Cleaning and disinfection in the GMP cleanroom

Introduction

GMP classified cleanrooms, for example in the biomedical or pharmaceutical industry, should have a controlled level of microbiological contamination. The standard for microbiological contamination in the air or on horizontal surfaces is of great importance and is closely monitored. This article gives an idea of how a good cleaning regime can make a successful contribution to achieving these important objectives.

Whether it concerns a cleanroom in a compounding pharmacy, a laboratory, or a high care room in the food industry, there are specific requirements for the cleanliness of the environment in the area concerned, as well as the machinery and interior of the work areas. This is achieved in a number of ways, including the physical operation of heating, ventilation and air conditioning (HVAC) systems. Considering that man is often the greatest polluter in a clean environment, a correct clothing regime must be followed. Of course, staff working in cleanrooms are trained in correct behavior. Particularly important is also the use of defined cleaning techniques, as well as the use of appropriate cleaning agents and disinfectants.

Purpose

The purpose of the cleaning and disinfection is to be able to continue to meet the requirements that apply to your specific process. Products and/or people must be protected from dust and/or germ contamination, so the process takes place in a room specifically designed and equipped for this purpose. Depending on the activities in the room, it must meet specific requirements. It goes without saying that there are fewer requirements for a space where cable harnesses are assembled for the automotive industry than in the pharmaceutical industry. There may also be a difference in the requirements in the country where the products you produce are exported to. Consider, for example, the very different food industry requirements in Europe and the US! We can’t go into all too much detail in this article about the range of different market segments and their specific requirements. However, we can present a brief overview of the various standards that often play a role in the design and construction of air-conditioned and controlled spaces.

In order to be able to define and check what qualifies as a cleanroom, clear agreements must be made. These agreements are laid down in guidelines, standards or even legislation. In a number of market segments, the focus is mainly on dust particles that may cause malfunctions or quality issues (e.g. in the semi-conductor, optical, or microelectronics markets). In markets such as pharmaceuticals, cosmetics, medical devices or microbiological companies, people are primarily interested in germ contamination in the air and on surfaces.

To give you a first impression, here follow a number of standard descriptions and important relevant tables:

ISO 14644 standard:

- Part 1: Classification of air cleanliness by particle concentration

Classification numbers Numbers (N)	Maximum concentration limits (particles/m³ of air) for particles equal to and larger than the considered sizes shown below					
	0.1µm	0.2µm	0.3µm	0.5µm	1µm	5.0µm
ISO 1	10					
ISO 2	100	24	10			
ISO 3	1 000	237	102	35		
ISO 4	10 000	2 370	1 020	352	83	
ISO 5	100 000	23 700	10 200	3 520	832	
ISO 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO 7				352 000	83 200	2 930
ISO 8				3 520 000	832 000	29 300
ISO 9				35 200 000	8 320 000	293 000

Table 1: Maximum particulate concentration per m3 air in various ISO 14644 classes.

Other parts in the ISO 14644 descriptions:

- Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration
- Part 3: specifies ancillary tests related to other aspects of cleanroom performance such as pressure difference, airflow, etc.)
- Part 4: Design, construction and start-up
- Part 5: Operations
- Part 7: Separative devices (clean air hoods, glove-boxes, isolators and mini-environments)
- Part 8: Classification of air cleanliness by chemical concentration (ACC)
- Part 9: Classification of surface cleanliness by particle concentration
- Part 10: Classification of surface cleanliness by chemical concentration

ISO 14698:

Since 2003/2004, specific cleanroom requirements have been described in the ISO 14698 standard, Parts 1 and 2, on data evaluation and interpretation, as well as monitoring, with regard to bio contamination data. However, this standard is not being applied internationally and was being revised at the time of writing this White Paper.

GMP (Good Manufacturing Practice):

EU GMP ANNEX I describes the EU guidelines for the GMP standard for medicines for humans and animals. Annex I contains the latest corrections specific to the production of sterile medicines.

- Four particulate cleanliness classes are described in the GMP standard:

Grade	At Rest		In Operation	
	Maximum permitted number of particles/m³ equal to or above			
	0.5µm	5µm	0.5µm	5µm
A	3 520	20	3 520	20
B	3 520	29	352 000	2 900
C	352 000	2 900	3 520 000	29 000
D	3 520 000	29 000	not defined	not defined

Table 2: Maximum particulate concentration per m3 air in various EU GMP ANNEX I classes.

