Basic Requirements For Aseptic Manufacturing Of Sterile Medicinal Products

A Comparison Between Europe And USA

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List of Abbreviations

ATCC	American Type Culture Collection
BSE	Bovine Spongiform Encephalopathy
CDER	Center for Drug Evaluation And Research
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CIP/SIP	Cleaning in Place / Sterilisation in Place
CPMP	Committee for Proprietary Medicinal Products
DGHM	Deutsche Gesellschaft für Hygiene Und Mikrobiologie
EMEA	European Medicines Evaluation Agency
EP	European Pharmacopoeia
EU	European Union
FDA	Food and Drug Administration
FIP	Fédération Intérnationale Pharmaceutique
GMP	Good Manufacturing Practice(s)
HACCP	Hazard Analysis and Critical Control Points
HEPA	High Efficiency Particulate Air
IPC	In Process Control
ISO	International Organisation for Standardisation
OOS	Out of Specification
PDA	Parenteral Drug Association
Ph. Eur	European Pharmacopoeia
PIC/S	Pharmaceutical Inspection Convention
QA	Quality Assurance
QWP	Quality Working Party
TOC	Total Organic Carbon
SAL	Sterility Assurance Level
SOP	Standard Operating Procedure
US	United States
USA	United States of America
USP	United States Pharmacopoeia
VPHP	Vapor-Phase Hydrogen Peroxide
WFI	Water For Injection

1 Introduction

In the early 1900s, the first parenteral drugs were manufactured on an industrial scale. The need arose to find suitable sterilisation methods for heat sensitive products that could not be autoclaved in the final container. So called aseptically manufactured drug products have to be sterile, although the sterilisation in the final container closure system is not possible.
Since then the standards for aseptic manufacturing of medicinal drug products have become very high and clearly specified because of the nature of the pharmaceutical form and / or the manner in which they are administered (for example injections, infusions, pharmaceutical forms for the eyes such as eye drops etc.). Parenteral products are intended to be nonpyrogenic too, additionally to the requirement to be sterile. Medicinal drug products that do not meet the requirement to be sterile / nonpyrogenic can otherwise cause severe harm or life-threatening health risk to the patient.
In a world where global effects in the supply chain become more and more important, it is necessary to know the differences in the requirements of the specific guidances and guidelines in the single markets of the world. Therefore this master thesis shall reveal the basic requirements of aseptic manufacturing of sterile drug products for the EU and US market. Knowledge of the differences in the requirements is important to guarantee the quality of the products and their supply in due time for the single markets.

To begin with, there is a short definition for example of sterility and aseptic manufacturing. Following it, is a summary of the requirements for aseptic manufacturing (environmental monitoring, sterile filtration and validation by media fill) along with the base of these requirements, keeping in mind the differences between Europe and USA. The outlook will present a rational approach to the complete qualification (rooms, equipment, supply systems, materials etc.) and validation with the help of HACCP (Hazard Analysis and Critical Control Points) concept.

1.1 Definitions

1.1.1 Sterility

Sterility[1, 2] means the complete absence of any viable microorganism in a drug product. The specification of sterility is unchanging and is independent of the kind of manufacturing process: sterilisation of the final product in its container closure system or aseptic manufacturing.
Terminal sterilisation usually involves filling and sealing product containers under high-quality environmental conditions. Products are filled and sealed in this type of environment to minimise the microbiological content of the in-process product and to help ensure that the subsequent sterilisation process is successful. In most cases product, container and closure have low bioburden but they are not sterile. The product in its final container is then subjected to a sterilisation process such as heat or irradiation. As terminally sterilised drug product undergoes a single sterilisation process in a sealed container thus is limiting the possibilities for error.

1.1.2 Aseptic manufacturing

Sterility is best achieved through sterile filtration of the bulk using a membrane filter (0.2 µm or less) in sterile container closure systems and working in a clean area. Drug product, container, and closure are first subjected to sterilisation methods separately and appropriately.

So this is a complex working procedure, which consists of several consecutive and necessary working steps, each of them contributing its part towards the aim of manufacturing an aseptic product (prevention of microbial contamination). Any manual or mechanical manipulation of the sterilised drug, components, containers or closures prior to or during aseptic assembly poses the risk of contamination and thus necessitates careful control.

Aseptic manufacturing is used in cases, where the drug substance is instable against heat, hence sterilisation in the final container closure system is not possible. Aseptic manufacturing[1, 2] means that the used drug substance and excipients were sterilised appropriately and all materials, equipment and container closure systems were used only after sterilisation. All working steps were performed in so called clean areas to avoid contamination. Therefore high standards have to be established concerning the manufacturing room, the personnel, the equipment and the supply systems[3, 4, 5] (air system, water for injection, sterile gases used in the working process; for example compressed air, nitrogen etc.).

1.1.3 Sterility Assurance Level, SAL

The grade of Sterility Assurance Level (SAL)[6, 7] is the probability of a non-sterile unit being present in a batch of sterile units. The required SAL according to the European Pharmacopoeia[6] is 1×10^{-6} and is deducted by extrapolation from the killing kinetic of resistant bacteria spores in a validated sterilisation procedure with a defined microbiological overload prior to sterilisation. A SAL cannot be calculated for aseptically manufactured drug products because the membrane filtration does not follow a killing kinetic which can be calculated. The sterility test of the final drug product aseptically manufactured is not statistically representative for the whole batch. Therefore some uncertainties exist as to how the sterility of aseptically manufactured drug products can be evaluated and verified.

1.1.4 Parametric release

Parametric release[8, 9] is based on evidence of successful validation of the manufacturing process and review of the documentation on process monitoring carried out during manufacturing to provide the desired assurance of quality of the product. It is a system of release that gives the assurance that the product is of the intended quality based on the information collected during the manufacturing process and on the compliance with specific requirements related to parametric release resulting in the elimination of certain specific tests of the finished product.

This means for products that are intended to be sterile that the microbiological quality of the batch of a medicinal product is stated by using the data from environmental monitoring and process data; a sterility test is not required in batch release. Parametric release however is restricted to products which are terminally sterilised in their final container closure system.[18] "When a fully validated terminal sterilisation method by steam, dry heat or ionising radiation is used, parametric release is the release of batch of sterilised items based on process data rather than on the basis of submitting a sample of the items to sterility testing may be carried out, subject to the approval of the competent authority. Parametric release can only be applied to products terminally sterilised in their final containers."

1.1.5 Isolators

Isolators[10, 11, 12, 13] consists of a decontaminated unit, supplied with class 100 or higher air quality that provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding clean room and personnel).
So aseptic processing using isolator systems minimises the extent of personnel involvement and separates the external cleanroom environment from the aseptic processing line. A very high integrity can be achieved in a well-designed unit.

1.1.6 Blow-Fill-Seal-Technology

Blow-Fill-Seal-Technology[11, 12, 13] is an automated process, by which containers are formed, filled and sealed in a continuous operation.

2 Issues Under Examination

2.1 Applicability of Specification of Sterility to Pharmaceutical Forms

Sterile drug products must meet the specification of sterility. The test methods for sterility (performing of the test, nutrient, incubation conditions) are described in the Pharmacopoeias.[6, 7] The FDA Draft Guidance[12] describes the limited sensitivity of the sterility test (statistic probability of detecting a positive unit), which is a destructing (and expensive) test method. That means that a 100% control of sterility of the batch is not possible. For batch release purposes it is important that an appropriate number of samples are tested and that those samples are representative for the whole batch (sampling at the beginning, mid, and end of the batch, in conjunction with processing interventions).

2.1.1 In Europe

Injections, infusions and pharmaceutical forms for application on eyes and on mucous membranes must meet the requirement to be sterile, but there is no general requirement for sterility of aqueous-based oral inhalation solutions, suspensions and sprays.[14]

2.1.2 In the USA

Additionally to the pharmaceutical forms mentioned in 2.1.1 aqueous-based oral inhalation solutions, suspensions and inhalation sprays must be sterile (21 CFR 200.51).[15, 16 ,17] Inhalation solutions, suspensions and sprays are intended for delivery to the lungs by oral inhalation for local and / or systemic effects and are to be used with a specified nebulizer. There were severe side-effects in patients after using such medicinal products when these consumed products were found to be contaminated by microorganisms. That was the reason for establishing the requirement of sterility for those pharmaceutical forms in the US.

2.2 Current Guidelines and Recommendations - Overview

In Europe aseptic manufacturing of sterile products is seen as a last resort which is only acceptable if all methods of terminal sterilisation in the final sealed container have been excluded. Such being not feasible or applicable, for example when the drug substance is instable against heat, the EU guidelines require the sterilisation in the final container closure system whenever possible. Only the stability of the drug substance is considered but not the container closure system.

The European Pharmacopoeia (EP)[18] prioritises the terminal sterilisation of the final container in manufacturing sterile drug products.

In Europe the "Guide to GMP for medicinal products and active pharmaceutical ingredients, Annex 1, Rev 1996, Manufacture of Sterile Products"[11] compiles the recommended procedures for sterile products and includes the aspects of aseptic manufacturing. The revision of 2000[19] increases the requirements (and so the time and costs) for the frequency of process validation with media fill ("initial validation with 3 consecutive satisfactory simulation tests per shift" .." repeated twice per year **per shift** and process ..."). Up until this revision, the process simulation was not required per shift, but only per filling line.

Annex 13 (Manufacture of investigational medicinal products) of the "Guide to GMP for medicinal products and active pharmaceutical ingredients, Rev 1996 resp. 2000"[20] fully

requires the validation for manufacturing sterile medicinal products for clinical trial. While using other manufacturing processes, it is possible to reduce the validation of the process. This fact again outlines the extraordinary importance of an aseptic manufacturing process even for medicinal products used for clinical trial.

In the USA basically, sterilisation in the final container is also seen as the method of choice according to the US Pharmacopoeia (USP[21]) to manufacture sterile medicinal products, but the approach to choose a manufacturing process (including sterilisation process) which is compatible with the product properties is more common there than to develop a medicinal product with regard to a special sterilisation procedure.[22] The US Guidelines require the final sterilisation, when the product is stable for final sterilisation and explain that, if necessary, the container closure system has to be changed. However, some final packaging may afford some unique and substantial advantage (e.g. some dual-chamber syringes) that would not be possible if terminal sterilisation methods were employed. In such cases, a manufacturer can explore the option of adding adjunct processing steps to increase the level of sterility confidence.
CFR 21, Current Good Manufacturing Practice in manufacturing, processing, packaging or holding of drugs / Current Good Manufacturing Practice for Finished Products, Code of Federal Regulations, Parts 210, 211 (1999, Subpart F Production and Process Controls)[15] is referred to in FDA Draft Guidance[12] and general requirements for example are stated in paragraph 211.113 (b) (written procedures to prevent microbiological contamination of sterile products, validation of any sterilisation process).[23]
For USA the FDA "Guidance for Industry Sterile Drug Products Produced by Aseptic Processing"[24] describes the expectations of the FDA for the validation of aseptic processing in a more detailed manner. A draft version[12] was published in 2003. This guidance updates the guidance of 1987[24] primarily with respect to personnel qualification, cleanroom design and isolators, air supply system, integrity of container closure systems, process design, quality control, environmental monitoring, and review of production records. The use of isolators for aseptic processing is also discussed. Blow-fill-seal-technology is mentioned too. According to this draft the acceptance criteria for the evaluation of media fill will be cancelled. Each contaminated unit of a media fill should be examined independent of the number of filled units. The microbial environmental monitoring (more frequency in testing) is accorded more importance to get more quality assurance. Additionally sterility testing of the finished drug product may use more samples - the number of which are not yet specified.
No extensive trends towards harmonising to the European Guide[11] are observed within this draft,[12] for example regarding the clean room classification.
Special details for the content of information and data which should be included in drug applications are found in the guideline entitled "Guideline for the Submission of Documentation for Sterilization."[25]

The most important international standards and recommendations are

The PIC/S is a working group who writes inspection guidelines. These recommendations [26, 27] for aseptically processing have to be seen as groundwork for the development of the EU GMP Guide[11] and as a basis of GMP inspections by the authority.
ISO (International Organisation for Standardisation) is a network of national standards institutes (non-governmental) from a lot of countries, working in partnership with international organisations, governments, industry, business and consumer representatives. ISO standards represents a reservoir of technology and are voluntary. The ISO norms series[28, 29, 30, 31] emphasise the statistical approach as evaluation of the process simulation

with media fill. The FDA's attitude is reserved as far as this approach is concerned. Otherwise the PIC/S guideline references to ISO 13408[28] hint that inspectors in Europe may use the ISO norms for inspections.
PDA technical reports[32 - 38] are publications of an expert group and have the character of recommendations.
In table 1 an overview of current requirements, guidelines and recommendations is presented.

