testing the regulation of all other critical parameters: Temperature, Humidity, etc.
• All other air-treatment equipment (other isolators, ventilated ceilings, RABS...) in the vicinity of the isolator must be operational when the test is conducted.

OQ TEST	Airflow measurement (airflow velocity, air exchange rate)	Sheet No. 5-OQ

SCOPE OF THE TEST

The objective of this test is to test three parameters:

- Air renewal rate of inlet air: ARR (useful for aeration, humidity)
- Air change rate (recycled air + new air): ACR
- Air speed (isolator with unidirectional flow): V

The determination of the hair change rate of new air in the isolator per hour (in V/h) corresponds to the ratio between the inlet flow rate of new air and the volume of the isolator.

– For a isolator supplied with new air only this will be the ARR.

– For an isolator with recirculated air: the three parameters parameters, ARR, ACR and V must be measured.

REFERENCE DOCUMENTS

- Standard ISO 14 644-3 (Test methods)
- Document CEA: P.M.D.S. Guide of the ventilation of nuclear installations

EQUIPMENT

According to the manufacturer's recommendations:
- Hot-wire or thermistor anemometer
- Pitot tube
- Helical anemometer (min 0.3 m/s)

TEST PROTOCOL

In the case of unidirectional flow, air speed measurements are taken at a distance of 150 mm to 300 m from the filter face at multiple points over the surface of the filter or membrane if fitted (CG panel).

Examples of the number of points to be checked per filter size are:

– 12 points for a 1200 mm x 600 mm filter,

– 9 points for a 900 mm x 600 mm filter,

– 6 points for a 600 mm x 600 mm filter,

– 3 points for a 300 mm x 600 mm filter.

In the case of turbulent flow air speed is measured using a hood to channel the air flow over the measurement sensor. The flow is obtained from a direct reading or from a calculation of the average speed.

Measurements can also be made in the isolator inflow or outflow ducts upstream of the absolute filters for example. In this case, take a measurement with the helical anemometer and estimate the air flow.

ACCEPTANCE CRITERIA

As detailed in the customer requirement specifications.

Comment :
According to BPF Annexe 1 and ISO 14644-3, average speeds for unidirectional flow must be in the range 0.36 m/s to 0.54 m/s (measured between 150 mm to 300 mm from the filter face). Higher speeds may be necessary, for example, to properly scavenge the work surface providing these are in accordance with the airflow diagrams for the isolator.

REMARKS

OQ TEST	Visualization of airflow in production mode	Sheet No. 6-OQ

SCOPE OF THE TEST

This test has the aim of verifying the airflow to identify any critical area under nominal operating conditions (areas that are insufficiently ventilated, dead zones, etc.).

The isolator is tested using normal operating parameters in two conditions, empty and loaded.

In non-unidirectional flow ('turbulent') isolators verify the direction of air flows, check that they are in line with pressure gradients, and in association with any interfaces present.

In unidirectional flow isolators, apart from checking the correct direction and uniformity of air flows, visualisation of airflows enables the testing of risky handling operations such as, for example, the unloading of a container using the isolator gloves. Tests can also be conducted at the upper and lower air speed limits. In this case, the uniformity of the inlet air flow is checked at the filter face the uniformity of the unidirectional airflow verified in the working or the 'critical area'.

As this test is performed under normal working conditions the results can be used to define the location of the 'worst case' measuring points for other tests such as particulate testing or , placement of chemical indicators and inoculated carriers (BIs).

REFERENCE DOCUMENTS

Standard ISO 14 644-3 (Test methods)

MATÉRIEL

Smoke generation: different methods can be used. In all cases appliance manuals must be readily available during testing:
DRÄGER smoke tube referenced CH00216® (see **FIGURE 6.4**)
• Compressed solid carbon dioxide, using a fogger (e.g. MSP Fogger 2001 & 2010 – Intertek Fogger PWC C02, Intertek)
• ultra-sound, using a generator (e.g. PMT France Systema AFM-V18, PMT France – Intertek Utility Fogger)

Recording results: photos or video recording; diagram enabling the visualization of results at the filter face and at working area level.

FIGURE 6.4 – Smoke tube referenced CH00216®

PROTOCOL

Prerequisites:
• A fully equipped isolator
• Operational air handling system (air change rate conform)
• During smoke generation it is recommended that the spatial characteristics of the airflows, the isolator load, and operating set up used during the test be noted.
Testing is conducted:
• empty and loaded,- all interfaces with the other chambers adjoining the isolator (MSC, LFH, RABS...) must be present,
• during operational simulations of using the which may impact on air flows.

Test sequence:
– **Isolator at rest in normal operating mode:**
* test of 'laminarity' under filter face to see if flow is unidirectional
* visualisation of airflow at the working area level
* test at the interfaces with other equipment, in the configurations representative of the use of the equipment.

Depending on the process, the isolator can also be tested at rest and under unfavourable conditions of pressure and/or speed (upper and lower limits).

– Isolator in normal operating mode:
* visualisation of airflow at the working area level
* tests during use of gloves, opening the airlock doors, use of the RTP system, etc.
Depending on the process, the isolator can also be tested under unfavourable conditions of pressure and/or speed (upper and lower limits).

Important: As the isolator airflow is being tested in different situations,
• the objective of each test and the operational conditions must be clearly defined
• the connection between each test and the corresponding video sequence or photos must be detailed in the test report. The video files or photos should be attached to the report.

ACCEPTANCE CRITERIA

In the case of unidirectional airflow, the criteria sought are:
• Uni-direction air flow at the filter face.
• Flows visualized at the working area level must demonstrate effective scavenging with minimal turbulence, no dead zones and no reversed flows (capable of 'conveyance' of particles from the isolator work surface to vials open for filling).

Where flow is turbulent the priority is the absence of dead zones.

OBSERVATIONS

Prerequisites:
• Complete mechanical installation (IQ approved...), availability of components, vials, stoppers and various consumables for operational tests.
• Approved test protocol with mapping of testing points at filter level (high) and work surface level (low)
• Availability of operating instructions for smoke generator (to generate smoke perpendicular to the air flow)
• For testing of hospital pharmacy, filling station or sterility test isolators, the load must be in place.

Comments on the methods and resources available:
• Some smoke tubes can introduce chemical contamination into the chamber so the use of non-contaminating smoke generators is recommended. In particular, the use of water-based smoke generation must be used where aseptic filling is carried out. If necessary, follow the test with an aeration phase (flushing) and manual cleaning of the isolator.
• The compressed solid carbon dioxide tracer injection method generates a sudden increase in the level of relative humidity. This must be taken into account before starting a surface sterilization cycle directly after the smoke test (as this may be affected by the humidity level).

Comments on test conditions:
• This test must be conducted within the normal operating range of the isolator: for example, for an isolator functioning at a pressure of 60 ± 20 Pa, airflow patterns can be established at pressures between 40 Pa and 80 Pa (upper and lower pressure limits).
• Equally if the isolator is connected to other chambers (equipment airlock), airflow testing will also need to take account of these (visualization of flows from the isolator to the other chambers).
• Operations representative of production under nominal conditions of speed and pressure must be clearly described (the type of operations challenged in the MFT) in the protocol. Only operations which have the greatest potential impact on airflow will be tested.

OQ Test	Non viable particulate measurement with the isolator	Sheet No. 7-OQ

SCOPE OF THE TEST

The objective of the test is to evaluate the concentrations of 0.5 µm and 5 µm particles in suspension in the isolator.
• This test verifies the classification of the isolator's particulate cleanliness at rest and if required by the application in activity.
• The test determines of 'worst case' points which will be selected to establish the recovery time of the isolator

REFERENCE DOCUMENTS

• Standard ISO 14644-1
• Good Manufacturing Practice Guide (Pharmaceutical industry)
• Good Preparation Practice Guide (French BPP) (Hospital pharmacy)

TEST EQUIPMENT

Particle counter, tubing and isokinetic probe

TEST PROTOCOL

Ideally the isokinetic probe is the only measurement appliance placed in the isolator.

The location of the testing points are defined by mapping the isolator (broken down by sector, by filtration element, by surface unit).

Preferred locations: points where the product is exposed, workstations, connections, junctions with gloves, the areas identified as performing poorly during the airflow testing.

In an isolator, the number of particle counting points in the OQ phase is considerably higher than the minimum required for a cleanroom for a similar surface area. This enables determination of the critical points, which will based on a risk analysis be subsequently used routinely.

NB: standard ISO 14644-1 defines the minimum number of points in a cleanroom. In all cases, the minimum number of locations is one. For isolators with an area less than 6 m2 generally between 2 and 3 points are used. For larger working areas, the minimum number of particle sampling points will be defined in accordance with the recommendations of standard ISO 14644-1 (see **CHAPTER 18** Appendix).

ACCEPTANCE CRITERIA

For example, for grade A aseptic filling operations, class A maximum of 3520 particles of 0.5 µm or larger per cubic meter of air and 20 particles of 5 µm or larger than per cubic meter of air.

REMARKS

• For fixed counters, the isolator requirements specification must include a sufficient number of connection points for the equipment and must take account of the performance characteristics of the particle counter (tubing length).
• According to the specific needs of a process (excluding pharma) other particle diameters may need to be measured.

OQ Test	Isolator particulate recovery time (isolators with 'turbulent' or non-unidirectional flow)	Sheet No. 8-OQ

SCOPE OF THE TEST

This test is conducted in order to determine the capacity of a 'turbulent flow' isolator to eliminate particulate following an operation that has generated particles.
The test consists firstly in artificially polluting the isolator, (for example by shutting off its ventilation, or by generating particles with a smoke tube), in order to obtain a particulate concentration peak. The isolator ventilation system is then turned on and the reduction in particle concentration monitored over time.

REFERENCE DOCUMENTS

Standard ISO 14 644-3 (Test methods)

TEST EQUIPMENT

- Particle counter, isokinetic probe, sampling tubing
- Smoke generator; e.g. CH00016 (DRAEGER) (see **FIGURE 6.4**)

TEST PROTOCOL

The recovery time is calculated from 100 to 1 and is defined as being the time required to reduce the particle concentration to less than 0.01 times the initial concentration.

Place the counter probe at the sampling point, (do not place the probe under the inlet filter) determined during the particle counts test. Set the measurement channel to 0.5 µm.

Contaminate the atmosphere in the isolator (smoke tube) and wait until the particulate concentration as measured by the optical counter reaches 100 times the level of the required cleanliness classification. If necessary, use a smoke generator to reach this criterion. Re-start the ventilation system and using a counter monitor the particle level until the particulate concentration reaches the target class level (the initial particulate concentration divided by 100).

ACCEPTANCE CRITERIA

The recovery time to be defined in the requirements specifications

OBSERVATIONS

This test is not recommended for installations with unidirectional flow (paragraph 4.2.9, ISO 14644-3) and for ISO 8 et ISO 9 class (paragraph B.12.1, ISO 14644-3)
Care must be taken not to saturate the particle counter.

OQ TEST	Sterilant generator testing	Sheet No. 9-OQ

SCOPE OF THE TEST

To verify that the generator and its alarms are functioning properly
- After connecting the equipment, the system functions and the cycle runs
- Activation of the alarms

The presence of the sterilizing agent in the isolator is verified, for example, by monitoring the following parameters: heating temperature of the agent, air flow and air pressure, consumption of the agent, injection rate, injection time, humidity level(H_2O_2).

REFERENCE DOCUMENTS

- Manufacturer's data on the sterilizing agent generator
- Sterilizing agent supplier's data
- Operator manual
- Calibration certificates

TEST EQUIPMENT

- Sterilizing agent and generator
- Personal protective equipment (mask and gloves)

TEST PROTOCOL

Prerequisites:
- The temperature of the room must be stable during the tests.
- During testing, the isolator must be connected to the exhaust system for extracting vapour or sterilizing agent aerosols.

Run a compliant cycle, with no alarms and then repeat cycles with alarms. Check alarms according to the manufacturer's data.
Checked that the generator carries out all phases of the cycle.
Also check that in the event of malfunction, the correct alarm level is activated (non-compliant cycle and making the equipment safe).
The functional parameters and the alarms must be recorded (digitally and/or on paper).

ACCEPTANCE CRITERIA

- No activation of the alarm during the running of a compliant cycle of the generator
- Consumption of the sterilizing agent

REMARKS

- Personnel must protect themselves against the effects of vapours or aerosols of the sterilizing agent in accordance with the recommendations of the safety data sheets.
- In some cases, according to the complexity of the installation (generator + isolator) or for safety reasons, a demineralized water cycle may be run prior to the test.

OQ Test	Noise level (emitted by the isolator into the surrounding area)	Sheet No. 10-OQ

SCOPE OF THE TEST

This test has the objective of checking that the sound level generated by isolator during normal use is sufficiently low not to disturb the operator.

REFERENCE DOCUMENTS

- Standard EN 12469
- Standard EN ISO 3744

TEST EQUIPMENT

Standard sound source, sound level meter, tape measure

TEST PROTOCOL

The sound level meter is set up 1 m from the isolator (in the centre of the equipment and in front of the working surface). The test equipment must be at least 1 m from all other equipment in the room. In the case of a half suit isolator, the noise level must be measured inside the half suit.

ACCEPTANCE CRITERIA

Background noise of 65 dB(A) corrected is recommended for rooms containing isolator systems, in line with the recommendations of standard EN 12469 for microbiological safety cabinets.

REMARKS

Where the acceptance criterion is exceeded, the installation must be revised or the operator equipped with ear defenders. Regulations indicate that from 80 dB(A), the employer must make ear defenders available for employees as well as setting up a testing programme.

OQ TEST	Light level map within the isolator	Sheet No. 11-OQ

SCOPE OF THE TEST

This test is to ensure a maximum of comfort for operations and manipulations carried out by an operator.

REFERENCE DOCUMENTS

- Standard EN 12469 (Appendix A: 750, Light)
- Standard EN 12464-1 (values to be observed)
- French standard NF X 35103 (testing methodologies)
- INRS Brochure ED85 (application guide)

TEST EQUIPMENT

Light meter; Tape measure

TEST PROTOCOL

The lighting of the isolator and room are turned on and measurement of light levels is carried out at several points over all of the working surfaces.

ACCEPTANCE CRITERIA

- For isolators with transparent walls, a Light level of 400 Lux measured at working level is sufficient (as prescribed by the French Labour Code).
- In the case of stainless steel isolators with a viewing window, average light levels in the work area must be at least 500 Lux. As stainless steel walls reflect light, the lighting level must be adjusted to take account of the operations being performed, levels of risk and operator age.

REMARKS

If the room is fitted with shutters or blinds, close them during the test as per French standard NF X 35 103.
NB: shutters or blinds also serve to reduce solar intensity.

5 DEVELOPMENT CYCLE (DC)

The objective of this section is to define for the bio-decontamination cycle
➲the best possible parameters
➲the best possible technical configuration (arrangement of the load, the location of injection points...).
➲the position of BI locations and if required positioning of thermocouples, hydrogen peroxide probes, temperature probes and relative humidity probes etc.

Typically cycle development studies are not considered as a qualification step but are classed as engineering studies.
However if cycle development testing is carried out using a GMP protocol the results of cycle development testing can be used to compliment performance qualification testing.

Cycle development testing is not always necessary.
In the case of a 'standard' isolator, that is to say one available from a manufacturer's catalogue predefined cycle parameters should be available from the manufacturer (these will not take into account individual load patterns).

Standard isolator applications:
In the case of a 'standard' isolator the verification of 'standard' bio-decontamination cycle parameters can be performed during the OQ phase, this verification must take into account:
- The load (for example the minimum, maximum and/or typical loads defined in the requirements specification or URS).
- Ambient conditions on site.
- The maximum duration of the cycle defined in the requirements specification or URS
- The supplier's recommendations for the use of the equipment.

Special isolator applications:
For special or non-standard applications or installations (isolator and/or load) a bio-decontamination cycle development study should be carried out (see sheets CD-1 TO CD-4).

Prerequisites for a bio-decontamination cycle development study:
- User requirements specification
- Documentation from the isolator manufacturer and if applicable the vapour generator manufacturer
- Installation Qualification and Operational Qualification completed without major non-compliances
- Room air conditioning qualified and operational (air temperature, pressure, relative humidity level, and hourly air change rate)
- Prevention plan and safety structures in place for the use of the sterilizing agent.

Bio-decontamination cycle development for a special isolator :

Reference documents:
User requirements specification: maximum admissible cycle duration, minimum load, maximum and standard load, frequency of sterilization, on site ambient conditions, prerequisites, procedures and internal standards to be applied.
Recommendations for implementing the cycle from the isolator manufacturer.
Theoretical calculations of cycle parameters from the generator manufacturer.
Engineering drawings, FDS, DDS and PID.

CYCLE DEVELOPMENT : DEFINITION OF CYCLE PARAMETERS

❶ **Distribution of the sterilizing agent test (1-CD)**
- Measurement of the distribution of the sterilizing agent using chemical indicators and concentration probes, air flow measurements and smoke tests to evaluate the distribution of the sterilizing agent. Measurement of the time taken for concentrations to rise in different zones and confirmation of the level of concentration possible (injection rate, air flow rate...).

❷ **Surface and air mapping (2-CD)**
- Mapping of the distribution of surface temperatures - evaluation of the homogeneity and range of the temperatures of surfaces and air

CYCLE DEVELOPMENT: DEFINITION OF CYCLE EFFICACY

❸ **Microbiological test to determine the efficacy and duration of the bio-decontamination cycle (3-CD)**
- Measurement of BI resistance using 'iterative 'or 'in situ system D value' methods -evaluation of the influence of isolator conditions on BI resistance.
- Calculation of the fractional validation cycle - demonstrated performance of the cycle + safety margin = validation cycle

❹ **Microbiological testing of the efficacy of surface sterilization (4-CD)**
- To verify the efficacy of a surface sterilization cycle– BI kill testing using the validation cycle.

Cycle parameters development

PERFORMANCE QUALIFICATION TESTS
- Microbiological tests and temperature mapping –
See sheets 1-PQ and 2-PQ with fractional cycle.
- Definition of production cycle - Validated fractional cycle + 'procedure' safety margin = production cycle
- Test of aeration time – see sheet 3-PQ with production cycle

ANNUAL PERFORMANCES REQUALIFICATION TEST
- Microbiological tests and temperature mapping
- Aeration time test

Cycle performance qualification

Cycle development test	Chemical mapping studies	Sheet No. 1-CD

SCOPE OF THE TEST

This test is to verify and define:

• The theoretical parameters of the cycle (injection rate, air flow rate…) that is to say the match between the initial cycle parameters and the configuration of the equipment.

• The distribution of H_2O_2 during sterilization, the influence of the load and the positioning of injection points on the homogeneity of the distribution of the sterilizing agent.

The test is performed using H_2O_2 probes or sensors, and chemical indicators.

REFERENCE DOCUMENTS

Manufacturer's data
Equipment status:
• Initial parameters of the isolator and generator
• Minimum and maximum loads
• Flow rates and flow diagrams in bio-decontamination mode

TEST EQUIPMENT

Chemical indicators of the type STERIS CCo6o® or BIOQUELL HPV-CI®
Chronometer
A measuring system for the sterilizing agent (remote sensor, transmitter/display unit) DRAGER, or equivalent

TEST PROTOCOL

The theoretical or initial parameters (for example, injection rate, air flow rate, start humidity, duration…) must be provided by the manufacturer.

