Aseptic Handling

31

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Contents

Abstract

Aseptic handling is the process to enable sterile products to be made ready to administer, using closed systems. The starting materials are sterile and must be kept sterile during the process. This chapter describes the conditions to do so (sterility assurance). The most important points are trained staff wearing special clothes and sterile gloves, working 'non touch in a Grade A zone (LAF cabinet, safety cabinet or isolator) and using materials and equipment with a low bioburden.

If antineoplastics (cytostatics) are involved requirements are not only to protect the product against contamination of micro-organisms, but also to protect the operator and the environment from these hazardous medicines.

Microbiological checks are carried out firstly to see if staff are sufficiently skilled in aseptic activities, secondly to determine the microbial risk from the environment and thirdly to validate the aseptic procedures.

This chapter does not cover the situation where medicines that cannot be sterilised in their final container are sterilised by aseptic filtration.

Keywords

Aseptic handling • Aseptic processing • GMP Annex 1 • Antineoplastics • Microbiological controls • Monitoring • Validation • Qualification

31.1 Definitions

Aseptic Processing	The process used for products that cannot be sterilised in their final container, i.e. cannot be terminally sterilised.

Based upon the chapter 25 Aseptisch Werken by Frits Boom, Hans van Doorne and Marco Prins in the 2009 edition of Recepteerkunde.

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Conflicting Definitions for the Term "Aseptic Preparation"
In the EU GMP Annex 1 "aseptic preparation" is used for the preparation of sterile products that cannot be sterilised in their final container [1]. In the UK the term "aseptic preparation" is used for aseptic handling without a manufacturing licence granted by the Competent Authority.

Aseptic Handling	The process to enable sterile products to be made ready to administer, using closed systems.
Closed Procedure	A procedure whereby a sterile pharmaceutical product is prepared by transferring sterile ingredients or solutions to a pre-sterilised container, either directly or using a sterile transfer device, without exposing the solution to the external environment [2].
Bioburden	Total number of viable micro-organisms on or in a health care product prior to sterilisation [3].
Colony Forming Unit (CFU)	One or more micro-organisms that produce a visible, discrete growth entity on a semisolid, agar-based microbiological medium [3].

31.2 Aseptic Processing

Medicines, which cannot be sterilised in their final container, are sterilised by aseptic filtration. The standards required for aseptic processing are laid down in Annex 1 of EU GMP [1]. Aseptic processing is called aseptic preparation in Annex 1 (see Sect. 31.1).

To reduce risks of microbial contamination, aseptic processing is executed in a controlled environment, in which the air supply, facility, materials, equipment and personnel are regulated to control microbial and particulate contamination to acceptable levels [3]. Contact between product and environment should be minimised, sterile equipment should be used, and there should be two consecutive filtration processes through sterile 0.2 μm filters. The first filter will minimise the microbial challenge to the second filter, which should be just before the sterile final container. The shelf-life of the product is often restricted and it may be stored in the refrigerator to further reduce the risks of microbial growth.

For more information about membrane filtration see Sect. 30.6.

31.3 Aseptic Handling

Within pharmacy, aseptic handling is carried out in a controlled environment by trained staff. In any hospital, however, aseptic handling also takes place in clinical areas such as wards and operating theatres [4, 5]. This chapter only discusses aseptic handling in pharmacy. Aseptic handling in clinical areas is described in Sect. 13.8.

31.3.1 Guidelines for Aseptic Handling

In 2008 the Pharmaceutical Inspection Convention published the PIC/S guide to good practices for the preparation of medicinal products in healthcare establishments [2]. Although this document is a stand-alone document and should be used for PIC/S related inspections, it is used more and more as a reference for preparation including aseptic handling in pharmacies in Europe.

USP Chapter <797> Pharmaceutical compounding – Sterile preparations describes the conditions and practices for all sterile preparations in compounding pharmacies in the United States [6]. The so-called compounded sterile preparations (CSPs) are divided into low-risk level, medium-risk level and high-risk level. Low- and medium-risk levels use closed systems and cover aseptic handling in controlled environments.

In the United Kingdom "Quality Assurance of Aseptic Preparation Services" are the national NHS standards for aseptic preparation in hospital pharmacies [7].

In 1996, the Dutch Association of Hospital Pharmacists wrote, in close cooperation with the Dutch Healthcare Inspectorate, a GMP guide for hospital pharmacy. The Chapter Aseptic Handling in this guide has been reviewed in 2005 and 2013 and covers aseptic handling in the hospital pharmacy as well as in clinical areas [8]. Different levels of product protection are defined.

In 2012 the German Organisation of Hospital Pharmacy published the ADKA guideline on Aseptic Preparation and Quality Control of ready-to-administer parenterals. All precautions to be taken to keep the products sterile are described systematically [9].

The differences between these guidelines and standards are not huge. They all focus on preventing microbiological contamination and more or less on medication errors, e.g. due to incorrect calculations. This chapter only discusses preventing microbiological contamination.