Table 1 Overview of International Requirements, Guidelines and Recommendations for Aseptic Manufacturing

Edited by	**Requirement / Guideline / Recommendation**
Ph. Eur, 4 ed., chapter 5.1.1, 2002[18]	Methods of Preparation of Sterile Products
USP <1211>, 2004[21]	Sterilization and Sterility Assurance of Compendial Articles (Manufacturing of Sterile Drug Products)
USP <1116>, 2004[13]	Microbiological Monitoring of Clean Rooms and Other Controlled Environment
EMEA CPMP/255/96, 1998[22]	Development Pharmaceutics
EMEA CPMP/QWP/054/098, 1999[22]	Decision Trees (choice of procedures for manufacturing sterile drug products)
EMEA CPMP/QWP/486795, 1996[51]	Manufacture of the Finished Dosage Form
CDER (1994) [25]	Guideline for the Submission of Documentation for Sterilization
US Food and Drug Administration (FDA) Center for Drug Evaluation and Research, 1987, [24] draft revised 2003[12]	Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing. Validation with media fill: requirement: in 1,000 filled units not more than 1 non-sterile unit (Confidential level 95%) and at least 3,000 units to fill. The draft (2003) of this guidance requires that every non-sterile unit has to be regarded as a problem and must be examined independent of the number of filled units. Higher frequency in environmental monitoring programme, testing and in end product's sterility testing is required. There is no harmonisation regarding the classification of clean rooms with European Standards (see table 15).
European Community[11]	Guide to GMP for Medicinal Products and Active Pharmaceutical Ingredients, Annex 1, Rev 1996, Manufacture of Sterile Products, 20003
International Standard Organisation ISO 13408-1 (1998) [28]	Aseptic Processing of Health Care Products Part 1: General Requirements
International Standard Organisation ISO 14644-1 (1999) [29]	Cleanrooms and Associated Controlled Environments, Part 1 (Specification for particles in air in clean rooms)
International Standard Organisation ISO 14644-2 (1999) [30]	Cleanrooms and Associated Controlled Environments, Part 2, (specifications for testing and monitoring to prove compliance with ISO 14644-1)
International Standard Organisation ISO 14644-4(1999) [31]	Cleanrooms and Associated Controlled Environments, Part 4, (Design construction etc. of clean rooms)
PIC/S[26]	Recommendation on the Validation of Aseptic Processes (2001)

Table 1 (continued) Overview of International Requirements, Guidelines and Recommendations for Aseptic Manufacturing

Edited by	**Requirement / Guideline / Recommendation**
PDA, (Parenteral Drug Association) Technical Report No 13 rev. (2000) Technische Monografie[34]	Fundamentals of an Environmental Monitoring Programme
PDA, (Parenteral Drug Association) Technical Report No 22 (1996) Technische Monografie[32]	Process Simulation Testing for Aseptically Filled Products
PDA, (Parenteral Drug Association) Technical report No 28 (1998) Technische Monografie[33]	Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals
PDA, (Parenteral Drug Association) Technical Report No 26 (1998) Technische Monografie[35]	Sterilizing Filtration of Liquids

Beside this basic literature presented in table 1, some other literature[39 40, 41, 42] is available, which provides a good overview.

2.3 Special Aspects in Aseptic Manufacturing

Aseptic manufacturing consists of a lot of single working steps. But the whole process is only as good as the worst single step.
To achieve the aim of a sterile product, several aspects have to be considered and have to be separately validated. At the end, the process simulation with media fill is the final validation measure and allows the final evaluation of the appropriateness of the whole process.

2.3.1 Monitoring at Aseptic Manufacturing Sites - Environmental Monitoring

It is state of the art to produce medicinal products under controlled conditions. This control requires monitoring of the environment. The design of the monitoring (frequency, number of sampling sites, method / equipment of sampling, procedure in regard of deviations etc.) is different in the companies. The common aim, however, is to recognise any deviation of the validated state. The necessity of monitoring environment as a key element of a quality assurance programme is widely accepted. Air, surfaces and personnel are identified as contamination risk for the environment.
To come to reasonable limits, the rooms of the production areas have firstly to be classified depending on the production step. Limits of air, surfaces and personnel are proposed under consideration of the official recommendations.

2.3.1.1 Clean Rooms

The specification for clean rooms is figured in the EU GMP Guide[11] and in the USP (chapter 1116[13]) as well as in the FDA Guidance.[12] The requirements for air particles are compiled in table 3, for viable organisms in table 4. The main differences are the different nomenclature for clean rooms, the time when the measurements are performed (at rest, in operation) and the consideration of mean values in the EU GMP Guide.[11] The risk of mean values however may be that spike values are levelled.

2.3.1.1.1 Room Classification

To monitor the quality of a room, proper standards have to be established. According to EU GMP Guide[11] limits are recommended but side by side warning (alert) limits and action limits have to be determined.
Different room classes are necessary for the different production areas as regards the different production steps. Critical steps need stronger requirements for room quality.

Table 2 Room Classification and Examples for Working Steps According to EU GMP Guide[11]

Area	Requirement
Critical Area A	sterile products - sterilised in the final container closure system - filling sterile products - aseptic preparations aseptic preparation aseptic filling transfer of partially closed containers (lyophilisation) preparation of creams, ointments, suspensions, emulsions filling of creams, ointments, suspensions, emulsions production steps with high risk: filling area, container for the stoppers, open ampoules and vials
Critical Area B	sterile products - aseptic preparations background areas for zone with class A transfer of partially closed containers (lyophilisation in sealed transfer rackets)
Controlled Area C	sterile preparations - sterilised in the final container closure system preparation of solutions (production step with high risk) filling process background area for zone with room class A for filling of preparations (production step with high risk) preparation of ointments, creams, suspensions, emulsions sterile preparations - aseptic preparations preparation of the solution to be filled background area for blow-fill-seal-machines
Area with Requirement D	sterile preparations - sterilised in the final container closure system preparation of solutions and components for filling background area for blow-fill-seal-machines sterile preparations - aseptic preparations handling of components after the washing process background area for an isolator

2.3.1.1.2 *Risk of Contamination*

Everything that can come into contact with the product is a potential risk causing contamination (active ingredients and excipients, process water, primary and secondary packaging material, rooms, technical installations, air, personnel). Limiting the duration of exposure of sterile product elements, providing the highest possible environmental control, optimising process flow, and designing equipment to prevent entrainment of lower quality air into the class 100 clean area are essential to achieving high assurance of sterility. Both personnel and material flow should be optimised to prevent unnecessary activities that could increase the potential for introducing contaminants to exposed product, container closures or the surrounding environment.

Some aspects should be regarded in more detail:

- air / purified air: is a main source for contamination.
  According to the EU GMP Guide[11] the airborne viable particles in clean areas have to be determined and these results have to be regarded in batch release. The air contamination reflects any microorganism which may come from personnel or surfaces even if there is no direct product contact between such surfaces or the personnel. So, air is an important indicator for the whole hygienic state of the production area. Deviations are easily to recognise and corrective measures can be initiated.
- surfaces: surfaces which get into immediate contact with the product are highly critical. But indirect transfer via air from surfaces without direct contact to the product must also be taken into account. The design of the facility (smooth surfaces without unevenness and tears (see EU GMP Guide[11]) are important to avoid contamination and to support the success of any sanitisation procedure.
- personnel: a special regard is the hygienic behaviour of the personnel (infectious diseases, open wounds, regular healthy checks). Personnel are one of the main sources of contamination. Important factors are protective garments, regular change of garment and regular change of gloves. Details are described in the EU GMP Guide[11] and the FDA Draft Guidance 2003.[12]

2.3.1.2 Monitoring Programme

The monitoring scheme has to be established and documented (standard operation procedure, SOP). The SOP shall contain limits, methods / equipment, frequency, measures in case of deviations, sampling plan (responsibility), performance, sampling sites, documentation.

2.3.1.2.1 *Particle Monitoring*

Particles are significant because they may enter a product and contaminate it physically or, by acting as vehicle for microorganisms, biologically. The air supply systems are characterised by special parameters (temperature, humidity, positive pressure - differential pressure relative to adjacent rooms of lower air cleanliness -, velocity, air changes per hour) which may influence the particulate matter.

The efficacy of protection measures in the clean room is verified by the content of airborne particles. Particles in the entry air are retained by HEPA filters and particles in the clean room are removed by laminar air stream. HEPA filter are able of retaining of at least 99,97 percent of particulates greater than 0,3 micron in diameter. The regular integrity testing of

the HEPA filter is absolutely necessary. Their efficacy (rating of the filter) can be determined too (normally part of the installation qualification) but this is not suitable for testing of filter leaks.
The requirements for content of airborne particles and their measurement are part of ISO 14644 part 1[29] and part 2.[30] There are no exactly common requirements between Europe and USA (see table 3). Additionally the direct comparison of values is difficult because the kind of measuring and the used equipment used may greatly influence the values (USP 1116[13]).

Table 3 Requirements for Air Particles – USA and Europe

Room Class				**Maximal Number of Particles / m^3 / ft^3**				
US FDA[24] / **USP**[13] in operation**	**ISO 14644-1**[20]	**ISO 13408**[28]	**EU**[11]	**USP**[13] > 0,5 µm	**ISO 14644-2**[30] **US FDA Draft**[12] > 0,5 µm	**ISO 14644-2**[30] > 5 µm	**EU guide**[11] in operation** (only EU) > 0.5 /5 µm	**EU guide**[11] at rest *** (only EU) > 0.5 / 5 µm
100 / critical / M 3.5	5	critical	**A**	3530 100	3 520 / 100	29	3 500/1****	3 500 / 1****
1 000*/ M 4.5	6	not defined	not defined	35 300 1 000	35 200 / 1000	293	not defined	not defined
10 000* / M5.5	7	other	**B**	353 000 10 000	352 000 / 10 000	2930	350 000 / 2 000	3 500 / 1****
100 000 / controlled / M6.5	8	support area	**C**	3 530 000 / 100 000	3 520 000 / 100 000	29 300	3 500 000 / 20 000	350 000 / 2 000
not defined	9	support area	**D**		35 200 000	293 000	not defined	3 500 000 / 20 000

* introduced with FDA Draft Guidance[12]

** in operation / dynamic conditions: with personnel present, equipment in place, and operations ongoing
carried out at representative locations normally not more than 1 foot away from the work site, within the airflow, and during filling / closing operations, upstream of the airflow.

*** at rest: equipment in place, personnel absent

**** the areas are expected to be completely free from particles of size greater than 5 µm; for statistical reasons the value is set to 1 particle / m^3.

2.3.1.2.2 Microbiological Monitoring

Microbiological contamination is a critical point in aseptic manufacturing procedures. Microorganisms in the air are, generally, in combination with particles, but there is no correlation between the count of particles in the air and the microbiological contamination, because the microbiological contamination of particles is quite different (skin particles, or by transport issued particles / shedding of primary packaging materials). Therefore, the bacteria count in the air has to be performed by microbiological tests.
Requirements for microorganisms in the air, surfaces and personnel are figured in table 4.

Table 4 Requirements for Viable Organisms (Air, Surfaces and Personnel) – USA and Europe

class	a. air active CFU / 10 ft³ b. settling plates (diameter 90 mm / CFU / 4 hours) c. US FDA 1987[24]			air passive CFU / plate		surfaces CFU / contact plate		glove CFU / 5 finger		gowning CFU / contact plate	
ISO / US / EU	USP[13] CFU / m³ air (CFU / ft³ air)	a. / b. US FDA 2003[12]	EU[11] CFU / m³	US[12]	EU[11]	US[12] (USP[13])	EU[11]	US[12] (USP[13])	EU[11]	US[12] (USP[13])	EU[11]
5 / 100 / A	< 3 / (< 0.1)	a. < 1 b. < 1 c. < 1	< 1	-	< 1	3 (3 including floor)	< 1	3	< 1	5	
6 / 1000 / n.d.	n.d.	a. 7 b. 3 c. n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7 / 10 000 / B	< 20 / (< 0.5)	a. 10 b. 5 c. n.d.	10	n.d.	5	5 10 (floor)	5	10	5	20	n.d.
8 / 100 000 / C	< 100 / (< 2.5)	a. 100 b. 50 c. 25	100	n.d.	50	n.d.	25	n.d.	n.d.	n.d.	n.d.
9 / n.d. / **D**	n.d.	n.d.	200	n.d.	100	n.d.	50	n.d.	n.d.	n.d.	n.d.

n.d. = not defined

2.3.1.2.3 Personnel, Training and Entry to Clean Rooms

Personnel are the main source of contamination of clean rooms with microorganisms. The education and training of the personnel, the garments and the procedure to dress (see EU GMP Guide Annex 1[11], ISO 13408-1[28], FDA Draft Guidance[12]), the rules for entry and the behaviour are important factors. The different guidances and guidelines (see table 1) consider this issue in detail.

