

Auditing an Aseptic Sterile Area

Title: Auditing an Aseptic Sterile Area					
Auditor Manual: 20					
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

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Auditing an Aseptic Sterile Area

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Controlled access areas: those facilities, or areas of facilities, which allow only authorized personnel to access. These may be also known as restricted access areas.

Endotoxins: toxic molecules consisting of lipopolysaccharide originating from the outer cell wall of Gram-negative bacteria. Endotoxins may cause fever reactions in humans.

Environmental monitoring program: a defined documented program which describes the routine particulate and microbiological monitoring of processing and manufacturing areas, and includes a corrective action plan when action levels are exceeded. This program provides meaningful information on the quality of the aseptic processing environment when a given batch is being manufactured as well as environmental trends of the manufacturing area. An adequate program identifies potential routes of contamination, allowing for implementation of corrections before contamination occurs.

Grade A: the normal air classification for an aseptic processing area. This classification means that there are not more than 3,500 particles measuring 0.5µm or larger in one cubic metre of air, when measure during activity. Grade A may be considered equivalent ISO class 5 and FDA class 100. Other grades/requirements can be found in the EU-GMP.

High efficiency particulate air (HEPA) filter: high efficiency particulate air filter with a minimum 0.3 micron particle retaining efficiency of 99.97 percent.

HVAC: heating, ventilation and air conditioning system.

Integrity test: a test to determine the functional performance of a filter or filter system and detect the presence of individual leaks in the filter media, frame, and seal.

Laminar flow (LAF): an airflow moving in a single direction and in parallel layers at a constant velocity from the beginning to the end of a straight-line vector. However, true laminarity is not achievable in clean room applications. “Unidirectional flow” is the more accurate description for clean room applications and is defined as; an airflow moving in a single direction, in a robust and uniform manner and at sufficient speed to reproducibly sweep particles away from the critical processing or testing areas.

Media fills: a method of evaluating an aseptic process using a microbial growth medium. (Media fills are understood to be synonymous to simulated product fills, broth trials, broth fills etc. The term “process simulation” is sometimes used interchangeably with media fill).

Media growth promotion test: a test performed to demonstrate that microbial growth media will support microbial growth.

Pyrogens: fever producing substances.

Sampling frequency: an established time interval for collecting samples, e.g. once per minute, once per week, once per month, etc.

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such products keeps focus on the key quality factors and potential hazards to the patient.

Key requirements for sterile products are sterility, apyrogenicity, low particulate counts (Note! Cotton fibres may be more dangerous than glass particles as they can clog capillaries and serve as vectors for microorganisms), chemical and biological purity and container integrity. Patient risks are generally greater for large volume products used for infusion, as the patient will be exposed to very large volumes intravenously.

The best way to assure sterility is to sterilise the product in its final packaging. Such products are often referred to as “terminally sterilised” products. Sterilisation is commonly achieved by steam sterilisation (autoclaving), dry heat, gamma or electron beam irradiation or ethylene oxide fumigation.

For terminally sterilised as well as aseptically prepared products the same basic principles apply. Challenge to the sterilisation step as well as the potential for recontamination of the product once it has been sterilised should be minimised.

In auditing you need to know the product (liquid or powder), whether it is aseptically prepared or terminally sterilised, what the container type is, whether the product is for single/multi-use and preserved/unpreserved. How it will be used must also be considered. Is the product administered as an injectable or infusion directly to the bloodstream, intrathecal (to the spine) or subcutanally? Is it for ophthalmic, topical or inhalation use? What are the indications and the health status of the patients?

Sterile products manufacture

General principles

Common steps in the manufacture of sterile products are preparation of solution, filtration, filling, closure, sterilisation if the product is not filled aseptically, container closure integrity testing, visual inspection and labelling. For some products aseptic filling may be followed by freeze drying. Another technique used is blow-fill and seal, where the container is formed, filled and sealed in a sequence in a blow-fill and-seal machine.