Levels of Product Protection in the Netherlands [8]
The sterility of the product is maintained by using a controlled environment and trained staff. Depending on how well these can be controlled, three levels of product protection can be distinguished [8]:

- Limited product protection is the lowest of these three levels, and refers to a doctor or nurse carrying out the aseptic activity on a clean worktop.
- Increased product protection is the middle of the three levels. This can be carried out in a clinical area in a separate room within a laminar airflow (LAF) or safety cabinet or isolator by a member of pharmacy staff or by a nurse or doctor.
- Maximum product protection is the highest of the three levels of product protection. In this case the aseptic activity is carried out within a LAF or safety cabinet or isolator sited within at least an EU Grade D of controlled background in the hospital pharmacy.

31.3.2 Complexity

Aseptic handling varies in complexity from drawing up the contents of a vial or ampoule into a syringe, to compounding a parenteral nutrition mixture from several separate starting materials. Complexity of aseptic activity has been defined in several texts [6, 10, 11].

As complexity increases so does risk of microbiological contamination, although there is little evidence to support this. Other risk factors for microbiological contamination include the extent to which the product is a good growth medium, and the time between its preparation and administration to the patient.

Some authors have developed risk assessment tools for injectable medicines [11–13]. These tools can be helpful in determining if a process is simple or complex, and hence a higher risk, see Table 31.1.

31.3.3 Staff and Personal Hygiene

The greatest source of contamination in any clean room is the operator [15]. He or she spreads micro-organisms directly into the surrounding air and either directly or indirectly onto surfaces in the room. This can be minimised by the operator donning suitable clean room clothing such as non-shedding suits or coats (depending on the EU Grade of environment), hair cover, shoe covers or dedicated clean room shoes, gloves, and a mask covering the nose and mouth.

The operator remains a source of microbiological contamination, nonetheless, and so aseptic technique is important to protect the product [16]. The principle is to avoid direct contact between the operator and the product (non-touch technique) and hence it is essential to have suitably trained operators whose competency is regularly assessed and who are appropriately supervised.

Before any aseptic activity, hands should be thoroughly washed and disinfected, usually with an alcohol-based gel. The skin flora is both transient and resident. Resident flora is difficult to remove, so it is recommended to always use gloves within the clean room suite. Whilst carrying out aseptic handling in a Grade A environment, sterile gloves are required.

31.3.4 Working Area

The working area is the immediate environment in which the aseptic handling is performed. It is the working surface (EU Grade A zone) of the LAF or safety cabinet or isolator.

Table 31.1 Examples of simple and complex activities [8]

Simple activities
Drawing an injection liquid from a vial or ampoule* into a syringe
Dissolving a powder for injection and drawing it into a syringe
Injecting a few injections into an infusion liquid
Complex activities
Preparing a medication cassette (several additions, de-aerating, long-term use at room or body temperature)
Preparing parenteral nutrition from components (several additions, mixing large volumes, good growth medium)
Preparing parenteral nutrition starting from a registered all-in-one commercial product with more than two additions (several additions, good growth medium)

*Transferring injection liquid from an ampoule involves more risk of microbiological contamination than from an injection vial as a vial is a truly closed system [14]

The background area is the room in which the LAF or safety cabinet, or isolator is housed. In the case of open-fronted cabinets there is a distinction in background requirements between the different guidelines mentioned before. The PIC/S guide [2] and the German ADKA guideline [9] require at least EU Grade C, the Dutch hospital pharmacy GMP [8] at least EU Grade D and the requirements in the UK are harmonised with Annex 1 of EU GMP, i.e. Grade B. For isolators the requirement for the background in all guidelines is at least Grade D [1].

Reference is made to Sect. 28.3 for more information on clean rooms, LAF cabinets, safety cabinets and isolators.

The precautions for the different kinds of product protection (see Sect. 31.3.1) as used in the Netherlands are summarised in Table 31.2.

Table 31.2 Level of product protection (Example from the Netherlands)

Level of product Protection	Clothing and hand hygiene	Working area	Background area	Air quality in background area
Limited	Clothing, daily cleaned Hands: cleaned and disinfected, single use gloves for each session	Table top, disinfected per session	Quiet	No requirements
Increased	Clothing: overalls, daily cleaned, hair cap, mouth-nose mask Hands: cleaned and disinfected, sterile single use gloves for each session	Horizontal LAF cabinet, safety cabinet or overpressure isolator	Separate, limited access; easy to clean	No requirements
Maximum	Clothing: overalls, daily cleaned, hair cap, mouth-nose mask, special shoes Hands: cleaned and disinfected, sterile single use gloves for each session	Horizontal LAF cabinet, safety cabinet or overpressure (positive pressure) isolator	In accordance with GMP Grade D: smooth surfaces, interlocked changing rooms etc.	Grade D