As this is a development study, parameters may evolve during testing. As a consequence, the protocol must allow for a change of parameters from one cycle to another.

Dependent on complexity it is advised to proceed in the order:

• Measurement of air flow rates

• Airflow study (flow patterns in sterilization mode)

• Test cycle on the empty isolator (without load) to serve as a reference

Chemical indicators are placed at process critical points (DPTE port, stopper bowl, airlock door, interfaces with temperature equipment such as autoclaves, freeze-dryers, cold-rooms, etc.) and in critical locations in the chamber (ceiling, walls, gloves, corners…).

Sterilizing agent measuring sensors(s) are placed in the chamber at strategic and critical points within the limitations of available access.

As a general rule, it is advisable to use a maximum number of positions in cycle development and to retain only the critical points in subsequent tests (such as the points which take longest to display a CI colour change these being the slowest to attain the required concentration of sterilizing agent).

Start the sterilization cycle with the initial parameters for the first cycle.

Note the change in colour of the chemical indicators (provide for a lighting system if the lighting in the isolator is switched off).

Record and analyze the sterilizing agent concentration curve (time to reach a concentration plateau, graph profile, etc.).

After completion of the first test(s),it may be possible to optimise:

• Distribution of the sterilizing agent (review the distribution of the load, and the addition of a nozzle or a distribution fan).

• Cycle parameters: concentration plateau not reached or not maintained = injection rate too low, chemical indicator not changing colour-= injection rate too low, condensation of sterilizing agent = injection rate too high etc.)

Cycle optimisation is repeated until the following are obtained:

• The best possible parameters for the bio-decontamination cycle.

• The best technical configuration (distribution of the load, injection points…).

The area where the rise in concentration is weakest, the profile of the concentration curve and maximum concentration are recorded for each cycle.

ACCEPTANCE CRITERIA

Acceptance criteria:
- The time for the last chemical indicator to change colour
- The time between the colour change of the first and the last chemical indicators
- The rate of the increase in concentration and the concentration plateau must be conform to the supplier's recommendations

REMARKS

Tests 1-CD and 2-CD are linked and can be conducted in parallel (see **FIGURE 6.5**)
The areas which perform worst with respect to rise in concentration are used to define:
- The position for the 'system D Value' test, sheet 3-CD
- The critical positions for biological indicators, sheets 3-CD and 4-CD
- The length of the fractional cycle, sheet 4-CD

Sterilizing agent distribution mapping data, can be used to justify the positioning of the concentration sensors and the sampling points for the verification of residual concentration (Aeration testing) to be used during routine operation.

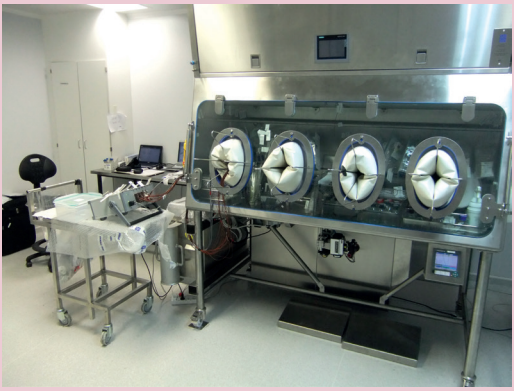

FIGURE 6.5 – Cycle development testing of an isolator

Cycle development test	Surface and air temperature mapping studies	Sheet No. 2-CD

SCOPE OF THE TEST

The objective of this test is to define the temperature conditions for correct operation of the cycle.

Depending on the process being used surface and/or air temperatures can be critical to the efficacy of H_2O_2 sterilization.

This test uses thermocouples to :

- measure temperature distribution and the reproducibility of surface temperatures .
- the ambient temperature in the surrounding room
- the air temperature in the chamber

REFERENCE DOCUMENTS

Manufacturer's data
Data recorder documentation and calibration certificate
Thermocouples calibration report

TEST EQUIPMENT

Fluke, Kaye or equivalent data recorder
Thermocouples
Adhesive (the adhesive should be chosen with care as sticky residues should not be left on isolator surfaces)

TEST PROTOCOL

The theoretical start parameters or initial parameters (for example, injection rate, air flow rate, RH%, duration, etc.) should be provided by the manufacturer.

As this is a development study, parameters may evolve during testing. As a consequence, the protocol must allow for a change of parameters from one cycle to another.

If the isolator is complex it is advisable to perform an empty cycle which may later be useful as a reference.

The thermocouples are placed in process critical locations (RTP, port, stopper bowl, airlock door, interfaces with temperature equipment such as autoclaves, freeze-dryers, and cold rooms, etc.) and in critical locations in the chamber (celling, walls, gloves, corners, etc.).

The thermocouples are held in contact with the surface using adhesive tape.

As a general rule, it is advisable to use a maximum number of positions in cycle development and to retain only the critical points in subsequent performance qualification tests (hottest and coldest points).

Start testing using the theoretical or initial parameters.

The coldest and hottest points included as locations for the placement of biological indicators.

Room and chamber air temperatures can be measured either with thermocouples or probes already installed for the same purpose.

The results of the temperature mapping are used to either modify cycle parameters or to modify the installation to reduce the difference between hot and cold spots.

ACCEPTANCE CRITERIA

The distribution of the minimum and maximum temperatures of surfaces must be compatible with the sterilization process.
The air temperatures of the room and the chamber must be compatible with the sterilization process.

REMARKS

Temperature mapping must be performed taking into account all the ancillary equipment that may be connected or placed inside the isolator during production where these are likely to have an impact on surface temperatures and therefore on the efficacy of the cycle.

Tests 1- CD and 2 -CD are linked and can be conducted in parallel.

Temperature mapping results can be used to locate the isolator temperature probes that will be used to monitor the process during routine use.

Cycle development test	Microbiological testing of the efficacy of surface sterilization	Sheet No. 3-CD

SCOPE OF THE TEST

The efficacy of the cycle is specific to the isolator, sterilization cycle parameters, the biological indicator, and the locations where indicators are placed.

The objective of this test is to:

- Evaluating the efficacy of a surface sterilization cycle.
- Defining the length of a fractional sterilization cycle (contact time with the sterilizing agent).

This test is conducted using BIs inoculated with at least 6 log of spores (see **CHAPTER 4**).

REFERENCE DOCUMENTS

The results from tests 1-CD and 2- CD will have defined:
- The cycle parameters (injection rate, flow rates, etc.)
- The 'worst case' test positions
- The time taken for concentration to rise, the average and peak concentration values.

TEST EQUIPMENT

- Surface sterilizer and surface sterilizing agent
- 10^6 BIs
- Incubator
- Sterile sampling tweezers if required
- Tubes of culture medium

Sufficient equipment and consumables should be provided to allow for repeat cycles.
BI carrier (or BI gun).

TEST PROTOCOL

The method used to carry out the efficacy and duration test will dependent on what means of manipulation (gloves/RTP) and transfer the chamber is equipped with :

- The "iterative" method is used when there is little or no means of manipulation.
- The system D value is used when sufficient means of manipulation are available.

- **The 'iterative' method:** Involves running several cycles of increasing or decreasing length, until the desired level of performance is obtained (for example a 6 log reduction at the 'worst case' points. Two techniques are used for this method:
  - The 'Total kill approach': Absence of growth after incubation
  - Use of 'Replicate BIs' approach (see acceptance criteria)

- **The 'system D value method:** The inactivation time is the time required to inactivate biological indicators inoculated with 106 Geobacillus stearothermophilus bacteria. Determining the inoculation time means verifying how long the cycle takes to reduce a population of spores by one log (D-value *in situ* or system D-value) and extrapolating this result to obtain the length of the fractional validation cycle.

Verification of the cycle using the 'iterative' method:

The objective is to expose a sufficiently large number of BI at critical positions to ensure that they are killed by the sterilization cycle.

Remove the BI from the refrigerator long enough in advance to allow them to reach the ambient temperature of the chamber.

Place the BIs at the 'worst case' points taken from the results of sheets 1-CD and 2-CD.

Dependent on the approach used place one or several BI at each position.

Close the isolator and start the surface sterilization cycle.

Once the cycle has ended place each BI (without packaging) using sterile tweezers if required in a tube of culture medium. Avoid any risk of generating false positives or false negatives.

Incubate along with controls, (refer to the paragraph on biological indicators) at the required temperature.

Repeat the test until the cycle duration is obtained which will allow the BIs to be reproducibly killed or if necessary until a 6 log reduction is obtained in accordance with the Halvorson-Ziegler equation (see acceptance criteria).

Verification of the efficacy of the cycle by the 'system D value' method:

The objective is to expose a sufficiently large number of BIs to allow the system D value to be calculated (fraction-negative method) for a given lot of BIs in the chamber under consideration. A BI carrier or a BI gun can be used to conduct the D value study.

One or a number of sterilization cycles are run (or several if necessary) to evaluate the D value.

Remove the BI from the refrigerator long enough in advance to allow them to reach the ambient temperature of the chamber.

The indicators are placed in sets on a carrier (or on the BI gun) which are/is placed in the isolator.

The choice of test area is crucial. It is a compromise between an area identified as critical in the results of sheet 1-CD and the ergonomics required to perform the test (gloves, DPTE or passage for the BI gun).

Close the isolator and start the surface sterilization cycle.

The exposed BIs are recovered at predefined regular predefined intervals and immediately placed into tubes of media and then incubated. The growth of the incubated BI is monitored and the time required to inactivate the biological indicators is calculated using the fraction-negative method or the Limited Spearman Karber Method. The inactivation time is used to define the efficacy of the bio-decontamination cycle.

Definition of the length of the fractional sterilization cycle:

The objective is to define a contact time that is sufficiently robust to ensure consistently compliant cycles for both the initial performances qualification cycles and the routine requalification cycles in the years to come.

With this in mind, it is advised to develop the sterilization cycle with a safety margin sufficient to take into account as a minimum:

- the distribution time of the sterilizing agent (see 1-CD)
- the natural variability in resistance between different lots of biological indicators

Other factors can also be added to the safety margin:

- The time for temperature homogenization
- Sensor tolerance (dosage etc.)
- Etc.

It should be noted that the approach described here concerns systems whose efficacy is based on contact time. Nevertheless a similar approach can be applied to systems which function by increasing the amount of sterilizing agent.

ACCEPTANCE CRITERIA

- 'Total kill approach': Absence of growth after incubation and 6 log reduction.
- 'Replicate BIs approach: 6 log reduction, using several BI per location and application of the Halvorson-Ziegler equation (Journal of Bacteriology);

Determine the "fraction-negative window" to be able to apply the method known as the 'Limited Spearman Karber Method'.

REMARKS

- It is important to reference the BI lots and to know the D values for the surface sterilization procedure employed (CHAPTER 4).
- The reference isolator used for testing can be either an empty or loaded.
- In case of a non-compliant result, open an investigation (see **CHAPTER 4**) and if needed, revise the parameters of the bio-decontamination cycle.

Cycle development test	Decontamination efficiency studies with BI's	Sheet No. 4-CD

SCOPE OF THE TEST

The objective of this test is to check the efficacy of a surface sterilization cycle (fractional or short).

The test is performed using BIs inoculated with a minimum of 6 log spores placed at different locations in the isolator (refer to CHAPTER 4).

The cycle parameters and the BI locations will have been determined in tests 1-CD, 2-CD, and 3- CD.

REFERENCE DOCUMENTS

The results of tests 1-CD-1, 2-CD, and 3-CD.

TEST EQUIPMENT

Surface sterilizer and surface sterilizing agent
10^6 BIs
Incubator
Sterile sampling tweezers (if required)
Tubes of culture medium
Thermocouples and system temperature probes (room and chamber)
Test load

TEST PROTOCOL

Remove the BI from the refrigerator long enough in advance to allow them to reach the ambient temperature of the chamber.
Place the BI in the chosen test locations. These should correspond to:
- An even distribution of biological indicators on all surfaces and in each corner;
- At points judged to be critical that is:
  - Process critical: bowl, needles, filling, RTP port (DPTE),
  - Locations judged to be 'worst case during the chemical indicator study of the distribution of the bio-decontaminating agent (see 1-CD).
  - High and low temperature critical locations recorded during temperature mapping (see 2-CD)

Close the isolator and start the surface sterilization using the fractional cycle (see 3-CD).

Once the cycle has ended place each BI (without packaging) using sterile tweezers if required in a tube of culture medium. Avoid any risk of generating false positives or false negatives.

Incubate along with controls, (refer to the paragraph on biological indicators) at the required temperature. Make a detailed record of the following in the test report:

- The cycle parameters
- The parameters of the room
- The parameters of equipment associated with or connected to the chamber
- The load pattern

ACCEPTANCE CRITERIA

'Total kill approach': Absence of growth after incubation and 6 log reduction, for three repeat tests
Replicate BI's approach: 6 log reduction using the Halvorson-Ziegler equation

REMARKS

It is important to reference the BI lots and to know the D values for the surface sterilization procedure employed (**CHAPTER 4**).

This CD test must be implemented as closely as possible to the performances qualification test that follow during the performances qualification phase.
The parameters recorded in the test report will serve as a basis for performance qualification.
It is advisable to include in the results:
- mapping of the distribution of the sterilizing agent
- surface temperature mapping
- A record of air temperature in the room and the chamber
- H_2O_2 concentration
- measurement of residual concentration after aeration (for information)

In case of a non-compliant result, open an investigation (see CHAPTER 4) and if needed, revise the parameters of the bio-decontamination cycle.

6 PERFORMANCE QUALIFICATION (PQ)

Performance qualification is conducted with the isolator configured as it would be for use in routine operation. The functional and operational parameters used are those determined during the operational qualification phase and/or the cycle development phase.

Prerequisites for PQ:

- IQ and OQ completed
- Knowledge of the critical parameters and the alarms associated with the process (isolator, generator, room, etc.) and in particular the parameters of the fractional sterilization cycle
- Availability of the machinery configurations and loading patterns for consumables
- Air handling qualified for the room in which the qualified isolator in housed
- Trained and qualified personnel to carry out the PQ tests.

Examples of Performance qualification tests are described in the following test sheets.

Deliverable data from the PQ steps:

- Procedures for use (sequence of steps, etc.)
- Routine revalidation protocols

PQ Test Bio-decontamination procedure	Microbiological surface sterilization test of the loaded isolator	Sheet No 1-PQ

SCOPE OF THE TEST

The test has the objective of verifying the efficacy of a surface sterilization cycle:
– Using inoculated carriers each containing at least 6 log of spores positioned at different locations in the isolator (refer to **CHAPTERS 4**, **6**, and **7**).
– the parameters of the fractional cycle (see CD)
The cycle is repeated 3 times.

REFERENCE DOCUMENTS

- USP 41
- European Pharmacopoeia 8th edition
- ISO 14161
- Cycle development test reports
- Supplier's documentation

TEST EQUIPMENT

- Surface sterilizer and surface sterilizing agent
- 10^6 BIs
- Incubator
- Sterile sampling tweezers (if necessary)
- Tubes containing culture medium

TEST PROTOCOL

Set up the inoculated carriers or BI at ambient temperature.

Place the BI at positions which correspond to:
– A spatial distribution of the biological indicators on the surfaces and in each corner,
– A distribution of biological indicators at the points judged to be critical, namely:
- Those of the procedure: bowl, needles, filling, RTP port (e.g. DPTE®), etc.
- Those corresponding to the unfavourable locations where temperature mapping recorded extreme high and low values
- The locations judged 'worst case' during the use of chemical indicators, during the study of the distribution of the sterilizing agent

Close the isolator and start the fractional surface sterilization cycle
When surface sterilization is finished, use a pair of sterile tweezers as needed for each inoculated carrier or BI and place each of them (BI without packaging) in a tube of culture medium avoiding all risk of false positives or false negatives.
Incubate at a temperature that will allow the growth of spores, and include controls (refer to **CHAPTER 4**).
Repeat the test at least three times

ACCEPTANCE CRITERIA

– 'Total kill approach': Absence of growth after incubation and 6 log reduction, over three consecutive tests
– Replicate BI'S approach: 6 log reduction over three consecutive tests, with several BIs per position, applying the Halvorson–Ziegler equation (Journal of Bacteriology).

REMARKS

- It is important to reference the lots of the inoculated carriers and to know the D value with the surface sterilization procedure used (see **CHAPTER 4**).
- During the initial validation, these performance qualification tests **'Surface sterilization microbiological tests, loaded isolator'** must be accomplished with three consecutive compliant tests.
- Routinely, this test must be repeated at regular intervals (routine revalidation) and after every significant modification of equipment or procedure.

- In the event of non-compliant biological indicator test proceed to the investigations (refer to **CHAPTER 4 PARAGRAPH 2.3**).

PQ Test (Bio-decontamination procedure)	Temperature mapping on the surface and air	Sheet No. 2-PQ

SCOPE OF THE TEST

In order to be in control of the H_2O_2 sterilization cycle, it is recommended that the distribution and the reproducibility of surface temperatures are verified. This is done using thermocouples held in direct contact with the surface using adhesive tape.

It is also advised that the ambient temperature of the room housing the isolator be verified (monitoring system for the area, failing that by thermocouple).

REFERENCE DOCUMENTS

- Manufacturer's documentation
- Development tests report

TEST EQUIPMENT

- Thermocouples and data loggers

TEST PROTOCOL

– The thermocouples are placed in locations that are judged to be :
  - Process critical: RTP port, stopper bowl, airlock door, interfaces with temperature equipment such as autoclaves, freeze-dryers, cold rooms etc.
  - determined as being the coldest and the hottest during cycle development tests.
  - The ends of the thermocouples are maintained in contact with surfaces using adhesive tape.

– The temperature of the air in the chamber and the room are recorded using system probes or failing these validation thermocouples
– Start the fractional validation cycle and the recording of the temperature data.
– At the end of the cycle, save the temperature data.

ACCEPTANCE CRITERIA

- The minimum and maximum surface temperatures must be compatible with the sterilization process.
- The air temperatures in the chamber and the room must be compatible with the sterilization process.

REMARKS

- The acceptance criteria must be defined with the supplier and must take production scenarios into account (functioning of interface equipment, clean in place etc.).

The temperatures recorded during the initial PQ tests, can be used to determine specifications for :
- Routine production sterilization cycles
- Periodic revalidation sterilization cycles

PQ Test Bio-decontamination procedure)	Aeration time qualification	Sheet No. 3- PQ

SCOPE OF THE TEST

The objective of this test is to determine (worst case), the time required to obtain a concentration of surface sterilizing agent following a production sterilization cycle compatible with:
- operator safety (for example opening the airlock door, hatches).
- the safety of the process

The isolator is in operating condition.