Terminally sterilized products

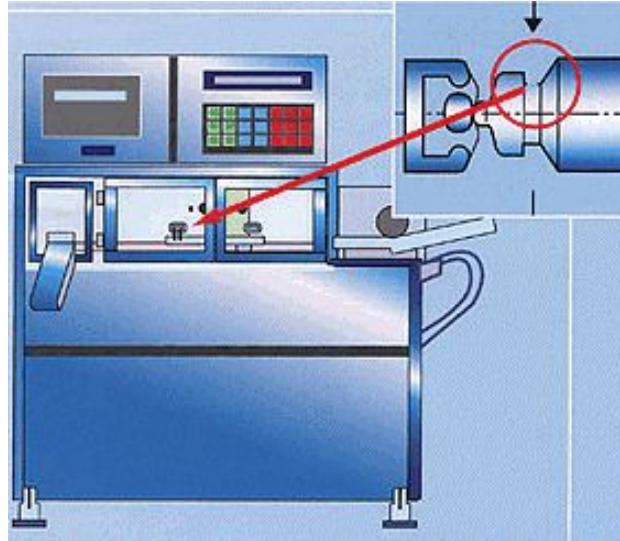
The bulk product is usually formulated by dissolving ingredients in water for injection. The solution is then filtered in order to remove particles and to lower the bioburden (number of microorganisms in the solution). The holding time for the solution must be validated to ensure control of bioburden. The filtered solution is filled into pre-washed containers and sealed to prevent recontamination. Filling and sealing is performed in a clean environment in order to minimise risks for particulate and microbiological contamination. Microbiological contamination levels should be kept to a minimum level of challenge to the sterilisation step. The filled containers are sterilised using a process demonstrated via validations to deliver a SAL of 10^{-6} or better.



Stainless steel tanks for preparation of solutions

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and cracks to be detected.



This is a schematic showing the point at which an ampoule may leak. The high-voltage leak detector relies on the ampoule contents being conductive.

For vials and bottles the fit between vial and stopper is of great importance and should be challenged by microbial intrusion tests as part of the validation. Commonly a suspension of *Br. Diminuta* is used. The products used in such challenge tests should have been exposed to worst-case handling and storage conditions. Vials, bottles and stoppers at extremes of dimension specifications should be used.

Lyophilization

Products with limited stability in solutions may be lyophilised (freeze dried), which once the containers have been closed constitute a hostile environment for microbial survival. The process itself includes freezing of a wet substance (which have been sterilised by filtration) causing the ice to sublime directly to vapour at low pressure whilst supplying heat. Lyophilization renders the product being chemically and/or biologically stable as well as making moisture levels too low to permit microbial growth.

Blow Fill Seal (BFS)

Generally BFS gives the potential for greater levels of sterility assurance during the filling process, as product exposure to the environment is minimal. The BFS process combines a solution filling system with extrusion blow moulding; typically pharmaceutical grade polymers (e.g. polyethylene or polypropylene) are used. The equipment used will sterilize the polymer granules, form the container to be filled, fill the solution into the container and finally seal the container. The process requires minimal operator intervention as it is highly automated. Therefore there is a reduction of the potential for microbial contamination.

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via validations and deliver a SAL of 10^{-6} or better.

4. *Gas sterilisation* through the use of ethylene oxide is the method of last resort. Wherever possible, gas concentration, relative humidity, temperature and duration of exposure must be measured & recorded. The effectiveness of the process must be checked via the use of a suitable biological indicator (see European Pharmacopeia 5.1.2 Biological Indicators of Sterilisation) and a representative sample of the batch must be tested for sterility prior to release.

Aseptic Processing

Where terminal sterilisation is not possible for example when a product is heat sensitive, aseptic processing is used. It is a manufacturing process designed to prevent the introduction of viable organisms and particulate contamination into the drug product. The material is rendered sterile by a filtration process that removes microorganisms. The filter must be challenged before and after filling to demonstrate its integrity has not been compromised. Primary packaging components are to be sterilised before use. Aseptic processing is not limited to filling of product; it may also include aseptic blending followed by filling.