Double gloves are often used in industrial practice, as a result of the dressing technique (the second pair of gloves is worn after finalising the dressing).

Some aspects of aseptic technique and behaviour in the clean room are mentioned in the FDA Draft Guidance 2003.[12] Important for aseptic manufacturing process, is the detailed description in SOPs of the industrial company.

2.3.1.3 Cleaning and Sanitization

The guidelines do not provide a complete overview of the requirements. Only some aspects are mentioned. All international guidelines / guidances require that the disinfectants used in clean rooms A and B should be sterilised. It is not clearly described if the sterility must be tested according to the pharmacopoeia, or if a validated sterilisation method and a parametric release are acceptable, or if a test result of a very small CFU (smaller than 1

CFU in 10 or 100 ml) is sufficient. The difference in efforts is great and therefore its industrial practice is uncommon.
The efficacy of the disinfectant should be validated. This requirement is part of the ISO 13408[28] and of the PIC/S recommendation.[26] It is not clearly described if the testing of efficacy is acceptable by an independent laboratory (for example a DGHM certificate) or if own validation studies are required.
The FDA Draft Guidance[12] states that sanitizing agents should be effective against spores too. Sanitization procedures should be described in sufficient detail (e.g., preparation, work sequence, contact time, etc.) to enable reproducibility. Once the procedures are established, their adequacy should be evaluated using routine environmental monitoring programme.

2.3.1.4 Methods of Microbiological Monitoring

There are different methods and equipment available for testing air, surfaces and personnel (see USP[13]). It is necessary that the methods used for sampling the environmental samples are clearly described in SOPs.
The active determination of microorganisms is the method of choice in the USA, in Europe beside filtration methods, settling plates also are used. For detecting microoganisms on surfaces contact plates are used. The sampling has to consider the use of disinfectants for sanitising (sampling is not allowed immediately after sanitising). A problem is that surfaces with product contact carry a high risk of contamination. According to FDA Guidance[24] contact samples of the filling needles are required. There is a high risk of contamination even if the sampling is performed at the end of the filling process. The benefit of such a result is critical even if the result is positive.

2.3.1.5 Limits and Methods of Sampling

2.3.1.5.1 Air

Limits are given in the EU GMP Guide Annex 1,[11] the USP chapter <1116>[13] and in the FDA Guidance[24] (see table 5; a proposal is also included[43]). Different methods (for example see USP[13]) may be used (sedimentation, filtration, impaction, impingement).
Sedimentation using settling plates is no quantitative method and the value of this method is limited even in laminar air flow environment. Tests show that the results are dependent on the method used. Therefore, it is important to use always the same method in (air) monitoring.

2.3.1.5.2 Surfaces (Room, Equipment)

In the EU GMP Guide Annex 1[11] states limits for the single classes. There is no indication if those limits are valid for personnel or surfaces and there is no differentiation (table, wall, floor etc). In general the limits are given for surfaces near the product. Sampling should be performed at the end of the production step to avoid risking the product. Methods used are contact plates and pads or swabs (see table 5; a proposal is given too[44]).

2.3.1.5.3 Personnel

Proposals for limits are given in the EU-GMP Guide Annex 1[11] and in the USP chapter <1116>.[13] No indications are found in the FDA Guidance[24] (see table 5; a proposal is given too[44]).

Sampling should be performed at the end of the production step to avoid risking the product. Methods used are contact plates, pads and hand washing method for personnel.

Table 5 Overview Limits of Viable Particles (Air, Surfaces, Personnel)

	limits of viable particles (CFU / m^3)											
Class	EU[11] mean value (calculated from 10 consecutive values)			USP 27[13] [CFU / $feet^3$ of air]			FDA[24] [CFU / 10 $feet^3$] FDA[12] [CFU / m^3]			proposal for practical usage[43, 44]		
	Air											
Critical Area A - 100	< 1			< 3 [0.1]			< 1[24] < 1[12]			W: 1 A: 3		
Critical Area B – 10 000	10			< 20 [0.5)]			< 7 (class 1000) [12] < 10 (class 10000) [12]			W: 3 A: 7		
Controlled Area C - 100 000	100			< 100 [2.5]			< 25[24] [88 / 10 m^3] <100[12]			W: 50 A: 88		
Area with Requirements of D	200			-			-			W: 200 A: 400		
	Surfaces											
Class	EU[11] mean value (calculated from 10 consecutive values)			USP 27[13]			FDA[24]			proposal for practical usage[43, 44]		
	contact plates (55 mm diameter) CFU / plate			CFU / personnel plate (24 – 30 cm^2)						(CFU / 25 cm^2)		
	pr	O	B	pr	O	B	pr	O	B	pr	O	B
Critical Area A	-	< 1	-	-	3	3	0	-	-	0	G: <1 W: 2 A: 3	W: 3 A: 5
Critical Area B	-	5	-	-	5	10	-	-	-	-	G: 5 W: 2 A: 5	W: 5 A: 10
Controlled Area C	-	25	-	-	-	-	-	-	-	-	G: 25 W: 25 A: 50	W: 25 A: 50
Area with Requirements of D	-	50	-	-	-	-	-	-	-	-	G: 50 W: 100 A: 200	W: 100 A: 200

Table 5 (continued) Overview Limits of Viable Particles (Air, Surfaces, Personnel)

	Personnel											
Class	EU[11] mean value (calculated from 10 consecutive values)			USP 27[13]			FDA[24]			proposal for practical usage[43, 44]		
	5 finger contact (CFU / Glove)			CFU / contact plate			Not defined			CFU / 25 cm^2		
	H	U	M	H	U	M	H	U	M	H	U	M
Critical Area A	< 1	-	-	3	5	5	-	-	-	W: 1 A: 2	W: 3 A: 5	W: 3 A: 5
Critical Area B	5	-	-	10	20	20	-	-	-	W: 2 A: 3	W: 5 A: 10	W: 5 A: 10

G: guidance value: mean value from 10 consecutive measures (EU)[11]
W: warning limit
A: action limit
pr: product contact
O: surfaces (table, machine, wall)
B: floor
H: hand
U: forearm
M: cap

2.3.1.6 Identification of Isolates

This is a requirement of the FDA guidance.[24] The FDA Draft Guidance[12] says this in a more detailed manner: "... routine identification of microorganisms to the species (or where appropriate, genus) level." To apply this requirement on a complete identification of all isolates in the clean rooms A and B means a high expenditure in costs and efforts. Table 6 provides an approach. The identification of isolates in positive units of media fill however is a necessity in case of investigation failure. The FDA Draft Guidance[12] even specifies the testing method using rapid genotype methods.

Table 6 Identification of Isolates[44]

Clean Room Class	Exceeding Limit	Investigation
Class A	action limit	Identification of all morphologically different colonies
	alert limit	Shortened identification of all morphologically different colonies
	no exceeding limit	Shortened identification of all morphologically different colonies
Class B	action limit	Identification of all morphologically different colonies
	alert limit	Shortened identification of all morphologically different colonies
	no exceeding limit	Shortened identification of all morphologically different colonies
Class C	action limit	Identification of all morphologically different colonies
	alert limit / no exceeding limit	Shortened identification of all morphologically different colonies if there is a suspicion of Pseudomonas or spore generating germs

2.3.1.7 Evaluation, Limits and Trends

It is usually distinguished between alert or warning limit and action limit.[13] An alert limit or level in microbiological environmental monitoring is that level of microoganisms that means a potential drift from operating conditions. Exceeding the alert limit is not necessarily grounds for definitive corrective action, but it should at least prompt a

documented follow-up investigation that could include sampling plan modifications. Alert limits are usually based upon historical information gained from the routine operation of the process in a specific controlled environment. An action limit or level in microbiological environmental monitoring is that limit or level of microoganisms that when exceeded requires immediate follow-up and, if necessary, corrective action.
A procedure in case of exceeding limits must be described. Thereby the identification of isolates is also discussed. The established action limits are not acceptance criteria but statistical guiding values during the monitoring programme. The exceeding of the action limit does not mean an Out Of Specification (OOS) result and does not mandatorily lead to the rejection of a sterile product manufactured on the day of the exceeding limit. The decision of the release of the product is based on a deviation report, which takes into account several points (additional sampling, the whole monitoring data, the rationale of the decision etc.) and not only a single value.
Trending means that all data of environmental monitoring will be evaluated in predefined intervals and changes are verified. The trending analysis of environmental monitoring data is a requirement within ISO 13408[28] and USP[13] recommends considering trends to worse data to initiate corrective actions in due time. PDA[34] recommends using statistic process control for trending. The FDA Draft Guidance[12] requires thorough trending analysis.

2.3.1.8 Sampling Sites

The critical sites must be taken into account. Examinations near the filling needle are expected.[24]

Examples for Sampling Sites

• air	filling line : open and / or filled containers near the working area
• laminar air	flow near the area with high activity
• surface (room)	floor, door handle, walls; curtains
• surface (equipment)	filling line, container of the stoppers
• personnel	fingerprint

2.3.1.9 Frequencies of Environmental Monitoring

According to EU Guide[11] sampling is required at the end of a critical processing step to avoid any risk from the product. FDA Guidance[24] requires measurements during the whole process. The USP[13] determines intervals of sampling (each operating shift for Class A or better and B; twice / a week for class C in case of product contact; once / a week in case of non-product-contact). But nothing is said regarding the number of sampling points. Details of monitoring programme are given in the PDA 13.[34]

Proposal for frequencies for environmental monitoring is presented in table 7.[45]

Table 7 Proposal for Sampling - Frequency[45]

	EU Productions	US Productions
Critical Area A		
Air	After the critical processing step of a batch (in case of several batches on the same day, only one measurement per day).	At the beginning of the batch, then every 2 hours.
Surfaces Working Areas, Equipment	For each batch after the critical processing step of a batch.	Several measurements on the day of production.
Wall	For each batch after the critical processing step of a batch.	Several measurements on the day of production.
Floor	Once a week till every quarter of a year (depending on production frequency and usage of the room).	Additional several measurements on the day of production.
Personnel Hand	For each batch after the critical processing step of a batch.	
Forearm	Monthly (after entry in airlock (at minimum once a year for each employee)).	
Cap (near mask)	Monthly (after entry in airlock (at minimum once a year for each employee)).	On production day complete sampling (hand forearm, hand of each personnel at the end of a shift).
Critical Area B		
Air	After the critical processing step or after usage, sampling provides no risk of contamination by measurement.	At the beginning, in the middle and at the end of a production batch.
Surfaces Working Areas, Equipment	After the critical processing step or after usage, sampling provides no risk of contamination by measurement.	Several measurements on the day of production.
Wall	After the critical processing step or after usage, sampling provides no risk of contamination by measurement.	Several measurements on the day of production.
Floor	Weekly till monthly (depending on production frequency or room usage).	Several measurements on the day of production.
Personnel Hand	For each batch after the critical processing step.	
Forearm	Monthly after entry in airlock (for each employee at least once a yer).	
Cap (near mask)	Monthly after entry in airlock (for each employee at least once a yer).	On production day complete sampling (hand, forearm, hand of each personnel at the end of a shift).
Controlled Area C		
Air	Daily till every three months (depending on production frequency or room usage).	
Surfaces Table	Daily till every three months (depending on production frequency or room usage).	
Wall	Daily till every three months (depending on production frequency or room usage).	
Floor	Daily till every three months (depending on production frequency or room usage).	
Area With Requirement D		
Air	Monthly till every three months (depending on the risk of the product).	
Surfaces Table	Monthly till every six months (depending on the risk of the product).	
Wall	Monthly till every six months (depending on the risk of the product).	
Floor	Monthly till every six months (depending on the risk of the product).	