31.3.5 Aseptic Handling of Antineoplastics

A relevant therapeutic group of active substances, handled aseptically, are parenteral antineoplastics. Many are classified as very toxic for the operator, mainly because of carcinogenicity and reprotoxicity [17], see also Sect. 26.3.3. Therefore, if antineoplastics are involved in aseptic handling, requirements are not only to protect the product against contamination of micro-organisms, but also to protect the operator and the environment from the product. The first measure however is a working procedure to minimise exposure to antineoplastics. This involves

- Working with closed systems (this is good practice for all aseptic handling)
- Using injection vials and needle-free devices, as far as possible, to minimise needle-stick injuries. If sharps cannot be avoided, their use should be minimised [18], see also Sect. 26.10
- Using an aeration spike with a hydrophobic filter to avoid overpressure in vials
- Attaching an infusion system, partly filled with Sodium Chloride 0.9 %, to the infusion bag with antineoplastics to reduce the chance of leakage of antineoplastics when the bag is attached to the patient)

To protect the operator and the environment, the most effective measure is working in a safety cabinet or in an isolator with underpressure (negative pressure). Ideally the exhausts of these cabinets should be connected to the open air. An isolator gives more protection than an open-fronted cabinet. See Sect. 28.3.

The safety cabinet or the isolator should be placed in a well-ventilated and classified background room. To protect surrounding rooms from airborne contamination of antineoplastics the USP advises negative pressure in the background room [6]. However, a study in the Netherlands, carried out by the Netherlands Organisation for Applied Scientific Research (TNO) concluded that airborne contamination from a grade D background (overpressure 15 Pa) to the surrounding environment is not a risk issue.

TNO Study: Risk During Aseptic Handling of Antineoplastics

Risk from airborne contamination of antineoplastics from a background room (with overpressure) to surrounding rooms is only a question of concern in the case of a huge calamity such as a spill or breakage involving a large amount of antineoplastics as a dry powder. Calamities are rare (less than once a year in a Dutch hospital pharmacy) and when it occurs, it is nearly always with a solution in which the antineoplastics are dissolved. The risk of transferring antineoplastic residues to the environment by hands or the outside of vials or finished products is much greater (see also Sect. 26.5.4). Measures to prevent this occurrence and also regular training in emergency procedures are most important to protect people and environment from the risk of antineoplastic residues.

Further protective measures are:

- Personal protection by wearing coveralls and sleeves made of impermeable material and sterile gloves into which antineoplastics do not easily permeate, see Table 26.6
- Working on a sterile preparation pad and removing the pad after each session as antineoplastic-contaminated waste

To prevent contamination with residues it is important to know that cross contamination of antineoplastics from one room to another by direct contact (outside of vials, outside of finished products) is a real risk [19, 20]. This risk can be significantly reduced by a validated cleaning procedure for the safety cabinet or isolator and the background room, and by properly packing the finished products before transport to nursing and treatment centres. Additionally a procedure for removing (potentially) antineoplastic-contaminated waste and a procedure on how to handle spillages or in an emergency are important to prevent contamination with residues. Environmental sampling by wipe tests should demonstrate that all those procedures are effective. In the Netherlands and in Germany a surface contamination limit of less than 0.1 ng/cm^2 is becoming used more and more as a guideline value, see Sect. 26.5.4.

31.3.6 Storage Periods

Wherever possible, aseptic products should be stored at 2–8 °C. The shelf life depends on the chemical stability of the product and the potential for microbial contamination [6, 8]. In the Netherlands, from a microbiological point of view, a shelf life at 2–8 °C for 1 month for simple and 1 week for complex aseptic handling is acceptable. In the UK, aseptic products made in pharmacy can only be given a shelf life of seven days unless the pharmacy is licensed with the MHRA (Medicines and Healthcare products Regulatory Agency) for these activities.

Table 31.3 Shelf life and administration period for aseptic handling [8]

	Shelf life		Administration period*	
Complexity	Time	Condition	Time	Condition
Simple	1 month	2–8 °C	7 days	Room temp.
Complex	7 days	2–8 °C	7 days	Room temp.

*Shelf life and administration period are two separate periods. For example: if the administration with a medication cassette is started 6 days after preparation of that cassette, the administration period still will be 7 days

Medicines produced by aseptic handling are sometimes administered for more than one day (medication cassette, parenteral nutrition etc.). During the administration the product temperature is higher than the storage temperature, which influences the shelf life. Therefore a second period has to be used, the administration period, which is defined as the maximum time from start to the end of the administration. Both, shelf life and administration period for simple and complex aseptic handling at maximum level of product protection as used in the Netherlands are summarised in Table 31.3 [8].

31.4 Cleaning and Disinfection

The emphasis on providing the correct level of cleanliness is to ensure that the properly designed and maintained area is clean and dry. Depending on monitoring results, the use of a disinfectant may need to be considered. However, disinfection is difficult to achieve in an area with even small amounts of dirt [21].