- The requirement for limitation of microbiological contamination in the air and on surfaces is also included:

Grade	Recommended limits for microbial contamination (a)			
	air sample cfu/m³	settle plates (diam. 90 mm), cfu/4 hours(b)	contact plates (diam. 55 mm), cfu/plate	glove print 5 fingers, cfu/glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Table 3: Maximum 'colony-forming units' relating to microbiological contamination on specific surfaces or in the air.

Government authorities and/or other controlling bodies regularly inspect or validate your working methods and organisation as well as your physical cleanroom space for microbiological and other forms of contamination, for example in accordance with specific standards as briefly described above.

However, the specific contamination requirements mentioned above are certainly not the only regulations that must be complied with, when, for example, designing or building a pharmaceutical production area. Examples include the GMP rules and regulations, which are also required by the FDA, on the commissioning process and the qualification requirements for critical 'direct impact' systems (direct impact on product quality). This subject is so complex and extensive that PP4CE will return to it in a future White Paper.

Understanding cleaning and disinfection:

Cleaning and disinfection are two different concepts, which can sometimes lead to confusion. The most important thing is that cleaning, with the help of a ‘detergent’ (cleaning agent with active ingredients), takes place prior to disinfection.

Detergents are normally used to remove dirt such as grease, dust or other ‘adhesive’ materials from a surface. The active ingredients reduce the surface tension of the water and improve the penetration of the water into the dirt. This allows the dirt to be dissolved and removed more readily.

A disinfectant is produced on the basis of a certain type of germicide, which makes it possible to eliminate bacteria, fungi or other germs on a surface, reducing the population of microorganisms to the desired level.

The better the cleaning is done prior to disinfection, the more optimal the disinfection step will be.

Selecting the most suitable means:

It is important to select the most suitable cleaning agents and disinfectants. A responsible choice must be made, tailored to the type of contamination and the desired disinfection level. It is also necessary to ensure that the various means are compatible with each other.

When choosing cleaning agents, it is important that:

- Detergent should have a neutral composition (not alkaline or acidic).
- The detergent must not contain any ionic components.
- The detergent must not foam when mechanical forces are applied.
- There must be no components in the detergent that inactivate the action of the disinfectant.

When selecting a disinfectant, it is important that:

- The disinfectant must have a broad spectrum of action. The spectrum of action refers to the properties of a disinfectant and its ability to kill various microorganisms, including gram-negative and gram-positive bacteria.
- To be able to comply with the GMP guidelines one must rotate with two different disinfectants to prevent germs from becoming immune to the germicide part. To this end, the two disinfectants must be chosen on the basis of different forms of activity.
- The speed of action depends on the contact time required for the disinfectant to destroy a microbial population. Contact time is how long it takes a disinfectant to dry completely.
  It goes without saying that the substance will stop working once it has dried up. The disinfection processes should preferably fit in with the cleanroom processing schedule.
- The proper functioning of the disinfectant should be tested against the prevailing temperature and RH within the cleanroom.
- The disinfectant should be tested against the chemical resistance of the applied materials on which the disinfectant is used. Remember that stainless steel can also be damaged by certain chemicals with prolonged exposure.
- The disinfectant must be safe for use by personnel and must be registered and comply with local health and safety legislation.

Sinner's circle

Sinner's circle or 'The Circle of Sinner' is a tool within the cleaning sector to determine the ratios needed to clean a certain surface, object, or part. When cleaning requires chemical and/or mechanical help, the so-called 'Sinner's circle' is used to determine the correct ratio for cleaning the contaminated surface. Sinner's circle can also be useful in the cleanroom for cleaning in general and disinfection in particular.

Sinner's circle consists of 4 function variables, as shown in the model below. The function variables are distributed evenly by default and when necessary, it can be easily determined whether the ratio should be different.

If dirt is firmly attached, the following may be required:

- Increase the cleaning time (i.e. allow the applied detergent solution to work longer)
- Raise the temperature (i.e. use warmer water to prepare detergent)
- Increase the chemical concentration (increase dosage of detergent in line with indicated concentrations on the packaging)
- Increase amount of mechanical effort (more intensive polishing, scrubbing and/or sanding)