2.3.1.10 Incubation of the Samples

USP 1116[13] indicates 22.5 ± 2.5 °C und 32.5 ± 2.5 °C for 72 respectively 48 hours as incubation conditions. This may be too short for microbiological monitoring samples, even for surfaces which are disinfected and partially damaged, microorganisms may occur and may have a quite long lag phase. It makes sense to adapt these conditions to European Pharmacopoeia (at least 5 days incubation). The FDA Guidance[24] recommends at least five days at 10 to 25 °C to detect bacteria and fungi. It is also necessary to get information for anaerobic microorganisms (to sample and incubate some sampling sites under anaerobe conditions).

2.3.1.11 Measures in Case of Exceeding Limits

Results are to be collected and to be documented. Investigations have to be performed in case of exceeding limits. These have to be documented too (protocol and report). It must be evaluated if there is a deviation in the hygienic state of the product or the result of the test is in doubt. During the investigation the following points should be considered

- influence on the product
- quarantining measures
- investigations to reveal the cause of the deviation (identification of the microorganism)
- testing after measures to demonstrate that the chosen measures are successful.

2.3.1.12 Documentation

A thorough documentation practice is required. The following must be clearly specified as regards documentation of procedures: “What“, “when“, “by whom“ and “with what“.

2.3.2 Aim of Environmental Monitoring - Discussion

The efficacy of cleaning and sanitization measures should be verified by environmental monitoring and the level of potential contaminants should be very low (FDA-1987,[24] USP 1116[13]: “Routine microbial monitoring should provide sufficient information to ascertain that the controlled environment is operating within an adequate state of control“). According to GMP Guide Annex 1[13] additional environmental monitoring of personnel and surfaces after critical working steps is required. According to PIC/S[27] sampling at worst case positions is necessary. The FDA Draft Guidance of 2003[12] raises the requirements regarding the number and frequency of sampling and also requires intensive trending (daily, weekly, monthly) and the depth of identification of isolates. This fails to consider that microbiological monitoring is based on conventional methods (choice of nutrient medium, sampling technique) that do not satisfy quantitative aspects of all present microorgansims. Additionally the clean room underlies dynamic variations (personnel, air fluctuation, use of disinfectants) so the sampling and the results are limited and only can provide a snapshot at the time of sampling for a defined point.

2.3.3 Sterilisation Procedures

Sterilisation can be achieved by different methods dependent on the material, the aim, and the possibility of residues of the used sterilising agent. There are different methods for sterilisations[11] namely: heat sterilisation (dry, wet [steam sterilisation]), sterilisation by gas (ethylene oxide, VPHP - vapor-phase hydrogen peroxide), sterilisation by radiation, sterile membrane filtration. The validation of the sterilisation of all components is a general requirement.

All methods have to be thoroughly validated. Sterilisation by filtration of bulk is the method of choice in aseptic manufacturing of a sterile drug product when the final sterilisation in the container closure system is not possible. The other methods (mentioned above but which are not described here in detail) are also used for the sterilisation of materials, of equipment, of primary packaging materials.

2.3.3.1 Sterilisation by Membrane Filtration

Sterile filtration is an important building block of aseptic processing of drug products and is part of the aseptic manufacturing because aqueous product is sterile filtered during the filling procedure. The used filter has to be validated for each filtered product. It is important to ensure as part of the process validation that the filtration step achieves the designed quality aspects in a reproducible and documented manner and that inadvertent side effects can be excluded. The most important quality target is of course sterility (bacterial retaining rate) whilst shedding of particulate matter or fibres from the filter into the solution, release of extractables or adsorption effects of the filter material regarding the active ingredients or excipients must also be avoided. The filter must be sterilised prior to use and filter integrity testing after sterilisation and after use are further requirements defined by EU GMP Guide.[11]

Validation of sterile filtration is a central quality step in aseptic manufacturing process. The filter specific properties and performance (obtained by the filter manufacturer) define its appropriateness for their usage. This contains the documentation of the correlation of bacterial shedding and non destructing integration test of the filter under standardised conditions. Pressures, flow rates, maximum use time, temperature, osmolality and effects of hydraulic shocks must be considered. Additionally tests on particle and endotoxin shedding, pH shifting, TOC, viscosity and conductivity of the solution to be filtered as well as microbiological challenges are necessary in filter qualification. Tests according to USP[46] (biological safety) ensure that the used plastics do not have acute or systemic toxicity. Additionally, product specific testing of the filter is required to take into account the specific interactions between product properties and filter properties.

2.3.3.1.1 Qualification of the Filter: Physical and Chemical Compatibility and Shedding of Particles

The compatibility of a given filter with the process conditions and the properties of the product have to be demonstrated.

Physical compatibility regards the maximal allowed temperatures and pressures.

This includes the preparation steps (sterilisation of the filter, rinsing of the filter, and the filtration itself). The process parameters of filtration (pressure, batch size, process time, etc.) will have to be reflected in the examination of bacterial retention time.

Chemical compatibility regards possible interaction of the solution with the filter materials depending on for example duration of action and temperature. The integrity test is also an indicator of chemical compatibility. Additional information for example non volatile extractable substances, can be obtained from the manufacturer of the filter. This information has to be regarded with the product specific properties (for example extreme pH values of the filtration solution may increase particulate shedding).

2.3.3.1.1.1 Extractable Substances

The quantity and quality of extractable substances into the filtration solution have to be determined. The possibility of shedding asbest fibres must be evaluated. In general, the rinsing of the filter with WFI after sterilisation is a means to minimise the extractable substances.[47]

2.3.3.1.1.2 Adsorption of Substances

Another interaction between filter and pharmaceutical product may be the adsorption of ingredients (active ingredient or excipients such as preservatives). In this case product specific examinations are required.

2.3.3.1.1.3 Microbiological Challenge - Bacterial Retaining Rate

A standard procedure for the qualification characteristic for a 0,22 µm (or less) sterile filter is the adding of at least 10^7 Brevundimonas diminuta (ATCC 19146) per cm^2 of filter area. The supplier of the filter will perform a lot of microbiological challenges. He will test the integrity of the filter by an appropriate filter integration test to get the correlation of both test procedures and to deduct the concrete testing parameters of the integrity which is a non-destructive test. The integrity test may be performed before and after the filtration step. According to FDA Guidance[24] the microbiological challenges using the pharmaceutical product as carrier solution is also an important aspect. The PIC/S[26] also indicates such testing.
This is to demonstrate that even under worst-case conditions, product properties of the filtration solution (ionic strength, osmolality, viscosity, surface tension, pH value) and the process parameters (temperature, pressure, filtration time, batch size, flow rate) do not have any effect on the performance of the filter. The temperature and the time for producing the batch will have to be considered. The PDA Technical report No 26[35] proposes to test at least three membrane batches regarding the specification for the K_L-value respectively bubble point testing of the corresponding membrane type. To reduce the effort, it is possible to summarize the validation studies on a scientific base. The determination of bioburden prior to filtration under qualitative and quantitative aspects is important and the amount should not exceed 10 CFU / 100 ml of the solution.

2.3.3.1.1.4 Specification of Filtration Time

Filtration (specification of filtration time): the filtration time, at standard batch sizes, indicates blockages or reduced filtration rates. As a criterion for an in-process control, the filtration time of the product should be analysed for the maximum filtration time (guaranteeing a well working process) and the filtration time should be specified in the batch manufacturing record.

The total time for product filtration should be limited to an established maximum to prevent microorganisms from penetrating the filter (bacterial retention time). Sterilising-grade filters should generally be replaced following each manufactured lot (or after one working day[11]) because they can provide a substrate for microbiological attachment. Maximum use times for those filters used upstream for solution clarification or particle removal should also be established and justified.

2.3.3.1.1.5 Testing of the Integrity of the Filter

An appropriate testing procedure for filter integrity is required prior to and after the filtration step to ensure that the filter is according to the supplier's specification and that the filter is not changed by sterilisation or damaged by other unforeseeable events. The forward flow test or the bubble point test is an example for such a testing.

2.3.3.1.1.6 Support by the Supplier of the Filter

Validation of sterile filtration requires an intensive co-working between the user and the supplier (manufacturer of the filter):

- Validated procedures for the sterilisation of the whole system regarding the filtration
- Design of the filtration process including the preparation of the filter regarding the specification of the filter and the handling instructions of the supplier of the filter
- Examination of possible interactions between filter and product to be filtered
- Implementation of a validated test procedure of filter integrity prior to and after the filtration step
- Close co-working uses the know-how of the product and reduces the effort for the process validation.

2.3.4 Validation of Aseptic Filling Procedure with Media Fill

2.3.4.1 Definition of Media Fill and Requirements of the Guidelines

According to all guidelines the process simulation with media fill is state of the art for the validation of aseptic manufacturing process. Media fill means that a microbiological nutrient media will be filled into a container closure system (ampoule, vials etc) instead of the product under simulation of aseptic standard procedure. The filled container closure systems are incubated under defined parameters and finally checked for microbiological contamination. This is to demonstrate that rooms, equipment and personnel are able to manufacture a product with very low contamination rate.

All manufacturing procedures in pharmaceutical industry must be validated. This requirement is stated in the European Pharmacopoeia:[18] "Process validation include ... checks on the process are regularly carried out by means of process simulation tests using microbial growth media which are then incubated and examined for microbial contamination (media fill tests)."

The EU GMP Guide[11] provides more details on this issue: "Validation of aseptic processing should include a process simulation test using a nutrient medium (media fill) ... The process simulation test should imitate as closely as possible the routine manufacturing process and include all the critical subsequent manufacturing steps."

The validation covers filling of media, environmental monitoring and incubation and evaluation of the filled vials. Additionally the growth promotion properties of the nutrient must be demonstrated. Microbiological examination of positive vials, bio-burden examination of the materials used and identification of contaminants are as well issues that need to be considered.

2.3.4.2 Microbiological Requirements on Validation of Media Fill

Media fills or process simulation technique is generally accepted as the procedure to validate aseptic manufacturing processes. Liquid nutrient growth medium, capable of supporting a wide range of microorganisms, is prepared, sterilised, and filled in simulation of a normal manufacturing process that includes compounding, sterile filtration, in-process controls, sterilisation of manufacturing process, materials (garments, primary containers, filling equipment), cleaning and sterilisation process (e.g., cleaning in place - CIP / sterilisation in place - SIP) and filling.
The sealed containers of medium thus produced are then incubated under prescribed conditions and subsequently examined for evidence of microbial growth. If the media fill reflects the standard procedure of product filling, the contamination rate or contamination probability may be used as indicator for the safety of the production process. Comprehensive control of production environment, personnel, and installations, influencing the overall hygienic state of manufacturing processes will be performed.
Since, in pharmaceutical production, validated methods have been already used for sterilising equipment, processing air and water and filtration techniques, media fill validation is very much focused on the aseptic technique of the human operator. Intensive training and education of personnel is required in order to ensure that media fill validation is recognised as a means of checking sterility level of aseptic processing.[12]

2.3.4.2.1 Production Environment

Media fill may be used together with large microbiological environmental controls of installation, of room air, of personnel to ensure the safety of an aseptic production process of sterile solutions, suspensions, lyophlisates etc. Before starting media fill, it is recommended to check that the status of all qualification, validation and calibration state must be valid.
For production materials and equipment maximal holding times have to be defined. During media fill equipment with maximal expiry date should be used.

2.3.4.2.2 Environmental Control in Media Fill

The environmental controls[48, 49 50] required during media fills are discussed in detail in chapter 2.3.1.
An enhanced monitoring (additional sampling points) is only suggested in case of start validation of a new sterile room. In case of revalidations routine sampling points are sufficient. The frequency of sampling can be increased during media fills. The limits of the routine monitoring should be the same for routine monitoring. In case of exceeding limits the usual procedure should be started.
The accompanying monitoring should be carried out by the same methods which are used for routine monitoring.

Incubation under anaerobe conditions for at least selected monitoring points (near filling needle) is recommended.
A special monitoring for fungi is not considered necessary if plates with trypticase soy agar are used (incubation minimum 5 days at 28 - 32 °C).
Environmental monitoring programmes demonstrate a state of control in the manufacturing areas for aseptic processing. The sampling is part of the process (contamination risk by sampling personnel) and should be part of the validation. This is required by the EU-GMP Guide:[11] "The process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all the critical subsequent manufacturing steps."