REFERENCE DOCUMENT S

- The supplier's documentation
- Development tests report
- French INRS FT 123 datasheet for H_2O_2
- French INRS FT 239 datasheet for PAA

TEST EQUIPMENT

- H_2O_2 Dräger detector tube No. 8101041 0.1/a (range from 0.1 to 3 ppm) and pump or any other systems described in **CHAPTER 5**:
- H_2O_2: Dräger Polytron 7000 with low concentration probe, ATI/ F12/, Dräger portable detector X-am5100

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FIGURE 6.6 – H_2O_2 Measurement System Polytron 7000®

TEST PROTOCOL

Run a production surface sterilization cycle (worst case).
Using a detector tube positioned in the isolator extraction pipework and or in the 'worst case' location, note the time required to obtain the desired residual concentration.

ACCEPTANCE CRITERIA

The acceptance criterion is dependent on the method of investigation used.

REMARKS

- The French INRS toxicological datasheet for H202 (FT 123), gives the OEL of 1 ppm (1.5 mg/m³) for an 8 hour period.
- A French INRS toxicological datasheet exists for PAA (FT 239).

Here, the worst case is understood in terms of the production cycle with the longest exposure time and/or the highest concentration of sterilizing agent.

- As a general rule, it is advisable to add a safety margin to the validation cycle to obtain the routine production cycle. This safety margin, depending on the process used, translates as increased contact time or increased amount of sterilizing agent.

- The position of the measurement point can be defined from the results of the distribution of the sterilization conducted during cycle development. Dependent on the complexity of the installation, several measurement points may be required.

PQ Test (permeation tests/studies)	Media fertility test (Sterility test Isolator)	Sheet No. PQ-4

SCOPE OF THE TEST

As with MFTs (Media fertility test), testing of culture media and sterility test bacteriostatic and fungistatic properties are carried out after PQ of the sterilization process. These tests are specific to sterility test applications.

This test has the objective of verifying that the nutritional properties of the culture media used for sterility tests or for testing the environment are not affected by the sterilization cycle or by the residues of sterilizing agents in the isolator.

NB: The manufacturers of culture medium must provide details of the nutritional properties of culture media.

REFERENCE DOCUMENTS

European Pharmacopoeia
USP
Japanese Pharmacopoeia

TEST MATERIAL

Culture medium*	Strains to be tested**
TS Broth for sterility testing	*Aspergillus brasiliensis* ATCC 16404 *Bacillus subtilis* ATCC 6633 *Candida albicans* ATCC 10231 Bacterium from the environment
Thioglycolate Broth for sterility testing	*Clostridium sporogenes* ATCC 19404 *Pseudomonas aeruginosa* ATCC 9027 *Staphylococcus aureus* ATCC 6538 P Bacterium from the environment***
TS agar settle plates or contact plates for environmental sample	*Aspergillus brasiliensis* ATCC 16404 *Bacillus subtilis* ATCC 6633 *Candida albicans* ATCC 10231 *Staphylococcus epidermidis* ATCC 12228 Bacterium from the environment***

**Media are cited by way of example and must be adjusted or supplemented depending on those present in the load.*
***The bacteria cited are given by way of example and must be adjusted or supplemented depending on the media the load.*
****The addition of non-reference bacterial strains, and environmental moulds and yeasts is strongly advised.*

TEST PROTOCOL

The permeability study is conducted on the media culture elements present in the load. It enables verification that these elements (TSB and TGB culture media, contact plates and sedimentation plates, the equipment used for sterility testing...) are not affected by their exposure to the surface sterilization agent during the surface sterilization cycle.
The study includes the following checks:
- Fertility verification, before using culture medium (on receipt)
- Fertility verification, after the sterilization cycle

This study is conducted using production cycle parameters with the longest exposure time and the highest concentration.
The tests are repeated at least 3 times for each of the culture media (agar media and liquid media).

ACCEPTANCE CRITERIA

The fertility of media is verified, in accordance with the European and/or American Pharmacopoeia, after incubation and reading of results.
- In liquid medium, a haze should appear before the end of the incubation period.
- In agar medium, a microbial growth should be visible before the end of the incubation period.

REMARKS

This protocol complies with the regulations in force. However, for solid media, a count and a comparison with non-exposed media enable the real impact of surface sterilization to be verified.

In some cases, the sterilization cycle is run twice before conducting the fertility tests.

PQ Test permeation (tests/studies)	Control of absence of inhibitory effect on the product / supplies for sterility testing	Sheet No. 5-PQ

SCOPE OF THE TEST

This test has the objective of verifying the absence of false negatives to ensure that surface sterilization does not exert a bacteriostatic and fungistatic action on the samples to be sterility tested.
False negative testing is carried out the products or items to be sterility tested. It enables verification that the elements of the load (product samples, culture media, equipment and small items used for the sterility test...) are not affected by their exposure to the sterilizing agent vapours during the sterilization cycle.

REFERENCE DOCUMENT

European Pharmacopoeia

TEST EQUIPMENT

Products or items subjected to sterility tests	Strain to be tested*
Vials, pipettes, syringes etc.	*Aspergillus brasiliensis* ATCC 16404 *Bacillus subtilis* ATCC 6633 *Candida albicans* ATCC 10231 *Clostridium sporogenes* ATCC 19404 *Pseudomonas aeruginosa* ATCC 9027 *Staphylococcus aureus* ATCC 6538 P Bacterium from the environment**

**The bacteria used for the study must be the same as those used to validate the sterility test.*

*** The addition of local bio-burden bacterial strains and environmental moulds or yeasts is strongly advised.*

TEST PROTOCOL

This is a test of the recovery of inoculated microorganisms on products or items subject to sterility testing, in order to verify that the original microbial contamination has not been removed by sterilization.

The sterility test must be conducted with the equipment and media that have been exposed to the sterilization cycle.

This study is conducted with the production cycle parameters that use the longest exposure time and the highest concentration.

The tests are repeated at least 3 times for each bacterium and for each product or item (vials, pipettes, syringes, etc.)

ACCEPTANCE CRITERIA

The sterility tests of items and/or inoculated products conducted in accordance with the pharmacopoeias in force are positive before the end of the incubation period.

REMARKS

PQ test (isolator ambient environment)	Performances qualification of viable and non-viable particulate grades/classifications in the chamber (Aseptic filling production isolator)	Sheet No. 6a-PQ

SCOPE OF THE TEST

The objective of this test is to show:
•the maintenance of viable and non-viable particulate classifications and,
•the maintenance of environmental conditions in the chamber,
in activity and at rest, over a defined routine period and for a defined routine application.
When performing this test the isolator is considered equivalent to a clean room.

REFERENCE DOCUMENTS

ISO 14644-1
ISO 14698-1 & 2

EQUIPMENT

- Air bio-collector
- Petri dishes with appropriate culture medium (air and surfaces) or possibly membranes (air)
- Swabs
- Optical particle counter
- Instrumentation for testing environmental conditions (pressure, temperature, humidity, etc.)

TEST PROTOCOL

- An evaluation of the bioburden of the isolator surfaces can be conducted after the cleaning step and before the isolator sterilization cycle.
- The at rest performance qualification test is conducted between the end of sterilization and the start-of production operations (setup, filling, etc.)
- The in activity performance qualification test must be as close as possible to the routine aseptic manufacturing procedure and include all the critical steps. It must also take account of the various operations which may take place during normal production as well as situations considered as 'worst case'.
- The duration of the in activity testing in activity must be longer than the total validated time of use of isolators in production.
- The test parameters for environmental conditions (e.g. temperature, relative humidity, pressure differential between CAA) are qualified over a time period representative of routine production/use.

ACCEPTANCE CRITERIA

- As a general rule, no criterion is applied to the bioburden evaluation test. This test is conducted with the aim of ascertaining the level and the nature of the bioburden of the surfaces and gloves.
- The microbiological sampling of the air and surfaces complies with the standards laid down for the target classification both at rest and in activity.
- The non-viable particle counts comply with the standards laid down for the target classification both at rest and in activity.

NB: There should be no alarm on the test parameters of environmental conditions over the test period.

REMARKS

– Locations for taking air and surface sampling (viable and non-viable particulate) of the isolator environment can be defined on the basis of a risk analysis. This risk analysis can, for example, consider criteria such as:

- the process: filling, crimp sealing, transport, interface, etc.)
- process flows (components, waste etc.)
- OQ data (visualisation of airflow, particle counts, air speed measurements, etc.)

– Routine viable and non-viable air and surface sampling locations can be derived from the results of this test.

– The isolator environment must be periodically requalified and after every significant equipment or procedural modification so as to demonstrate conformity with the initial qualification.

Performance qualification or requalification testing of the isolator can be done in parallel with the media fill test (MFT) as the requirements of the two studies in terms of activity and environmental sampling are similar.

PQ Test (isolator ambient environment)	Performances qualification of viable and non-viable particulate grades/classifications in the chamber (Sterility test isolator)	Sheet No 6b-PQ.

SCOPE OF THE TEST

This test has the objective of this test is to show:
- the maintenance of particle and microbiological viable and non-viable particulate classifications and,
- the maintenance of environmental conditions in the chamber,

in activity and at rest, over a defined routine period and for a defined routine application.
When performing this test the isolator is considered equivalent to a clean room.

REFERENCE DOCUMENTS

- ISO 14644-1
- ISO 14698-1 & 2

TEST EQUIPMENT

- Air bio-collector
- Petri dishes with appropriate culture medium (air and surfaces) or possibly membranes (air)
- Swabs
- Optical particle counter
- Instrumentation for testing environmental conditions (pressure, temperature, humidity, etc.)

TEST PROTOCOL

Environmental samples are taken at each position each time the isolator is used or once a day
- The samples to be taken are the following:
  - Air samples via sedimentation using an exposed agar plate (4h) during operations/activities
  - Active air samples (1m3) using an air bio-collector and an agar plate during operations/activities
  - Microbiological surface samples at the end of each sterility test campaign or once a day at each position
  - Particle counting during operation/activities and at rest every six months or in accordance with ISO 14644-1 and 2

ACCEPTANCE CRITERIA

- The non-viable particle counts comply with observe the standards laid down for the target classification concerned both at rest and in activity.

NB: There should be no alarm on the test parameters of environmental conditions over the test period.

REMARKS

- Locations for taking air and surface sampling (viable and non-viable particulate) of the isolator environment can be defined on the basis :

- of the supplier's recommendations (small chambers)
- of a risk analysis. This risk analysis can for example consider criteria such as:
  - the process (filling, crimp sealing, transport, interface, etc.)
  - flows (components, waste. Etc.)
  - OQ data (airflow visualization, particle counts, air speed measurements, etc.).

- Routine sampling locations can be derived from the results obtained during the initial qualification study (test in activity) and this test.

NB: As it is difficult to modify pass throughs in the isolator after manufacture it is advised to consider this point during isolator design.

- The isolator environment must be periodically requalified and after every significant equipment or procedural modification so as to demonstrate conformity with the initial qualification.

Performance qualification or requalification testing of the isolator can be done in parallel with the media fill test (MFT) as the requirements of the two studies in terms of activity and environmental sampling are similar.

PQ Test (Media Fill Test)	Aseptic filling test (Media fill test under production isolators)	Sheet No. 7-PQ

SCOPE OF THE TEST

Objective: To confirm that aseptic conditions are maintained during the manufacturing process (preparation, filling).

REFERENCE DOCUMENTS.

GMP, Annex 1 : Validation of aseptic manufacturing processes must include simulation of the process using culture medium' (paragraph 66)

TEST EQUIPMENT

The choice of culture medium will depend on the pharmaceutical form of the product, and on the selectivity, clarity, concentration and suitability for sterilization of the medium (GMP).

Casein-soybean culture medium is generally used for process simulation (media fill).

TEST PROTOCOL

Paragraph 42 of GMP :

The manufacturing process simulation test must reproduce the routine aseptic manufacturing process as closely as possible and include the critical stages. It should also take account of the various operations which may take place during normal production and ' worst case' situations.

Simulation tests must be conducted for the initial validation with three consecutive compliant tests for each of the teams and must be repeated at regular intervals and after every significant modification of the air filtration system, equipment, process or the number of teams. Simulation tests must normally be repeated twice a year, for each team and each process.

ACCEPTANCE CRITERIA

Objective: 0 contaminated unit

REMARKS

Principal concepts:
This is a study that is broader in scope, which challenges the aseptic process as a whole. In an isolator production line the process simulation test is adapted to include all the operations inherent in isolation technology to confirm the isolator maintains aseptic conditions.

Validation approach:
• After sterilization of production isolators by sterilizing agent vapour, activities are conducted over the period of production. If isolators do not have any production activities during this period, simulation activities will be carried out until the end of the period or of the process to be validated (this may be a period of time or a quantity).
• Production line operators carrying out the activities must be qualified.
• The activities to be carried out under isolation must be defined in the protocol.

Production activities under isolation:
• filling/loading or unloading of a production lot.
OR
• filling/loading or unloading of flasks filled with culture medium
OR
• a simulation of activity representative of production

A simulation of activity representative of production consists of:
• setting in motion the isolator systems for a period representative of production
AND
• the performance a human operation under isolation.

The carrying out of these 2 operations ensures the representativeness of production conditions.

Isolator operations:
Everything that is carried out by an operator on the manufacturing line is considered as an operation. An activity carried out via the RTP port can be considered as an operation.
• Inherent operation: a regular operation carried out for most of the production runs.
• Corrective operation: a non-regular operation which appears in one or several production runs. In the event of corrective interventions, a system enabling the tracing and recording of these non-regular interventions should be put in place (for example, an annual review).
• Operations to be carried out when working with during culture media

In industrial pharmacy, operations chosen as representative of critical parts of the aseptic procedure, are carried out during the phases of loading of the isolator, filling vials with culture media and removing the vials.

'Multi-product' production line under isolation:
With respect to the choice of format to test, the solution is either to define an unfavourable configuration or to carry out an alternating media challenge on the different products/formats filled in the isolator under consideration.

In hospital pharmacy, validation of procedure consists in showing that no unit is contaminated, using the components/items and procedure as envisaged.

Test conditions:
Except in specific cases, the parameters of the isolator and machines (e.g. filling speed) chosen for the procedure simulation study are the routine nominal production parameters.

Environmental tests
– Microbiological tests:
On the days when culture medium is being replaced, the environmental tests in the isolators will be carried out according to a predefined sampling plan, which can be specific to the study but which generally will be based on a routine sampling plan.
– Particle tests:
Particle sampling (0.5 and 5 µm) will be carried out throughout activities, as in production (culture medium, simulations, production), in each of the isolators.
– Qualification of the ambient environment of production isolators:
During procedure simulations of full production runs, the ambient environment of the isolators can be requalified in parallel with procedure simulation.

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* Test outside the BELAC accreditation scope

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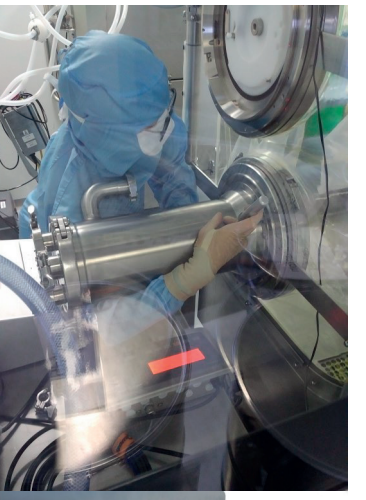

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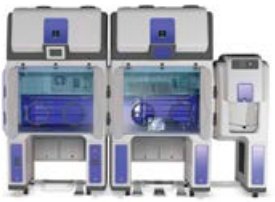

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7

VALIDATION OF THE SURFACE STERILIZATION PROCEDURE (BIO-DECONTAMINATION)

1 DEFINITION OF THE LOAD 101

2 PHYSICO-CHEMICAL VALIDATION 103

3 MICROBIOLOGICAL VALIDATION 105

3.1 Validation empty 105

3.2 Validation loaded 106

Validation of the surface sterilization procedure in isolator technology encompasses 'sterilizing agent/generator/isolator/load'.

Any change to an element in this group requires a new risk analysis to validate the absence of impact (equivalence) or the need for a new validation.
Control of aeration after sterilization is also a factor to be assessed during validation of the surface sterilization procedure.

According to the standard ISO 14937, validation corresponds to a *written operating mode used to obtain, record and interpret the results necessary to establish that a procedure will consistently provide a product that complies with predetermined specifications.*

1 DEFINITION OF THE LOAD

In the pharmaceutical industry, validation of an isolator is linked to its contents. Validated loads are defined as for autoclaves.
Very often, a defined maximum load is validated. Routinely, the user may then sterilize all loads which do not exceed the maximum load.

Any new element (if it is not equivalent) that is integrated into a load must be qualitatively and quantitatively validated. The location of the product/and or the item forms part of the definition of the load (see **FIGURES 7.1** and **7.2**).

Légende charge consommable

	7 bottles of 1 l, Or 4 bottles of 1 l and 3 bottles of 500 ml		44 bottles of 100 ml : 22 BTS + 22 THIO
	11 syrup bottles of 500 m		2 packs of filtration units
	5 stainless steel boxes for scissors and forceps		1 wipes pack
	1 basket of 20 vials Or Biocollector + grid		1 settle-plate bag
	7 bottles of 100 ml		1 Agar count-tact bag
	Alcohol spray Or 1 bottle of 1000 ml		1 count-tact tool
	3 clamps for Agar Count-tact		1 biocollector + grid
	1 test tube of 25 ml		1 stainless steel tray
	1 basket of 20 vials Or 1 bag of settle- plates + 1 bag of count-tact		1 basket of 20 vials Or 8 bottles of 100 ml, upper level

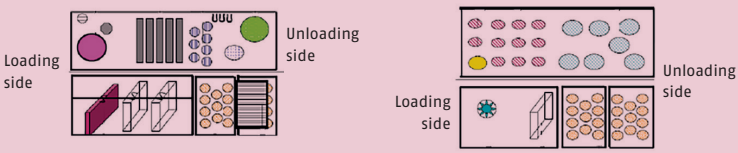

FIGURE 7.1 – Example of a sterility test isolator load (top shelf, lower left and right shelves)

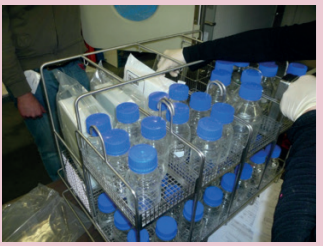

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FIGURE 7.2 – Example of a load for a sterility test isolator (shelves shown above)

Hospital Production

It is difficult to define a load typical for hospital production because of the large variety in type and quantity of equipment and consumables that need to undergo surface sterilization. During validation of the surface sterilization process, suffice to define one or several loads representative of routine.

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FIGURE 7.3 – Hospital pharmacy preparations using isolation technology in hospital pharmacy

Qualitatively the **load** must be a mixed load incorporating the different materials used during production, for example:

➲**Glass:**
- Vials, glass ampoules, containing active ingredients or solvents

➲**Plastics:**
- PE/PP syringes from 1 to 50 ml
- Solvent pouches from 100 to 1000 ml
- Giving sets
- Empty pouches

➲**Non-woven:**
- Drapes
- Compresses

➲**Mixed:**
- Needles
- Air-intake needles

➲**Paper:**
- Labels

➲**Miscellaneous:**
- Agar media
- Culture media

Quantitatively the **load** must be equivalent to a maximum of 80% of the volume of the chamber to be sterilized with sufficient space between each element to allow circulation of the sterilizing agent.
The choice of the shape and size of the different items included in the load will depend on the type of activity. To apply the results of the validation routinely the mix of the validated load must be adhered to without using more that 80% of the chamber volume.