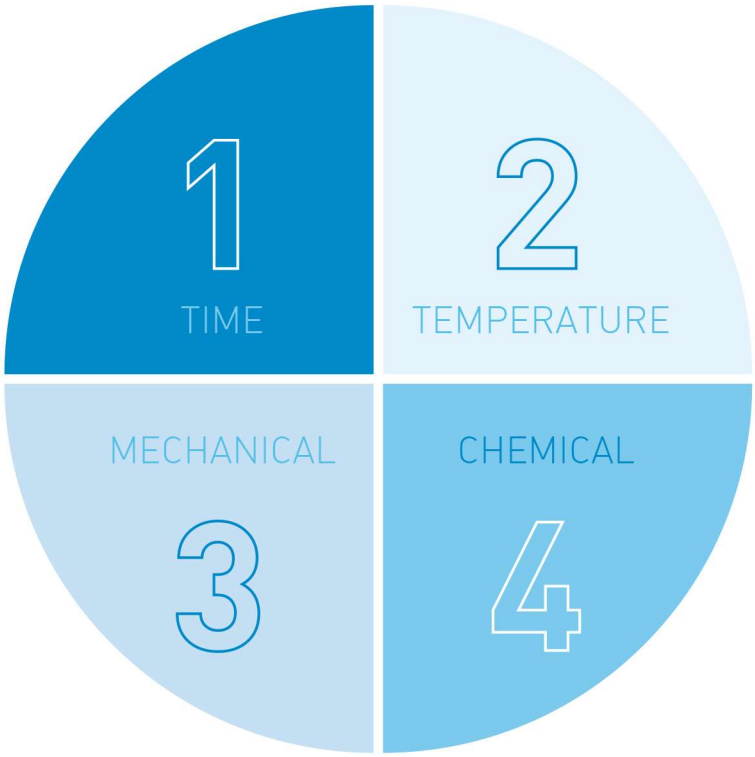

Figure 4: Sinner's circle.

What is the effect of a disinfectant?

There are different types of disinfectant, with different modes of action, and varying levels of effectiveness against microorganisms. Understanding the distinction between different disinfectants is important when choosing a disinfectant. The distinction between non-oxidising and oxidising chemicals is often important when choosing. Non-oxidising disinfectants include alcohols, aldehyde, amphoteric substances, biguanide, phenols and quaternary ammonium compounds. Oxidising disinfectants include halogens and oxidising agents such as peracetic acid and chlorine dioxide. If necessary, PP4C will seek the advice of a biologist on the basis of the expected microbiological contamination, in order to optimally determine which disinfectant can be used in a specific room.

Qualification of applied disinfectants

For use in pharmaceutical facilities, the disinfectants used must be classified and validated. The disinfectant can be tested on a laboratory scale, in accordance with standardised processes, in relation to the various surface materials and the nature of the microbiological contamination. While most of these tests can be performed by the disinfectant manufacturer, a few may need to be performed in house by the cleanroom user.

Factors influencing disinfecting efficacy

There are a number of factors that influence the effectiveness of a disinfectant in practice. It is important to understand these and thus optimise the results of a disinfection process.

- Concentration: This is the optimal dilution of the disinfectant to achieve the largest microbial kill. It is a misconception to think that by increasing the concentration of the disinfectant, it is possible to accelerate or improve the killing of the germs.
- Time: The amount of time the disinfectant is active is of great importance. Sufficient time is needed to allow the germicide to bind itself to the microorganism, penetrate the cell wall, and then carry out its lethal action in the nucleus.
- Temperature and pH: Each disinfectant has an optimal pH and temperature to achieve the most effective result. If the temperature or pH lands outside this optimal curve, the reaction speed (i.e. ‘kill time’) can be affected.
- Determining the germicide: Ideally, the numbers and types of microorganisms should be identified in order to choose the best type of germicide to use in the disinfectant.

The cleaning technique

Cleaning and possibly disinfecting is a serious matter in a GMP cleanroom. Not only should the cleaning agents and disinfectants be suitable, but the tools and other equipment used for cleaning should also be determined before the cleanroom is put into operation. For the correct and properly diluted application of cleaning agents and any disinfectants on floors, walls and other surfaces in climate-controlled production areas, they must be applied with equipment certified for cleanroom use, which does not cause contamination during application. Depending on whether work is carried out in a completely sterile environment, the equipment will have to be adapted accordingly. This could include equipment that can be sterilised in an autoclave. The supply of materials and equipment will also differ in a normal cleanroom, in comparison with a sterile working environment. Because Wero is a partner of Dastex Cleanroom Products, we can advise you on your choice of disposables and cleaning equipment, and offer you a wide choice of high quality products.

Finally, in this section we would also like to say something about the type of water that will be used: PUW (Purified Water) or WFI (Water for Injection). The table below shows the different properties of these two qualities of water for the pharmaceutical industry. Naturally, the correct type of water must be used in the cleaning programme, in accordance with the URS.

