Best Practices for Cleaning and Disinfection

White Paper

Author: Dr Tim Sandle

Contents

Operator entering a cleanroom to conduct cleaning and disinfection (Image: Tim Sandle)

Introduction

Cleaning and disinfection practices are an essential part of contamination control in the healthcare and pharmaceutical industries. These practices can be divided into two key parts:

- Cleaning and disinfection of cleanrooms
- Cleaning of equipment (which requires cleaning validation to verify the effectiveness of the cleaning)

The focus of this paper is on the cleaning and disinfection of cleanrooms, a process that should form a central part of a pharmaceutical organisation's bio-contamination control strategy[1].

An effective cleaning and disinfection programme in pharmaceutical grade areas of a Good Manufacturing Practice (GMP) facility is critical to assure the quality of the products. It is not sufficient to simply have a programme in place for regulatory agencies – a GMP facility requires validation data to support sanitisation and disinfection procedures.

Within the pharmaceutical organisation, microbial contamination may arise from many sources such as water, raw materials, environmental failures and people, as well as from inadequate cleaning. Due to these multiple sources of contamination and the different types of microorganisms associated with them, disinfectants used in the pharmaceutical industry must have a broad spectrum of activity and be effective against a wide range of organisms in different physiological states.

An important step towards achieving microbial control within a cleanroom is the use of defined cleaning techniques, together with the application of detergents and disinfectants. The objective of cleaning and disinfection is to achieve appropriate microbiological cleanliness levels for the class of cleanroom for an appropriate period of time. This involves the application of detergents (which 'clean') and disinfectants (which inactivate or destroy microorganisms, depending upon the type of disinfectant). Detergents are cleaning agents and are deployed to remove 'soil' from a surface. The removal of soil is an important step prior to the application of a disinfectant, for the greater the degree of soiling remaining on a surface, the lesser the effectiveness of disinfection. A disinfectant is a type of chemical germicide which is capable of eliminating a population of vegetative microorganisms (although some disinfectants are sporicidal, a chemical does not need to be sporicidal to be classified as a disinfectant)[2].

Disinfectants vary in their effectiveness against different types of microorganisms, a variation relating to both the intrinsic resistance of different microorganisms and the range of different types and formulations of disinfectants. Furthermore, different disinfectants act in different ways depending upon their active ingredients[3].

The focus of this white paper is upon the regulations and procedural requirements, and the qualification requirements for introducing a disinfectant into use.

What is meant by 'Cleaning' and 'Disinfection'?

Cleaning is the process of removing residues and 'soil' (such as dirt, grease, protein residues and so on) from surfaces to the extent that they are visually clean. This involves defined methods of application and often the use of a detergent. Importantly, the act of cleaning is necessary prior to the application of a disinfectant as a surface needs to be properly cleaned before the application of a disinfectant. This is in order for the disinfectant to work efficiently[8], as disinfectants can either be inactivated by organic residues or the soil can create a barrier which prevents the disinfectant from reaching all of the microbial cells.

A detergent is a chemical used to clean equipment or surfaces by removing unwanted matter (soil). Detergents generally work by penetrating soil and reducing the surface tension (which adheres soil to the surface) to allow its removal (in crude terms, a detergent increases the 'wettability' of water). Many detergents are synthetic surfactants (an acronym for Surface Active Agents). Whilst "cleaning" is not "disinfection" the cleaning process can remove or dilute microbial populations. Furthermore, many detergents have chemical additives that can 'disinfect.' However, a cleaning agent will not meet the criteria for the disinfection required by the European and United States standards for disinfectant efficacy testing in terms of reducing a microbial population of a defined range by the required log reduction[4].

The term 'disinfection' is normally applied to an inanimate object (sometimes the term 'biocide' is used, although this relates to a larger group of chemical agents). The term 'antiseptic' is used to describe the reduction of a microbial population on living tissue. Thus an antiseptic is a disinfectant which can safely be applied to the surface of the skin (sometimes the terms 'hand sanitiser' or 'hand disinfectant' are used interchangeably). In turn, the term 'disinfectant' is usually reserved for liquid chemical germicides which cannot be applied to tissue because of their corrosive or toxic nature.

Disinfectant solutions are chemical or physical agents that destroy or remove vegetative forms of harmful microorganisms when applied to a surface. The types of disinfectants selected must be effective for use within cleanrooms and must be controlled. In making this choice there are various types of disinfectants available[5]. Types of disinfectants include alcohols, aldehydes, amphoterics, biguanides, halogens, phenolics, quaternary ammonium compounds and peroxygens. Different types of disinfectants have different spectra of activity, modes of action (the sites within the bacterial cell targeted by the disinfectant) and differing efficacies. Some disinfectants are classed as bacteriostatic, where the ability of the bacterial population to grow is halted. Here the disinfectant can cause selective and reversible changes to cells by interacting with nucleic acids, inhibiting enzymes or permeating into the cell wall. Once the disinfectant is removed from contact with bacteria cells, the surviving bacterial population could potentially grow. It is also possible for bactericidal disinfectants to become bacteriostatic if they are over-diluted.

Other disinfectants are bactericidal in that they destroy bacterial cells through different mechanisms, namely, causing structural damage to the cell, autolysis, cell lysis, or by the leakage or coagulation of the cytoplasm. Within these groups, the spectrum of activity varies, with some disinfectants being effective against vegetative Gram-positive and Gram-negative microorganisms only, while other disinfectants are effective against fungi. Other disinfectants have a broader spectrum and are sporicidal in that they can cause the destruction of endospore forming bacteria. Bacterial spores demonstrate particular resistance to disinfectants due to the outer layer creating a relatively impermeable barrier to the biocide. However, a chemical agent does not have to be sporicidal in order to be classed as a 'disinfectant'[6]. The bacteriostatic, bactericidal and sporicidal properties of a disinfectant are influenced by many variables.