The microbiological environmental control during media fill simulates the conditions of the actual manufacturing process. This include:

- Microbiological examination of the air (under laminar air flow (class A / 100), in the surrounding area (class B / 10 000).
- Microbiological examination of the surfaces (filling needle, equipment).
- Microbiological examination of the personnel.
- Examination of the particulate matter under laminar air flow and in the surrounding area (along with the direction of the laminar air stream; at critical points of the filling and sealing process, during each shift: at the beginning and at the end of the working steps (batch).
- The determination of the microorganisms in the used gases.

All hygienic controls in the critical area (class A / 100) should be measured near the place of exposition: "measured not more than one foot away from the work site, and upstream of the air flow, during filling / closing operations" (FDA Guidance[24]). All hygienic controls have to be executed in operation.

Table 8 Overview of different Guidances / Guidelines about Frequency of Microbiological Monitoring

Reference	**Requirement**
EU GMP Guide Annex 1[11]	Frequent controls are required for aseptic manufacturing procedure. Additional microbiological monitoring is necessary besides the production process (for example after validation of systems, cleaning and sanitization).
ISO Norm 13408-1[28]	14.3.1.1 The aseptic processing area shall be routinely monitored for presence of microorganisms, i.e. environmental flora / isolates.
USP 27 <1116>[13]	Microbiological monitoring programmes for controlled environments should assess the effectiveness of cleaning and sanitization practices by and of personnel that could have an impact on the bioburden of the controlled environment. ... routine microbial monitoring should provide sufficient information to ascertain that the controlled environment is operating within an adequate state of control.
FDA Guidance[24]	In aseptic processing, one of the most important laboratory controls is the establishment of an environmental monitoring programme.
FDA Draft Guidance[12]	The environmental monitoring programme is an integral asset of the quality control unit's charge to ensure ongoing control of an septic process.

2.3.4.2.2.1 Air Condition System

The integrity of HEPA filters must be checked every 6 months. The uniformity of the flow and its speed should be checked regularly. The examination that the qualification is valid should be done and documented prior to media fill.

2.3.4.2.2.2 Qualification of Personnel

Media fill may be used to qualify new personnel according to hygienic training. Requalification is required once a year.

2.3.4.2.2.3 Gas Supply Systems

Using inert gas (nitrogen) or pressurised air in routine aseptic manufacturing process has to be simulated during media fill. Inert gas may stop or at least inhibit microbial growth in the filled container. Therefore sterile air will be used (exemption: usage of nitrogen in case of anaerobic conditions). The sterility of the inert gas has to be separately demonstrated by filtration procedure or by introduction of gas into the nutrient.

2.3.4.2.2.4 Cleaning and Sterilisation of Equipment

The used procedure (method of sterilisation, aseptically combining of equipment, CIP/SIP-procedure) has to be validated in each single case according to the specified conditions.
This ought to be part of the media fill validation.

2.3.4.2.3 Necessity of Environmental Controls

All environmental monitoring locations should be described in SOPs with sufficient detail to allow reproducible sampling of a given location surveyed (frequency, time of sampling, i.e. during or after conclusion of operations, duration of sampling, sample size [surface area, air volume]), specific sampling equipment and techniques, alert and action levels, appropriate response to deviations from established limits).
Aim of monitoring is to recognise deviations from the validated state.

- ISO 13408-1[28] emphasises:
  "A media fill is a point in a time-representation of the capabilities of an aseptic processing system including environment, equipment and personnel ... Media filling in conjunction with comprehensive environmental monitoring can be particularly valuable in demonstrating that the aseptic processing of sterile solutions, suspensions, and powders is functioning as intended."

- A general requirement is given in PIC/S PE 002-1 (1999):[27]
  "6.1 air borne microbial and non-viable particle monitoring
  It is important to state that the monitoring activity itself should not compromise the product quality. Worst case scenarios of simulation tests should also include monitoring activities."

- The PDA-Technical Report No 22[32] provides more details:
  "In accordance with GMP requirements, microbiological environmental and personnel, monitoring should be carried out during process simulation testing using routine operation procedures. This must include the set-up period and, specifically, set-up personnel."

This illustrates that the main source of contamination is the environment, but even performing the environmental controls risks contamination. Therefore environmental sampling is to be simulated during process validation. This explains the FDA Guidance[24] warning to keep the process validation cleaner than the routine production process: "Media fills should be conducted under environmental conditions that simulate actual and preferably 'worst case' conditions established as quality limits for production. To the extent such stressful conditions are permissible within standard operating procedures, it is vital that they exist during some media fills used to assess the process covered by those procedures. An inaccurate assessment (making the process appear 'cleaner' that it may in fact be) may result from conducting a media fill under extraordinary air particulate and microbial quality, and under production controls and precautions taken expressly in preparation for the media fill. Rather, the system should be challenged at the established limits for such things as number and activity of personnel, temperature, and humidity" (FDA 1987). The draft of the FDA Guidance[12] chooses a clearer formulation: "An accurate assessment (making the process appear 'cleaner' than it may in fact be) may result from conducting a media fill under extraordinary air particulate and microbial quality, and under production controls and precautions taken expressly in preparation for the media fill. Therefore, media fills should be constructed under environmental conditions that simulate actual as well as "worst case" conditions established as quality limits for production. To the extent standard operating procedures permit such stressful conditions, it is vital that they regularly exit during media fills in order to support the validity of the manufacturing procedures and process."

2.3.4.2.4 Extended Environmental Controls Versus Normal Environmental Controls

There is the question if extended environmental controls should be performed during media fill. A recommendation is given in PIC/S PE 002-1:[26] "Microbial monitoring should be performed in and around areas of high operator activity. It is not unusual to see settle plates and air sample locations well away from such areas. A typical example is where settle plates are located well to the rear of the filling machine where there is a little or no operator activity. The same may be true for air sampling. It is important, therefore, to observe operator activity over a period of time and ensure that the monitoring sites are so located as to monitor operator activity. The process simulation test provides an ideal opportunity to confirm that worst case locations have been identified by the use of additional monitoring during the test." Extended monitoring must be defined and may refer to location, frequency and additional examinations.

2.3.4.2.4.1 Site Locations

Extension of the sampling sites makes sense for the start validation of a new sterile room. At this time the locations for routine monitoring are often undefined. For the every six-month revalidation, only the normal sampling locations have to be regarded, otherwise uncertainties for the routine monitoring programme are revealed. Additionally regular

hygienic controls assist in recovering weak points which may lead to the incorporation of such points into the routine monitoring programme.

2.3.4.2.4.2 Frequency

During media fill the frequency of monitoring is recommended to be higher (FDA Guidance[24]): "it is especially important to monitor the microbiological quality of the aseptic processing area to determine whether or not aseptic conditions are maintained during filling / clothing activities ... The use of such devices should be used at least daily during production. In case of multiple production shifts, daily monitoring should cover each shift."

2.3.4.2.5 Additional Tests

Anaerobic microorganisms are routinely examined during media fill.

2.3.4.2.6 Limits

The results of the environmental controls during media fills ought to be considered for establishing the limits. ISO 13408-01:[28] "15.1 Development of alert and action levels - Alert and action levels shall be developed for all sampling sites in the APA (aseptic production area).
NOTE 1 Alert and action levels should be derived from and be consistent with results obtained during the aseptic process validation. Historical data from routine monitoring may also be appropriate for use in setting alert and action levels...
NOTE 3 Adjustments of alert and action levels could be appropriate, based upon results of the periodic media fill-re-evaluation and associated environmental monitoring data."

2.3.4.3 Documentation

Major elements for example that need to be considered in the contribution of environmental monitoring to media fill should include (validation protocol):

- Bioburden of bulk preparation and holding time: testing of starting materials, bulk on present microorganisms. The microbiological load (bioburden) of the intermediate prior to sterile filtration must be closely limited.[41] This enables a comparison using reference strains proposed by the respective pharmacopoeia between product-specific in-house populations and method validation.
- Training of personnel, hygiene conditions of the processing environment (e.g. rooms and filling lines), particulate situation in class A and B should be checked during the validation of the manufacturing process or established as a routine environmental control. The hygiene situation in the area of class A and B should be checked as part of validation of manufacturing process, or established as routine environmental control.
- Manipulations during filling procedure: a list of manipulations during filling should be created, stating how to simulate critical measures like stoppages in production, entry to class A only through an airlock system, entrance, change of personnel.
- Sterilisation of production equipment: the product sterilisation parameters should be validated in terms of temperature distribution and temperature penetration. The heat

distribution is recorded in a full chamber load of the autoclave. If indicated, product specific validation of part chamber loads of autoclaves should also be performed.

- Visual inspection: The quality of the optical inspection of filled containers should be validated for each product.
- Temperature mapping during storage: Sensors should be introduced into the product load in order to document and evaluate the conditions while the filled containers are stored.
- The disinfection regime covers environmental particulate and microbial monitoring, trend analysis of hygiene conditions (e.g., in house populations), sterilisation processes used in validation practices, including sanitization practices, systems of water, steam, compressed air, and other process gases.
- Validation methods and data for media fills and container/closure systems: filtration (bacterial retaining rate).

A validation protocol should be created considering the following additional points

- sampling, (responsibility)
- transport to microbiological laboratory
- sampling sites (responsibility, time restriction between sampling and test performing)
- sampling sites and time of sampling (identification of samples)
- test methods, (sampling, nutrient, incubation; results depending on methods)
- incubation
- limits and evaluation.

The media fill validation is finalised by a validation report. The results of the environmental monitoring must be included into the report. This is mentioned in PDA:[36]
”Protocol preparation
Once the process has been clearly defined, the simulation protocol or procedure can be written. This document should include but not be limited to the following information; ...
Environmental monitoring to be performed.“

2.3.4.4 Qualification and Requalification of Media Fill

The first qualification with media fill is done three times. It is recommended to regard worst case conditions for example short duration time of heat in depyrogenisation / sterilisation of primary packaging material, highest speed in filling, in number of personnel, interventions in filling process.
Requalification has to be done every six months.

2.3.4.5 Media fill and Detection of Specified Organisms

Nutrient media suitable for the tests are suggested and the conditions of incubation are discussed.
Soya-bean casein digest medium is the most used nutrient which is appropriate to detect molds and yeasts. USP[7] provides a lot of nutrients but there are no details under which aspects which nutrient should be used. The FDA Guidance[24] requires that the used nutrient must detect yeasts and molds. The detection of anaerobe microorganisms is only required if this is justified by the special product / process.

2.3.4.6 Cleaning, Heat Sterilisation and Depyrogenization of Container Closure Systems

Water of high quality (water for injection, WFI) has to be used for the final rinsing of the primary packaging material (European Medicines Evaluation Agency (EMEA) Note for guidance on quality of water for pharmaceutical use, 2001.[47] FDA Draft Guidance[12] establishes the same requirement.
Glass containers for sterile products often are sterilised by dry heat and depyrogenized at the same time (temperature above 210°C).
Rubber stoppers often are sterilised by autoclaving. Special caution has to be taken for the depyrogenization of rubber stoppers. In this case the final rinsing of the rubber stoppers using WFI has great importance.

2.3.4.7 Description of Media Fill

2.3.4.7.1 Choice of the Media Fill

A microbiologically appropriate nutrient is to be used. Within pharmaceutical industries Soya-bean casein digest medium (aerobe microorganisms) and thioglycollate (anaerobe microorganisms) have been verified. Both media relate to the quality control of pharmaceutical medicinal products because of their use in sterility testing. The growth promotion proprieties for a lot of microorganisms such as bacteria, yeasts and moulds have been proved very often. The anaerobe simulation is restricted for filling lines which are used for products filled in an atmosphere where oxygen is excluded.
The absence of germs of Bovine Spongiforme Encephalopathy (BSE) is to be mandatorily demonstrated in case of every nutrient.

2.3.4.7.2 Media Fill and Filling Volume

Some points regarding the filling volume have to be considered (all surfaces and stoppers must be wetted; microbiological growth must be ensured; this means that at least half of the nominal volume of the container should be used, the filling volume has to be taken into account the physiological preconditions of the microorganisms [aerobic conditions: half the nominal volume; anaerobic conditions: full nominal volume]).

2.3.4.7.3 Preparation of Media Fill

The nutrient medium must be processed, handled, and filled in a manner that precisely simulates the normal manufacturing process. Thus, the normal manufacturing process has to be analysed. In media fill validation, dried nutrient medium base and Water for Injection (WFI) are used as the compounding starting materials. Bioburden counts and determinations of endotoxin content, as well as analysis of pathogenic contaminants, for e.g. Pseudomonas aeruginosa or Echerichia coli, are performed as in-process controls on the raw materials.
Media fill should be prepared considering the instructions of the manufacturer regarding the usual manufacturing process (for example using the sterile filter systems as appropriate). The preparation should be performed analogously as the drug product (facility, monitoring activities, equipment, materials, personnel).