Properties of the perfect filter:

- Compatibility with products and non-extractables.
- Can cope with unlimited flow rates,
- Is inert to all chemicals,
- Is a total sink for microorganisms
- Is physically & thermally stable.

Both terminal sterilisation and aseptic processing are applicable to liquid products, although care must be taken to ensure that product quality is not affected by the chosen method if terminal sterilisation is used.

Clean Rooms

There are special requirements for premises used for the manufacturing of sterile products. The environment needs to be controlled with respect to microbial and particulate contamination as well as temperature and humidity.

Different means of protecting the room from contamination is put in place, e.g. special HVAC systems to provide highly filtrated air, the room is kept an overpressure to the exterior at all times (including when doors are opened), sterilisation/sanitation of material/equipment entered into the clean room, separation of premises into different areas/rooms in which different activities are performed. The air in the room is changed (different rates for different grades) frequently (e.g. at least 20 times/hour for grade B rooms) in order to keep particulate levels low.

The correct pressure differential between different areas and grades of cleanliness are monitored as well as microbial and particulate contamination levels, air flows are visualised to confirm the correct movement of air (i.e. smoke studies), and recovery tests are performed in order to confirm how quickly a room meet the accepted criteria after contamination.

Qualification of rooms includes: air supply capacity, air velocity/uniformity, air change rate, air flow patterns, HEPA filter integrity, pressurisation, unidirectional flows (LAFs), particle count, recovery rate, microbial counts (air and surface), temperature and humidity. The correct performance of a room is also addressed during process simulation tests (media fills) performed regularly for aseptic processes.

Clean Room Gowning

The greatest risk for contamination in a modern, well designed clean room is people. Introduction of people into a pharmaceutical clean room may seriously compromise the integrity of the facility with regards to particulate and microbial contamination. Despite efforts

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verify that the information you have received regarding the facility is accurate and assure that it is appropriate for the operations you observe. You may accomplish this through review of as-built diagrams and cleanroom certification reports and interviews with personnel. Also ensure that blackout procedures exist (that is procedures to return area to sterile to guarantee products).

Observe operator aseptic technique and gowning practices during the performance of the aseptic filling operation. Observe position and movement of the operator in relation to the exposed product and component areas. Breaches of the LAF air in these areas are unacceptable. Assure that the operators are properly gowned, with no exposed skin.

Observing the movement of personnel is of particular importance throughout the aseptic process area to assure that movements are appropriate, including hand sanitizing practices, vial and stopper jam corrective actions. Is equipment in the area sanitized and constructed of materials that will not cause contamination (i.e. non-porous, easily cleanable, non-reactive)? Is the facility/area constructed in a manner to assure that it can be sanitized, will not easily disintegrate or shed particles, and will not be a source of contamination? Also observe the sampling points for environmental monitoring and review the rationale for choosing them.

As you perform your walk through, review available documentation where possible and identify equipment, batch records and personnel that you wish to gather more information about later in the audit. For example, identify staff to be included in your training record review and select equipment for IQ/OQ and PQ review.

Ensure that you include the Purified and Water for Injection (WFI) systems in the walk through and review all monitoring and trends for this system.

Key Parameters for Auditing an Aseptic Processing Facility

Prior to the audit

- Find out what sterile products are produced at the site.
- Find out if they are terminally sterilised or aseptically produced and the methods used.
- Review previous audit/inspection and actions taken.
- Review listed reference materials to assure that you are familiar with worldwide regulatory expectations.
- Ensure activities will be ongoing during your audit
- If processes in the cleanest rooms cannot be viewed from outside, find out what needs to be done to allow you access

During the audit

- Inspect the facility for appearance and cleanliness.
 - Surface finishes should be smooth, impervious, unbroken, minimise shedding, easily cleanable and resistant to cleaning agents.
 - Windows should be non-opening and flush fitted.
 - Door sealing should not prevent appropriate air movement (e.g. from clean rooms to support areas). Sliding doors should not be used. Where part of an air lock (e.g. changing room) doors should be interlocked or alarmed unless both are visible at once.
 - Look for cracks in the ceilings (false ceilings should be sealed in place), walls, and floor (epoxy screed, terrazzo, sheet vinyl), dirt.
 - Conveyors should not pass from “dirty” to “clean areas” unless part of a sterilising tunnel.
 - Flush mounted and easily cleanable intercoms or window mounted speech panels

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Ensure appropriate and timely actions are taken.