Many disinfectants are effective against fungi although, like bacteria, the cell wall chemistry and internal cellular structure differ. Thus fungal susceptibility to disinfectants differs. To add to this, susceptibility also relates to the physiological state and stage of growth[7]. Hence a disinfectant effective against a target preparation of bacteria may or may not be effective against fungi. In addition, disinfectants used in the pharmaceutical industry may need to demonstrate virucidal properties. In certain sectors, like biotechnology, this is a concern. Here non-enveloped viruses tend to be more resistant than enveloped forms[8].

There are various approaches to the categorisation and sub-division of disinfectants including grouping by chemical nature, mode of activity or by microstatic and microcidal effects on microorganisms. Within the pharmaceutical industry, categorisation by chemical properties is more typical. The two principal categories

Preparing a disinfectant solution for dilution (Image: Tim Sandle)

are grouped in two classes, oxidising and non-oxidising chemicals.

Understanding the chemical basis is paramount to conform with GMP expectations. Pharmaceutical manufacturers are expected to use at least two disinfectants with different modes of activity[15]. Whilst the two disinfectants do not need to be sporicidal, the use of a sporicidal disinfectant is recommended for aseptic processing areas, at least on an occasional basis, even where such a disinfectant does not form part of the standard set. Therefore, a third disinfectant is often held in reserve and deployed at a lower frequency to the two primary disinfectants.

The phrase "cleaning and disinfection" not only distinguishes between cleaning agents and disinfectant, it also describes the order in which surfaces are treated. In order for a disinfectant to work effectively, 'soil' (such as grease and dust particles) must be removed first using a suitable cleanroom grade detergent.

The GMPs

The use of detergents and disinfectants, and the need to keep cleanrooms clean, is a regulatory requirement within the pharmaceutical sector. The main regulatory documents relating to the use of disinfectants in pharmaceutical manufacturing are:

- Code of Federal Regulations (CFR): 21 CFR 211.56b and 21 CFR 211.56c (which refer to sanitation), CFR 211.67 (which refer to equipment and maintenance), CFR 211.182 (which describes the need for a cleaning programme) and CFR 211.113b
- FDA Aseptic Processing Guide
- USP (General Chapter <1072> Disinfectants and Antiseptics)
- Annex 1 to the EU Guide to Good Manufacturing Practice

Although there are some differences between the global GMP regulations, a number of similar areas are covered. In summary, the regulations require[9]:

- The need to have written procedures (CFR / EU GMP)
- That cleaning responsibilities are assigned (CFR). Often this is interpreted as the need to have independent cleaning staff separate from those involved in product manufacture
- That staff must be trained in cleaning techniques and have a training record (CFR/EU GMP)
- The details of cleaning frequencies, methods, equipment and materials to be recorded in written procedures (CFR). This may relate to an approved supplier specification
- The cleaning of equipment and materials to take place at regular intervals (CFR)
- That when designing a disinfectant protocol for the sanitisation of floors, walls and surfaces, a pharmaceutical organisation would normally select two or three disinfectants for the same application. This is a requirement of regulatory bodies and the strongest pressure for it has come from Europe with the EU GMP Guide stating that "where disinfectants are used, more than one type should be employed" (Annex 1, paragraph 3714). This is normally interpreted as the need for disinfectant rotation (which is discussed on page 6)
- That disinfectants should be rotated (EU GMP / FDA warning letters)
- That inspection of equipment for cleanliness before use should be part of routine operations (CFR)
- That a cleaning log should be kept. The purpose is to keep a record of the areas cleaned, agents used and the identity of the operator (CFR)
- The microorganisms isolated (the microbiota) from environmental monitoring programmes should be examined for resistant strains (EU GMP). The inference here is that such isolates are incorporated into disinfectant efficacy studies[19, 20]
- The monitoring for microbial contamination in disinfectant and detergent solutions should be periodically undertaken (EU GMP)
- The storing of disinfectant and detergent solutions should be for defined (and short) periods (EU GMP)
- That disinfectants and detergents used in Grade A and B cleanrooms should be sterile before use (EU GMP)
- That room use should be recorded after each operation (CFR / EU GMP)
- That disinfectants should be 'qualified' (validated) (CFR)
- That there should be a technical agreement with the company who supplies the disinfectant. Ideally the disinfectants purchased should be lot tracked (EU GMP)

