1. Cleanrooms for the Production of Cytotoxic Drugs Chapter (Heading)

1.1. DEFINITION

Cleanrooms are controlled environments specifically designed to minimize the introduction, generation, and retention of airborne particles and microorganisms. In the context of cytotoxic drug preparation, cleanrooms ensure a sterile and safe setting to protect both the product and personnel.

A cleanroom typically meets the following conditions :

It has controlled levels of contamination, defined by the number of particles per cubic meter.

It is built and maintained according to specific ISO classifications (e.g., ISO Class 5, ISO Class 7).

It includes filtered airflow systems (HEPA or ULPA filters) to trap contaminants.

It follows strict procedures for personnel behavior, gowning, and cleaning.

Cleanrooms are vital in cytotoxic drug production due to the high risk of product contamination and the toxic nature of the drugs, which could harm staff and patients.

1.2. Which Cleanrooms Are Required

The production of cytotoxic drugs requires a structured suite of rooms, each serving a distinct purpose. These may include:

Airlocks (Personnel and Material Locks :

Airlocks are used to keep contaminants out of cleanrooms when personnel enter or exit a cleanroom or when material is brought into or taken out of cleanrooms. Only 1 door is opened at a time to prevent contaminants from having a straight path into the cleanroom. Air locks typically have HEPA filtration to remove contaminants from inside the airlock and interlocked doors to ensure that only one door can be opened at a time.

Types of airlocks include personnel airlocks, material transfer airlocks, pass thru's, and air showers.

Table 1 — ISO Classes of air cleanliness by particle concentration

ISO Class number (*N*)	Maximum allowable concentrations (particles/m³) for particles equal to and greater than the considered sizes, shown below[a]					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
1	*10*[b]	d	d	d	d	e
2	100	*24*[b]	*10*[b]	d	d	e
3	1 000	237	102	*35*[b]	d	e
4	10 000	2 370	1 020	352	*83*[b]	e
5	100 000	23 700	10 200	3 520	832	d, e, f
6	1 000 000	237 000	102 000	35 200	8 320	293
7	c	c	c	352 000	83 200	2 930
8	c	c	c	3 520 000	832 000	29 300
9g	c	c	c	35 200 000	8 320 000	293 000

[a] All concentrations in the table are cumulative, e.g. for ISO Class 5, the 10 200 particles shown at 0,3 µm include all particles equal to and greater than this size.

[b] These concentrations will lead to large air sample volumes for classification. Sequential sampling procedure may be applied; see Annex D.

[c] Concentration limits are not applicable in this region of the table due to very high particle concentration.

[d] Sampling and statistical limitations for particles in low concentrations make classification inappropriate.

[e] Sample collection limitations for both particles in low concentrations and sizes greater than 1 µm make classification at this particle size inappropriate, due to potential particle losses in the sampling system.

[f] In order to specify this particle size in association with ISO Class 5, the macroparticle descriptor M may be adapted and used in conjunction with at least one other particle size. (See C.7.)

[g] This class is only applicable for the in-operation state.

Preparation Room: A Grade C or D area where materials are unpacked and wiped down before transfer.

Weighing Room (Optional): May be included for precise weighing of active substances under contained conditions.

Production Room: Typically a Grade B environment with Grade A laminar airflow hoods or isolators, where aseptic compounding of cytotoxic drugs takes place.

Waste Handling Area: Separated zone for disposal of cytotoxic waste, with proper airflow to avoid cross-contamination.

These rooms must be designed to support unidirectional workflow (from less clean to more clean areas) and must comply with Good Manufacturing Practice (GMP) standards.

1.3. Technical Requirements for Cleanrooms

To maintain a clean environment suitable for cytotoxic drug preparation, specific technical parameters must be controlled and regularly monitored:

Pressure Conditions: Differential pressure must be maintained (e.g., positive pressure in Grade A/B areas relative to adjacent areas) to prevent backflow of contaminated air. For hazardous substances, negative pressure isolators may be used to protect staff.

Temperature: Must be controlled and generally maintained between 18–22°C to ensure product and personnel comfort.

Humidity: Controlled between 30% and 65% to prevent static charge and ensure comfort.

Particles: Particle counts must meet ISO standards (e.g., ISO 5 for laminar airflow zones), especially during "at rest" and "in operation" conditions.

Air Changes per Hour (ACH): Typically, high-efficiency systems provide 20–40 ACH or more, depending on room classification.

These parameters ensure that the cleanroom remains within qualified conditions, minimizing contamination risks during the handling of high-risk cytotoxic agents.

1.4. Cleanroom equipment according to classification – equipment and furnishings (e.g., workbenches, isolators, other equipment, furniture, etc.) – Zs.Oras

1.4.1. Equipment

The basic requirements for equipment used for the preparation of cytostatic mixed infusions is the protection of:

- the product
- the personnel
- the environment and
- the protection against cross-contamination

1.4.2. Product protection

During aseptic compounding (filling, opening sterile products) within the device ISO Class 5/ Grade A environment must be provided using HEPA filtered, laminar air flow (LAF) velocity to sweep particles away from the product. But LAF hoods only protect the product, they do not offer operator protection. It is not permitted for hazardous materials, so additional ventilation technology must be introduced.