Disinfectants and detergents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilised. Disinfectants and detergents used in Grades A and B areas should be sterile prior to use.

31.4.1 Cleaning of Clean Rooms

A suggested cleaning regime is that floors and work surfaces are cleaned daily and walls, ceilings and storage shelving at least monthly [6]. All cleaning materials, such as swabs and mops, shall be nonshedding and must be disposable or suitably washed after each cleaning session. Mops and swabs used in Grade A or B areas must be sterile.

Swabs and Mops Used in Clean Rooms
Clean rooms have to be wet cleaned with the aid of polyester or microfibre swabs or mops. Polyester is used for light cleaning and disinfecting. The polyester fibres adsorb dirt and if wetted with a disinfectant the disinfectant will be evenly spread out on the surface. Microfibre swabs and mops are used for cleaning only. The microfibres ensure that particles are not only removed from the cleaned surface but are firmly captured within the fibres. Dry polyester swabs or mops are only used for removing wet product or other wet waste.

Some general remarks on cleaning of clean rooms:

- Approved standard operating procedures should state how the various rooms are to be cleaned, what materials have to be used and how adequacy of cleaning is checked.
- Cleaning materials for clean rooms should not be used in other rooms.
- Thorough rubbing is important for the effectiveness of the cleaning process.
- Spilt materials must be removed immediately with a non-shedding absorbing cloth.
- Floors and walls must be cleaned in a fixed order from cleanest to least clean. The air stream and position of the exit determine where to start and where to finish.
- Cleaning of clean rooms requires specially trained staff.
- The same clothing regulations apply for cleaning staff as well as for staff preparing the product.
- The effectiveness of the cleaning should be checked.
- Cleaning must be recorded in a log which gives details of the cleaning agent used in addition to the person who has performed the cleaning.

Regularly (monthly) a general check is advised on the level of cleanliness, paying particular attention to corners and ridges. The cleanliness of the surface is best assessed by floodlight. Specialist ultraviolet lamps are also available for this purpose. If necessary, however, the cleanliness may be checked with a white non-shedding cloth. Contact the person responsible for the cleaning process if the background room or changing rooms are not sufficiently clean.

31.4.2 Cleaning of LAF Cabinets, Safety Cabinets and Isolators

It is advisable to leave LAF cabinets, safety cabinets and isolators running (possibly in standby mode if this can be validated) to avoid dirt and micro-organisms accumulating on the clean side of the HEPA filters, i.e. in the Grade A working zone. Because of the frequent disinfection of these devices, separate cleaning with a detergent is not necessary. Any spills must be cleaned as quickly as possible with a non-shedding cloth and, if necessary, with sterile water.

31.4.3 Disinfection of Clean Rooms

Using good cleaning procedures, the disinfection of a Grade D room should not be necessary. Disinfection of a Grade C room can be a necessity, depending on monitoring results. Grade B rooms need a precise disinfection procedure.

Clean room disinfection should be performed with a broad spectrum (non aggressive) disinfectant. Most commonly used are alcohols, chlorine compounds, hydrogen peroxide, phenolic compounds and quaternary ammonium compounds. Table 31.4 gives an overview of the microbiological inactivation. Alcohols and hydrogen peroxide do not leave residues after evaporation. Sodium hypochlorite is very corrosive towards many materials, including stainless steel. For more information about the disinfectants in Table 31.4 see [6] and [22].

Normally two disinfectants are used alternately to prevent accumulation of resistant micro-organisms, however there is little evidence to support this [23].

Table 31.4 Classes of commonly used disinfectants [6, 22]

Chemical class	Examples	Common activity range
Alcohols	70 % ethanol	Bactericidal, fungicidal (limited range) virucidal (limited range)
	70 % isopropanol	
Chlorine compounds	0.5 % sodium hypochlorite	Bactericidal, fungicidal, mycobactericidal, virucidal, sporicidal
Hydrogen peroxide	0.5 % hydrogen peroxide solution	Bactericidal, fungicidal, mycobactericidal, virucidal, sporicidal
Phenolic compounds	0.4–1.6 % chlorocresol	Bactericidal, fungicidal, mycobactericidal, virucidal
	0.4–1.6 % orthophenyl phenol	
Quaternary ammonium compounds	0.4–1.6 % benzalkonium chloride	Bactericidal (not all gram-negative types), fungicidal, virucidal (limited range)

31.4.3.1 Disinfection of LAF Cabinets, Safety Cabinets and Isolators

Commonly used disinfectants are ethanol 70 % or isopropyl alcohol 70 %. The disinfectant must be sterile and spore-free. This can be achieved by adding 0.125 % hydrogen peroxide, sterilisation by 0.2 µm filtration, or sterilisation by gamma radiation. Neither ethanol nor isopropyl alcohol are sporicidal, i.e. they are not effective against bacterial spores (see Table 31.4). Unless there are validation data from the manufacturer, it is recommended that the maximum in-use period for sterile disinfectants is limited to one week after opening. This should be noted on the container after it is opened. (Often the contents will be used well before then.)