Disinfectant Rotation

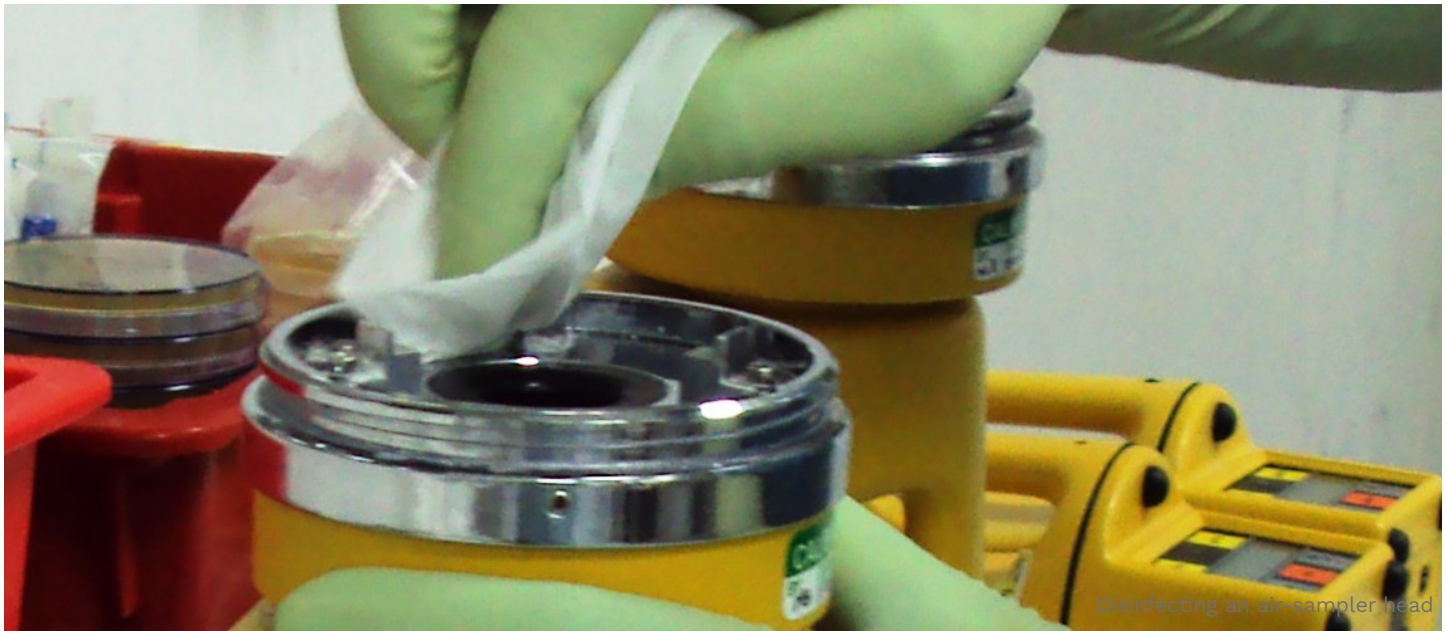
It is typical to rotate between two different disinfectants with different modes of activity. Within Europe, the rotation between two disinfectants of differing modes of activity is a regulatory expectation. The argument for rotating two disinfectants is to reduce the possibility of resistant strains of microorganisms developing. Whilst the phenomenon of microbial resistance is an issue of major concern for antibiotics, there are few studies which support development of resistance to disinfectants[10].

The frequency of rotation needs to be defined by the user and supporting data can be supplied through field trials. Here microbial populations and microbial species are tracked. Due to the theoretical differences in resistance between microorganisms, greater focus is given towards endospore forming bacteria and Gram-negative bacteria rods[11].

Key Factors to Assess when Selecting a Disinfectant

Deciding the types of disinfectants to be used in cleanrooms is an important decision for microbiologists. When selecting disinfectants, there are a number of different criteria to consider. Importantly, new

disinfectants should be introduced through a change control process. A disinfectant must have some characteristics or properties[12], such as a wide spectrum of activity, a fairly rapid action, be noncorrosive and compatible with both the surface to be disinfected and the detergents used. Further characteristic considerations include having sporicidal properties, a range of formats available, not being neutralised by residual matter and being safe for operator use. Optimal environmental conditions, its environmental impact and the temperature of use, costs and health and safety requirements also need to be considered. A brief description of the influence of each characteristic or property is examined below.



Disinfecting an air-sampler head

a) A wide spectrum of activity

The spectrum of activity refers to the properties of a disinfectant being effective against a wide range of vegetative microorganisms including Gram-negative and Gram-positive bacteria. For use in cleanrooms, disinfectants should be bactericidal (that is rather than simply inhibiting microbial growth, it should be capable of killing bacteria). A separate decision to be made is whether the disinfectant is required to be sporicidal. Furthermore, in some facilities the disinfectants should also be virucidal.

b) A fairly rapid action

The speed of action depends upon the contact time required for the disinfectant to destroy a microbial population. Thus the 'minimum' contact time is the time required for the disinfectant to be effective after its application. The contact time is sometimes referred to as the 'action time' or 'dwell time' (the latter is more applicable to disinfectants used for gaseous decontamination). Given the requirements of most pharmaceutical manufacturers and healthcare facilities, a disinfectant should ideally have a contact time of 10 minutes or less, although certain sporicidal disinfectants can require longer contact times. The reason for seeking a short contact time is one of practicalities. Many pharmaceutical facilities operate to lean manufacturing principles, where downtime is considered to be an economic problem. In addition, many cleanrooms have rapid air-change rates, which mean that disinfectants dry quickly. In most cases, the contact time infers that the surface remains 'wet' for the duration of the time. Prolonged contact times would most likely lead to reapplication of the disinfectant - a situation which many cleanroom users would be keen to avoid.

c) Disinfectants should not be neutralised by residual matter

Although detergents and effective cleaning practices can remove the majority of soil, including organic matter, some traces will remain. It is important these organic residues do not interfere with the active ingredient of the disinfectant and reduce its efficacy.

d) Optimal environmental conditions

Some disinfectants require certain temperature and pH ranges in order to function properly. One type of disinfectant, for example, may not be effective in a cold room due to the lower temperature. The reason for this is the validation standards for disinfectants measure the bactericidal activity at 20°C, therefore the disinfectant may not be as effective at higher or lower temperatures.

e) Non-corrosive

Disinfectants should be non-corrosive. If the disinfectant causes extensive abrasion of a surface, it will either degrade the material or cause cracks and recesses which can harbour microorganisms. It is recognised, however, that the most efficacious disinfectants, especially those which are sporicidal, through repeated applications over time will cause some corrosion. This is particularly so for chlorine based disinfectants in relation to stainless steel surfaces.