1.4.3. Personnel and environmental additional protection

Only safety cabinets (safety workbenches) and isolators (glove boxes) ensure both person and product protection from contamination using negative pressure and balanced vertical air flows. (Hoods with horizontal air flow are not suitable for CMR drugs, because they do not protect the operator and do not prevent cross contamination.)

Inward air is directed downwards into a plenum below the work surface and is filtered before being redirected into the work area as a laminar down flow of clean air. The balance of this laminar down flow with the incoming air provides an air curtain at the open front which provides the operator protection. The split between exhausted and recycled air on each cycle is normally in the range 20/80 to 30/70.

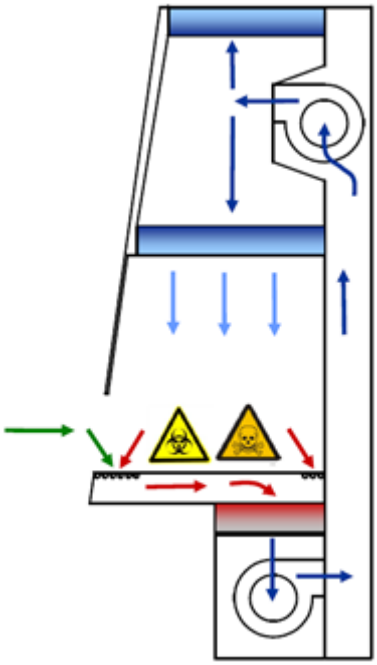

Figure: Airflows (room, clean and contaminated air) in a safety cabinet

Based on these regulations a safety workbench for anticancer drugs (SWC) according to DIN 12980, Isolators for Cytotoxics (IFC) and biohazard safety cabinets (BSCs) according to PIC/S guide can be used.

SWCs and BSCs have to operate in class B room or class C if the process is validated. An isolator may be used in a class D room.

A total exhaust air extraction system is mandatory using a SWC/BSC as
an additional safety measure. If this is not feasible, the air returning to the room must be recirculated through a two-stage HEPA filter system.

1.4.4. Furnishing

Cleanroom furniture is furniture that has been designed to be non-particulating (should be made of non-shedding, nonporous material) and easily cleaned.
Materials like cloth, paper, cardboard, and wood are never used in cleanroom furniture. It has to be always considered, that harsh disinfectants can cause damage to some materials, so it is wise to choose furniture made from aluminium or stainless steel or furniture with powder-coated finishes.
Choosing the right furniture for the cleanroom ensures the environment is kept sterile at all times while also providing practical surfaces and ergonomic use.

- (stainless steel) cleanroom tables (for easy cleaning)
- cleanroom rolling chairs (designed to be easily cleaned and non-particulating)
- carts
- benches
- shelving
- garment cabinets, etc.

1.5. Qualification and classification of cleanrooms (Zs.Oras)

Aseptic drug preparation, including anticancer drug compounding requires a cleanroom environment to minimize the risk of contamination. Common contamination includes dust particles, moisture, bacteria, fungus, human skin, cosmetics, lint, fibers, and many other contaminants. These contaminants could be introduced into the environment by the air entering the environment, cleaning products or by personnel and process equipment used in the environment.
Cleanroom classification is the determination of the cleanliness level of an environment based on the maximum permissible particle size (diameter) and the maximum permissible number of airborne particles per unit volume.

It is globally standardized by the International Organization for Standardization (ISO) 14644-1 standard, which defines nine ISO classes, from ISO Class 1 (the cleanest) to ISO Class 9 (the least clean, equivalent to ordinary room air). ISO uses a logarithmic scale based on the number of particles (≥0.1 μm to ≥ 5 μm) per cubic meter of air.

Other classification standards are the US Federal Standard 209E (but now it has been largely superseded by ISO) and GMP Annex European Community Cleanroom Standards. According to these Annex, there are four grades defined as Grade A, Grade B, Grade C, and Grade D, each corresponding specific cleanliness requirements, and applies ISO principles to pharmaceutical environments and introduces additional microbial limits, gowning expectations, and operational state distinctions ("at rest" and "in operation").
The level of contamination in each standard is based on the number of particles in a defined particle size range that is allowed in the air.