	Purified Water		Highly Purified Water		Water For Injection	
	USP	EP	USP	EP	USP	EP
Process	Distillation,reverse osmosis and any other suitable process	Distillation, ion exchange, reverse osmosis and any other suitable process	N/A	Double-pass reverse osmosis coupled with other suitable techniques such as ultrafiltration and deionisation, for example	Distillation or reverse osmosis	Distillation
Conductivity	≤ 1,3 µS/cm @ 25°C	< 4,3 µS/cm @ 20°C	N/A	≤ 1,1 µS/cm @ 20°C	≤ 1,3 µS/cm @ 25°C	≤ 1,1 µS/cm @ 20°C
Bacteria	100 cfu/ml (suggested)	< 100 cfu/ml	N/A	< 10 cfu/100ml	< 10 cfu/100ml (suggested)	< 10 cfu/100ml
Endotoxin	N/A	< 0,25 IU/ml (only for bulk water for dialysis)	N/A N/A	< 0,25 IU/ml	< 0,25 IU/ml	< 0,25 IU/ml
TOC	500 ppb	≤ 0,5 mg/l	N/A	≤ 0,5 mg/l	500 ppb	≤ 0,5 mg/l
pH	5-7	5-7	N/A	5-7	5-7	5-7
Nitrates	N/A	< 0,2 ppm	N/A	< 0,2 ppm	N/A	< 0,2 ppm
Heavy metals	N/A	≤ 0,1 ppm	N/A	≤ 0,1 ppm	N/A	≤ 0,1 ppm
Aluminium	N/A	≤ 10 ppb (if intended for use in the manufacture of dialysis solutions)	N/A	≤ 10 ppb (if intended for use in the manufacture of dialysis solutions)	N/A	≤ 10 ppb (if intended for use in the manufacture of dialysis solutions)

Table 5: Specific properties of various water systems.

Cleaning regime

A cleaning regime should be drawn up based on the different processes, the required classification, and the relevant market segment in which the cleanroom will be used. This can be divided into two parts. On the one hand, there is the regular recurring cleaning, which is usually carried out by our own personnel. On the other hand, it is necessary to implement periodic cleaning in a classified area in a specific cycle. The kind and frequency of these activities is described in Guideline 4, drawn up by the Association of Contamination Control Netherlands (VCCN). Below you will find an overview of the tables in this Guideline.

It should be noted that the regular cleaning is often carried out by specialised companies. This has to do not only with the qualifications of the trained cleaning personnel, but also with the materials and equipment that are needed to carry out all work correctly and safely.

Wero is happy to offer you a tailor-made cleaning regime in which we guarantee a high quality of regular cleaning and, if desired, a training programme on daily cleaning for your own staff.

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Work schedule for low-dust areas			
Dust Class according to ISO 14644	**ISO 5**	**ISO 7**	**ISO 8**
Action	**Recommended annual frequency**		
Floors			
• damp wiping of hard floors	520	260	260
• wet cleaning of hard floors	52	24	24
• wet cleaning of mats	520	260	260
• repairing floors (if applicable)	12	12	12
• preservation of floors (if applicable)	1	1	1
• vacuuming	260	260	260
please note: for perforated floors, adapt work programme			
Furniture and equipment			
• emptying paper / waste bins and pedal bins	260	260	260
• damp wiping of any furniture and equipment within reach	260	260	260
• damp wiping of parts with hard finish	260	260	260
• damp wiping of furniture and equipment out of reach	260	52	12
• complete wet cleaning of outer surfaces of furniture and equipment	52	24	12
• damp cleaning of sinks	520	260	260
Walls / ceilings			
• damp wiping of edges / ledges within reach	260	260	260
• damp wiping from edges / ledges out of reach	260	52	12
• removing stains from doors incl. window frames / tracks	260	260	260
• damp cleaning of doors including window frames / tracks	52	24	24
• damp wiping of walls	12	12	12
• damp wiping of complete ceiling	1	1	1
please note. for perforated walls or ceilings, adapt work programme			
Other			
• cleaning of fire-extinguishing equipment	260	260	260
• cleaning of light switches in direct contact areas	260	260	260
• cleaning of communication equipment	260	260	260
• cleaning of technical facilities (traps, etc.)	1	1	1
General			
• damp wiping of surfaces that frequently come into contact with cleanroom staff	520	520	520
The above frequency is recommended by the Dutch Association of Contamination Control Netherlands (VCCN). Of course, frequencies will have to be adjusted if the production process gives cause for this. Complete cleaning, including the entire ceiling, is necessary when the cleanroom has been shut down (whether or not on purpose!)			