A post-disinfection step to remove disinfectant residues, such as a sterile water rinse or wiping with a milder disinfectant like 70% (v/v) isopropyl alcohol (IPA), can minimise material surface damage.

f) Operator safety

Many disinfectants are toxic or irritant and unpleasant for staff to use. Consideration must be given to safety requirements, material safety data sheets, label information, the toxicity upon human health and to the protective measures required for staff to use them (such as avoiding contact with exposed skin or the need to use a disinfectant in a well-ventilated area).

g) Compatibility with the surface to be disinfected

Certain disinfectants may be less effective with certain materials or may cause excessive damage to certain materials, as with the aforementioned reaction of chlorine dioxide against stainless steel.

h) Compatibility with detergents used

It is important that the disinfectant and the detergent are compatible and that detergent residues do not inactivate the active ingredients in the disinfectant solution.

i) Residual activity of the disinfectant

Most disinfectants, with the exception of alcohols, leave residues. On one hand, this could prolong antimicrobial activity. However, most pharmaceutical microbiologists regard residues as undesirable. Residual activity of the disinfectant may lead to resistant strains or cause problems when an alternative disinfectant is applied. While the total effect will depend upon the types of disinfectants and the drying time, it is good practice to remove disinfectant residues with a water rinse. Residues also tend to render surfaces sticky, which causes operational issues.

j) Sporicidal properties

If isolates from the environmental monitoring programme include the recovery of endospore forming bacteria on a frequent basis or in high numbers, then the use of a sporicidal disinfectant is essential, with the frequency determined by a review of the environmental monitoring programme.

Applying a disinfectant spray to a wipe
(Image: Tim Sandle)

k) Range of formats available

The cleanroom facility will require a disinfectant to be available in several formats[13]. For example, a type and formulation of disinfectant may be required in a ready-to-use format, as a concentrate, or an impregnated wipe, and so on, so that the most convenient and effective method of cleaning can be used. The pharmaceutical manufacturer, when selecting cleaning agents, should aim for products which are produced in different presentations.

For smaller spillages and the cleaning of process area surfaces and laboratory benches, sterile disinfectants in trigger sprays are the most effective design.

With slightly larger areas, ready-to-use disinfectant solutions in bigger volumes are desirable. For cleaning larger areas, such as process area floors, it is more practical and cost effective for disinfectants to be supplied in the concentrate form (for this the disinfectant is prepared by

l) Temperature of use

As the temperature increases, the rate of microbial kill increases until a plateau is reached. The degree that this happens is a factor of the active ingredient. This phenomenon relates to the temperature coefficient (Q10)[14]. This is assessed by determining the time to kill a known population of microorganisms at two temperatures differing by 10°C. Understanding this factor is important when using disinfectants outside of 'room temperature' conditions, especially in lower temperature environments, such as pharmaceutical cold rooms. Temperature is one of the constants in disinfectant efficacy testing, which is discussed later.

m) Cost

The calculation of cost needs to include not just the price of the disinfectant, but also other cost factors such as the time taken to prepare or apply the disinfectant, protective clothing requirements, wastage and the steps needed for the removal of residues.

n) Health and safety

The safety aspects of a disinfectant are an important consideration and standard operating procedures should contain appropriate health and safety requirements for using detergents and disinfectants. This should include reference to appropriate personal protective equipment. In particular, contact with eyes, skin and mouth is to be avoided. Safety data sheets must be examined for all disinfectants and detergents and appropriate measures taken to ensure that they are applied properly, in well-ventilated areas.

o) Environment impact

The effect of the disinfectant on the environment needs to be considered. This includes the extent of the biodegradability and the safe method of disposal. Disinfectants can be harmful to the environment through various actions or effects, in particular[15]:

- By causing resistant bacteria
- By causing higher tolerance (formaldehyde)
- By killing sensitive bacteria

- By affecting sewage treatment performance
- By forming organic halogen compounds (AOX) (especially sodium hypochlorite)
- By contaminating surface water
- By forming mutagenic substances

Control of Detergents and Disinfectants

Bulk chemical disinfectants and ready-to-use preparations should be controlled in the same manner as other incoming raw materials. As a minimum, a certificate of analysis should be inspected and an identity check performed.

Once received into the pharmaceutical facility, efficient stock control and rotation should be practiced. This includes discarding any disinfectants that have passed the manufacturer's stated expiry time. The disinfectant should be stored as per manufacturer's instructions - typically this is between 18-25°C and avoiding exposure to direct sunlight[16].

An important consideration for application of cleaning agents is whether sterile chemicals are required. This is ordinarily by filtration of a prepared solution using a filter with a porosity of 0.22µm. Normally, agents for use in EU GMP Grade A and B/ISO 14644 class 5 and 7 (in operation) environments must be sterile, whereas agents for use in EU GMP Grade C and D/ISO 14644 class 8 (in operation) environments are not normally required to be sterile. However, with such lower grade areas it is recommended that the solutions should be qualified or periodically monitored for microbiological contamination. A time limit should also be imposed on how long in-use dilutions be used for, with controls in place so that they are discarded after use.

Validation of Disinfectants

There is a regulatory expectation that disinfectants used in the facility have been qualified. For the qualification there are different test standards. Unfortunately, there is no international harmonisation, with competing standards in Europe (CEN norms) and in North America (AOC and ASTM). However, there is commonality on the types of qualification that needs to be undertaken, even if methodologies differ.

The following stages should be considered as part of a validation strategy:

- Disinfectant screening tests
- Disinfectant surface challenge tests
- Validation of the neutralising effect of contact plates
- Qualification of hold times for diluted disinfectants
- Field trial of cleaning and disinfection regime

It is incumbent on companies to provide a reasoned and justified approach to their disinfectant validation studies. A quality risk management approach should be applied. It may not be appropriate to perform all of the aforementioned steps and the extent of testing that is performed will be dependent on where and how the disinfectant is used.

The most common type of testing performed for the purpose of validation of cleanroom disinfectants involves the application of a known number of organisms mixed with an interfering substance onto a hard, non-porous surface (the 'surface test'). After the inoculum has dried, the disinfectant is then applied for a set contact time and, following neutralisation of the disinfectant and recovery from the surface, the number of organisms inactivated is calculated. There are a variety of internationally recognised test methods available, both qualitative and quantitative, with and without mechanical action. The reduction of the number of test organisms caused by a disinfectant is generally expressed as decimal logarithm (log), commonly referred to as the log reduction. In order to demonstrate compliance with the pre-determined acceptance criteria specified in terms of a log reduction, it is necessary to use a quantitative test method. The incorporation of mechanical action into the method is significant as disinfectants used in the cleanroom will usually be applied using some form of mopping or wiping. Test methods that do not incorporate mechanical action could therefore be considered as "worst case".