Environmental monitoring, comprising airborne counts, particle counts, and hygiene status of personnel and materials - e.g., balances and compounding vessels - is conducted during the weighing and compounding of materials.
Prior to filtration, the pH-value of the nutrient broth is checked and in-process controls (IPC) on identity, clarity, and bioburden are conducted. Samples are controlled for analytical and microbiological controls like that of any other product.

Holding and process times are documented and may be prolonged for validation purposes. The bulk solution is sterile-filtered using the same filter material as in normal aseptic processing. Filter integrity is checked prior to and after use. Environmental monitoring is conducted at this processing step.
Prior to filling, primary containers are sterilised and depyrogenized, the filling line is cleaned and sterilised (CIP/SIP) or transfer lines and dosage pumps are sterilised separately. Environmental monitoring is performed during the filling procedure.
A change in personnel is carried out during filling, as are several, pre-defined investigations and in process controls, for e.g. filling volume.

2.3.4.8 Worst Case Simulation

The simulation should consider such conditions which simulate the highest risk (worst case) of maximum expected and permitted loads. Examples for worst case conditions are defined in ISO 13408.[28] PIC/S[26] requires simulation of all interventions which may occur during a shift (refilling of closures, adjustments of filling needles) Not only should these kinds of interventions be regarded, but also their frequency (FDA Draft Guidance[12]).
For vial dimension and filling speed the worst condition is the biggest vial with the longest filling time, the widest-neck vial and the smallest vial with the highest speed.

2.3.4.8.1 Interventions

All interventions and measures of the usual process should be simulated in media fill. For example manual control of the filling volume, interventions in class A / 100, change in personnel, performance of environmental monitoring. Even technical interruptions should be considered (lack of air system, stopping of the machine). The approach is done during risk analysis with cooperation from the following departments: production, quality, validation, quality assurance.

2.3.4.9 Number of Filled Units and Duration of the Filling Process

According to FDA Guidance[24] the minimum number of filled units of media fill is 3,000 units (to reach a confidence level of 95% for demonstration of a contamination rate of less than 0.1%). For batch sizes smaller than 3,000 units, smaller numbers are acceptable (requirements are given in ISO 13408[28] and EU GMP Guide Annex 1[11]). For small batch sizes (for example products used for clinical trials) at least the actual batch size should be simulated during media fill. [11] For very large batches, it is recommended to simulate media fill with 1% till 10% of the actual daily batch size.[12, 40] The vials with the smallest and the biggest size should be regarded in media fill.
The units in media fill shall be enough to simulate worst case conditions.

2.3.4.10 Duration of Process, Holding Times and Stopping Times

Time limits should be established for each phase of aseptic processing. Time limits should include for example the period between the start of bulk product, compounding and its filtration, filtration processes, product exposure while on the processing line, and storage of sterilised equipment, containers and closures.[30] Bioburden and endotoxin load should be assessed when establishing time limits for stages such as formulation processing stage.
The total duration of the procedure consists of the time needed for the preparation of the bulk, time between the beginning of the preparation and the end of the sterile filtration. PIC/S[26] recommendation gives information about the duration of a media fill run. The whole filling time should be simulated, but it is possible to stop the machine to avoid excessive numbers of filled units. PIC/S[26] recommends simulating the process per shift and per filling line and not only per filling line. This is integrated in the EU GMP guide Annex 1[11] and also in the FDA Draft Guidance 2003.[12]
Holding times (e.g. materials, bulk, equipment) up to the beginning of the filling process etc. are usually defined within the manufacturing process. The validation of such times is performed during media fill and then simulates worst case conditions.
For small batch sizes (for example products for clinical trial) at least the actual batch size should be simulated during media fill (GMP Guide Annex 1[11]). ISO 13408-1[28] requires at last 5,000 units for the primary qualification of the process with three or more runs. The number of units for media fill is increased in FDA draft Guidance[12] (at least 5,000 units) compared with the current FDA Guidance.[24]

2.3.4.11 Selection of Units

It is recommendable to incubate all units of media fill. In any case the thorough documentation of all filled units is necessary. It is possible to select damaged units prior to incubation according to routine processing. But the accurate reconciliation of all units is a general requirement. It is not acceptable to select positive units after incubation because the checking reveals defects for example in the container closure system. The FDA draft Guidance[12] clarifies that intervention in the aseptic manufacturing process during media; that is to say an interruption of the aseptic barrier does not mean that those units have to be incubated, but it must be assured (SOP) that during routine manufacturing process such units are rejected.
Although no guideline mentions that the samples for fertility testing should not be taken prior to incubation of media fill, it is recommended to perform the fertility test after the evaluation of the media fill.

2.3.4.12 Acceptance Criteria in Media Fill

2.3.4.12.1 Warning Limits and Action Limits

All guidelines regard any positive unit during media fill as a potential problem and the source of contamination should be evaluated thoroughly.
ISO 13408-1[28] contains a table which gives a statistical approach of the evaluation of media fill (see table 10). Warning limits and action limits have to be defined. Acceptance criteria for the successful validation of an aseptic manufacturing process is a contamination rate of 0.1% in at least 3,000 filled units with a confidential level of 95%. The sterilisation assurance level SAL is 10^{-3}. The current discussion of the contamination rate aims at

negative detection of contaminated units. A contamination rate of 0.1% will no longer be tolerated by FDA's inspectors. Any positive unit has to be examined thoroughly and could be a reason for the failed media fill. FDA's acceptance of this probability in test results (0.1% contamination rate) does not mean that an aseptically processed lot of drug product purporting to be sterile may contain one non-sterile unit per thousand count.
According to PIC/S[26] the contamination rate should be ideally zero but the statistic approach refers to ISO 13408-1.[28]

2.3.4.12.2 *Action Limits / Procedure in the Case of Failed Simulations*

Measures for analysing the cause of contamination and an investigation thereafter have to be established. On exceeding the action limit, a requalification is immediately required.
According to ISO 13408-1[28] an investigation should be performed in case of exceeding the warning limit (1 contaminated unit up to 10 250 units) and the run has to be repeated. If the warning limit is exceeded again, it implies that the media fill has failed and the complete primary qualification has to be repeated (three consecutive runs of media fill must be successful). In the case of requalification (usually every 6 months one successful media fill) exceeding of the warning limit in two consecutive runs has to be evaluated as exceeding the action limit. This is clearly said in the ISO 13408-1[28] and in PIC/S:[26] "Exceeding the action limit means that a thorough investigation into the failure has to be performed and a complete requalification must be initiated. All produced batches since the failure must be quarantined until the cause for failure of the media fill is identified." PIC/S[26] recommends that all produced batches since the last successful process simulation have to be taken into account. Table 9 illustrates the limits of first qualification and requalification in media fill.

Table 9 First Qualification and Requalification: Warning and Action Limits in Media Fill According to ISO 13408-1[28]

production batch number of units	**minimum number of medium fill runs**	**minimum number of total filled units**	**warning limit / run**	**action limit / run**
first qualification				
< 500	≥ 3	5,000	1	2
≥ 500 - 2,999	≥ 3	5,000	1	2
≥ 3,000	3	9,000	1	2
requalification				
< 500	≥ 3	maximum batch size	1	2
≥ 500 - 2,999	1	maximum batch size	1	2
≥ 3,000	1	3,000	1	2

Table 10 Statistical Approach for the Evaluation of Media Fill: Warning and Action Limits in Media fill according to ISO 13408-1[28]

units per media fill run	warning limit per run	action limit per run
3,000	not acceptable	1
4,750	1	2
6,300	1	3
7,760	1	4
9,160	1	5
10,520	2	6
11,850	2	7
13,150	3	8
14,440	3	9
15,710	4	10
16,970	4	11

In case of any contamination, it is necessary to identify the corresponding microorganism. The FDA Draft Guidance[12] recommends the following:

- 5,000 - 10,000 filling units in media fill: 1 contaminated unit

investigation required including consideration of a repeat media fill

- < 10,000 filling units in media fill: 1 contaminated unit

investigation required

2 contaminated units

are considered as cause for revalidation following investigation

Table 11 illustrates a list of points for such an inspection.

Table 11 Example for Inspective Points in Case of Contamination

Data from environmental control including trending
Personnel hygiene and trending data
Data from particulate matter in the air
Count and kind of microorganisms in the preparation solution
Count and kind of microorganisms in process water and trending
Sterilisation parameters of the media (cycles of autoclave, filtration conditions)
Calibration state of the sterilisation material
Integrity data of pre and post filtration
Assembly of the filtration equipment
HEPA filter: change, maintenance, integrity
Change of air rate, air stream
Training of the personnel
Any conspicuous fact during the filling procedure
Storage conditions, holding time of equipment and containers, sterilisation conditions of the containers
Identification of the micoorganisms causing the contamination
Rejected containers during the filling procedure

Production manager and quality manager have to be informed in case of exceeding limits. The filling line should be rejected until a successful revalidation has been performed. The investigation may be extended on all drug products which have been produced since the last successful media fill run.
In general the repetition of a media fill validation becomes more and more difficult (zero contamination rate) and invalidation of media fill run is generally considered inappropriate. Supporting documentation and thorough justification should be provided in such truly rare and exceptional case (FDA Draft Guidance[12]).

2.3.4.13 Incubation Conditions

The container filled with media fill shall be incubated for 14 days at temperatures which allow the growth of a wide microbiological spectrum. The FDA Draft Guidance[12] recommends a temperature range from 20 to 35 °C and maintenance within 2.5 °C of the target temperature. A link to sterility testing[6, 7] is recommendable so as to have a rationale for the choice of these temperatures. Usually the containers are stored for 7 days at 20 to 25 °C followed by 7 days at 30 to 35 °C (the temperatures are defined in the European Pharmacopoeia). The incubation conditions have to be documented. (PDA[32])
The change in incubation temperature requires a change in storing of the filled containers. Here, during change in storing it is recommendable to inspect the containers and to wet the whole surface of the container with media fill.

2.3.4.14 Checking of Growth Promotion

The filled containers are taken off at different times. This is to assure that all parameters of the manufacturing process are regarded and not only the influence on the nutrient (temperature, filtration, introduction of gas) but also on the containers (washing, sterilisation, depyrogenisation, special treatment of the surfaces etc).

The growth promotion characteristics should be demonstrated with reference microorganisms but also with microorganisms typically for the manufacturing area (in house population, bioburden). Beside these microorganisms of the process systems it is recommended to take into account the room and personnel hygiene as well as microorganisms from the air should also be used. For growth promotion (fertility) testing the filled containers have to be inoculated with 10 to 100 colony forming units (CFU) and to be appropriately incubated (temperatures according to European Pharmacopoeia).
Anaerobic nutrient media such as thioglycollate should be used so when the containers are incubated under anaerobic conditions.

2.3.4.14.1 Growth Promoting Properties of the Used Nutrient Media

The used nutrient should allow growth of a wide spectrum of microorganisms. An overview of requirements is presented in table 12. USP <71>[7] and European Pharmacopoeia[6] use Soya-bean casein digest medium: „Soya-bean casein digest medium was primarily intended for culture of aerobic bacteria but is also suitable for fungi. Other media may be used provided that they have been shown to sustain the growth of a wide range of microorganisms."
FDA Draft Guidance[12] recommends Soya-bean casein digest medium: "Generally, a microbial growth medium that supports the growth of a broad spectrum of aerobic microorganisms, such as soya-bean casein digest medium should be used."

Table 12 Nutrient Media for Process Simulation

Guideline	**Requirement**
FDA Guidance on Sterile Drug Products Produced by Aseptic Processing[24]	Before a medium is chosen for validation runs, it should be demonstrated capable of supporting microbiological growth. ... Generally, a microbiological growth medium that supports the growth of a broad spectrum of aerobic ,microorganisms, such as soybean-casein digest medium, is acceptable.
FDA Guideline on Sterile Drug Products Produced by Aseptic Processing - Draft[12]	The most important aspect of media is its ability to promote microbiological growth. Before any medium is chosen for process simulation runs, it should be demonstrated capable of supporting microbiological growth.
USP 27 <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environment[13]	In general, an all-purpose, rich medium such as soybean casein broth that has been checked for growth promotion with a battery of indicator organisms.
Recommendation on the Validation of Aseptic Processes, PIC/S PE 002-1[26]	The medium should have a low selectivity i.e. capable of supporting growth of a wide range of microorganisms.
Aseptic processing of Health Care Products - Part 1: General Requirements, ISO 13408-1[28]	The media selected for media fill runs shall be capable of growing a wide spectrum of microorganisms and supporting microbiological recovery and growth of low numbers of microorganisms, i.e. 100 colony-forming units (CFU) / unit or less.