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FIGURE 7.4 – A loaded Airlock ready for sterilization

2 VALIDATION OF PHYSICAL AND CHEMICAL PARAMETERS

Validation of a sterilization process depends on the ability to control of the physical parameters described in **TABLES 7.1** and **7.2**.

Pressure, time, surface temperature, airflow, sterilizing agent concentration, air change rate and in some cases ambient temperature and humidity need to be controlled.

It is also necessary to check that all surfaces are reached by the surface sterilizing agent (in particular sleeves and door aperture seals, for example).

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FIGURE 7.5 – Glove supports in a production isolator (optimal exposure to the sterilizing agent)

TABLES 7.1 and **7.2** show physical and chemical parameters as a function of the sterilizing agent utilized

TABLEAU 7.1 : Physical and-chemical parameters to be verified during surface sterilization when using H_2O_2

LOCATION	PARAMETERS	THRESHOLD LIMIT VALUES	COMMENTS
Isolator	Humidity (initial)	In accordance with the manufacturers recommendations technology	– influences sterilizing agent concentration – this parameter must be controlled as it has an impact on overall cycle time can and affect the reproducibility of cycle values – check that the installed probes can tolerate the sterilization conditions
	Concentration of the sterilizing agent	0.5 to 2 mg/l, measured in the isolator 350 to 1400 ppm (for an ambient temperature of 25°C)	AT 20°C, 1.41 MG/L corresponds to 1015 ppm
	Surface temperature	± 3°C (maximum desirable difference between the initial temperature and the temperature during the sterilization cycle)	– Materials such as stainless steel, plastics, rubber, behave differently at the same surface temperature – Measurements taken at the beginning and end of the cycle
	Air change rate* (aeration phase through the HEPA filter)**	> 20 Vh^{-1} (for example) Aerate to a level compatible with non–interference with the conditions of use	Parameter influencing aeration time
	Differential pressure of the isolator during the sterilization cycle	40 to 80 Pa (average of 60 Pa)	
Ambient conditions	Temperature	19 to 26°C (in accordance with NF S 90351)	Do not position the isolator under an air supply inlet
	Humidity	50 % ± 10 %	Parameter influencing dehumidification phase duration

**: or hourly air change rate*

***: The compressed air of the sterilizing agent generator can be used to aerate the isolator.*

NB: The values given in the above table are examples for information only.

TABLEAU 7.2 : Physical and-chemical parameters to be verified during surface sterilization when using PAA

LOCATION	PARAMETER	THRESHOLD LIMIT VALUES	COMMENTS
Isolator	Humidity	30 to 65%	Non-limiting factor The humidity level does not reduce the efficacy of surface sterilization.
	Concentration of sterilizing agent	Initial concentration: 1 to 5 %	There is currently no direct means of measuring the concentration of peracetic acid during surface sterilization
	Surface temperature	Closest to Ta ≥ 18°C	Condensation does not reduce the efficacy of surface sterilization.
	Isolator pressure during the sterilization cycle	40 to 70 Pa under positive pressure (-200 Pa for MSC type 3, according to EN 12469)	Do not exceed the maximum pressure of the leak-tightness test
	Air change I rate* (aeration phase through the HEPA filter)**	Injection with continuous and stable airflow (isolator ventilation or compressed air of the generator)	Parameter influencing aeration time
Ambient conditions	Temperature	19 to 26°C (according to NF S 90351)	Cold walls carry a condensation risk
	Humidity	Not controlled	Non-limiting factor

**: or hourly air change rate*

***: The compressed air of the sterilizing agent generator can be used to aerate the isolator and/or using the isolator ventilation.*

NB : The values given in the above table are examples for information only.

3 MICROBIOLOGICAL VALIDATION

3.1 VALIDATION EMPTY

For standard equipment, the manufacturer's studies can serve as a reference. The need to validate the empty isolator will dependent on the configuration encountered.
For special or bespoke equipment, two configurations can arise.
- Using H_2O_2: Airflow studies, temperature mapping, visualization of gas distribution by chemical indicators will determine critical points which must be used for inoculated carrier locations.
- Using PAA, airflow studies will determine critical points which must be used for inoculated carrier locations.

Regardless of the sterilizing agent used, the **critical points** most often selected are sleeves, gloves, interfaces between different types of material, the doors, the refrigerator, the airlock, areas of low airflow.

3.2 VALIDATION LOADED

The load must be evenly distributed (see **FIGURE 7.6**).

The recommended sampling plan includes:

– The distribution of biological indicators on the surfaces and in each corner;

– The positioning of biological indicators at the points judged to be critical, that is:

• Process critical: bowl, needles, filling area, RTP port (DPTE®), etc.

• Maximum and minimum temperature locations.

• Worse case locations identified during gas distribution studies using chemical indicators.

The complexity of the load must be taken into account and the 'Worst Case' approach given preference.

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FIGURE 7.6 – Standard isolator load for a sterility test isolator

The following precautions must be observed:

Before being placed in the isolator, each element which constitutes a load must be thoroughly cleaned on all surfaces to minimalise bio-burden. As the bio-decontamination process is a surface bio-decontamination process only all **equipment and products placed in the isolator must be sterile**.

Sterilization of containers and consumables/accessories in industrial pharmacy:

RTP containers, tools, consumables and accessories are sterilized in autoclaves and transfer isolators before their aseptic transfer into the working chamber. The maximum time between sterilization and use of these items of equipment and consumables must be validated.

In hospital pharmacy: Items, components and raw materials must undergo complete sterilization using a method described in the European Pharmacopoeia, before being introduced into the isolator. E.g. Radio-sterilized pens, medical devices, bags of consumables, etc.

Good practice to be observed:

– It is always best to validate **a maximum load which is as representative as possible of the manipulations which will be carried out in the isolator.**

– The load must be distributed so that no item is in contact with another. **A minimum distance of around 2 cm must be observed between each vial, syringe, items containing culture medium, and all other devices.**

– The load pattern must take into account the gas distribution in the chamber (smoke test, chemical indicators, etc.).

- All sterilized items on the work surface must be positioned on a grid or wire frame so that all surfaces of this item are in contact with the sterilizing agent.
- If the load is large, perforated multi-tiered shelving must be provided.
- Items can be suspended (for example from a hanging bar), to improve surface sterilization.
- No residual water must be present as this will absorb the sterilizing agent.
- In the case of H_2O_2, it is advised that low temperature items should not be introduced just before or during surface sterilization, to avoid all risk of condensation. All items in the load must be at ambient temperature when the sterilization cycle begins.

This does not exclude running conducting the sterilization cycle with different thermal profiles if necessary, provided that these conditions are also validated during performance qualification.

8

CLEANING AND DISINFECTION

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The Aspec, March 2015 french guide on '*Cleaning and Disinfection: Rooms and external surfaces of equipment*', gives details on the majority of themes mentioned in this chapter including cleaning and disinfection products, monitoring tools and cleaning validation,...

1 CONTEXT AND INTRODUCTION

This paragraph concerns cleaning and disinfection operations for an isolator and its accessories (intermediate operations carried out between production runs, batch changeovers, maintenance, shutdown...). The operations are carried out in accordance with written procedures. It is essential that these operations be carried out in order to achieve sterilization.
The outside of the isolator must be cleaned in the same way as the cleanroom in which the isolator is housed.

Cleaning and disinfection operations are carried out:
• Before a maintenance operation in order to protect the operator from biological and/or chemical risks and possibly in order to facilitate the operation.
• After a maintenance operation, in order to make the equipment clean for the user and to avoid the introduction of external contaminants into the interior of the isolator as a result of the maintenance operation.

Cleaning after maintenance of the isolator must be even more thorough as the bio-decontamination stage which follows requires clean and dry surfaces.
The operations carried out before a maintenance operation will focus either on cleaning and/or disinfection, depending on the use of the isolator.
Disinfection must be adapted to the requirements and processes encountered. Disinfection may be:
- Optional depending on the processes,
- Partial (limited to parts that are critical to the process, surfaces which are hidden during sterilization, etc.),
- Complete and carried out on all surfaces.

Cleaning may be carried out at the same time as disinfection by using a disinfectant which also has detergent properties or in two stages carried out consecutively with a cleaning stage followed by a disinfection stage.

2 OPERATIONAL PREREQUISITES

Regulatory requirements:
Cleaning/disinfection operations are conducted in accordance with a biological and/or chemical risk assessment. The general regulations for the prevention of chemical risks fall within the scope of **articles R. 4412-1 to R. 4412-57 of the French Labour Code**. The specific preventive measures to be implemented when using agents which are carcinogenic, mutagenic or reprotoxic are laid down **by articles R. 4412-59 to R. 4412-93 of the French Labour Code**. The preferred preventative measure is substitution. Where this is not possible, minimal exposure should be sought, giving priority to collective preventive measures.
Regulations related to external companies are set out in the French Labour Code in **articles 4511-1 to 4514-10**.

There must be **a cleaning and disinfection procedure in place for the isolator** with detailed instructions and illustrated with photos. Depending on the organization structure the procedure will be signed off by the Head Pharmacist, the Production Manager, the Quality

Manager, the Head Microbiologist for the site or the Maintenance Manager. If there are several isolators in the clean room, where a minimum of production activity has to be maintained, the procedure must set out the minimum precautions to be taken in the event of only one section of the isolators undergoing maintenance.
It is desirable to have a **record sheet for the cleaning operations carried out prior to the maintenance operation**. This sheet should mention, as a minimum, the date, the name of the person who carried out the cleaning operation and the reference of the protocol used or, where applicable, the protocol itself.

The French Regulation of 16 July 2007 (biological risk containment areas) also mentions: "*Decontamination of material and equipment likely to be contaminated (centrifuge, fermenter, microbiological safety cabinet, ventilation and air conditioning system...) before any maintenance operation which may involve a biological risk for the operator. A document certifying the decontamination must be made available to Maintenance*".

The cleaning–disinfection team must include:
– The user of the equipment, who knows the products to be used and the procedures to be implemented (particularly in the case of biological and chemical risks);
The user must be present at least at the beginning and at the end of the maintenance operation, as they alone know how the cleaning and disinfectant product interacts with their activities.
– The operator in charge of maintenance who is familiar with the equipment and its materials (seals, structure...) and therefore with its critical points.

NB: An external operator must not be left unattended during preliminary cleaning.

3 GENERAL NOTES ON PREPARATION FOR MAINTENANCE OPERATIONS

Before any maintenance operation, the isolator must be cleaned in order to remove, for example, debris linked to the activity: glass, plastic, product, etc. This operation requires the isolator to be emptied in advance ('line clearance') to make surfaces accessible and access safe before the operation.

Cleaning operations must be considered during the design of the isolator and its interfaces in order for them to take place in the best conditions. These are validated operations.

Cleaning operations may differ in different situations:
➲**Line maintenance – without closing down production isolators.**
➲**Periodic maintenance with programmed interventions and stoppage of isolators or a section of them.**

Cleaning operations may require the isolator to be opened, which may lead to specific risks for the operator.
Depending on the defined level of risk, the operations prior to cleaning to empty the isolator ("line clearance") are carried out with the isolator closed using transfer systems. When the isolator has to be open (**FIGURE 8.1**) for a maintenance operation, the cleaning and disinfection operators must use PPE.

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FIGURE 8.1 – Example of production isolator undergoing maintenance (doors open)

To avoid cross-contamination, several "good practice" rules can be put in place (for example, disposable clothing, dedicated operators, decontamination mats,...) and incorporated into a formal procedure.

4 SEQUENCE OF OPERATIONS ACCORDING TO TYPES OF ACTIVITY

The sequence of operations (cleaning-disinfection, maintenance, bio-decontamination, etc.) will depend on the activity carried out inside the isolator.
Three main situations may be encountered. These are listed in TABLE 8.1:

TABLE 8. 1 List of activities, risks and order of execution in an isolator

ACTIVITY CARRIED OUT INSIDE THE ISOLATOR	RISKS IDENTIFIED	SERIES OF OPERATIONS OR SEQUENCE
Handling of pathogens	Microbiological for the staff and the environment and possibly for the operation and the process	Bio-decontamination with an sterilizing agent effective against the pathogens identified as a risk→ Cleaning → Maintenance → Cleaning → Bio-decontamination For example: aseptic filling or handling of highly pathogenic substances (in the case of P_4 laboratories or activities relating to highly pathogenic microorganisms and toxins)
Handling of hazardous and sterile chemical agents *ED769, INRS , Handling of genotoxic substances used in the laboratory*	Chemical risk to staff and the environment and microbiological for the operation or for the process	Cleaning → Maintenance → Cleaning/disinfection → Bio-decontamination
Handling of sterile products which are chemically non-hazardous and non-pathogenic	Microbiological for the operation or for the process	Cleaning → Maintenance → Cleaning/disinfection → Bio-decontamination with PAA or H_2O_2

Note 1: Isolator empty of content (or line clearance) before cleaning
Note 2: Waste is double-bagged and disposed of via the appropriate means (see **CHAPTER 15***).*
Note 3: In the case of hazardous chemical substances or pathogenic biological substances, precautions are taken when changing filters and in certain cases, these are placed into a safety cabinet which allows a safe change using the Bag-In/Bag-out technique. The maintenance of filters requires particular precautions to be taken by the maintenance operator, when this is performed in a clean room (dedicated tools personal protection equipment in the cleanroom or where applicable, cleaned and disinfected before the maintenance operation on the isolator).
Note 4: Advanced therapy medicinal products may use both hazardous chemical agents and pathogenic biological agents.

Once all operations have been performed, a procedure for checking microbiological and disinfectant residues can be implemented.

5 CLEANING AND DISINFECTION PRODUCTS

5.1 PRODUCT FAMILIES AND SELECTION CRITERIA

The product families most commonly used are:

➲**Cleaning detergents** particularly for the removal of chemical contaminants on surfaces

➲**Disinfectants or detergent-disinfectants for disinfection**

A rinsing operation (purified water, highly purified water or WFI) is generally carried out between the application of the detergent and the disinfectant.

Whatever the cleaning or disinfection product, it is essential to follow the manufacturers' recommendations for use.

The criteria for choosing a product are based on the following characteristics:

- **Spectrum of activity** in the case of a disinfectant or a detergent-disinfectant: as a minimum bactericidal, fungicidal and possibly sporicidal, in accordance with EN 13697 (surface test: killing of bacteria and fungi – to be adapted for killing of spores). Virocidal action may also be required. **Composition of the cleaning or disinfection product**
- **Possibly, the capacity to break down the product that is handled or manufactured**, in the case of a detergent formula
- **Compatibility with the materials of the isolator, the load and equipment**
- **Residues of cleaning and/or disinfection agents (risk of interaction with the product to be manufactured)**
- **Method of application** of the cleaning and disinfection product (generally, wet wiping)
- **Compatibility with the sterilizing agent**

Additional criteria to be taken into account:

- **For Grade A and B areas** (aseptic filling), products must be **sterile and double bagged**.
- When working on a closed isolator, cleaning and disinfection products and cleaning and disinfection materials and tools must also be sterile.
- For maintenance operations on an open isolator, products do not necessarily have to be sterile (but this duplicates management operations: non-sterile products for maintenance cleaning and sterile products for routine cleaning)
- **Ready-to-use products** are convenient to use and recommended as they minimize the risks of errors linked to dilutions.
- Products used for cleaning and possibly disinfection **should preferably be the same as those used routinely** in production to avoid as much as possible the addition of new formulations.
- **The cleaning and disinfection products for the isolator are often the same as those used for the cleanroom**; as a validation study has already been performed on the products.

- When several cleaning and disinfection products meet the technical requirements (compatibility with materials and the activity exercised in the isolator), **the product should be chosen which presents the lowest risks for the operator and the environment**.

To determine this, refer to the product label and the safety datasheet which accompanies all cleaning and disinfection products.

Detergent products:
Cleaning products may be:
- Detergent agents
- Solvents
- Water (WFI)

The mechanism of action is either direct or indirect by the use of a detergent. **It should be noted that to date there does not exist one single cleaning agent that is effective against cytotoxic molecules**.

Detergent products must be tested in advance in order to ensure that the agents are compatible with the surfaces being treated (materials and equipment) and also for risks of interaction with the sterilizing agent.

For further information, several references for specific studies which have dealt with cleaning in hospital pharmacy are quoted in the bibliography (CHAPTER 17, PARAGRAPH 4.1)

Disinfection products:
As *a minimum*, the disinfection products used should have a spectrum of activity that matches the targets in question:
- Bactericidal
- Fungicidal
- Sporicidal
- Virocidal

Disinfection products must be tested in advance in order to ensure that the agents are compatible with the surfaces being treated (materials and equipment) and also for risks of interaction with the sterilizing agent.

Sporicidal action is required after a maintenance operation to prepare for bio-decontamination. The action of disinfectant products must be tested both on reference strains but also on the environmental strains of the isolator. As with cleanrooms, the principle of alternation may be implemented in the isolator.

Note on diluted isopropyl alcohol (IPA):
- IPA in 70% dilution is a biocidal product commonly used in cleanrooms. It meets the requirements of Biocides Regulations n° 528/2012 and of REACH, European Regulation n° 1907/2006.
- IPA does not have a cleansing effect on proteins and fixes them.
- Alcohol may however be used in the maintenance of isolators in order to remove residues and traces left after the application of detergent and/or disinfectant solutions.

6 USE AND STORAGE CONDITIONS

Cleaning operations should be performed with clean equipment, cleaned in advance comprising of:
- Wall mops with telescopic handles for reaching difficult areas,
- Wipes (100% polyester in order to avoid the release of fibres, particles and chemical contaminants),
- Cotton buds (100% polyester heads) to clean areas that are difficult to access, cracks and cavities, recesses and corners,
- Cleaning and disinfection products.

Surfaces within arm's reach should be cleaned using wipes. For other surfaces, wall mops can be used.

It is preferable to clean isolator manipulation equipment in situ: this is fragile equipment made from polymer materials, which is best not dismantled.
Wipes may be dry for impregnation or pre-impregnated with a detergent or disinfectant solution. They must be sufficient in number to avoid spreading contamination, (change wipe during the operation).

Operations are carried out using overlapping parallel movements moving:
- from the "cleanest" areas to the "dirtiest" areas,
- from top to bottom (ceiling then partitions),
- from opposite surfaces towards evacuation areas.

The cleaned and disinfected surfaces of the isolator and its interfaces must be dry before the application of the bio-decontamination agent.
Storage of isolator cleaning and disinfection in a room identical to that for bio-decontamination agents (PAA, H_2O_2) must be evaluated.
French ED 753 of the INRS makes a few recommendations (**TABLE 8.2**)

TABLE 8.2 Storage conditions for chemical products

PRODUCT	SPECIFIC CABINET OR ROOM	CONTROLLED AND LIMITED ACCESS TO THE ROOM	ADDITIONAL PRECAUTIONS
Label T+: very toxic	X	X	
Label E: explosive	X	X	
Label O: combustive	X		To be kept away from combustible products, in particular those labelled as extremely or easily inflammable
Incompatible with water	X	X	Avoid the presence of piping in the room
Label F+ or F –: extremely or easily inflammable	X		The storage enclosure must be ventilated
Concentrated bases			Storage must be separate from that of acids
Concentrated acids			Storage must be separate from that of bases

SOURCE : ED 753, INRS

7 COMPATIBILITY

7.1 COMPATIBILITY OF CHEMICAL CLEANING PRODUCTS AND/OR DISINFECTANT PRODUCTS WITH ISOLATOR MATERIALS

Compatibility with materials depends on the frequency of cleaning and disinfection operations and the age of the surface concerned.