- Ensure the pressure differential between rooms of different classifications is at least .05" water or 10 – 15 pascals.
 - Check that a maintenance program is in place for HVAC-systems and LAF-units
 - Blackout procedures
- Ensure that airflow directions are appropriate.
 - Review air pressure differentials.
 - Review flow pattern data.
 - Review smoke studies.
 - Determine if personnel flow and material flow are designed to minimize the potential for contamination.
 - Ensure that all material entering the processing area is disinfected with an appropriate disinfectant.
 - Inspect the processing area while production is in progress.

Note: in some of the cleanest areas you may not be allowed access due to the risk of contamination. It may however be possible to observe through a window. If you cannot observe work being carried out it may be very difficult to draw any conclusions. The issue has to be discussed/resolved.

- Observe personnel and determine if they are using proper aseptic technique.
 - Ensure that all personnel, including mechanics and QC inspectors, working in the area have had adequate training including participation in media fills.
 - Ensure that all personnel working in the area are gowned properly.
 - Observe personnel performance, including but not limited to: set-ups and adjustments of machines, correction of vial jams, stopper jams and selection of production samples (if required).
 - Observe if environmental monitoring sampling equipment is placed properly.
 - Ensure sampling points are representative
 - Determine if there is adequate control, while production is in progress, to prevent cross-contamination.
 - Determine if sterile components are used within their expiration dating.
 - Determine if there is adequate and clearly marked storage to prevent mix-ups of sterilized and unsterilized processing components/products.
- Filling process
 - Layout of filling line. Evaluate whether the design of the line is appropriate (filling level compared with level of protection walls, for aseptic filling design should be confirmed with smoke studies and possibilities for operator interference limited to what is necessary)
 - Following filling integrity of products should be checked (container integrity validation and 100% integrity testing of ampoules closed by fusion, e.g. high voltage inspection methods)
 - Visual inspection
 - Ensure visual inspection is preformed as required
 - In manual operations check that the time operators perform visual inspection is regulated
 - Check that personal is checked for eye-sight regularly

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- Audit the manufacturing process and documentation.
 - Choose a product and include the following in your data review:
 - Review Process Validation reports and identify critical process parameters
 - Review Manufacturing Process Description or batch records and assure the process is currently being performed according to the validated process.
 - Review time limits for various process steps.
 - Request data to support the sterilization process
 - Ensure filters are checked before and verified after filtration. Filter testing should be validated for the products in scope.
 - Review deviation documentation to assure that the validated process was followed.

- Inspect the packaging and labeling area.
 - Ensure that final units are inspected to remove damaged/defective units.
 - Ensure that line clearances have been performed prior to and after the labeling and packaging of lots.

- Review the release packet for a recently released lot.
 - Ensure environmental monitoring data and investigations, including personnel data, were reviewed.
 - Ensure that all investigations of deviations were properly performed and sound lot disposition decision was made.

- Review all documentation associated with the following:
 - Facilities and equipment
 - State of repair and maintenance of facilities and equipment
 - Personnel, material and process flow
 - Compounding area
 - Filtration processes
 - Filling process
 - Lyophilization process if applicable
 - Inspection process
 - Product testing
 - Terminal sterilization if applicable
 - Personnel (including observation of aseptic technique and gowning practice)
 - Media fill program, practice and performance
 - Ongoing environmental monitoring, including personnel monitoring
 - Training and qualification of personnel, including gown qualification
 - Sterility testing area and data
 - Microbiology laboratory (see Laboratory Training unit)
 - Water systems including Purified, Water for Injection, clean steam