LAF cabinets, safety cabinets and isolators should be disinfected from cleanest to less clean areas, e.g. from back to front for a horizontal LAF, in overlapping strokes. The non-shedding cloth (polyester is advisable due to its low particle load) has to be wetted regularly to make sure there is a constant film of liquid on the surface, and this will dry in the airstream. An extension to hold the cloth may be used to disinfect surfaces that are difficult to reach.

31.4.3.2 Disinfection of Materials and Equipment

The initial microbiological surface contamination (bioburden) of materials and equipment used in LAF cabinets, safety cabinets and isolators (Grade A zone) should be as low as possible. Surfaces of sterile devices (tubes, syringes, needles etc.) are presented sterile. Surfaces of ampoules, vials and bottles however are not presented sterile and must be disinfected before being transferred into the Grade A zone. Although 70 % alcohol is widely used for disinfection, it is non-sporicidal. The most effective method with liquid disinfection is a combination of spraying and wiping [24]. The physical movement of the wipe over the surface will help to remove the organisms. Nevertheless, spore forming bacteria will not be totally eliminated [24, 25]. Beware of excessive spraying, as the maximum allowable concentration of alcohol in the air can easily be exceeded, see Sect. 26.7.2.

There are no formal regulations on how often and where the transfer disinfection has to be carried out. In general, regular microbiological monitoring of gloved hands, materials and equipment has to show that the chosen disinfection procedure is effective (see Sect. 31.6.1). In the pharmaceutical industry, disinfection between each clean room level is common. In the UK, during aseptic handling, materials and equipment undergo at least two separate disinfection steps. In the Netherlands only one step is common.

Just before use, critical spots (vial stoppers, ampoule necks) should be disinfected again.

31.5 Aseptic Work Session

Remove as much secondary packaging as possible before transfer into the background area to minimise dust and bioburden. Transfer materials into clean rooms via interlocking hatches. Minimise storage of starting materials and components in the background area and, if stored, use closed cupboards. Documentation and labels should be generated in outer support areas.

Work with a ‘sterile area’ where materials can be placed after they have been disinfected and before they are put into the grade A zone if the quantity of materials to be processed is too large to have in the grade A zone simultaneously. A ‘sterile area’ may be (part of) a work top or the top of a trolley.

It is recommended that two people perform an aseptic work session [26]. One (the preparer) works in the Grade A zone and the other (the helper) carries out the disinfection and assists the preparer with getting materials to and from the working area.

Staff should be fully trained in good aseptic techniques. Special attention has to be given to ‘non-touch’ manipulation, which means that critical places like syringe tips should never touch non-sterile surfaces. Disinfected surfaces (work top) and hands (even when sterile gloves are worn) must be considered as non-sterile surfaces.

An example of a working procedure step by step:

- Remove secondary packaging as much as possible before materials are placed in the interlocked hatch (in UK the first disinfection stage is at this point).
- Prepare documents and labels outside the background room.
- Wash hands in or adjacent to the changing room.
- Change clothes according to the clothing procedure in the changing room, disinfect hands and put on non-sterile gloves.
- Enter the background room.
- Collect all necessary materials and check these according to the preparation document.
- Hang preparation document in an easy-readable place.
- Disinfect the work top in the grade A zone and if necessary disinfect the ‘sterile area’ outside the grade A zone.
- Disinfect the non-sterile gloves and
  - Disinfect the outside of the materials and place them in the grade A zone or on the ‘sterile area’.
  - Remove the outer layer of the wrapped, sterilised disposable equipment and place them in the grade A zone or on the ‘sterile area’.
- Disinfect hands.
- Put on sterile gloves (this can be done outside the grade A zone).

- Place materials in the correct order in the grade A zone.
- Disinfect critical spots (vial stoppers and ampoule necks).
- Carry out the aseptic handling.
- Label the product.
- Remove the product and waste material from the grade A zone.

Keep Sterile Gloves Sterile!
It is essential to keep the outside of the gloves sterile or at least low bioburden. Thoroughly disinfecting the outer surface of the materials before transferring to the LAF cabinet, safety cabinet or isolator and keeping the gloved hands in the LAF cabinet or safety cabinet are most important. Sterile gloves can be disinfected, but disinfected gloves are slippery. So, don't disinfect too often and dry gloves in the sterile airflow. A disinfection frequency of 30 min is satisfactory in general, however gloves should be disinfected after removal from the work station before returning to the Grade A working zone. Every pharmacy should monitor this process using finger dabs (see monitoring). Check gloves constantly for damage (at least every 30 min as a minimum) and change gloves immediately if they are damaged. Avoid changing gloves during a session unless they are damaged, however.