TABLEAU 8.3 list of the principal materials of an isolator

MATERIALS OF THE CHAMBER AND EQUIPMENT	SEALS	MATERIALS FOR MANIPULATION EQUIPMENT
PVC	Silicone	PVC/PE (Divetex, white...)
Stainless steel 316L	PVC	Neoprene
PMMA	EPDM[1]	CSM or chlorosulphonyl polyethylene formerly called Hypalon®)
Polycarbonates	Viton®	Neoprene and CSM
Glass	PTFE	Polyethylene (RAC 100)
Polyéthylène (PEHD,...)		Rigid polycarbonate
PEEK		White PVC
		Butyl rubber
Polyurethane		Latex
Aluminium (anodised)		

[1] *A material which is becoming increasingly present in isolators*

TABLES 8.4 AND **8.5** detail some incompatibilities between materials and cleaning and disinfection products

TABLEAU 8.4 Incompatibilities between cleaning/disinfection products and materials

MATERIAL	CLEANING OR DISINFECTION PRODUCT
Silicones	Alcohols
Plexiglass	Alcohols
Polycarbonates[1]	Alcohols
Aluminium alloys	Phosphoric acid
Aluminium alloys	Alkalis with pH > 12

[1] *Material becomes opaque or cracks*

SOURCE : ASPEC Biocontamination Guide , 2008

TABLEAU 8.5 Compatibilities/incompatibilities between glove materials and isopropyl alcohol

MATERIAL	IPA
Polyisoprene: Natural Rubber	++
Polychloroprene: Neoprene	++
Polyisobutylene: Butyl rubber	+++
Chlorosulphonyl polyethylene: CSM	+++
Ethylene-propylene-diene terpolymer: EPDM	+++

Caption: -Not recommended +Usable under certain conditions ++Suitable +++ Recommended

FIGURE 8.2 – Examples of gloves : natural rubber (top left) and CSM (top right and bottom)

The criteria to be taken into account in compatibility studies are the concentration of the cleaning and disinfection products used and their method of application.

For more information on compatibilities between cleaning and disinfection products and the materials of the isolator, refer to the isolator manufacturers recommendations.

7.2 COMPATIBILITY OF GLOVE MATERIALS AND STERILIZING AGENTS

TABLEAU 8.6 Incompatibilities between glove materials and sterilizing agents (H_2O_2 and PAA)

GLOVE MATERIALS	H_2O_2	PAA
Polyisoprene: Natural Rubber	+	–
Polychloroprene: Neoprene	+	+
Polyisobutylene: Butyl rubber	++	++
Polyurethane	+	+
Chlorosulphonyl polyethylene: CSM	++	+
Ethylene-propylene-diene terpolymer: EPDM	+++	+++

Caption: -Not recommended +usable under certain conditions ++ Suitable +++ Recommended

7.3 COMPATIBILITY OF CHEMICAL PRODUCTS (DETERGENTS/DISINFECTANTS/DETERGENT-DISINFECTANTS) AND STERILIZING AGENTS

After cleaning and disinfection operations, no deposits or residues of the cleaning and disinfection products should remain.

Traces may appear during bio-decontamination if cleaning is insufficient (traces of detergents and residues of the products manufactured). In addition, residues not removed before resumption of activity may cause problems for aseptic filling and sterility testing.

You should make sure in advance of compatibility between the cleaning and disinfection products used based on the data supplied by isolator equipment suppliers, manufacturers of cleaning and disinfection products and results of experimental studies.

8 EFFICACY OF CLEANING AND DISINFECTION

8.1 EFFICACY OF CLEANING

Cleaning serves to eliminate residues of the products manufactured or used. There are two approaches to cleaning:

❶ The products are soluble in a solvent and are eliminated by dilution. In order to increase the solubility of products, surfactants and co-solvents can be added.

❷ The products are not soluble or are too hazardous to be handled as they are. A suitable chemical product is used to neutralize them or make them soluble.

The first cleaning approach ❶ can then be applied.

Unlike disinfectant products, the detergent efficacy of a cleaning product does answer to any standard. For optimal cleaning of the isolator, it is preferable to use a detergent product rather than a detergent-disinfectant product.

The efficacy of a cleaning operation is dependent on several factors including:
• the detergent solution used
• the volume of the solution: sufficient to dissolve surface contamination but also recoverable by wiping with a suitable wipe
• the contact time which may be different from that used for a disinfectant (around 5 minutes)
• the method of application
• the number of wipes/wiping cloths used.

The monitoring of cleaning operations and in particular checking of the absence of residues is carried out via:
• Lighting of surfaces with an raking white light;
• 100% polyester wiping cloths. The last wiping cloth should be checked for the absence of residues.

Cleaning validation should be carried out on the product (active agent, excipient, etc.) the most difficult to remove of the complete procedure (see Aspec French guide, *Cleaning and disinfection*).

Acceptable residue limits (product manufactured, detergents, possible breakdown by-products of the product) on critical surfaces (direct or indirect contacts with the product), after cleaning, must be established and justified on the basis of a risk analysis and then validated. e.g.: Weighing, handling and formulation preparation isolators.

The acceptable limits will take into account in particular the subsequent bio-decontamination, the risks of cross contamination between products, and the toxicity for maintenance operators. An analysis method specific to the residues (such as HPLC coupled with mass spectrometry, or mass spectrometry) is preferable to non-specific general methods (TOC, etc.).

8.2 EFFICACY OF DISINFECTION

As previously indicated, disinfection of an isolator is:
- Either combined with a detergent,
- Or is a specific operation carried out after surface.

A risk analysis of the situation encountered (routine or maintenance, etc.) will allow the required level of disinfection to be determined.

9

PRODUCTION

Although the isolator represents the highest level of separation between the process and the operator, its efficacy depends on the level of training of the personnel working on or in the isolator.

The efficacy of surface sterilization (or bio-decontamination) depends on the condition of the surfaces. It should once again be stated that we are dealing here with the sterilization of clean exposed surfaces. So the cleanliness of surfaces and their temperature are essential to ensure good surface sterilization.
As a consequence, the cleaning and preparation process prior to the surface sterilization cycle must be the subject of a written procedure that is understood by the personnel involved. 'Good practice' in cleaning must be applied to isolators: the cleaning of isolators must be thorough and as a minimum be confirmed by visual inspection.

Operations which may influence the temperature of surfaces to be sterilized must be analyzed and their sequence made the subject of a written protocol regarding surface sterilization (for example, cold water functional test, cleaning *in situ*, freeze-drying etc...).

The validated loading plan must be documented in a protocol and complied with to ensure a correct distribution of the sterilizing agent.
The loading plan in unidirectional flow isolators is also important in production mode as any deviation from the validated plan can disrupt airflow patterns.
In another equally critical category for aseptic processes cross contamination between products must be controlled, this includes cross contamination between the product and the Trypticase Soy Broth used during MFT.

Manual operations in isolators using gloves must be performed by trained personnel, as bad practice can generate pressure alarms and/or particulate. Bad practice can also reduce the operating life of gloves (scratches, holes...) with a risk of breach of sterility as the ultimate consequence.
Inspection of gloves (including sleeves) must be carried out regularly. Checks of gloves should include, if possible, both visual inspections (as regularly as possible) by trained operators and physical test, for example with glove testers (as a minimum once in every production run...).

The management of gloves: Gloves should be scrupulously monitored, for example the monitoring of defects (holes, scratches, deformation etc.) is strongly recommended. This monitoring should allow improved practices and equipment with the aim of reducing the frequency of defects (elimination of sharp edges, ergonomic improvements etc.). **Still on the subject of the management of risks linked to glove defects, the wearing of fine latex inner gloves (or nitrile or equivalent) is highly recommended**. Particular attention must also be paid to the bioburden inside isolator gloves on the operator side, (disinfection of inner gloves each time hands are introduced into the isolator) with the aim of reducing the impact of a glove defect (hole) on the aseptic process as much as possible. The use of sterile tools (tweezers...) must be preferred in areas of aseptic filling.

Management of hidden surfaces (surfaces not exposed to the sterilizing agent) which could be uncovered either accidentally or during a non-routine maintenance (repair) during the course of production must also be managed by process and periodically evaluated in MFT.

For safety reasons, **storage of sterilizing agent** must follow scrupulously the manufacturer's instructions. It is strongly advised that the storage conditions should be analyzed precisely (proximity to combustives, means of retention and disposal in the event of spillage). The use of a cupboard for dangerous chemical products is strongly advised. Expiry dates must be adhered to in all procedures.

Systems for personnel safety (such as eyewashes and/or PPE) must also be in place as a prerequisite for the first tests with the sterilizing agent.

Changes excluding preventive/corrective maintenance must be analysed and evaluated as to their impact on:
- Sterilization performance (material and surface condition, the amount of surface to be sterilized, creation of surfaces or areas difficult for the sterilizing agent to reach, modification of surface temperature, etc.).
- Aeration performance (material, changes in the surface to be sterilized, etc.)
- Aeraulic performance (geometric modification, modification of pressure, flow, etc.)
- Cleaning performance (material and surface condition, etc.)
- Permeation performance (packaging of sensitive consumables and product containers for the purpose of the sterility test).

For maintenance of doors and RTP containers as well as gloves refer to CHAPTER 13.

10

EXAMPLE OF RE-QUALIFICATION PROGRAM

In the case of uni-directional flow isolators:

- HEPA filters are tested periodically (integrity test, check for leaks air speed measurements, uniformity of air speed and air change rate)
- Verification of air flow patterns (smoke test) should be:
  - Carried out periodically during the life of the equipment
  - Systematically whenever there is a significant change to the equipment.

One the equipment is in regular use PQ tests for verifying the sterilisation cycle using biological indicators and measurements of the residual levels of the sterilisation agent at the end of the sterilisation cycle should be carried out at regular intervals and whenever there is a significant change to the equipment. Only one test is necessary when performing a routine requalification.

NOTE : A check for the residual levels of sterilising agent at the end of the cycle can be performed systematically at the end of every production cycle. If this is the case a periodic check becomes unnecessary.

In the case of grade A zones:

Requalification of the particulate classification is generally carried out every six months. Particulate classification must be at minimum done in accordance with ISO 14644-1 and 2 (once a year). This might differ for enclosures equipped with continuous particulate monitoring systems where the historical data can be used to monitor the performance of the enclosure over time.

The need to requalify the air quality inside the enclosure should be systematically evaluated after any significant change to the equipment or process.

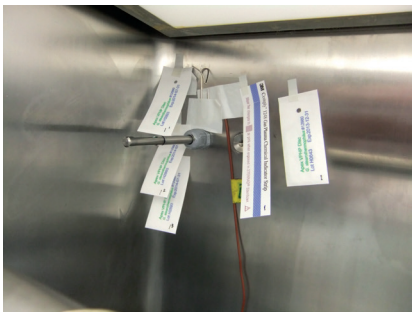
The periodic requalification of the air quality inside the isolator can be carried out in parallel with media fill testing or during production.

TABLE 10.1 shows a suggested requalification and routine test program.

TABLEAU 10.1 : Program for requalification and routing testing

LISTE DES PARAMÈTRES	REQUALIFICATION	ESSAIS DE ROUTINE	FRÉQUENCE	OBSERVATIONS
HEPA filter integrity	X	/	Once or twice per year for critical filters or once per year or once every two years	
Leaktighness of the enclosure (leak test)	/	X		
Functional test of pressure regulation and alarms	/	/		Pressure regulation test only
Air flow performance (air speed, uniformity, air change rate and make up air flow)	X (in general air speed only)		Once per year	The air change rate per hour is calculated by dividing the volume of air calculated from the air speed measurements by the volume of the enclosure
Verification of airflow patterns (smoke test)	See Chapter 10	/		To be repeated during the life of the isolator
Test of the sterilising agent generator	/	X		After maintenance and calibration of the generator
Microbiological test of the sterilisation of the loaded isolator	X		Once per year	
Temperature mapping and vapour distribution test using chemical indicators	X		Once per year	Must be done according to the sterilisation process used
Aeration time test	X (it is not necessary to do this during routine production for each cycle)		Once per year	
Performance qualification of the viable and non- viable particulate levels in the enclosure	X	Particulate monitoring	Twice per year (if grade A or grade A at rest)	Frequency can be extended based on a risk assessment, the level of monitoring systems in place and the measured performance against pre-defined acceptance levels
Aseptic fill test (Media fill test for a production isolator)	X		Twice per year	

CHEMICAL INDICATORS (IC), BIOLOGICAL INDICATORS (BIs) AND THERMOCOUPLES POSITIONED IN AN ISOLATOR



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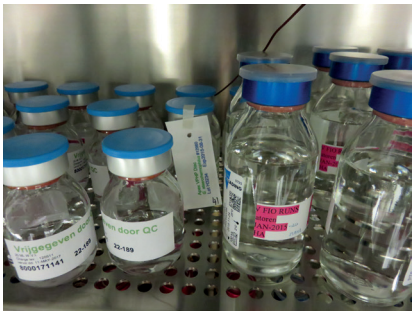

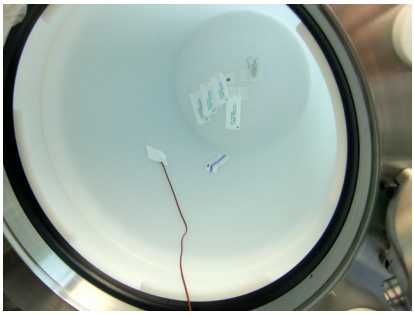

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11

MAINTENANCE STRATEGY AND SEQUENCE OF OPERATIONS

1 THE OBJECTIVE OF MAINTENANCE 127

2 DIFFERENT TYPES OF MAINTENANCE 127

3 PRE-REQUISITES FOR MAINTENANCE OPERATIONS 127

4 FREQUENCY OF MAINTENANCE OPERATIONS 128

1 THE OBJECTIVE OF MAINTENANCE

The goal of maintenance is to ensure the performance criteria obtained during installation and start up are the same as those obtained during the operational qualification.

2 DIFFERENT TYPES OF MAINTENANCE

The maintenance operations described are common to all types of isolator and sterilisation agent generator.
Two types of maintenance are performed on isolators:

- Preventative maintenance
- Corrective maintenance

The different definitions associated with each type of maintenance can be found in the glossary of terms (**CHAPTER 16, SECTION 2**).

Preventative maintenance of an isolator can be defined as follows:
– As a function of the number of run time hours (as per the strategy used on pharmaceutical sites)
– Maintenance operations programed according to a schedule or timetable regardless of run time (as per the strategy applied by healthcare establishments which have to be operational 365 days per year)

The criteria to take into account when establishing the frequency of operations in a maintenance programme are:
- The number of production runs.
- The number of bio-decontamination (sterilisation) cycles.

Maintenance of the isolator can be carried out either by the user or by the isolator supplier. In the case where maintenance is carried out by the user it should be according to the recommendations of the supplier.

Maintenance is an integral part of the running costs of the isolator.
In the case of a batch failure during production all maintenance operations are systematically investigated.

3 PRE-REQUISITES FOR MAINTENANCE OPERATIONS

- A maintenance request from the user, or a temporary or annual prevention plan (see example at the end of chapter 2)
- The maintenance technician (including sub-contractors) must have the appropriate training and certification (electrical, electro mechanical, hazardous chemicals) and must be qualified for the operation to be executed.
- Prior to entering the cleanroom the technician must have all the appropriate personal protective equipment (chemical, biological etc.) and must also be in compliance with site procedures.
- The Maintenance technician is responsible for ensuring the work area is clean and that the maintenance operation can be carried out safely.
- Maintenance operations carried out on isolators require particular precautions when done in clean areas (use of tools kept inside the clean area or suitably cleaned and disinfected before being transferred into the area).

4 FREQUENCY OF MAINTENANCE OPERATIONS

Maintenance operations can be carried out periodically by the user as a function of checks performed at each use of the equipment for:

- Changes in the physical appearance of: Seals, tubing, liquid levels, consumption
- Checks of cycle times

Isolator maintenance must be carried out at least annually by the appropriate department, manufacturer or a competent sub-contractor. Maintenance must be performed according to the manufacturer's recommendations:

- Replacement of seals
- Calibration of sensors
- Measurement of flows
- Measurement of volumes flow rates
- Check of sterilising agent consumption
- Others...

NOTE: Periodic replacement of leak tight seals must be done in accordance with the manufacturer's recommendations.

12

MAINTENANCE OF THE STERILIZING AGENT GENERATOR

1 DESCRIPTION OF A GENERATOR 131

2 SPECIFIC PRE-REQUISITS FOR GENERATOR MAINTENANCE 133

3 THE MAINTENANCE PROGRAM 133

3.1 List of operations 133

3.2 Example of a maintenance technician's check-list 135

1 DESCRIPTION OF A GENERATOR

Either an integrated or a freestanding mobile unit the generator serves to inject either a vapour or a spray (nebulisation) in order to bio-decontaminate the enclosure.

The generator can be broken down into six modules:
- Ⓐ Storage of the sterilizing agent
- Ⓑ The liquid circuit (sterilizing agent)
- Ⓒ The Sterilizing Agent Generation System
- Ⓓ The ventilation system (carrier gas)
- Ⓔ The device for connecting to the isolator
- Ⓕ Measuring, monitoring and control systems

Note: In the case of a critical and complex operation, its maintenance must be carried out carefully.

FIGURES 12.1 and **12.2** show examples of sterilizing agent generator scheme.