Individual companies need to consider the available methods and how they may be modified in order to meet their individual requirements. A summary of the most common EN, AOAC and ASTM methods is provided at the end of this section.

The following test parameters should be considered when designing a study:

- Test surfaces
- Test organisms
- Contact time
- Acceptance criteria
- Water quality
- Temperature
- Neutralisers

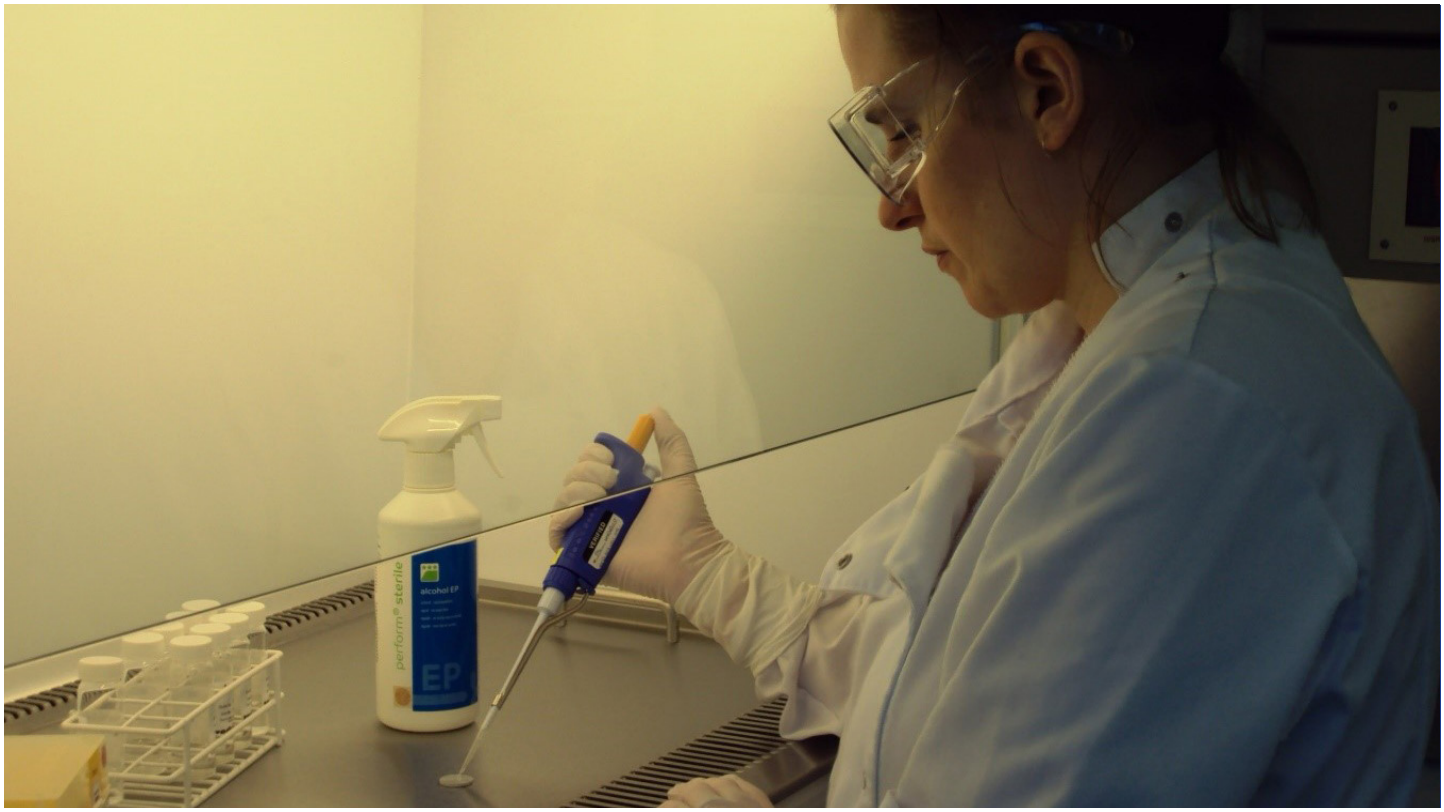
While modifying the test methods may more accurately reflect actual usage conditions, they may also adversely affect test reproducibility.

With the above list, the contact time is defined as the total time an organism is exposed to the antimicrobial action of a disinfectant.

The contact time necessary to achieve a desired level of reduction in microbial viability may differ according to the target organism.

The type of surfaces to be included in the study should be selected according to risk assessment considering:

- Predominance - total surface area
- Surface finish - how easy/difficult they are to clean/disinfect
- Criticality - proximity to critical process steps, potential for indirect product contact
- Suitability - the surface must be non-porous and possible to cut into coupons of a suitable size for testing
- Availability - in older facilities it may not be possible to obtain sufficient samples of the surface for testing



Technician performing a surface disinfection study (Image: Tim Sandle)

The selection of organisms for testing should be made according to risk assessment considering:

- Manufacturer's claims for biocidal activity – e.g. it is not appropriate to test a non-sporicidal product using a spore suspension
- The spectrum of organisms that could reasonably be expected to occur in the cleanroom – i.e. Gram-positive cocci, spore-forming bacilli, Gram-positive non-spore forming rod, or fungi
- A historical review of environmental monitoring data – each facility will have unique microbial flora with only a few species representing the majority of organisms recovered from the environment

The reduction of the number of test organisms caused by a disinfectant is generally expressed as decimal logarithm (log) and is commonly referred to as the log reduction. In the EN standard methods, this is calculated by comparing the number of survivors from the test article to the number of survivors in a control (test performed simultaneously without the disinfectant) in order to take into account any reduction in viability that has occurred as a result of the testing process itself. Typical values are:

- Vegetative bacteria - 3 log reduction
- Bacterial spores – 2 log reduction

Whilst in vitro testing can cover some practical usage conditions, it does not take into account all factors that may influence the success of the cleaning and disinfection regime as a whole. Such factors may include methods of application, frequency of disinfection and rotational patterns followed. As such, a field trail (also known as in situ testing) should be performed.