Sterile filtration is not by definition included in aseptic handling. For aseptic handling, the starting materials and all equipment are sterile and closed procedures are used. When drawing up from glass ampoules (risk of glass particles) a sterile filter straw or filter needle should be used. Replace the filter straw or needle with a fresh needle before adding the solution to another container. In the case of complex aseptic manipulations like filling a medication cassette, sterile filtration (0.2 μm) may be used as an additional precaution, however.

31.6 Microbiological Controls

Although aseptic handling differs significantly from aseptic processing, the principles for microbiological controls, like monitoring and process validation, are the same. As most aseptic work is done manually, the aseptic technique of the operators has to be checked with additional microbiological controls.

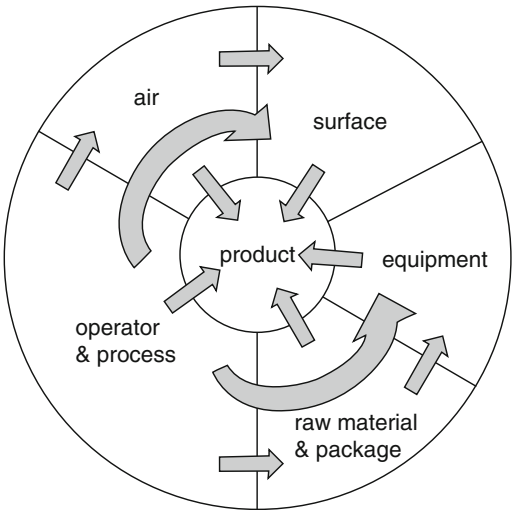

Fig. 31.1 Microbiological threat to the product. Source: Recepteerkunde 2009, © KNMP

31.6.1 Microbiological Monitoring

Microbiological monitoring is applied to determine the extent of environmental contamination. Figure 31.1 states which environmental factors are important: the nearer to the (open) product, the more risk of contamination.

It is most important, therefore, to monitor the areas nearest the product for microbiological contamination. Monitoring generally focuses on counting the numbers of micro-organisms. Where products with a shelf life of several months are concerned, monitoring results have to be considered before product release. In the case of aseptic handling, however, monitoring results are often not available at the point of release of the aseptic product.

31.6.1.1 What has to be Monitored?

For monitoring the air, settle (sedimentation) plates and volumetric air samplers are used. The latter come in various types [27]. Most of them suck up a fixed volume of air and the micro-organisms are deposited on a growth medium. With settle plates, the micro-organisms fall onto an opened 90 mm Petri dish containing an agar medium (see Fig. 31.2).

The opening time has to be 4 h [1]. If the preparation time is shorter (usual in case of aseptic handling) the settle plates have to be closed at the end of the preparation.

Monitoring of flat surfaces is carried out with contact plates of agar medium in a 55 mm dish. The medium has a slightly convex surface which can be gently pressed on the surface to be examined, see Fig. 31.2. These plates are sometimes known as RODAC plates (RODAC is the brand

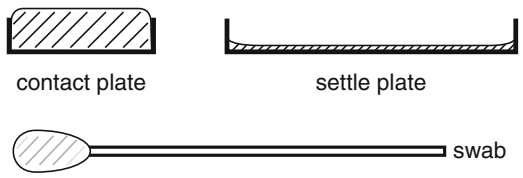

Fig. 31.2 Techniques for monitoring

name meaning Replicate Organism Duplicate Agar Contact). Remnants of the growth medium may stay on the sampling spot so cleaning and disinfection after sampling must be a standard component of the sampling procedure.

If the surface is not flat and or accessible, a swab has to be used. This is a small wad of cotton on the end of a short rod, see Fig. 31.2. First this has to be wetted with sterile water. After that it can be swabbed onto the surface to be examined and finally it has to be wiped across an agar medium in a petri dish. The recovery from a contact plate is 30–50 % and from a swab around 10 % [24].

Monitoring of the gloved hand is done by fingerprints (finger dabs). At the end of each session the tip of the five fingers of the gloved hand should be pressed gently but firmly on an agar surface in a petri dish. Use one plate per hand. In Dutch hospital pharmacies printing is only done with the most used hand [8]. In the UK and USA both gloved hands are monitored [6, 27].

31.6.1.2 Media and Incubation Time

The agar medium in the petri dishes and the contact plates is Tryptone Soy Agar (TSA) on which most micro-organisms that we can expect in the environment grow. Incubation temperature is 30–35 °C and the incubation time is at least 3 days. Especially for yeast and moulds Sabouraud dextrose agar is used. The incubation temperature and time for this medium are 20–25 °C and 5 days. Investigation in Dutch hospital pharmacies has shown that a broad spectrum of bacteria, yeast and moulds grow well on TSA at 30 °C within 3 days [28].

Colonies, which may be counted, grow from the micro-organisms on the agar surface. As it is not known whether the colony has developed from one or several micro-organisms, the expression colony forming units (CFU) is used. The results after incubation are expressed as CFU per plate.