Observation of special problems during monitoring or in sterility testing necessitates second special media. (Sabouraud medium in case of problems with fungi, thioglycollate in case of problems with anaerobic microorganisms).

2.3.4.14.2 *Overview of Requirements for Referenced Microorganisms in Checking of Growth Promotion*

The guidelines generally require that referenced microorganisms from the Pharmacopoeia are used. Besides this, isolates from monitoring are named.

Table 13 Overview of Referenced Microorganisms used in Growth Promotion Testing

Guideline	**Performing**	**Microorganisms**
FDA Guidance on Sterile Drug Products Produced by Aseptic Processing[24]	In this regard, it is valuable to incubate positive control units along with media fill.	• Microorganisms referenced in USP <71>[7] growth promotion tests • types of microorganisms that have been identified by environmental monitoring ((regularly - every 6 months - updating is necessary)) • types of microorganisms that have been identified by positive sterility test results ((caution because of the probability of secondary contamination))
FDA Guidance on Sterile Drug Products Produced by Aseptic Processing - Draft[12]	When performing a process simulation run, it is valuable to incubate positive control units inoculated with < 100 CFU challenge.	• Microorganisms referenced in USP <71>[7] growth promotion tests • types of microorganisms that have been identified by environmental monitoring ((regularly - every 6 months - updating is necessary)) • types of microorganisms that have been identified by positive sterility test results ((caution because of the probability of secondary contamination))
USP 27 <1116>[13] Microbiological evaluation of Clean Rooms and Other Controlled Environment[13]	In general, an all-purpose, rich medium such as Soybean Casein Broth that has been checked for growth promotion with a battery of indicator organisms ... at a level of below 100 CFU / unit, can be used.	• Indicator microorganisms <71>[7] • Isolates from the controlled environment where aseptic processing is to be conducted may also be used ((regularly - every 6 months - updating is necessary))

Table 13 (continued) Overview of Referenced Microorganisms used in Growth Promotion Testing

Guideline	Performing	Microorganisms
Recommendation on the validation of Aseptic Processes, PIC/S PE 002-1[26]	Growth promotion tests should demonstrate that the medium supports recovery and growth of low numbers of microorganisms, i.e. 10 - 100 CFU/unit or less. Growth promoting testing of the media used in simulation studies should be carried out on completion of the incubation period to demonstrate the ability of the media to sustain growth if contamination is present. Growth should be demonstrated within 5 days at the incubation temperature as used during the simulation test performance.	• Bacillus subtilis • Staphylococcus aureus • Candida albicans • Aspergillus niger • Clostridium sporogenes ((process simulation with thioglycollate medium)) • In-house flora (e.g. isolates from monitoring etc.) ((regularly - every 6 month - updating is necessary)).
PDA-Technical Report No. 22[32]	Finally, the medium should be tested for growth. Samples may be tested initially upon production. It may also be tested concurrent with incubation and / or after 14 days of incubation. The units used for growth testing must be subjected to the same processing steps (e.g. cleaning, depyrogenisation, sterilisation, filtration, filling, lyophylization, reconstitution) up to the point at which they are placed into incubation.	• Pharmacopoeial organisms • environmental organisms ((regularly - every 6 month - updating is necessary)) or • Organisms isolated from sterility positives ((caution because of the probability of secondary contamination)).
PDA-Technical Report No. 28[33]	Confirmation of the media's growth promotion properties is an essential element ... The growth promotion units should be inoculated with a low concentration (less than 100 organisms per container) ... Media growth promotion studies can be performed prior to, concurrent with or after the completion of the process simulation incubation period.	• The USP growth promotion organisms - Bacillus subtilis & Candida albicans. ((it makes more sense to use the whole range of microorganisms referenced in the Pharmacopoeias than to choose only two representatives)). • Other organisms commonly found in the aseptic processing area environment such as organisms isolated during personnel monitoring ((regularly - every 6 month - updating is necessary)), sterility testing ((caution because of the probability of secondary contamination)), etc.
Aseptic Processing of Health care products - Part 1: General requirements, ISO 13408-1[28]	Verification of growth promotion of media used in specific media-fill runs shall be conducted following the run.	•Test organisms in conformance with pharmacopoeial requirements.

(()) critical explanation to the guideline

The time for performing the growth promotion test is given in PDA Technical Report No. 28[33]: “Media growth promotion studies can be performed prior to, concurrent with or after the completion of the process simulation incubation period. When growth promotion is performed before incubation, the acceptability of the media is confirmed prior to the simulation. Pre-simulation testing cannot confirm the acceptability of the media used in the actual trial and either concurrent or post-incubation growth promotion must be employed as well. The use of concurrent testing appears preferable as the results will then be available prior to completion of incubation. Post-incubation growth promotion provides a similar degree of assurance, but the delay in obtaining results effectively extends the length of time before the process simulation results are definitive.“

The Technical Report No. 22[32] does not indicate the time of testing: ”Finally the medium should be growth tested. Samples may be tested initially upon production. They may also be tested concurrent with incubation and / or after 14 days of incubation.“

FDA Guidance[24] describes testing concurrently and after incubation: “When performing a process simulation run, it is valuable to incubate positive control units inoculated with < 100 CFU challenge concurrent with media fill runs. Another widely accepted approach is challenging the media following the 14 day incubation period.“

The PDA Technical Report No 22[32] emphasises this requirement: “The units for growth testing must be subjected to the same processing steps (e.g. cleaning, depyrogenisation, sterilisation, filtration, filling, lyophilisation, reconstitution) up to the point at which they are placed into incubation.“

This means that filled vials from media fill are inoculated. This is possible if the growth testing is performed after incubation of the filled vials. This timing is an advantage because it allows evaluation of all media fill units.

The temperature for incubation performing the growth promotion test is described in ISO 13408-01:[28] “17.6.2 The incubation temperature shall be the same as that used for the media filled units ...

17.7.3 Incubation temperatures shall be appropriate for the specific growth requirements of microorganisms that are anticipated in the aseptic filling area.

NOTE Environmental monitoring data can assist in identifying the optimum incubation temperatures. Frequently-used temperatures ranges for incubation are 20 °C to 25 °C and 30 °C to 35 °C or 28 °C to 32 °C.”

The whole incubation time is 14 days. Only if the conditions of the growth promotion test are met the validation can be declared as valid. FDA Guidance[24] says: “For instance, in which the growth promotion testing fails, the origin of any contamination found during a process simulation should nonetheless be investigated and not merely discounted. This run may not have detected all potential contamination and should be repeated.“

It is self-explanatory that such invalidation has to be thoroughly documented.

2.3.4.15 Inspection of the Filled Containers

The filled container may be leaked-tested prior to incubation under defined temperature conditions (which are checked). Damaged filled containers must not be incubated but are part of the reconciliation of media filled units.
The incubation is followed by visual inspection of the filled containers. Each container with signs of turbidity is a circumstantial evidence of bacterial growth and hence, contamination of the nutrient growth medium. In case of turbidity the microorganisms are to be identified (microscopically etc.).

2.3.4.16 Identification of Microorganisms from Positive Units

The guides recommend identifying the microorganisms found in media fill.

Table 14 Requirements for Identification of Microorganisms

Guideline	**Requirement**
FDA Guideline on Sterile Drug Products Produced by Aseptic Processing[24]	-
FDA Guideline on Sterile Drug Products Produced by Aseptic Processing - Draft[12]	Where findings are adverse or indicate the process may not be in control, a comprehensive investigation should determine the origin of the media contamination, and should generally be followed by multiple repeat of process simulation runs.
USP 27 <1116> Microbiological evaluation of Clean Rooms and Other Controlled Environment[13]	Following incubation, the medium filled containers should be inspected for growth: Media filled isolates are identified by genus and, when possible by species in order to investigate the source of contamination.
Recommendation on the Validation of Aseptic Processes, PIC/S PE 002-1[26]	The microorganisms present in the containers of the simulation test should be identified to genus but preferably species level to aid determination of the possible sources of the contamination.
PDA-Technical Report No. 22[32]	All positive (from integral containers) should be identified to at least genus, and to species whenever possible.

2.3.4.17 Microbiological Testing in Media Fill - Summary

Besides the environmental controls required during media fills in order to validate aseptic processes, additional microbiological investigations have to be carried out.
The growth promotion qualities of the media used have to be demonstrated in the original containers at the end of the incubation, under the use of the reference organisms of the Pharmacopoeia for sterility tests

- Staphylococcus aureus ATCC 6538 for fluid medium with Casein and soya-bean casein digest medium;
- Clostridium sporogenes ATCC 11437 for fluid thioglycollolate medium

and

- isolates from environmental controls of sterile room (inoculation quantity 10 (-100) CFU / unit).

Therefore, addition of inactivating agents for neutralisation to antimicrobial substances is absolutely necessary (for example rest of disinfectants). Additionally the materials used for

monitoring should be incubated for demonstrating the growth promotion quality (inoculation with not more than 100 CFU Staphylococcus aureus ATCC 6538).
All contaminants from contaminated bottles should be immediately identified at least to genus, or better still to species.
Bioburden investigations of the medium serve as a determination of the bioburden of species, and also other possible causes of contamination, for example wetting fluid from membrane filter (WFI), liquid of water bath from ultrasonic treatment etc. Used primary packaging materials (bottles, stoppers etc.) should be tested for sterility if necessary.
The isolated organisms must be identified in order to find contamination sources. The frequency of identification is in relation to the result of media fill.
The necessity of identification of microorganisms from the monitoring is recommended in PDA Technical Report No. 22:[32] "The identity of microorganisms from the contaminated units should be determined. The identification of the contaminant should be compared to the database of organisms recently identified. The biochemical (genus / species) profile of the contaminating microorganisms can then be compared to that of microorganisms obtained from the sterility tests and bioburden and environmental monitoring programmes, in order to help identify the potential sources of the contaminant. These isolates should be checked for possible identification matches, as should isolates for any areas which exceed their count limits or are trending upward. In addition, literature references detailing possible sources of the organism may be helpful in locating its point of entry into the process."
ISO 13408-1[28] points that such data must be provided in case of positive units for the investigation: "17.11 Media fill run exceeding action levels
17.11.1.2 If action levels are exceeded, there shall be a prompt review of all appropriate records relating to aseptic production between the current media fill and the last successful one. The investigation should include ... consideration of the following: ...
j) identification of contaminants as a clue to the source of the contamination."

3 Results and Discussion

The available guidelines in Europe and USA often supplement each other and one guideline gives more details on a special issue than the other. On the one hand the current requirements in Europe and USA are not yet harmonised in each topic (for example the requirement of sterility for special pharmaceutical forms, environmental monitoring etc). So no one unique guideline exists and all guidelines should be considered and they must be used in a rational sense for practical use.
The revision of the guidelines shows that there is a trend in enforcing the requirements. Some details presented in FDA Draft Guidance[12] are in line with the EU GMP Guide (for example general requirements for facility design, personnel training, presentation of new technologies as blow-fill-seal-technology and Isolator technology). So efforts has been undertaken to harmonise the requirements in the two regions.
It is a progress that the requirements in the establishing of limits for environmental monitoring are based on ISO clean room classification in the FDA Draft Guidance[12] (numbers for limits are harmonised; a comparison between classification of cleanrooms is easier using the same reference unit). But in the details differences are still as always existing: for example class room 1,000 is only defined in the US.[12, 13] The measurement is only required at operation in the US. In the EU[11] limits for two particle sizes are established and limits for measurements in operation and at rest are presented. Mean values in evaluating the monitoring data for establishing the limits are only used in Europe.
FDA sees here a risk of levelling spiked values. On the other hand presenting more details there is a risk to establish new requirements (see table 15; for example disqualification of personnel who, repeatedly, fails the media fill qualification; video tape recording of media fill etc.) and to bring up more restrictions for the manufacturer (for example description of name of agents for air flow pattern studies). The more details are presented in the guidelines the more probability of deviation in specific details is given and requires more intensive reading of the guidelines.