GMP cleanroom classes and ISO equivalents:

- **Grade A** is reserved for the most critical operations, such as aseptic filling, compounding, and open handling of sterile products.
  - **ISO Equivalent**: ISO Class 5 (both at rest and in operation)
  - **Particle Limit**: ≤ 3,520 particles ≥ 0.5 µm/m³
- **Grade B** areas serve as the background environment for Grade A zones and support aseptic processing.
  - **ISO Equivalent**: ISO Class 5 (at rest), ISO Class 7 (in operation)
  - **Particle Limits**:
    - At rest: ≤ 3,520 particles ≥ 0.5 µm/m³
    - In operation: ≤ 352,000 particles ≥ 0.5 µm/m³
- **Grade C** cleanrooms are used for less critical stages, such as preparing solutions and handling components before sterilization.
  - **ISO Equivalent**: ISO Class 7 (at rest), ISO Class 8 (in operation)
  - **Particle Limits**: ≤ 352,000 particles ≥ 0.5 µm/m³
- **Grade D** areas are designated for the least critical stages of sterile drug production, including initial handling and washing of components.
  - **ISO Equivalent**: ISO Class 8 (at rest and in operation)
  - **Particle Limit**: ≤ 3,520,000 particles ≥ 0.5 µm/m³

1.6. Which activities are carried out in which rooms (Zs.Oras)

1.6.1. General principles

Compounding sterile cytostatic preparations should only be carried out in a department, designated solely for this process, separated from other rooms and marked with adequate warning symbol.

This department consist of a minimum of three or more rooms forming a functional unit with each other. In each room only the validated, dedicated work process can be performed. The rooms should be separated from each other and equipped with appropriate airlocks. The design should take into account the separate flow of personnel and products, preferably with a one-way flow.

The design, configuration, and layout of the rooms must be designed to reduce the contamination by microorganisms, particles, and cytotoxic substances to a minimum.
The required rooms are the following:

1.6.2. Stock receiving room

This is a clearly defined and marked area for:

- Receiving, unpacking and checking medications consisting CMR drugs (checking primary package)

Marking should be clear:

- different coloured surface/ demarcation by tape, etc.

Protective equipment should be available:

- working mats, protective gloves, mask
- Spill Kit in case of accident

Receiving shipment only by well-trained working staff; accident procedure, flowchart and competent person contact should be available.

1.6.3. Preparation/storage room

In this area medication, personal protective equipment and infusion solutions are stored according to good storage practice and prepared for use in the production room.
The required medicinal products and other materials are:

- laid out, disinfected and fed through an air lock into the production room.

The finally prepared parenterals are checked and signed off here and prepared for delivery using unbreakable, leakproof, sealable containers.

The following equipment, devices are required in this room:

- a refrigerator or cool box (with uninterrupted power supply) to store medicinal products and possibly opened bottles.
- suitable containers, shelves for stocking providing adequate space
- containers for delivering or
- pneumatic tube terminal (optional)

- tools for disinfection, decontamination, preparation: solution, mats, gloves, protective clothing
- transfer airlock into the production area
- computers, printers, telephone

This make-ready room should be clean and dry and maintained within acceptable temperature limits. Storage conditions (room, refrigerator or freezer temperature) should be consistently monitored and recorded with a temperature monitoring alarm system.

1.6.4. Production room

This buffer area is defined as an area where:

- the „Primary Engineering Control" device (biological safety cabinet, isolator) is located and where
- activities such as preparation, compounding and staging of sterile products occur.

The solution preparation room should be adequately separated from other rooms (by one or more airlock) to ensure aseptic and safe work processes. Its design should be designed to guarantee optimal function of the safety cabinet. This area should provide adequate space for the cabinet and may include a limited amount of shelving and/or carts for staging of compounding (not for storing stock).

Workers only can enter this room through the personal airlock after disinfection and putting on personal protective equipment.

Different airlock should use for entering materials to the production room.

1.6.5. Gowning area/room

Because of personnel is the main source of the potentional contamination, the first step in minimizing particles and a risk of bacteria is hand washing and gowning properly.

The sanitizing-gowning room's requirements:

- used for storing and changing outer (unclean) and sterile (clean) clothing and
- this is where hand washing and hand-disinfecting must be occurred
- unclean and clean area should be separated by a visible line or bench

- cabinets both for street clothes and sterile PPE
- Sink with elbow tap (stainless steel)
- Liquid soap dispenser (foot-operated)
- Electrical hand dryer
- Full length mirror

Staff with infectious diseases are not allowed to participate in compounding. Visible jewellery, cosmetics, personal electronic devices (e.g. cell phones) and any associated attachments must be removed prior to hand hygiene and garbing.

When working with CMR substances separate gowning rooms are recommended to reduce spreading these materials.

1.6.6. Documentation room

A documentation room in a cleanroom environment is a dedicated space, typically adjacent to the cleanroom or else in the clean room D zone, where paperwork and related activities are handled to minimize the risk of contamination within the controlled environment. This room helps maintain the cleanliness of the cleanroom by keeping paper and other potential contaminants outside, while still allowing for necessary record-keeping and documentation. There should be a communication link with the preparation area, and visual contact between the two areas is also advisable. If labels are printed in the office and transferred into the preparation area, an airlock must be used to maintain cleanliness.

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