31.6.1.3 Environmental Sampling Plan

An appropriate sampling plan has to be part of the environmental monitoring programme. It consists not only of the sampling locations but also of the sampling frequency. The locations should be based upon a risk analysis to determine the spots most likely to be contaminated during the aseptic activities. For settle plates and contact plates in the Grade A working zone, this is underneath the place where the aseptic activities are carried out.

The sampling frequency during/after aseptic handling in the Netherlands consists of every working day one settle plate and one contact plate in the Grade A working zone and immediately after the session one gloved finger print with the most used hand [8]. The frequency of monitoring the background Grade D room can be lower. A sampling plan of contact plates for the critical spots on the bench top (s) and several settle plates both every month, will give enough information about the contamination levels.

Before starting aseptic handling in a new facility or after a major process change, initial validation should be carried out. Part of this is frequent monitoring of all the locations to determine the average contamination level. After that, monitoring results should be reviewed on a periodic basis as a means of evaluating the overall control of the aseptic process. A graphical representation will help to determine whether there is an upward increase (trend) in the level of contamination present. An example of a graphical representation is shown in Fig. 31.3.

31.6.1.4 Limits, Alert and Action Levels

What are the criteria for monitoring results during aseptic handling? Table 31.5 gives the recommended limits for microbiological monitoring of clean areas during operation from the EU GMP guide [1].

As stated before, the sampling frequency during/after aseptic handling in the LAF cabinet, safety cabinet or isolator in the Netherlands is only one settle plate, one contact plate and one finger print. When there is growth, the average will be 1 CFU or more and that is above the limits for Grade A in Table 31.5. However, when aseptic handling is performed in the right way the frequency of samples with growth is low and the average CFU over a longer period will be far below one [29].

When the average contamination level (in CFU) is known, alert and action levels have to be determined. The alert level is the early warning level; a drift from normal conditions. When this level is exceeded it is recommended that the previous results are checked (is there a trend?) and that the following results are monitored more closely. When the action level is exceeded a thorough investigation should be made into the nature of the contamination, including identification of the isolates, and subsequent corrective actions should be implemented immediately. This may lead to adjusting the procedure and/or retraining the staff. More intensive monitoring will be necessary to be able to quickly assess if the adjustments have been successful. Action may also be required if the trend exceeds 50 % of the base line [30].

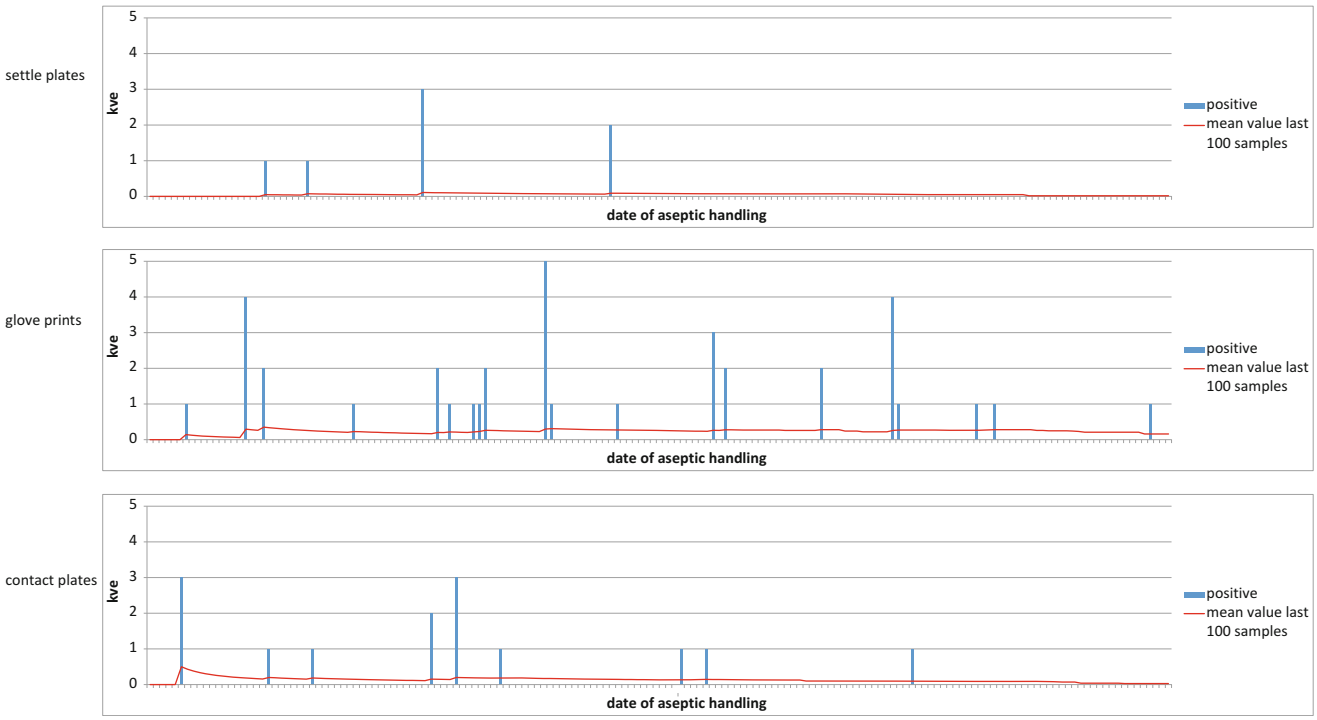

Fig. 31.3 Monitoring results over 1 year during/after aseptic handling in a Dutch hospital pharmacy. Every bar is a positive, the continuous line is the mean values over the last 100 samples