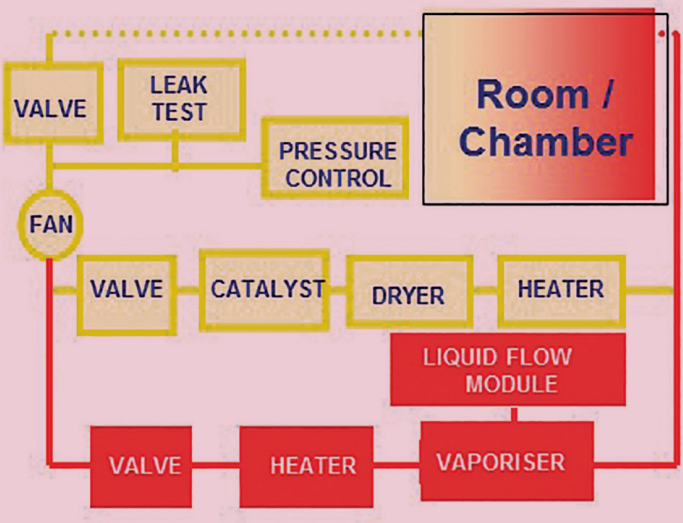

FIGURE 12.1 – Example of a diagram of a H_2O_2 generator

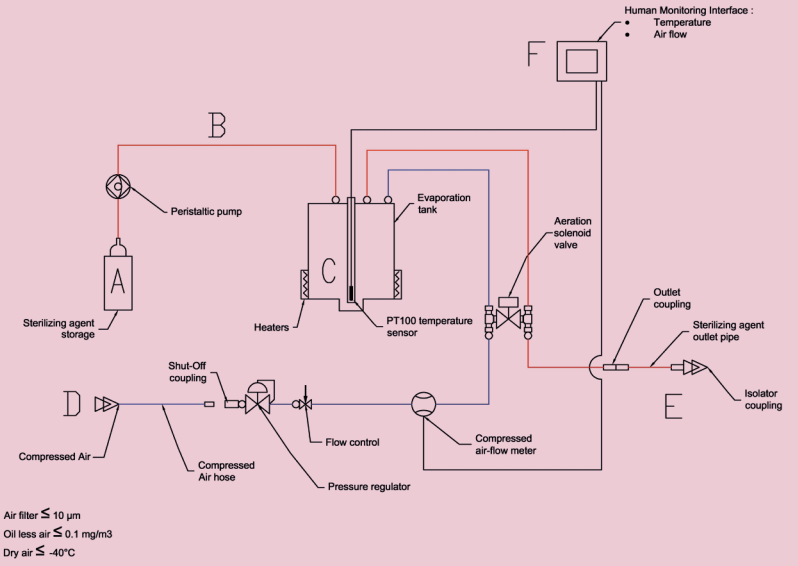

FIGURE 12.2 – Exemple d'un générateur APA

In the case of a sterilant compressed air generator, Class 2 according to ISO 8573-1 is recommended.

FIGURE 12.3 illustrates a sterilizing agent generator.

FIGURE 12.3 – Example of mobile sterilizing agent generator

Serial generators are illustrated in **FIGURE 12.4**.

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FIGURE 12.4 – Example of serial sterilizing agent generator (left) with filling station of the sterilizing agent (right), in a dedicated room (industrial application)

An example of a sterilizing agent installed in a technical gallery is shown in **FIGURE 12.5**.

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FIGURE 12.5 – Example of a remote gallery generator

2 SPECIFIC PRE-REQUESTS FOR GENERATOR MAINTENANCE

- **The technician will ensure, prior to his intervention, that the generator is purged of the biocidal products.**
- **At the end of the maintenance, the generator will have returned to its initial position and the technician will ensure that the system is restored, without risk to the user.**

3 THE MAINTENANCE PROGRAM

3.1 LIST OF OPERATIONS

It is essential to follow the manufacturer's equipment user's manual and recommendations for the bio-decontaminant agent generator.

TABLE 12.1 provides an example of a list of maintenance operations for two types of generator. Each generator will have its own list of operations based on the specific features of the machine. The following table is a resume of the minimum requirements to be considered.

TABLEAU 12.1 Summary of the maintenance operations to be carried out by users and maintenance technicians on the PAA and H_2O_2 generators (non-exhaustive list)

WHO	PAA GENERATOR	H_2O_2 GENERATOR
Ⓐ Storage		
User	• Rinse the bowl (if a manual generator) • Run a cycle using demineralised water • Check the drain valve • Check the expiration date of the chemical agent	• Check the expiration date of the chemical agent
Maintenance Technician	• Visually check the condition of the bowl • Clean the bowl	• Clean the reservoir
Ⓑ Liquid circuit (sterilising agent)		
User	• Drain the condensate • Visually check the condition of the pipework	• Check for leaks
Maintenance Technician	• Visually check the sterilising agent circuit: connections, check for leaks • Change the peristaltic pump tube (if applicable)	• Visually check the sterilising agent circuit: connections, check for leaks • Change the H202 filter • Change the peristaltic pump tube (if applicable)
Ⓒ Evaporation/nebulisation		
User	–	–
Maintenance Technician	• Change the seals • Check the heating elements and visually check the temperature • Check the evaporation circuit for leaks	• Check the heating elements • Check the evaporation circuit for leaks • Visually check the injectors • Visually check the condition of the seals and change if needed • Clean the vaporiser to eliminate possible residue
Ⓓ Ventilation system (Vapour)		
User	• Check the air supply pressure	–
Maintenance Technician	• Change the seals • Check the heating elements and visually check the temperature • Check the evaporation circuit for leaks	• Check the heating elements • Check the evaporation circuit for leaks • Visually check the injectors • Visually check the condition of the seals and change if needed • Clean the vaporiser to eliminate possible residue
Ⓔ Connections		
User	• Visually check pipes and connections • Verify the presence and the condition of the connections	• Visually check the pipes and connections • Verify the presence and the condition of the connections
Maintenance Technician	• Replace the compressed air tube and seals • Inspect the tubes and connections • Check the leak-tightness of the circuit	• Replace the air tube and the seals • Check the resistance of the trace heaters
Ⓕ Moyens de mesure, contrôle et pilotage		
User	• Check the temperature probe(s) display Check the level sensor • Check the timer • Change printer paper and the printer «ribbon» (if present)	• Change printer paper and the printer «ribbon» (if present) • Check the temperature, pressure, air flow rate, relative humidity readings, the weight measurement, the injection rate and check the solenoid valves and the fan operate
Maintenance Technician	• Check solenoid valves function • Check flow rate using calibrated flow • Check the temperature probe an temperature regulator (using a calibrated reference sensor) temperature regulator • Check the electrical voltage(s) Perform a grounding and continuity test • Check operating current.	• Check, using calibrated instruments, the temperature, pressure, air flow rate, relative humidity, scales, injection rate. Check operation of solenoid valves and the fan • Check the : – Tube limit switches – Bottle detector switch • Check the electrical voltage(s) Perform a grounding and continuity test

The technicians' equipment must be calibrated to ensure the verification of the measurement probes and actuators.

3.2 EXAMPLE OF A MAINTENANCE TECHNICIAN'S CHECK-LIST

Prerequisite: Before starting work, the technician must be equipped with personal protective equipment (PPE), such as gloves and safety glasses, suited to the risks involved (chemical, biological, etc.), and in compliance with site rules.

3.2.1 MECHANICAL CHECKS (POWER-OFF)

Mechanical checks begin with a by a visual inspection of the generator and the connection systems.

Ⓐ If the equipment is mobile, ensure that it is stable and that the brakes work correctly.
Ⓑ Verify that the sterilising agent reservoir is drained or made safe.
Ⓒ Check that there are no leaks visible to the naked eye.
Ⓓ Identify any potential signs of corrosion.
Ⓔ Ensure that the isolator connection systems (e.g. Camlock connections) are tight enough to ensure leak-tightness.
Ⓕ Check that the limit switches are working correctly.
Ⓖ Check the condition of the biocide circuit and condensate tubes (if there is condensate), tightening hose clamps as needed.
Ⓖ Check the condition of the H_2O_2 pre-filters. Check HEPA filters integrity with an Emery integrity test

3.2.2 CHECKS AFTER THE POWER HAS BEEN SWITCHED ON

➲ Electrical tests

- Check the LEDs on the solenoid valve connectors, switches and warning lights
- Check the low voltage levels
- Check the circuit breakers (relays, breakers and fuses)

➲ Mechanical tests

- Check that the pumps work within the tolerances
- Check the solenoid valves (polarity test)
- Functional testing of the motorized valves
- Check positioning of the measurement probes
- Check the condition of the heating plates (if heaters are used) and burn off potential residue with the heating cycle
- Test for the presence of refrigerant in the cooling column
- Check the effectiveness of the pressure regulator if compressed air is part of the circuit

3.2.3 METROLOGY

This involves checking and calibrating the different control sensors to ensure the proper functioning of the generator and alarms set by the manufacturer. These are presented in **TABLE 12.2**.

The technician should have all the necessary equipment for recalibrating the equipment at his disposal and be capable of supplying traceable calibration certificates for all reference instruments used during calibration.

TABLEAU 12.2 Instrumentation calibration checks for a chemical agent generator

CALIBRATION CHECK	PAA GENERATOR	H_2O_2 GENERATOR
Temperature probes (Supply air air, heating plate)	Thermometer	Recalibrate the temperature probes with the following: • reference thermometer • temperature block
Relative humidity probe	N/A	Saline solution kit ($MgCl_2$, NaCl, K_2SO_4)
H_2O_2 probe	N/A	With H_2O_2 simulator or reference gas (SO_2 + Azote)
Injection rate	Measurement: • by weighing with calibrated scales • with mass flowmeter	Measurement: • by weighing with calibrated scales • with pressure sensors located at the bottom of the reservoir Density correlation chart, if water is used for the calibration of the dosing pump
Air flow rates (rate during gassing and during aeration)	Calibrated flowmeter Measurement of the blower voltage	Calibrated flowmeter Measurement of the blowervoltage
Pressure sensor	Calibrated manometer (scale adapted to measurement range)	Calibrated manometer (scale adapted to measurement range)
Compressed air flow rate	Calibrated flowmeter	Calibrated flowmeter
Time	Calibrated chronometer	Calibrated chronometer Imperative correlation between the times that are displayed, printed, and measured
Alarms and set points	Check alarm response times using a chronometer and the corresponding reference instrument	Check alarm response times using a chronometer and the corresponding reference instrument
Leak-tightness test	Check the circuit of the sterilising agent generator for leak-tightness	This test is imperative after every filter change (including catalytic filters). The equipment will also be tested with the isolator.
Check the Man-Machine/ User-Machine Interface	Ensure that the versions are correct and perform updates as needed	Ensure that the versions are correct and perform updates as needed

FIGURE 12.6 illustrates a few tests.

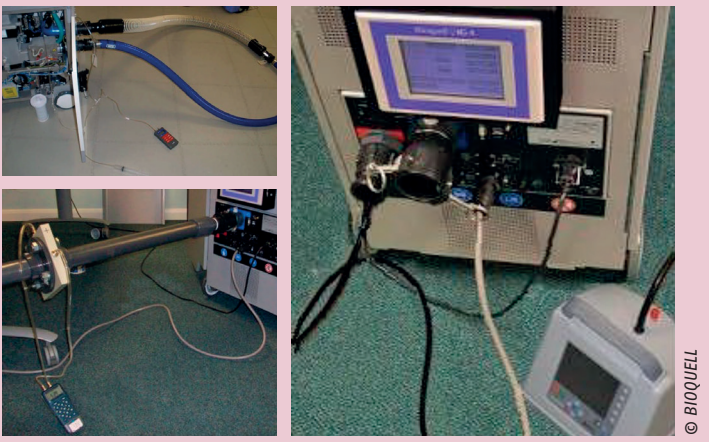

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FIGURE 12.6 – Calibration checks for a sterilising agent generator (Top left: Leak-tightness, bottom left: Air flow rate, Right: Particle counting)

3.2.4 END OF INTERVENTION AND FINAL REPORT

- A test cycle must be run in order to ensure that the generator functions correctly. This may be one the user's routine cycles or a cycle specified by the manufacturer.
- After setting the generator up for routine use, the technician checks that all waste has been removed from the test area.
- A dated intervention report must be given to the client and integrated into the equipment logbook.
- A written test report is delivered whilst on-site or sent after of all interventions and parts that were replaced during the maintenance.

13

MAINTENANCE TEST SHEETS

1 LEAK-TIGHTNESS 139

2 AIR TREATMENT 143

3 INSTRUMENTATION 147

1 LEAK-TIGHTNESS

The following test sheets 1 to 7 constitute a list of maintenance operations. The test sheets are organised according to two essential technical characteristics of the isolator:
- Leak-tightness
- Air treatment

Test sheet 1 : Leak-tightness of the enclosure

Why?	Guarantee the integrity of the enclosure
Who?	Authorised maintenance technician or any authorised production or test operator
What?	• All wall and work surfaces and interfaces including the quality of welds and seals • All interfaces involved in a procedure (for example, oven, autoclave, freeze-dryer, filing machine) including the valves, and means of handling and transfer The isolator must be in standard configuration
How?	**Initial test, visual inspection:** Localisation holes, cuts, damaged welding (stainless steel, PVC etc.), seals in poor condition; **Followed preferably by a pressure drop test** (using the H_2O_2 or PAA agent generator or by inflation with compressed air using the automated leak test function of the isolator when available. If the required leakage rate is not achieved proceed to a localisation of leaks using ammonia. Other gases may be used such as SF6 and He. Ultrasound may also be employed for rigid enclosures.
When?	• Routinely for the visual inspection • When not in routine operation for a pressure drop test (manual or automatic) and if required, the ammonia test • At least annually otherwise frequency based on the supplier's recommendations, use and application,
Where?	*In situ*, when not in use or while operating depending on the test
Acceptance criteria?	**Visual inspection:** • Check for holes or damage to isolator walls • Ensure that seals are not deformed, crushed or missing (clamp) • Check for wear and tear of welds **Leak-tightness test by measuring a drop in pressure:** • criteria defined during the qualification tests Leakage localization test: no leak detected by the system **For leaks with a dimension under or equal to 0.5cm** (or leak diameter that is critical for the process): seal the leak using compatible materials and sealants. **For leaks in the seals or valves:** replacement of leaking component
Checking of remedial action	Repeat the leak test and localise the leak as necessary with the ammonia test or equivalent method
Report(s) (contents)	• Numbers of parts replaced on the work sheet • Date and description of the remedial action
Reference document(s)	• Identification of parts replaced); Supplier documentation • Material certificates and batch number of parts replaced

Test sheet 2: Leak-tightness of gauntlets, gloves/sleeves and half suit

Why?	**Ensure the integrity of gloves; primary source of contamination**
Who?	Authorised maintenance technician or any authorised production or test operator
What?	• Gauntlets, two-piece gloves/sleeves, half-suit and their installation • Glove installation on the cuff ring and sleeve on the shoulder ring and the O-rings used for assembly
How?	**Visual inspection by a properly operator:** – Check for correct installation and verify the presence and position of the O-rings – Visual inspection: • for gauntlets: absence of damage, cuts and impact • for glove/sleeve assemblies: absence of damage, cuts and impact and no damaged welds – no deterioration of material **Physical test using a measurement system** NB: Half suits and gauntlets are tested according to the method described in test sheet 1. In the case of biological or chemical contamination risk, avoid all integrity tests for gloves outside of containment.
When?	• Before and after production: visual inspection and physical test of gaunlets, two-piece gloves/sleeves and half suit • During production: visual inspection of gaunlets, two-piece gloves/sleeves and half suit at each use and as often as possible (at least once per shift).
Frequency?	Check and replacement of gloves and sleeves: to be determined based on the criticality of the process, the nature and criticality of the glove/sleeve location ➲ Visual inspection – At the beginning and the end of each production run – At each shift change, after each intervention ➲ Physical test check – At the beginning and/or at the end of each production run Depending on the technology (by pressure drop test), the physical integrity test for gloves will only be done in nonsterile conditions (between production runs).
Where?	*In situ* for visual observation and physical test
Acceptance criteria?	**Visual observation** – Installation conformity, verification that the seal is firmly in place: • For gauntlets, the glove/sleeve and half suit: no damage, cuts or impacts • No damaged welds – No physical deterioration of the material (swelling, deformation, peeling of the glove bilayer) – Absence of chemical deterioration (sticky aspect) **Physical test** No oxygen rise or fall in pressure outside the manufacturers acceptance criteria.
Treating non-conformities	Replace the defective elements: gauntlets, gloves/sleeves, seals, half suit **For all isolator applications, avoid recycling gloves. Autoclaving gloves may change their physical-chemical characteristics. Defective gloves and sleeves are disposed of.**
Checking of remedial action	• Visual inspection • Physical test by measurement system
Report(s) (contents)	• Number of parts replaced on the work sheet • Date and description of the remedial action
Reference document(s)	• Identification of parts replaced; Supplier documentation • Batch number and material certificates of parts replaced

FIGURE 13.1 shows the component parts and installation of a glove-sleeve assembly.

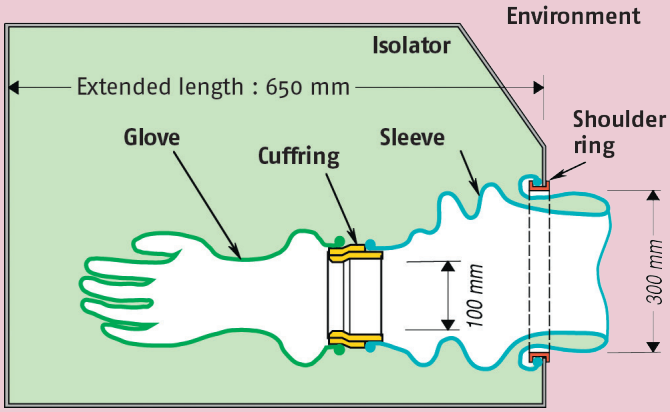

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FIGURE 13.1 – Drawing of glove - sleeve assembly

FIGURE 13.2 shows examples of sleeves

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FIGURE 13.2 – Examples of gloves-sleeve

FIGURE 13.3 shows an example of one-piece EPDM gauntlet

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FIGURE 13.3 – Example of one-piece EPDM gauntlets

Test sheet 3: Leak-tightness of connection systems (Removable and fixed)

Why?	System leak-tightness	
Who?	Authorised maintenance technician or any authorised production or test operator	
What?	All connections on all work surfaces and walls including fixed stationary connections (wall pass-through type)	
How?	• **Visually check seal integrity** (on the fixed and removable parts) and their position • **Physical test** by physical system fixed to the removable and the stationary parts of a transfer port, and container	
When?	• Routinely, by visual inspection, before each connection • In the event of general maintenance program, at least once a year	
Where?	*In situ*, installation operating or at rest depending on the test	
Acceptance criteria?	**Visual observation:** No damage to flat seal. No ondulations or cuts on lip seal. Correct material texture. **Physical system test:** the leakage rate measurement must be at least equal to that of the isolator	
Treating non-conformities	Replacement or seals and defective parts (**FIGURE 13.4**)	 © CAPE EUROPE **FIG 13.4 Example of a damaged lip seal**
Checking of remedial action	• Visual inspection • If the leak tightness rate is not compliant (leaks), run ammonia test	
Report(s) (contents)	• Number of parts replaced part(s) on the work sheet • Date and description of the remedial action	
Reference document(s)	• Identification of parts replaced part(s); Supplier documentation • Batch number and material certificates of parts replaced	

The connection systems taken into account for this form are the following:

- **RTP type systems (e.g. DPTE®) (see FIGURES 13.5 and 13.6)**
- **Disconnectable fluid connections**
- **Systems for the evacuation of liquids**

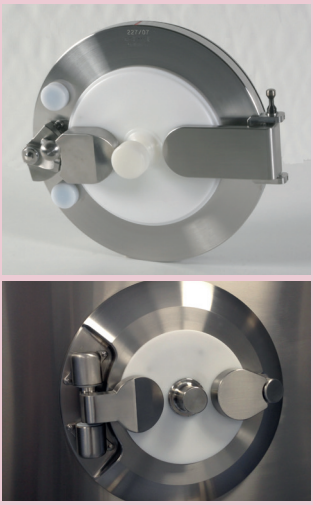
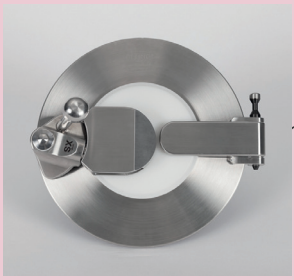
© GETINGE LA CALHÈNE

FIGURE 13.5 – Examples of transfer systems DPTE® -S190 (top left), DPTE®-XO (bottom left), DPTE®-XS 105 (right)

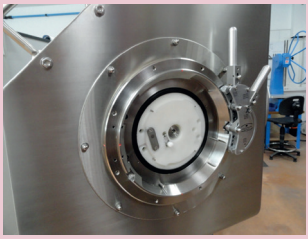
© CAPE EUROPE

FIGURE 13.6 – an example of a transfer system operated from outside the isolator

2 AIR HANDLING

The maintenance operations relating to the isolator air handling unit consists of carrying out visual inspections and the following operations:

- **Pre-filters:** criteria and replacement of pre-filters
- **Highly efficient filters (HEPA/ULPA):** Check (two aspects: loading and air tightness) and replace as needed
- **Fan:** Check motor rotation speed, power consumption, mechanical checks, vibration checks, cosϕ(phase difference), fan clogging
- **Air ducts:** Visual inspection in order to examine the cleanliness of ductwork and cleaning of connections, etc.
- **Humidification/Dehumidification**
- **Heat exchanger**

All air handling related maintenance operations are confirmed by the following tests:
- HEPA filter integrity testing, using either an aerosol generator and photometer the generator and aerosol photometer, or by the optical particle counter method
- A map of the air speeds from which it is possible to deduct the air flow rate and the air change rate of the enclosure.