Typically, this involves increased environmental monitoring, prior to and sometime after incorporation of a new disinfectant into the hygiene regime. Assessment would be based on analysis of the trends of microbial counts and the types of microorganisms recovered.

Preparation of Detergents and Disinfectants

The application of a detergent solution, made in hot purified water (when hot water is needed this is normally > 25°C), is the primary method of cleaning and removing particulate matter from floors and surfaces in cleanrooms. In some areas, such as an aseptic filling area, hot water is unlikely to be available due to the restriction of water in such areas in order to maintain contamination control. Instead it is more common for detergents to be prepared using higher grade pharmaceutical water – water for injections (WFI)[17]. It is important that the correct amount of detergent, as defined by the manufacturer's recommendation, is added to the appropriate quantity of purified water. Once prepared, detergents should have defined holding times from preparation to last usage.

When preparing disinfectant concentrates as ready-to-use solutions, the correct amount of disinfectant (as defined by the manufacturer's recommendation), must be added to the appropriate quantity of purified water or water for injections. For most disinfectants, hard water is not suitable for use as a diluent. This is because the main ions of the water hardness, such as magnesium and calcium, will cause interference with certain active ingredients in the disinfectant formulation. The water added should be of the correct temperature. If the water is too hot, it will cause certain disinfectants to be unstable (such as hydrogen peroxide, which becomes unstable if the water added is at a temperature of 60°C or greater).

Assessing microbial contamination (Image: Tim Sandle)

A temperature range of 20-25°C is suitable for most types of disinfectants (and thus is close to the temperature at which validation of disinfectants is commonly performed).

Any dilutions performed on the disinfectant must be clearly stated in a standard operating procedure. The dilution should be expressed as either: % weight/weight (w/w), % weight/volume (w/v), or % volume/volume (v/v).

Like detergent solutions, disinfectants should have defined times between preparation and usage. This is either a time applied to disinfectants prepared 'in-house,' or the addition of an expiry time to pre-prepared disinfectants. For pre-prepared disinfectants (wipes, trigger sprays and hand sanitisers) the expiry time is often for no more than one month after opening unless supporting data from the manufacturer is available. An in-use shelf-life must be assigned once a disinfectant has begun to be used. With ready-prepared solutions, this will be based on the reliability of the trigger spray to avoid ingress of air. For concentrates, the shelf-life begins once a disinfectant has been diluted for use. This is an important consideration as a disinfectant will begin to lose activity after a period of time has elapsed.

Procedures

All cleaning and disinfection procedures start with a comprehensive standard operating procedure. The SOP should contain a general section of basic principles for cleaning and sanitisation. The operational section must include a comprehensive tools and equipment list. The SOP must specify the approved techniques for all surfaces with pictorial directions. The final section will list the frequency of the cleaning and disinfection and the sequence of that cleaning.

The procedures should cover the following aspects:

- The type of detergents and disinfectants to be used (which are compatible)
- The frequency of rotation of disinfectants
- A list of suitable cleaning materials
- Cleaning techniques
- Contact times
- Rinsing
- Frequency of cleaning and disinfection
- Procedure for the transfer of cleaning agents and disinfectants into and out of clean areas (including the procedure for sterilisation of disinfectants)
- Holding times for detergents and disinfectants (for how long, after preparation, can a detergent or disinfectant be used?)

When writing cleaning procedures it is good practice to involve the staff who will undertake the cleaning so that the procedures are clear and easy to follow.

The list of tools for cleaning and sanitising cleanrooms include vacuums, mops, buckets, wipes, specialty tools, cleaning agents and disinfectants.

The vacuum must contain a current certified high efficiency particulate air (HEPA) filter with the appropriate tools that will not damage the surfaces. HEPA filtered vacuums are generally used to remove any broken glass, particulate debris and for any construction or shutdown residue.

They could be used prior to sanitisation if the tool heads are sterilised. Some vacuums are portable floor models, backpacks or centralised systems. Mops and buckets must be compatible with the disinfectants. The mops selected must be sterile and should be capable of thoroughly coating a surface.

The mop should be of non-shedding materials with a handle constructed of stainless steel or reinforced plastic.

The bucket design is generally a 2-bucket or 3-bucket system. The buckets should be manufactured of plastic or stainless steel, with a capacity of 11–19 litres.

The buckets should be autoclaved prior to each use. A sterile liner could be used if autoclave cycles are not available. However, buckets and carts should be autoclaved with a prescribed frequency.

Two methods for floor mopping are the "pull-lift" method and the modified figure "8".

Areas to which a detergent is applied must be allowed to dry before the application of the disinfectant step.

Disinfecting a cleanroom (Image: Tim Sandle)

The cleaning and disinfection process consists of:

- Sweeping away dust and debris (if applicable)
- Application of a detergent solution through wiping or mopping
- Application of a disinfectant solution through wiping or mopping
- Removal of disinfectant residue (if low residue disinfectants are not used) through wiping or mopping with water for injections or 70% (v/v) isopropyl alcohol (IPA)

During the cleaning process, care should be taken not to damage any HEPA filters, especially by wetting them with cleaning agents.