Table 15 summarises the main requirements which are new in the FDA Draft Guidance[12] compared with FDA Guidance of 1987.[24]

Table **15** Overview[54] of New Requirements in FDA Draft Guidance[12] compared with FDA Guidance[24] (Examples)

New Requirement	**Comment**
Regular monitoring of air particles should be performed during each shift. Recommendation of sampling equipment is also given. Definition of Class 1,000 and class 10,000. Definition of positive pressure differential and their monitoring. Description of membrane filters for air and gas filtration.	This is a more detailed description and often linked with more requirements than in the current Guidance (introduction of class 1,000 and class 10,000..
Constant overpressure for bulk vessels	Sterility can be achieved by other means (for example appropriate filter, validation of the integrity of the bulk vessel).

Table **15** (continued) Overview[54] of New Requirements in FDA Draft Guidance[12] compared with FDA Guidance[24] (Examples)

New Requirement	**Comment**
Air changing rate for room class better than 100,000: more than 20 per hour	According to practical experience this is not necessary and this cannot be achieved by technical installation.
Air pattern testing agents are specified: Dioctyl phathalate (DOP) and Poly-alpha-olefine (PAO).	Such a restriction is not necessary because other substances can be also used after qualifying.
Leakage testing by photometric method.	Other methods (for example laser particulate counter) may be more sensitive.
Testing of air velocity for HEPA filter every 6 months.	This does not seem to be appropriate because changes in air velocity are considered over a longer period.
Requirement for limiting the duration of exposure of sterile product elements. Recommendation for reducing personnel interventions for example using robotics. Further details for design of the facility and equipment are presented.	This is a more detailed and helpful description of this issue but often linked with more requirements than in the current Guidance.
Detailed description of personnel training in an own section, even till to procedures for reassigning a person to other areas after repeated failure in qualification. Frequency of personnel monitoring is described.	This is a more detailed description.
More frequent microbiological tests for personnel who intervents in aseptic processing.	This is not recommendable to avoid two classes of personnel.
Endotoxine testing of all surfaces that have product contact before and after sterile filtration.	For routine process this does not seems ought to be performed in practice.
Study Design of media fill is presented.	This is a more detailed description.
During Media Fills even operator fatigue should be considered in the study design of media fill.	For routine process this does not seems ought to be performed in practice.
Activities / interventions of each shift, and shift changeover should be considered in the study design of media fill.	This is enforcing the current requirement but is conform with EU Guide.[11]
Size of runs is defined as 5,000 to 10,000 units.	This is enforcing the current requirement.
Detailed requirement for change control and revalidation	This is a more detailed description.
Video recording is recommended for media fills.	This does not seems ought to be performed in practice.
Invalidation of media fill is a very rare occurence and restricted on cases which lead to rejection of commercial lots.	This is a more detailed description.
More details for qualification / validation of the filter are presented.	This is a more detailed description.
The same type of filter should be used in routine process as in media fill.	Until up to now the same membrane type was required; this issue must be clarified.
More details for qualification / validation of a steriliser are presented.	This is a more detailed description.

Table **15** (continued) Overview[54] of New Requirements in FDA Draft Guidance[12] compared with FDA Guidance[24] (Examples)

New Requirement	Comment
The age of the equipment should be considered in revalidation programme of an sterilisator.	The age should not be linked with validation procedures; any failure must be eliminated as soon as possible.
Identifying of microorganisms is based on rapid genotype methods using nucleiic acids.	This detailed point is a very strict requirement compared with USP[55]
The fertility test for incubated media should be demonstrated over the whole duration of incubation (each day of incubation).	This exceeds the requirements of the Pharmacopoeias.
The background area of an isolator should be class 100,000.	This requirement is not harmonised with EU.

Independent of the discussions around the FDA Draft Guidance[12] it is the responsibility of the manufacturer resp. the marketing authorisation holder that an aseptically manufactured drug product meets the requirement to be sterile. The sterility assurance of a membrane filtration cannot be extrapolated from measurement parameters and the function of segregation and protection measures can be demonstrated but their efficacy is not demonstrable for the single case. Important for the aseptic manufacturing process is that all single working steps and therefore the whole process is under control. Sterility is a scientific concept that is not demonstrable for each final drug product by testing. The sterility assurance level (1 non-sterile unit per 1 million of manufactured units) is an empiric parameter which has been established for drug products sterilised in the final container closure system. It may be extrapolated from physical parameters of a sterilisation process with known and demonstrated (validated) efficacy of resistant microorganisms. This cannot be carried over to aseptically manufactured drug products because it cannot be demonstrated that this requirement has been met.
The whole process is only as good as the worst step. Therefore, a thorough qualification of the facility / equipment and personnel and validation is necessary in aseptic manufacturing. A detailed risk analysis concept will help to work out an optimal programme for qualification and validation taking into account the necessary requirements of the specific process.

3.1 Risk analysis (HACCP - Hazard Analysis and Critical Control Points)

Since media fill validation should simulate a normal manufacturing process with the same exposure to the risk of contamination, e.g., from operators, environment, and equipment as would occur during routine aseptic processing, a risk analysis[52, 53] should be carried out.
The association between, and the mutual dependence of, aseptic processing and external conditions (environmental influences) and the desired hygiene situation require analysis in order to enable this broad division to be split further into directly and indirectly involved process and environmental factors. A risk assessment must be made for each individual process step and deregulating environmental factor with the aim of recognising risks, describing the problem, evaluating and eliminating it, or - if this is impossible - constructing suitable management mechanisms.

The hygiene situation, as the specialized case of a controlled process, can thus be viewed as a closed loop. A given state (hygiene situation) is influenced by a variety of environmental factors (generally speaking, deregulating factors). Regular controls continuously analyse the condition of hygiene situation - or compliance in general - by comparing nominal and actual states. Corrective measures are enacted as soon as warning values detect a tendency toward divergence from the predefined state. An integrated control system analyses the effects of corrective measures on other parts of the system. The appreciation of importance of microbiological factors in the manufacture of sterile products in a biologically non-sterile environment requires a proactive concept and management strategy to ensure constant hygiene conditions in aseptic processing.

In aseptic processing, HACCP (Hazard And Critical Control Points) can serve as a management tool that provides a structured and logical approach to the control of identified hazards. It has the potential to identify areas of concern where failure has not yet been experienced. It is therefore useful for the establishment of a validation protocol for media fill.

The HACCP concept is based on a structured approach that involves the identification and analysis of potential realistic hazards associated with all stages of pharmaceutical production from raw materials to the distribution of finished products. Microbiological, chemical and physical hazards, documentation, qualification, and validation should all be considered if they affect product safety or quality, e.g., in aseptic processing. Critical control points have to be defined as processing steps at which necessary action can be applied to ensure and maintain compliance with specified conditions Those points are identified with appropriate measures that can be applied to control each hazard. If no such critical control points can be established, product specific validation should eliminate potential risks of a certain process step. Alternatively, additional subordinated critical control points may be identified or installed. For each critical control point, criteria have to be established, which separate acceptability from unacceptability (definition of limits). In defining limits for a critical control point, action levels e.g., a value well below the actual limit which provides an opportunity to react to a potential or realistic hazard prior to the state of unacceptability.

A scheduled series of measures, e.g., in-process control, observation, testing of product samples and critical control points should be established. With respect to action values and limits, corrective action is applied to ensure specified conditions are met.

In the event that monitoring of critical control points indicates a trend towards loss of control, corrective action has to be defined and initiated.

Those uncontrolled conditions, needed for qualification and / or product-specific validation efforts, etc., identified during HACCP analysis are summarized in a report. This report may serve as a product-specific validation protocol and as the instrument within a pharmaceutical QA-system.

4 Conclusion and outlook

A lot of health care products such as biologics cannot be sterilized with steam or dry heat. Therefore aseptic manufacturing will retain its importance in the future.
A general harmonisation in the requirements for the clean rooms (particulate matter, microorganisms, averaging of values in EU) between Europe and USA does not yet exist. The fact that the FDA Guidance of 1987 is just the current one and a revision has been announced several years ago but only a draft Guidance has been edited demonstrates that the whole issue is much and (intensively) discussed.
However some concerns expressed by the authorities speak against aseptic manufacturing process, because there exists some uncertainty relating to a number of variables that can influence the process. The consequence is an increase in the requirements and subsequent increase of automatism in the aseptic manufacture.
One of such approaches of automatism are isolators and the blow-fill-seal-technology. These techniques mean a separation of personnel from the aseptic manufacturing process. But high requirements are also established for isolators regarding their qualification and validation. But between Europe and USA the requirement for the background environment of an isolator are not yet harmonised. For example USP 27 describes the requirements for validation of isolator systems.[56]
A detailed risk analysis concept will help work out an optimal programme for qualification and validation taking into account the necessary requirements of the specific process.
Aseptic manufacturing requires extensive environmental monitoring activities and this data are considered in batch release usually in addition to sterility testing. Although the value of sterility testing is limited in aseptic manufacturing, as explained above, the parametric release requires major efforts and the approval by the authority and is restricted to terminally sterilised products. So on the one hand the importance of the monitoring data in aseptic manufacturing is without doubt. However no harmonisation exists between these requirements in Europe and USA. (evaluation of limits, in EU using average values is accepted). In a global world where usually several markets are supplied by the same manufacturing facility the industrial companies have to take into account the current guidelines / guidances of Europe and US in for example establishing of warning (alert) and action limits in environmental monitoring. This is a precondition to satisfy the markets in due time and to guarantee constant quality of the drug products and last but not least to save costs and resources. Personal experiences in one's own company should also be considered. Standard operation procedures must not only provide precise information about the limits, but also about responsibilities, conditions of testing, method of testing, method of sampling, time of sampling, sampling sites, and so on. Efforts are underway to harmonise environmental monitoring, but the process is slow. Caution is advised in interpreting guidance as requirements. Companies are inspected against their own SOPs.
The whole process has to be thoroughly validated and documented. This is usually performed by media fill validation. A very helpful approach to the validation protocol can be performed using HACCP.
Thorough and detailed documentation is a further requirement and should have the same depth in validation as batch record of the drug product. It is absolutely important to describe the whole process (including monitoring activities) step by step. The monitoring data has to be considered in batch release. It is a fact that the monitoring data from microbiological testing as well as the result of sterility testing is available only after some delay. So the detailed documentation of the production process (see batch record) is a precondition in investigation of exceeding limits as well as investigation of failure during process validation

(media fill). It is also clear that a well qualified and reliable microbiological laboratory is very important to avoid false positive results because the validity of data generated by the laboratory may not be in doubt. A well functioning quality assurance system is a key element in aseptic manufacturing process and any (minor) weakness in this system may have a great impact on the quality of the product and with consequences for the company (for example rejection of batches, complaints, recall of batches). A well functioning and reliable microbiological laboratory and an effective change control system (any [minor]) change requiring requalification and revalidation) are very important parts of such a quality assurance system.

5 Summary

Health care products that must be sterile but are unsuitable for terminal sterilisation have to be processed under aseptic conditions. For example, all product parts or components that are in direct contact with aseptically-filled sterile product during the manufacturing process require pre-sterilisation. In addition, production has to take place in a controlled manufacturing environment where microbiological and particulate levels are maintained at defined low levels and where human intervention in the manufacturing process is minimised. In aseptic processing maximum efforts must be expended to use consistently qualified equipment and materials and validated systems, to use adequately trained personnel, and to control the environment. Furthermore a well documented systematic process is needed which impacts risks on product quality using the Hazard Analysis and Critical Control Points (HACCP) concept considering all aspects of qualification of equipment and materials and validation of the process (for example facility design, environment, materials, equipment suitability, supply systems, filter qualification, personnel, incubation conditions for the samples, identification of positive units, demonstration of growth promotion, process validation and quality control). Usually the validation of the whole process is performed by media fill (simulation of the manufacturing process by using nutrient media).
A lot of requirements have to be met to ensure that the aseptically manufactured drug product can be regarded as sterile.
The manuscript depicts international guidelines of EU and US and microbiological measures which should be implemented in development and performance of validation of aseptic manufacturing procedures. The whole process is only as good as any single step. The requirements in Europe and USA (for example sterile requirement for aqueous based inhalation pharmaceutical forms, clean room classification) have not yet been harmonised. In a global world the careful establishing of the requirements regarding the requirements in both regions - Europe and USA - is a precondition to supply different markets with the adequate quality of the drug products.

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Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

Mannheim, den _____ Unterschrift: _____
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