Table 31.5 Limits for microbiological contamination [1]

	Recommended limits for microbiological contamination (a)			
Grade	Air samples CFU/m^3	Settle plates (diameter 90 mm)	Contact plates (diameter 55 mm)	Glove print 5 fingers
		CFU/4 h (b)	CFU/plate	CFU/glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Notes: (a) these are average values; (b) individual settle plates may be exposed for less than 4 h

31.6.2 Microbiological Validation of the Process

The goal of microbiological validation of the process is to demonstrate that the procedures used during aseptic handling and the staff undertaking aseptic processes, are capable of maintaining the sterility of the product [31]. In this validation the aseptic handling is simulated with an appropriate broth solution, typically Tryptone Soya Broth (TSB). The final product is incubated for 7 days at 20–25 °C and 7 days at 30–35 °C successively and should not show any growth (in some countries, such as the Netherlands, 14 days at 30 °C only). The simulation should comprise all critical steps that occur in standard aseptic handling like withdrawing a solution from a vial or an ampoule, dissolving a powder in a vial and adding a solution to an infusion bag or a vial. Working with double or quadruple strength TSB can be helpful to simulate aseptic handling.

The simulation can be carried out daily at the end of a work session or periodically with a number of simulations together. Bringing all the results together, in the long run, provides good information about the overall quality of aseptic handling, however the total number of TSB simulations is limited in comparison to aseptic production in industry.

In judging the results, a validation curve can be drawn [31].

Validation Curve [32]

For judging the results of the microbial validation of aseptic handling by TSB simulation, a validation curve has been developed. The curve rises one unit after a simulation without growth and falls 100 units after a simulation with growth. The maximum is fixed at 299 and the minimum at 0. Three levels of performance are distinguished, see Fig. 31.4.

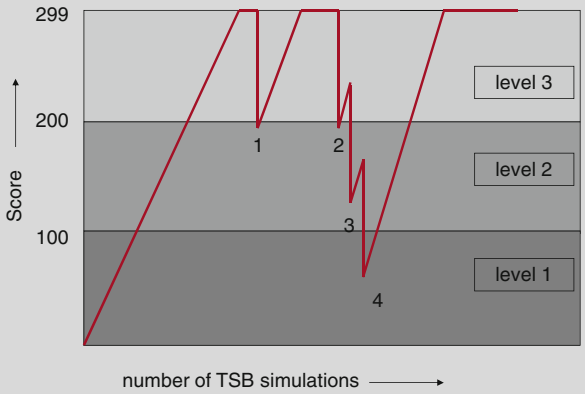

Fig. 31.4 Example of a validation curve

Like the validation of aseptic manufacturing according to GMP Annex 1 [1], corrective measures in the case of growth are made, depending on the total number of simulations without growth. This will be indicated by the level in the validation curve. For example:

- Positive simulation 1 (see Figure above), the curve falls down to 199. The corrective actions belonging to level 2 have to be executed. For example: identification of the micro-organism(s) found in the positive simulation and careful checking of the monitoring results.
- Positive simulations 2 and 3 (see Fig. 31.4), again corrective actions belonging to level 2 have to be executed.
- Positive simulation 4 (see Fig. 31.4), the curve falls down to level 1. The corrective actions belonging to this level have to be executed. For example: as well as the corrective actions for level 2, an audit of aseptic handling has to be performed and TSB simulations should be undertaken more frequently for a specified period.

31.6.3 Individual Qualification

As microbiological validation of the process will not generate sufficient data to give assurance that the aseptic technique of each individual operator is satisfactory, standard assessments for operator technique have been developed. In the Netherlands this assessment is known as "Individual Qualification" and in the UK as "Universal Operator Broth Transfer Validation" [8, 32]. Both tests consists of a repeated number of key techniques such as withdrawing a solution from a bag, vials or ampoules and adding it to empty sterile vials or infusion bags. The solution used is a growth medium (mostly TSB) and the filled vials or bags are incubated for 7 days at 20–25 °C and 7 days at 30–35 °C successively (some countries like the Netherlands, 14 days at 30 °C only) and must not show any growth to pass the test. Qualified operators have to be re-qualified at least every year with the same test.

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