The cleaning technique used for cleanrooms should be defined and standardised. It does not matter how effective the cleaning agents selected are if the cleaning technique practiced by cleanroom operators is poor. Thus the training of personnel is important. This partly relates to the techniques used and partly to the quality of the cleaning materials, for improperly cleaned areas where organic matter remains, or areas which have a detergent residue, could potentially confer some protection to microbial populations and impair the efficacy of the subsequently applied disinfectant. In many ways, the physical treatment of a surface or item of equipment is as important as the chemical.

Frequencies for Cleaning and Disinfection

Cleaning and disinfection sessions are performed at different frequencies for different areas. This is based on the criticality of the area and this will vary between facilities. The frequency is ideally established through risk assessment or based on empirical data, such as through a field trial. Cleaning and disinfection frequencies are often expanded through different parts of the cleanroom being cleaned or disinfected at different times ('levels of cleaning'). That is, floors and working surfaces will often be cleaned at a higher frequency than walls or ceilings.

Environmental Monitoring for Continuous Assessment

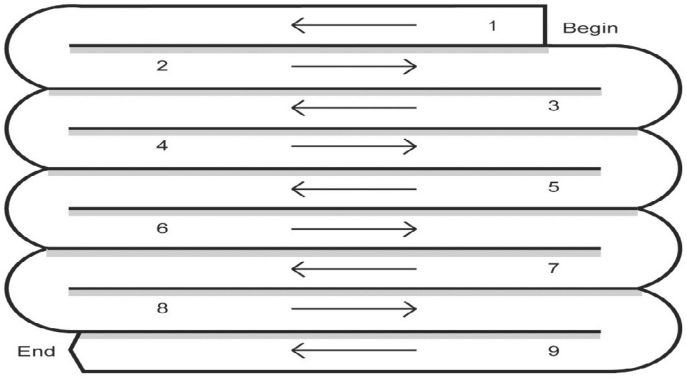
To ensure the effectiveness of a cleaning and disinfection programme, microbiological environmental monitoring of surfaces and equipment is necessary. The primary methods for conducting these tests involve the use of cotton swabs (with a recovery diluent and later plating onto agar or dissolving prior to membrane filtration) and contact plates. The agars used should contain appropriate neutralising agents in order to eliminate any disinfectant residues and thus allow any recovered microorganisms to grow. An appropriate general agar, such as soya-bean casein digest medium, is normally recommended. This agar, onto which swabs are sub-cultured or used in the contact plates, should have a dual incubation step designed to pick up a range of environmental microorganisms. A typical regimen would be 20–25°C for 48 hours followed by 30–35°C for 72 hours. However, the time and temperature selected must be qualified[18].

It is a regulatory requirement that the results of an environmental monitoring programme are examined at regular intervals for change in trends. This includes a check on the numbers of microorganisms recovered and for the development of resistant strains[20]. How the pharmaceutical organisation reacts to the presence of a resistant strain varies and the definition of a resistant strain (in terms of numbers, detection rate and spread of detection) will vary. Resistance is different to an "objectionable" microorganism. Some pharmaceutical organisations have lists of particular microorganisms that present a greater risk to products than others. Resistance, in this context, is about those microorganisms that are harder to kill or may be entirely resistant to the biocide applied[19].

Addressing Cleanroom Contamination

Contamination events are often representative of poor gowning techniques of the cleaning staff, barrier breach of the cleanroom garments, or poor cleanroom behaviour. Assuming the total number of colony forming units is below the alert limit, training should be the corrective action.

However, surface counts for spore-forming microorganisms, infectious organisms and moulds indicate a serious condition. These risks and the actions needed to return the cleanroom to the design level of control should be carefully understood.



The figure of '8' cleaning method

Aspects to consider in regards to causes of contamination include:

Testing disinfectant solutions (Image: Tim Sandle)

- Did someone enter the facility in ill health?
- Were the gloves or garments compromised by touching surfaces that were contaminated?
- Are the surfaces of the cleanroom breached?
- Is the rotation of disinfectant adequate?
- Is the organism resistant to the disinfectants selected?
- Has the air system (HEPA filters) been compromised?

There are other similar questions like these that must be addressed when the results of the monitoring plates are positive for growth outside what is "normal" for cleanrooms.

Summary

This white paper has, in the context of the pharmaceutical environment, examined the importance of the application of detergents and disinfectants within the pharmaceutical facility. The paper has emphasised the importance of observing GMP and the consistent and careful use of such agents in accordance with standard operation procedures. There has been an emphasis in this paper on the importance of a well-written policy to describe the agents which have been selected as well as the need for a clear procedure, to describe the application of cleaning and disinfection agents. The precise content of such documents will depend upon the actual facilities and the requirements of regulatory authorities.

The important point with disinfectants used in the pharmaceutical industry is taking care with their selection, ensuring that rotation is practiced, qualifying the disinfectants used and undertaking regular monitoring to note the continued efficacy. Covering these aspects ensures that an effective contamination control programme is maintained.

Cleaning in action (Image: Tim Sandle)