Table 6: Cleaning and disinfection in accordance with VCCN Guideline 4 for low-dust areas.

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Work schedule for low-germ areas			
Dust Class according to ISO 14644	**ISO 5**	**ISO 7**	**ISO 8**
Action	**Recommended annual frequency**		
Floors			
• damp wiping of hard floors	520	260	260
• disinfecting the floor	104	52	52
• wet cleaning of hard floors	52	24	24
• wet cleaning of mats	520	260	260
• repairing floors (if applicable)	12	12	12
please note: for perforated floors, adapt work programme			
Furniture and equipment			
• emptying paper / waste bins and pedal bins	260	260	260
• damp wiping of any furniture and equipment within reach	260	260	260
• damp wiping of parts with hard finish	260	260	260
• damp wiping of furniture and equipment out of reach	260	52	12
• complete wet cleaning of outer surfaces of furniture and equipment	52	24	12
• damp cleaning of sinks	520	260	260
• disinfecting furniture and equipment within reach	104	52	52
• disinfecting furniture and equipment out of reach	24	12	12
Walls / ceilings (excl. filters)			
• damp wiping of edges / ledges within reach	260	260	260
• damp wiping from edges / ledges out of reach	52	52	12
• removing stains from doors incl. window frames / tracks	260	260	260
• damp cleaning of doors including window frames / tracks	52	24	24
• damp wiping of walls	12	12	12
• damp wiping of complete ceiling	1	1	1
• disinfecting edges / ledges within reach	104	52	52
• disinfecting edges / ledges out of reach	24	12	12
please note. for perforated walls or ceilings, adapt work programme			
Other			
• cleaning / disinfecting fire-extinguishing equipment	104	52	52
• cleaning / disinfecting of light switches in direct contact areas	260	260	260
• cleaning / disinfecting of communication equipment	260	260	260
• vacuuming pre-filters of the LAF cabinets	52	52	52
• cleaning of technical facilities (traps, etc.)	1	1	1
General			
• damp wiping of surfaces that frequently come into contact with cleanroom staff	520	260	260
• disinfecting elements that frequently come into contact with cleanroom staff	260	260	260

The above frequency is recommended by the Dutch Association of Contamination Control Netherlands (VCCN). Of course, frequencies will have to be adjusted if the production process gives cause for this. Complete cleaning, including the entire ceiling, is necessary when the cleanroom has been shut down (whether or not on purpose!)

Table 7: Cleaning and disinfection in accordance with VCCN Guideline 4 for low-germ areas.

Monitoring of cleaning and disinfection

In order to be able to properly assess whether the cleaning programme meets the requirements set within the standard, it is required to carry out measurements on a regular basis. This measurement can be carried out partly automatically, for example by measuring the number of living microorganisms in a standard pre-determined amount of air at regular intervals, at a number of locations.

On surfaces, this can be done by measuring the number of living microorganisms on a predetermined surface area. Such measurements can be made using different sampling methods (stamping technique, sample plates, or swabs).
If the results obtained do not meet the standards, this may indicate a problem with the cleaning technique in general or the quality of execution or frequency of regular cleaning and/or disinfection. The results of these measurements must be documented and the necessary measures must be taken in case of deviations from the standards described in the applicable regulations.

If you have determined your cleaning regime, whether or not with external help, this must be laid down in an SOP (Standard Operating Procedure). The employees active in the cleanroom must be trained and become clearly aware of the importance of correct cleanroom behaviour. As soon as a facility proves to be under control, it is of paramount importance to carry out any further cleaning and disinfection with the right techniques and resources at specific frequencies. This will keep your cleanrooms clean and prevent contamination problems.

PP4CE (Professional Partners for Controlled Environments) is a strategic alliance between a number of specialised companies. They are active in the design, construction and maintenance of cleanrooms and laboratories in a broad spectrum of market segments dealing with high care areas in the food industry.

White Paper author: Geerd Jansen

Geerd is General Manager of the Brecon International B.V. and as such is strategically involved with the PP4CE partner WERO Controlled Environment Reiniging.

For further information, please see: www.werocleanroomreiniging.nl or http:/ www.pp4ce.com

References: Cleaning and disinfectant. Eight Steps for Success by Dr. Tim Sandle

Table 1-2-3: from ISO 14644 and Eudralex GMP Volume 4 Annex I

Table 5: Pure Water Group

Table 6-7: from ‘Guideline 4’ of the Association of Contamination Control Netherlands (VCCN)