Performing these tests ensures that the criteria, defined during qualification, are respected after each maintenance operation.

FIGURE 13.7 Air handling schematic for a production isolator with unidirectional flow.

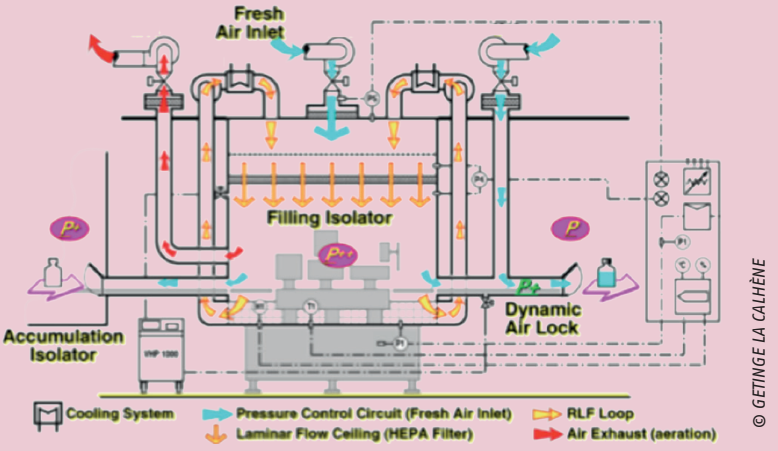

FIGURE 13.7 – An example of an air treatment diagram for a filling isolator with unidirectional flow

Test sheet 4: Pre-filter maintenance

Why?	Ensure the good condition of pre-filters to prevent premature clogging of the HEPA/ULPA filters (pierced pre-filter) or blower malfunctioning in order to guarantee an optimal air flow rate (clogged).
Who?	Authorised maintenance technician or any authorised production or test operator
What?	Pre-filters for all blowers
How?	Visual inspection and replacement.
When?	Preferably when isolator is switched off
Frequency?	Depending on the particulate level in the surrounding atmosphere: • At least once per year if the isolator is in a clean room • 2 to 6 times a year if in classified clean room Frequency should be based on historical data
Where?	*In situ* for replacement or in maintenance workshop if cleaning is possible
Acceptance criteria?	The pre-filter media and its holder must be in good condition with no, holes or tears, nor any permanent stains
Treating non-conformities	Change the pre-filters
Checking of remedial action	Visual inspection
Report(s) (contents)	• Number of parts replaced part(s) on the work sheet • Date and description of the remedial action
Reference document(s)	Number of replaced part; supplier documentation

Test sheet 5: HEPA/ULPA Filter maintenance

Why?	Ensure the quality of the air filtration
Who?	Authorised maintenance technician or any authorised production or test operator *NB: people previously trained to carry out the filter integrity test*
What?	Frame or casing mounted HEPA/ULPA filters
How?	• Using an aerosol generator (according to the methods proposed in the ISO 14644-3, paragraph B.6) • Test for local leaks by performing a full scan of the filter surface and frame seals • Check the isolator supply airflow before generating the aerosol
When?	After each filter change with the isolator in standby mode
Frequency?	1: carry out an inspection twice a year or once every two years 2: Replace the filter according to the following criteria: • Detected non-conformity: presence of leaks, maximum pressure drop, partial loading of filter, speed, non – uniform airflow • Supplier recommendations *NB: the activity that takes place inside the isolator may require more frequent inspections and replacements of the filters. On average this types of filter will last several years before the increase pressure drop makes replacement necessary*
Where?	imperatively *In situ*
Acceptance criteria?	• Local leakage rate below or equal to 0.01% (for a test using the generator and photometer method). • In the even that an aerosol generator and optical particle counter are used, the conformity criteria are given in appendix B (paragraph 6) of the ISO standard 14 644-3.
Treating non-conformities	• Replacement of the filter and visual inspection of installation • Investigation to assess the impact on production
Inspection of remedial action	Integrity test using a test aerosol (e.g. EMERY 3004® and list presented in appendix C, ISO 14644-3) to ensure the proper installation of the filters (media, seals, frame). Check of the pressure drop of the HEPA/ULPA filter during start-up of the on the installation
Report(s) (content)	• Identification of replaced filter on the work sheet(number of replaced filter and its batch number) • Date and description of remedial action
Reference document(s)	• ISO standard 14 644-3 (Clean rooms and associated controlled environments – Part 3 : Test methods); • Supplier documentation including the manufacturer's individual inspection certificate for each HEPA/ULPA filter, according to the EN 1822 standard.

Test sheet 6: Fan maintenance

Why?	Ensure airflow performance conforms to the validated airflow schematic
Who?	Authorised maintenance technician (electrician, electro-mechanical technician), or any authorised production or test operator
What?	All fans used on the isolator (recirculation, extraction, air circulation) and associated equipment
How?	• Check the speed of the distribution fans using a stroboscope. • Check the airflow rates/set points on the extraction, intake and recirculation blowers Then visually inspect the following: • Check the condition of the rotor blades (especially for the blowers in contact with the oxidizing agent) • Check the leak-tightness of all blowers • Check the leak-tightness of the connections • Check the condition of all mechanical fasteners • Check the electrical connections (retightening) • Check the rolling bearings (wear and tear...)
When?	at least once a year, with the isolator in standby
Where?	*In situ* or in the workshop depending on the work to be done
Acceptance criteria?	**Speed checks:** Value between 60 and 70% of the nominal speed (manufacturer's data) **Airflow measurements:** Value between 60 and 70% of the nominal airflow rate **Visual inspection:** Absence of defects (for example: leaks, Play or noise in the rolling bearings corrosion)
Treating non-conformities	• Repair or replace the fan • Other equipment (airflow rate measurement probe, variable speed drive and pressure control) which make it possible to verify the proper functioning of the fans. The non-conformities must not have an impact on the procedure, otherwise an investigation is necessary
Check of remedial action	• Check that the fan is functioning (direction of assembly) • Check the supply airflow rate
Report(s) (content)	• Identify the parts replaced on the work heet • Date and description of the remedial action
Reference document(s)	• Number of replaced fan • Supplier documentation

Test sheet 7: Air duct maintenance

Why?	• Maintain air ducts in clean condition, consistent with the process • Removal of materials likely to catalyse the disinfecting agent
Who?	Authorised maintenance technician or any authorised production or test operator
What?	If applicable, all supply, recirculation and air extraction ducts
How?	*Prerequisite: thorough knowledge of the air circuit* **Visual inspection:** • Check the air duct supports • Check the noise and vibration dampers • Check the leak tightness of the sample points, connections, and bellows • Check the cleanliness of the inside of the ducts (endoscopes...)
When?	Regularly throughout the equipment's life cycle, depending on the process, during shutdown of the isolator (isolator switched off).
Where?	*In situ*
Acceptance criteria?	**Visual inspection:** • The inside of the ducts must not contain any signs of product, stains, or debris generated by the process (e.g. glass), etc. • Thermal insulation must be clean, dry and in place • Final inspection, using an endoscope if applicable
Treating non-conformities	• Clean away impurities • Put insulation, supports, etc. back in place
Check of remedial action	Visual inspection
Report(s) (content)	• Identification of the parts replaced if necessary, on the work sheet • Date and description of the remedial action
Reference document(s)	Number of the replaced part; supplier documentation

NB:
- The heat exchangers and humidifiers/dehumidifiers are maintained in accordance with the equipment manufacturer's maintenance task list.
- All motorised dampers in the air handling circuit, must also be monitored.

3 INSTRUMENTATION

The measurement sensors and their installation must not interfere with the bio-decontamination process.
There are two types of sensors:

➲ Bio-decontamination process sensors

- Pressure sensor
- Possibly, a temperature sensor for the product or the bio-decontamination process
- Humidity sensor
- Speed sensor for unidirectional airflow

➲ Environmental sensors:

- Specific to the isolator: particle monitoring probe, viable particulate monitor, etc.
- Clean room environment: temperature, humidity, etc.

14

TREND ANALYSIS CONCEPTS (PREVENTIVE MAINTENANCE)

Trend analysis is primarily linked to environmental monitoring (non-viable and viable particulate).

Inside the isolator, the parameters that can be trended are:
- Pressure, when this parameter is not regulated
- Leak-tightness
- Consumption of sterilising agent
- Temperature, etc.
- H_2O_2 concentration
- Surface temperature
- Particle and microbiological controls

NB: For monitoring requiring microbiology culture methods, results after 3 to 7 days.

In the event of a change in deviation rate **the frequency of maintenance operations** is associated with trend analysis.
- trend analyses can be used to modify the frequency of maintenance operations (objective: increased system availability); maintenance operations may be more frequent or, on the contrary, less frequent.
- In collaboration with the quality department inspection and calibration of all sensors must be carried out

All users should compile a review of past maintenance reports.
Each user should set up his own list of the critical parameters requiring regular verification (e.g. pressure, sterilizing agent consumption).

Ref.: PDA, TR 68: Risk-Based Approach for Prevention and Management of Drug Shortages.

15

MAINTENANCE WASTE MANAGEMENT

The waste covered in this section is limited to that which results from isolator maintenance operations and the waste from cleaning operations before and after maintenance.

This includes the following:

➲**liquid cleaning products (detergents and disinfectants) as well as the associated cleaning fabrics**

➲**worn and replaced parts:**

- ventilation (filters)
- handling (gloves, sleeves, half suits...)
- operating (mechanical parts such as, for example, seals, valves, etc.)

The project manager is responsible for the waste produced during a maintenance operation and is responsible for disposing of it. Risk assessment, performed before the operation, makes it possible to judge the types of dangers presented by the waste. All waste must be disposed of according to the type of hazard identified. The sub-contract maintenance company must correctly handle hazardous waste and may offer to dispose of the waste or have it disposed of. It is important not to forget to dispose of overalls as well as single use gloves.

There are two types of hazardous waste:

➲**Chemical hazard:**

- The waste is identified in France by a 6-digit code, indicating their nature and origin, using a hazard warning label.
- Liquid effluents are collected in air-tight chemically compatible containers.
- Solid waste is wrapped using strong and chemically compatible packaging.

Depending on the activity in the laboratory, chemical hazardous waste could include filters, contaminated personal protection equipment. These must also be disposed using the disposal procedure for hazardous chemical products.
Waste is disposed of in compliance with applicable regulations

➲**Biological or infectious hazard:**

The regulations regarding the disposal of infectious medical waste (DASRI, déchets d'activité de soins à risques infectieux) that apply to diagnostic activities or to medical treatment, education, research and production, human or veterinary medicine and thanatopraxy[1] must be followed.
Depending on the procedures performed in the laboratory, the filters may be contaminated by microorganisms, as may the single use personal protection equipment used by the maintenance personnel. These items will be placed in resistant packaging immediately after use and disposed of with the potentially infectious clinical waste.

[1] *Articles R1335-1 to R1335-12 of the Public Health Code; amended French decree of 7 September, pertaining to the inspection of the disposal methods for potentially infectious clinical waste and anatomical parts; amended decree of 7 September 1999, pertaining to the methods for storing of potentially infectious clinical waste and anatomical parts.*

The DHOS/E4/DGS/SD.7B/DPPR n°2006-58 french Circular makes a distinction between two sets of procedures for the incineration of waste generated from anti-cancer treatment:
- **That of concentrated anti-cancer medication**, disposed of through a set of methods specific to hazardous waste, and therefore incinerated at 1,200°C. The same is true for ventilation filters, particularly those found in isolators.
- **That of DASRI** for the other waste **disposed of at a temperature of 800°C**.

The PPE worn by technicians and assistants during the isolator maintenance operations will be disposed of using the procedures for household waste.

FIGURE 15.1 shows an example of the disposal of DASRI waste produced in an isolator.

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FIGURE 15.1 – RTP transfer system DASRI waste container

French National Institute for Research and Safety (INRS) Bibliography (see CHAPTER 17):
• *ED 824, Hazardous industrial waste. Think safety 2010 Déchets dangereux dans l'entreprise. Pensez sécurité ! 2010*
http://www.inrs.fr/accueil/produits/mediatheque/doc/publications.html?refINRS=ED%20824
• *ED 918, Infectious watse disposal Déchets infectieux. Disposing of DASRI and associated waste Elimination des DASRI et assimilés, 2013*
http://www.inrs.fr/accueil/produits/mediatheque/doc/publications.html?refINRS=ED%20918

16

GLOSSARY AND ABBREVIATIONS

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1 SUMMARY OF ISOLATOR DEFINITIONS

1.1 APPLICABLE REGULATORY DOCUMENTS

French Best Practices for Preparations, 2007 (paragraph 6.4.3.1): «*The isolator is a sealed enclosure that does not exchange unfiltered air and contaminants with the surrounding environment, thereby maintaining the sterility of the inside of the enclosure. It creates a physical, airtight barrier between the preparation, the operator and the environment.*»

Sterile Drug Products – Produced by Aseptic Processing – Current Good Manufacturing Practice (glossary): Isolator is "*A decontaminated unit, supplied with Class 100 (ISO 5) or higher air quality, that provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding cleanroom air and personnel). There are two major types of isolators:*
– **Closed isolator systems** *exclude external contamination from the isolator's interior by accomplishing material transfer via aseptic connection to auxiliary equipment, rather than use of openings to the surrounding environment. Closed systems remain sealed throughout operations.*
– **Open isolator systems** *are designed to allow for the continuous or semi-continuous ingress and/or egress of materials during operations through one or more openings.*
Openings are engineered (e.g., using continuous overpressure) to exclude the entry of external contamination into the isolator".

1.2 STANDARDS

NF ISO 14644-7 standard: An isolator is a separative device defined as equipment utilizing constructional and dynamic means to create assured levels of separation between the inside and outside of a defined volume.
NB: Examples of separative devices specific to the industry are clean air hoods, containment cabinets, glove boxes, isolators and mini-environments.
(See **TABLE 16.1**)

TABLEAU 16.1 Extract from the stable of separative devices (4 principles applicable to the isolator), according to ISO 14644-7.

SEPARATION APPROACHES	MEANS	DEVICE DESCRIPTOR	EXAMPLES OF TERMS IN COMMON USAGE AND SYNONYMS
Unrestricted air overspill	Aerodynamic measures and filtration	Open – no curtains or screens. Operator equipped with normal cleanroom garments and gloves may reach into device for access and transfert. Clean zone is at positive pressure	Clean air device, laminar flow hood, clean air hood
Restricted air overspill	Aerodynamic and physical	Access severely restricted by curtains or fixed screens	Laminar-flow hood, clean air hood, directed air hood, clean work station
Nominaly enclosed – not capable of contained/controlled atmosphère operation	Aerodynamic and physical	Nominaly enclosed, may incorporate access devices and transfer devices	Point-of-fill device, filling tunnel
Nominaly enclosed – may be capable of contained/ controlled atmosphère operation – single or dual mode	Aerodynamic and physical	Large degree of physical separation in design. May be capable of controlled/ contained atmosphere operation	Filling tunnel, point-of-fill device, laminar-flow tunnel, clean tunnel, sterilising oven, mini-environments for electronics
Closed/undefined pressure integrity – performance may be hourly leak rate or other parameter	Physical	Closed devices with undefined integrity. May have flexible film walls	Isolators, glove bags, powder transfer control or hopper, flexible film/half-suit isolator, mini-environments for electronics
Low pressure integrity/ high hourly leak rate enclosure – positive or negative pressure operation	Physical	Rigid construction allows pressure integrity test of leak rate. May be operated under negative pressure	Isolators, glove-boxes, powder transfer control or hopper, animal test house isolator, biochemical instructional isolators, containment enclosures
Medium pressure integrity/medium hourly leak rate enclosure – positive or negative pressure operation	Physical	Medium pressure integrity	Isolators, glove-boxes, containment enclosures
High pressure integrity/ low hourly leak rate enclosure – positive or negative pressure operation	Physical	High pressure integrity, vacuum and inert gas operation, containment at molecular level	Isolators, glove-boxes, nuclear glove boxe, low molecular containment enclosures

SOURCE : ISO 14 644-7

ISO 13408-6 standard: An isolator is: a sealed enclosure capable of preventing ingress of contaminants by means of total physical interior/exterior separation, and capable of being subject to reproducible interior bio-decontamination.