References and Further Reading

1. Sandle, T. 'Selection and use of cleaning and disinfection agents in pharmaceutical manufacturing.' In Hanlon, G. and Sandle, T. (Eds). Industrial Pharmaceutical Microbiology Standards and Controls, Basingstoke: Euromed Communications, pp9.1-9.32, 2015
2. Sandle, T. Pharmaceutical microbiology: essentials for quality assurance and quality control, Cambridge: Woodhead Publishing, pp185-198, 2016
3. Sandle T, Vijayakumar R, Al Aboody M, Saravanakumar S. In vitro fungicidal activity of biocides against pharmaceutical environmental fungal isolates. Journal of Applied Microbiology. 2014;117(5):1267-73
4. Sandle, T. Ensuring Contamination Control: The validation of disinfectants, European Medical Hygiene, November 2012 edition, pp33-39.
5. Vina, P., Rubio, S. and Sandle, T. 'Selection and validation of disinfectants.' In Saghee, M.R., Sandle, T. and Tidswell, E.C. (Eds.) Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices, Chapter 11, New Delhi: Business Horizons, pp219-236, 2011.
6. Russell, A. D.: Assessment of sporicidal efficacy, International Biodeterioration and Biodegradation, 41, 1998, pp281-287.
7. Vijayakumar, R., Kannan, V.V., Sandle, T., and Manoharan, C. In vitro Antifungal Efficacy of Biguanides and Quaternary Ammonium Compounds against Cleanroom Fungal Isolates, PDA Journal of Pharmaceutical Science and Technology, 2012, 66 (3): 236-242
8. Rabenau HF, Steinmann J, Rapp I, Schwebke I, Eggers M. Evaluation of a virucidal quantitative carrier test for surface disinfectants, PLoS One. 2014; 9(1):e86128
9. Sandle, T. Application of disinfectants and detergents in the pharmaceutical sector. The CDC Handbook: A guide to cleaning and disinfecting cleanrooms, Grosvenor House Publishing: Surrey, UK, pp168-197, 2012
10. Sartain. E. K. 'Regulatory Update: Rotating Disinfectants in Cleanrooms: Avoid Going in Circles,' A2C2 Magazine, Vol. 8, No. 3, March, 2005, pp. 32-33
11. Buffet-Bataillon S, Tattevin P, Maillard JY et al. Efflux pump induction by quaternary ammonium compounds and fluoroquinolone resistance in bacteria, Future Microbiol. 2016;11:81-92
12. Sandle, T. Application of disinfectants and detergents in the pharmaceutical sector. The CDC Handbook: A guide to cleaning and disinfecting cleanrooms, Grosvenor House Publishing: Surrey, UK, pp168-197, 2012
13. Sandle, T. A review of cleanroom microflora: types, trends, and patterns, PDA Journal of Pharmaceutical Science and Technology, 2011; 65 (4): 392-403
14. Alenitsyn, Alexander G.; Butikov, Eugene I.; Kondraryez, Alexander S. Concise Handbook of Mathematics and Physics. CRC Press. pp. 331–332, 1997
15. Dettenkofer M, Block C. Hospital disinfection: efficacy and safety issues. Current Opinion in Infectious Diseases. 2005; 18(4):320-5
16. Black & Veatch Corporation. White's handbook of chlorination and alternative disinfectants, Hoboken: John Wiley & Sons, 2011, pp70-72
17. European Pharmacopeia. Water for Injections 01/2009: 0169, European Pharmacopeia Edition 6.3, Brussels: European Commission, 2009, pp4339-4341
18. Sandle, T. Examination of the order of incubation for the recovery of bacteria and fungi from pharmaceutical cleanrooms, International Journal of Pharmaceutical Compounding, 2014; 18 (3): 242 – 247
19. Abdallah M1, Benoliel C, Ferreira-Theret P, Drider D, Dhulster P, Chihib NE Effect of culture conditions on the resistance of Pseudomonas aeruginosa biofilms to disinfecting agents, Biofouling. 32015; 1(1):49-59

About the author

Dr Tim Sandle

Dr. Tim Sandle has over twenty-five years' experience of microbiological research and biopharmaceutical processing. He is a member of several editorial boards and he has written over six hundred book chapters, peer reviewed papers and technical articles relating to microbiology. Dr. Sandle works for a pharmaceutical manufacturer in the UK, and is a visiting tutor at both the University of Manchester and UCL.

Excellence in Science and Service

Reading Scientific Services Limited (RSSL) is a cutting-edge Contract Research Organisation (CRO) and winner of Business and Employer of the year at the 2021 Thames Valley awards. We pride ourselves on our excellence in science, quality and service.

For over 30 years, we have been providing support to the Pharmaceutical Sterile Manufacturing Industry and recently launched Sterility Testing (membrane filtration and direct inoculation), with Mycoplasma Testing to be offered soon. Our expert team can also support with raw material, vial and stopper testing to microbial analysis such as TAMC/TYMC and endotoxin (LAL).

We work in partnership with our clients to ensure that they meet the regulatory requirements both with routine testing as well as more complex projects such as cleaning validation and environmental monitoring, using the wealth of experience from our multi-disciplinary team of technical experts and consultants.

Sterile Manufacturing Support Services:

- Sterility Testing
- Endotoxin Testing
- Environmental Monitoring
- Raw Materials
- Vial and Stopper Testing
- Mycoplasma (coming soon)
- Investigative Problem Solving
- 24/7 Emergency Response Service
- Training and Consultancy

To find out more about how we can support your Sterile Manufacturing or to discuss your needs further, please contact us on: **+44 (0)118 918 4076**, email **enquiries@rssl.com**, or visit **www.rssl.com**

About Reading Scientific Services Ltd (RSSL)

We're a cutting-edge Contract Research Organisation (CRO), pushing the boundaries of science and innovation to support our clients developing life-changing treatments for patients. Our clients trust us to deliver innovative solutions to real-world problems facing the global life sciences, pharmaceutical, healthcare and personal care sectors.

From our state-of-the-art facilities in Reading, UK, our multi-disciplinary team work hand in hand with our clients to develop and supply drug products that are safe, innovative and capable of transforming lives around the world. We offer a diverse range of biological, microbiological, chemical and physical R&D and analytical services across all phases of clinical development into commercial release. We also provide bespoke training and consultancy to deliver tailored solutions.

Contact us to find out how we can support your sterile manufacturing goals.

Reading Scientific Services Ltd
The Reading Science Centre,
Whiteknights Campus,
Pepper Lane,
Reading,
Berkshire RG6 6LA

Tel: **+44 (0)118 918 4076**
Email: **enquiries@rssl.com**
Web: **www.rssl.com**