Note 1: An isolator can range in size from a small box to a large room.
Note 2: Physical separation can be achieved by an absolute solid wall completely surrounding the entire interior, where any discontinuities in such wall are equipped to physically prevent ingress of contaminants. Examples of such physical protection include pass-through air locks for sterile or bio-decontaminated goods, (HEPA)-filtered (high efficiency particulate air-filter) or sterilized inflow air, and high flow rate of outflow air through a minimal-sized orifice. Operators always remain totally separated from the interior of an isolator by means of an absolute physical barrier. (paragraph 3.3)

Standard ISO 13408-6 also defines the isolator system as:
Isolator with transfer systems and ancillary equipment used for aseptic processing (paragraph 3.4)

ISO 10648-2 standard: This standard defines a containment enclosure as: "*Enclosure designed to prevent the leakage of the products contained in the environment concerned into the external environment, or the penetration of substances of the external environment into the internal environment, or both at the same time.*"

Standard ISO 10648-2 defines 4 classes of leak-tightness presented in **TABLE 16.2**:

TABLEAU 16.2 Leak tightness classes according to Standard ISO 10648-2

CLASS	HOURLY LEAKAGE RATE T_F H^{-1}	EXAMPLE
1 *)	≤ 5 x 10^{-4}	Controlled atmosphere containment enclosure under vacuum or inert gas
2 *)	< 2,5 x 10^{-3}	Controlled atmosphere containment enclosure under vacuum or inert gas or with constant dangerous atmosphere
3	< 10^{-2}	Containment enclosure with constant dangerous atmosphere
4	< 10^{-1}	Containment enclosure with potentially dangerous atmosphere

1.3 RECOMMENDATIONS

ISPE, 2012 : *A leaktight enclosure designed to protect operators from hazardous/potent processes or protect processes from people or detrimental external environments or both. A basic enclosure consists of a shell, viewing window, glove/sleeve assemblies, supply and exhaust filters, light (s), gauge (s), Input and Output openings (equipment door airlocks, Rapid Transfer Ports (RTPs), etc.), and various other penetrations.*

There are two types of isolators

1. Closed Isolators – *Isolators operated as closed systems do not exchange unfiltered air or contaminants with adjacent environments. Their ability to operate without personnel access to the critical zone makes isolators capable of levels of separation between the internal and external environment unattainable with other technologies. Because the effectiveness of this separation, closed isolators are ideally suited for application in the preparation of sterile and/or toxic material. Aseptic and Containment isolators are two types of closed isolators.*

2. Open Isolators – *Open isolators differ from closed isolators in that they are designed to allow for the continuous or semi-continuous egress of materials during operation, while maintaining a level of protection over the internal environment. Open isolators are decontaminated while closed, and then opened during manufacturing. Open isolators typically are used for the aseptic filling of finished pharmaceuticals.*

NOTE: Containment, barrier isolation and isolation all refer to the same technology, which is enclosing an environment. In the interest of clarifying the existing confusion between the terms "isolators" and "barriers", and providing authoritative implementation and validation of isolation technology, the Parenteral Drug Association (PDA) published in October 2000 the Draft for Technical Report No. 34 "Design and Validation of Isolator Systems for the Manufacturing and Testing of Health Care Products".

Publication Source: ISPE Good Practice Guide: Quality Laboratory Facilities

➲ **ISPE, 2011 :** *A decontaminated unit meeting Grade 5 conditions that provides uncompromised, continuous, isolation of its interior from the surrounding environment. Isolators can be "open" or 'closed".*
– Isolator, Closed
An isolator that may exchange air with the surrounding environment only through microbially retentive filters.
– Isolator, Open
An isolator that transfers air directly to the surrounding environment through openings (e.g., "mouseholes") that preclude the ingress of microbial contamination.

Publication Source: ISPE Baseline® Guide, Vol. 3: Sterile Product Manufacturing Facilities (Second Edition)

2 GLOSSARY

STERILIZING AGENT: Physical or chemical entity, or a combination of entities, that have sufficient microbicidal activity to achieve sterility under defined conditions.
ISO 14937, 3.31, 2009 (ISO/TS 11139, 2006, definition 2.50)

BACTERICIDE: Product that irreversibly inactivates vegetative bacteria under defined conditions.
Note 1 to entry: The adjective derived from "bactericide" is "bactericidal".
EN 14885, july 2015

BIO-DECONTAMINATION: Removal of microbiological contamination or its reduction to an acceptable level.
ISO 13408-6, 3.1, june 2005

BIOLOGICAL INDICATOR: Biological indicators are:
Biological indicators are standardised preparations of selected micro-organisms used to assess the effectiveness of a sterilization procedure. They usually consist of a population of bacterial spores placed on an inert carrier, for example a strip of filter paper, a glass slide or a plastic tube. (European Pharmacopeia-9th edition – 5.1.2).

Or a *test system containing viable microorganisms providing a defined resistance to a specified sterilization process, ISO/TS 11139, 2006, 2.3*

CARRIER: Supporting material on or in which test microorganisms are deposited.
ISO 11138-1, 3.2

CHEMICAL INDICATOR: Test system that reveals change in one or more pre-defined process variables based on a chemical or physical change resulting from exposure to a process, *ISO 14937, 3.4, 2009. (ISO/TS 11139, 2006, definition 2.6).*

CLEANING (OF A SURFACE): The series of operations to ensure the correct level of cleanliness, appearance, comfort and hygiene, that uses, in various proportions the following: chemical action, mechanical action, temperature and exposure time, French NF X 50-790, *December 1995*

CONDITION-BASED MAINTENANCE: Preventive maintenance based on assessment of physical condition, *ISO 14224, 2016*
Or, Preventive maintenance based on the

results of a combination of equipment monitoring, inspections and tests, *EN 13306*
NB: Monitoring, inspections and testing can be scheduled, continuous or when requested, *EN 13306*

CORRECTIVE MAINTENANCE: Maintenance carried out after fault detection to effect restoration, ISO 14224, 2016
Or, Maintenance that is carried out after a failure and intended to restore an item to a state in which it can perform a required function, *EN 13306*

CRITICAL SURFACES: *Surfaces in direct contact with the product or the operation.*
In the GMP, the adjective «critical» is defined as «describing a procedure step, a procedure condition, a control requirement, or any other parameter or important point that must be controlled within pre-determined criteria in order to guarantee that the active substance complies to specifications.".

D VALUE: is the value of a parameter of sterilisation (duration or absorbed dose) required to reduce the number of viable organisms to 10 per cent of the original number, *European Pharmacopeia- 9th edition – 5.1.2.*
Or, D_{10} Value: time or dose required to achieve inactivation of 90 % of a population of the test microorganism under stated conditions, *ISO 11138-1, 2017*

DEFERRED CORRECTIVE MAINTENANCE: Corrective maintenance that is not carried out immediately after the detection of a failure, but is delayed, in accordance with a defined maintenance procedure, *EN 13306*

DESIGN QUALIFICATION (DQ): Verification that the proposed specification for the facility, equipment or system is suitable for the intended use, *ISO 13408-1, 2008, 3.15.*
Or,
The next element in the qualification of equipment, facilities, utilities, or systems is DQ where the compliance of the design with GMP should be demonstrated and documented. The requirements of the user requirements specification should be verified during the design qualification, *Annex 15 EU*

EMERGENCY CORRECTIVE MAINTENANCE: Corrective maintenance that is carried out without delay after detecting a failure in order to avoid unacceptable consequences, *EN 13306*

FUNGICIDE: Product that irreversibly inactivates fungi (moulds and yeasts) and their spores under defined conditions.
Note 1 to entry: The adjective derived from "fungicide" is "fungicidal".
EN 14885, july 2015

INOCULATED CARRIER: Supporting material on or in which a defined number of viable test organisms have been deposited, *ISO 11138-1, 2017, 3.8.*

INSTALLATION QUALIFICATION (IQ): Process of obtaining and documenting evidence that equipment has been provided and installed in accordance with its specification, ISO 13408-1, *2008, 3.25 (ISO14937, 2009. 3.11)*
Or,
3.8. IQ should be performed on equipment, facilities, utilities, or systems.
3.9. IQ should include, but is not limited to the following:
i. Verification of the correct installation of components, instrumentation, equipment, pipe work and services against the engineering drawings and specifications;
ii. Verification of the correct installation against pre-defined criteria;
iii. Collection and collation of supplier operating and working instructions and maintenance requirements;
iv. Calibration of instrumentation;
v. Verification of the materials of construction.
Annex 15, EU

NOMINAL CONDITIONS: Routine operating conditions of the isolator

OPERATIONAL QUALIFICATION (OQ): Process of obtaining and documenting evidence that installed equipment operates within

predetermined limits when used in accordance with its operational procedures, *ISO 13408-1, 2008, 3.27 (ISO 14 937, 2009, 3.14)*
Or
3.10. OQ normally follows IQ but depending on the complexity of the equipment, it may be performed as a combined Installation/ Operation Qualification (IOQ).
3.11. OQ should include but is not limited to the following:
i. Tests that have been developed from the knowledge of processes, systems and equipment to ensure the system is operating as designed;
ii. Tests to confirm upper and lower operating limits, and /or "worst case" conditions.
3.12. The completion of a successful OQ should allow the finalisation of standard operating and cleaning procedures, operator training and preventative maintenance requirements.
Annex 15, EU

PERFORMANCE QUALIFICATION (PQ): Process of obtaining and documenting evidence that the equipment, as installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined criteria and thereby yields product, *ISO 13408-1, 2008, 3.28 (ISO 14937, 2009, 3.16)*.
Or
3.13. PQ should normally follow the successful completion of IQ and OQ. However, it may in some cases be appropriate to perform it in conjunction with OQ or Process Validation.
3.14. PQ should include, but is not limited to the following:
i. Tests, using production materials, qualified substitutes or simulated product proven to have equivalent behaviour under normal operating conditions with worst case batch sizes. The frequency of sampling used to confirm process control should be justified;
ii. Tests should cover the operating range of the intended process, unless documented evidence from the development phases confirming the operational ranges is available.
Annex 15, EU

PLANNED MAINTENANCE: Preventative maintenance carried out according to a pre-determined schedule or a set number of operations regardless of the condition of the equipment. NB: The time intervals or the number of operations can be established by a failure mode analysis of the equipment, *EN 13306*

PREDICTIVE MAINTENANCE: Condition-based maintenance based on the results of a continuous monitoring or known characteristics and monitoring parameters critical to the deterioration of an item, *EN 13306*

PREVENTIVE MAINTENANCE: Maintenance carried out at predetermined intervals or criteria intended to reduce the probability of failure or a drop in the operating performance of an item, *EN 13306*

QUALIFICATION: Action of proving that any equipment works correctly and actually leads to the expected results. The word validation is sometimes widened to incorporate the concept of qualification, GMP
Or,
Documented process used by the health care manufacturer product to assure the reliability and capability of equipment and/or processes before approval for use in manufacturing, *ISO 13408-1, 2008, 3.30*.

AIR EXCHANGE RATE: rate of air exchange expressed as number of air changes per unit of time and calculated by dividing the volume of air delivered in the unit of time by the volume of the space, *ISO 14644-3, 2005*
NB:
- For an isolator supplied with fresh air only, the inlet air flow per hour determines the volume air exchange rate.
- For an isolator with both fresh air and recycled air (or re-circulation air), the volume air exchange rate is commonly called the air change.

SCHEDULED MAINTENANCE: Preventive maintenance carried out according to a predetermined schedule or according to a defined number of operations.
NB: Deferred corrective maintenance can also be scheduled, *EN 13306, 2010*.

SHORT TERM EXPOSURE LIMIT (STEL): According to INRS (French National Safety Institute), the occupational exposure limit of a chemical refers to the permissible concentration in the air that a person can breathe during a determined period of time. *The limit aims to provide protection against deterioration of the health of employees in contact with the product in question. The value is expressed in volume (ppm or part per million), in weight (mg/m³) or in fibre by volume unit (f/m³). Short term exposure limit values are based on a reference period of 15 minutes. They are all intended to avoid the toxic effects from short term exposure. The STEL replaces the former TLV measured over a maximum of 15 minutes.*

STERILE: Free from viable microorganisms, *ISO 13408-1, 2008, 3.34*
(ISO/TS 11139, 2006, definition 2.43)

STERILISATION: A process that destroys or eliminates all forms of microbial life (including spores) and is carried out by physical or chemical methods. Steam under pressure, dry heat, ethylene oxide gas, hydrogen peroxide gas plasma, and liquid chemicals are the principal sterilizing agents used in healthcare settings. *(USP <800>).*
Or,
Validated process used to render a product free from viable microorganisms, *ISO 14937, 2009, 3.28*

STERILISING AGENT: Physical or chemical entity, or combination of entities, that havesufficient microbiocidal activity to achieve sterility under defined conditions, *ISO/TS 11139, 2006, definition 2.50*

STERILITY TEST: Technical operation performed as part of development, validation or requalification to determine the presence or absence of viable microorganisms on product or portions thereof, *ISO/TS 11139, 2006, definition 2.54*

OPERATIONAL EXPOSURE LIMIT (OEL): According to French INRS (National Safety Institute), the operational exposure limit is measured over an 8 hour period. The OEL is intended to protect the employees from the delayed effects of contaminants. *The OEL is based on the average value over the 8 hours.*
The OEL can be exceeded for short periods of time, provided the exposure does not exceed the corresponding STEL if it exists for the product. Caution: 2 substances with the same OEL do not necessarily have the same level of hazard.

VALIDATION: Documented procedure for obtaining, recording and interpreting the results required to establish that a process will consistently yield product complying with predetermined specifications, *ISO 14937, 2009, 3.35*
Or
Action of proving, in accordance with the principles of Good Manufacturing Practice, that any procedure, process, equipment, material, activity or system actually leads to the expected results, GMP

WORST-CASE: Condition or set of conditions that encompass the upper and lower limits, within the limits of the operating procedures and which carry the greatest risk of failure of the product or process that compared with ideal conditions. Worse case conditions will not necessarily cause failure of the product or the process,
Or,
Set of conditions which represent the highest challenge to product integrity and safety which will be accepted during validation and routine production, *ISO 13408-6, 2005, 3.13.*

3 ABBREVIATIONS

ACH Air change per hour
ACR Air change rate
ANSM Agence Nationale de Sécurité du Médicament et des produits de santé (French Agency for the Safety of Drug and Healthcare Products) (http://ansm.sante.fr)
ARR Air renewal rate
ARRH Hourly air renewal rate
ATCC American Type Culture Collection (Private organization that manages and distributes a collection of microorganism strains.)
ATMP Advanced therapy medicinal products
BI Biological Indicator
BPF Bonnes Pratiques de Fabrication et de productions pharmaceutiques (Good Manufacturing Practices and pharmaceutical production)
BSC Biosafety cabinet
CCTP Cahier des Clauses Techniques Particulières (Special technical specifications)
CD Cycle development
CFU Colony-forming unit
CI Chemical indicator
CIP Clean in place
CMR Carcinogenic, Mutagenic and Reprotoxic
CNRS Centre National de la recherche Scientifique (www.cnrs.fr) (The French National Centre for Scientific Research)
CSM Chlorosulphonyl Polyethylene
DASRI Déchets d'Activités de Soins à Risques Infectieux (Infectious medical waste)
DDS Detailed Design Specifications
DEHS Diethylhexyl Sebacate
DPTE Double porte de transfert étanche (Leak-tight double door transfer port)
DQ Design Qualification
EP European Pharmacopeia
EPDM Ethylene Propylene Diene Monomer
FAT Factory Acceptance Test
FDA Food and Drug Administration (www.fda.gov)
FRS Functional Requirements Specifications
GMP Good Manufacturing Practice
HDPE High-density polyethylene
HEPA High Efficiency Particulate Air filter
HPLC High Performance Liquid Chromatography
H_2O_2 Hydrogen peroxide
INRS Institut National de Recherche en Santé et Sécurité au travail (French Institute) (www.inrs.fr)
IPA Isopropyl alcohol
IQ Installation Qualification
ISPE International Society for Pharmaceutical Engineering (www.ispe.org)
LFH Laminar flow hood
MFT Media Fill Test
MOT Micro-Organismes et Toxines hautement pathogènes (highly pathogenic micro-organisms and toxins)
OELs Occupational Exposure Levels
OQ Operational Qualification
PAA Peracetic acid
PE Polyethylene
PEEK Polyetheretherketone
PID Piping and Instrumentation Diagram
PMMA PolyMethyl MethAcrylate
PP Polypropylene
PPE Personal protective equipment
PPI Préparations Pour Injectables (Injectable preparations)
PQ Performance Qualification
PTFE Polytetrafluoroethylene
PVC Polyvinyl chloride
RABS Restricted Access Barrier System
RTP Rapid Transfer Port
SAL Sterility assurance level
SAT Site Acceptance Test
SLR Spore log reduction
STEL Short Term Exposure Limit (15 min.)
TLV Threshold Limit Value for 8 hours of work
TOC Total organic carbon content
TS Trypticase Soy
ULPA Ultra Low Penetration Air Filter
USP United States Pharmacopeia
WFI Water for injection
WHO World Health Organization (www.who.int/fr)
ZAC Zone d'Atmosphère Contrôlée (Cleanroom)

17

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<800> Biological Indicators for Sterilization
<1035> Biological Indicators for Sterilization
<1116> Microbiological evaluation of clean rooms and other environments
<1208> Sterility testing – Validation of isolator systems
draft chapter 1229.11 – Vapor phase sterilization–

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2.6.1. Sterility
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5.1.2. Biological indicators of sterilisation

The Biocidal Product Regulation (BPR, Regulation (EU) 528/2012) concerns the placing on the market and use of biocidal products, which are used to protect humans, animals, materials or articles against harmful organisms, like pests or bacteria, by the action of the active substances contained in the biocidal product.

The CLP Regulation ensures that the hazards presented by chemicals are clearly communicated to workers and consumers in the European Union through classification and labelling of chemicals.

The consolidated version of the Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) incorporates all of the amendments and corrigenda to REACH until the date marked in the first page of the regulation. REACH is a regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be

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EXAMPLES OF ISOLATORS

STERILITY TEST ISOLATOR

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Sterility test isolator (ISOFLEX-S, 3 gloves and ISOFLEX-S, 4 gloves)

ISOLATOR DESIGNED AND MANUFACTURED BY GETINGE LA CALHÈNE

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APPENDIX: PARTICLE LOCATIONS ACCORDING TO ISO 14644-1

TABLEAU 18.1 - sampling points based on the surface area of the clean room (according to ISO 14644-1) – up to 1,000 m²

SURFACE AREA OF THE CLEAN ROOM (M^2) LESS THAN OR EQUAL TO	MINIMUM NUMBER OF SAMPLE POINTS (N_L)
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10
56	11
64	12
68	13
72	14
76	15
104	16
108	17
116	18
148	19
156	20
192	21
232	22
276	23
352	24
436	25
636	26
1 000	27

Note 1 If the surface area of the zone in question falls between two of the table values, the greater value should be used.

Note 2 In the case of unidirectional flow, the surface area in question can be considered as being the perpendicular cutting plane with respect to the airflow direction. In all other cases, the surface in question will be the horizontal plane of the clean room or the clean zone.

SOURCE : ISO 14644-1, TABLE A1

ISBN 978-2-910218-21-8
EAN 9782910218218
Dépôt légal march 2017

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GUIDE **ASPEC**

ISOLATORS: QUALIFICATIONS AND MAINTENANCE

CLEAN ROOMS, CONTROLLED ENVIRONMENTS AND CONTAINMENT ZONES

The isolator is a barrier system, a close protection device, but also an air treatment device. In certain cases, it is also a containment enclosure. Isolators are now well established in many industrial sectors (pharmaceutical industry, fine chemistry, biotechnology, food-processing industry...), in hospital pharmacies, as well as in laboratories (specifically in biohazard containment zones). Isolators are used to protect employees and/or for aseptic production or microbiological tests. This publication will be of particular interest to isolator designers and those using isolators for the preparation of sterile medical products. However, the extensive content and the proposed approach can be applied to isolators used for other activities.

This publication is an update of first French edition published in 2002 and the work of multidisciplinary team. This edition focuses on qualification operations and features new chapters on maintenance. Surface sterilisation (or bio-decontamination), not to be confused with core sterilisation, are also addressed with a focus on the isolator qualification (DQ, IQ, OQ, and PQ).

This publication is a truly educational tool. It contains roughly 20 practical information sheets. This second edition constitutes a reference tool for isolator technology professionals.

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ASPEC © march 2017
64 rue Nationale – 75013 PARIS
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ISBN 978-2-910218-21-8
EAN 9782910218218
Prix :
- 99 euros TTC (French paper edition)
- free (English edition)