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Fundamentals of Aseptic Pharmaceutical Engineering

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Fundamentals of Aseptic Pharmaceutical Engineering

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Course Content

Introduction

Aseptic Pharmaceutical Engineering is perhaps the most interesting to an engineer compared to other pharma/biotech projects. (Someone once said Engineers really aren't boring people, they just like boring things.) There are two primary reasons it is a favorite. The first is the technical challenge. Things that can be overlooked in non-sterile manufacturing will present significant issues with Aseptic. The second is that there is clearer direction in regulatory directives as to fundamental scope requirements. Engineers like to begin with a firm scope. There is less to debate, and clearer expectations as to the end product.

This course provides an introduction to Aseptic operations in the Biopharmaceutical industry. Due to the ever-changing regulatory environment, general practices will be discussed without specific reference to the predominant FDA and EU guidances as much as possible. The goal is to provide the student with a well-rounded introduction to Aseptic operations. However, refer particularly to FDA's 21 CFR parts 210 and 211, as well as latest guidance documents. As a further and necessary disclaimer, you must evaluate each project on its own merits, and nothing herein should be considered "engineering consulting" for your specific project.

Content

What is Aseptic, and why is it needed?

What do we mean by Aseptic? Aseptic simply means there are no microorganisms that can cause infection in the patient. Unlike products that are terminally sterilized (the preferred method by major regulatory agencies), an Aseptic operation maintains acceptable sterility at critical steps of the manufacturing process (when sterile filtration or other means are not possible) and filling operations (when terminal sterilization is not an option). When the product can be

terminally sterilized (autoclaving the most common method), Aseptic processing is not necessary. Aseptic processing is common for parenterals (injectible drugs.)

Whether produced in an Aseptic manner or terminally sterilized, parenterals must be sterile in their final form to avoid problems for the patient. Products that are not sterile may contain pyrogens (“an agent capable of inducing an increase in body temperature; usually refers to fever caused by bacterial endotoxins.”)¹ An Endotoxin is “cell wall debris (lip polysaccharide) from Gram-negative bacteria.”² These may include bacteria such as E. coli, Salmonella, Shigella, Haemophilus, Pseudomonas, and Neisseria as well as other pathogens. Whereas drugs such as OSD’s (Oral Solid Dosage) do not require sterility since the body’s natural defense mechanisms engage after ingestion, parenterals are injected intramuscularly (I.M.) or intravenously (I.V.) and bypass the defense mechanisms. A simple example of this is normal drinking water. If you drink safe water, there is no ill effect. But if you were to inject the same water with a syringe, you could get extremely sick.

Especially careful formulation of parenterals is also important. A parenteral is formulated to have the same osmolarity of the blood (approximately 300 milliosmoles per liter or mOsmol/L). Solutions that have different osmolarity can cause damage to red blood cells or tissue irritation, and cause pain.

It is critical, therefore, to produce such products in an environment that mitigates contamination and to a rigid spec. Sources of contamination include the following:

1. The product
2. The environment/HVAC
3. Equipment
4. Packaging components and materials
5. And mostly, people. As we will study later, extreme care is required to protect the product from the natural contamination of the worker. As well, it is important to design Aseptic areas that minimize the number and effort of workers.

The Manufacturing Process

If the product can be sterilized prior to fill/finish¹, the manufacturing process is not required to be in an Aseptic environment. However, care must be taken to minimize the

¹Fill/Finish refers to filling the product in the final container, stoppering, labeling, etc.

bioburden – we wouldn't want to manufacture the product in the parking lot. It is common to produce products in class 100,000 cleanrooms that will be rendered sterile later.

A cleanroom class is measured by the quantity of viable (produced from living matter) and non-viable particles. The class may be referred to as other designations by regulatory agencies (for example, the EU classifies by letters A, B, C, and D), or ISO designations. (Be aware of the EU designations since they are different for at-rest and in-operation.)

What does the class mean quantitatively? For class 100,000, for example, there must be less than 100,000 particles of 0.5 micron and larger particles in a cubic foot of air (there are 25,400 microns in an inch, and 1,000 in a millimeter). Although the particulates may be nonviable (non-living), they still can be an “extraneous contaminate”³ to the product, and can contaminate it biologically by acting as a microbial vehicle. Class 100,000 can be used for non-Aseptic and less critical activities. (There is no specific general cleanroom classification requirement for all non-sterile drugs.) However, in the direct Aseptic area (exposed sterile product) the class must be 100, which we will discuss later. See Figure 1 below for comparative sizes of particulate.

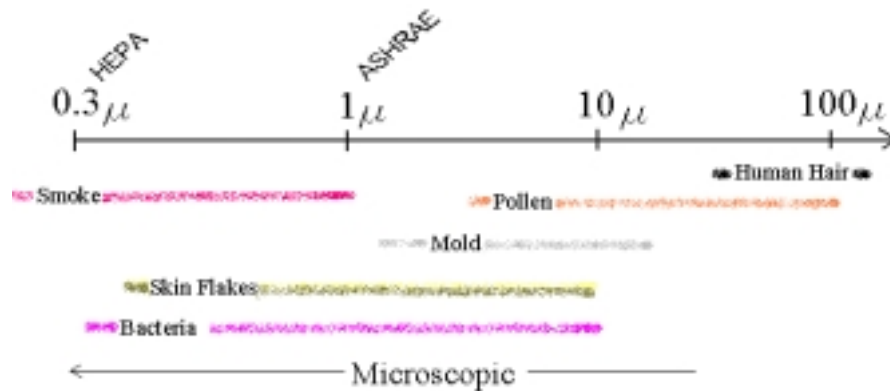


Figure 1 – Comparative Particle Sizes

Typical sterilization techniques of the product prior to fill include heat, irradiation, and most commonly filtration through a 0.22-micron filter (or less) which is sufficient to remove most bacteria and molds (but may let viruses and mycoplasmas through). Such filters should be validated that they repeatedly remove viable microorganisms from the sterilized process stream. These filters should be capable of a 10^{-3} SAL (we will discuss SAL later). Filters are tested to remove 10^7 *Brevundimonas diminuta* microorganisms per cm^2 while producing a sterile effluent.⁴ Filters should be pre/post-bubble tested to confirm integrity.

Once the product is sterilized, it is protected in a sterile state and packaged. Tanks holding or processing sterile products should be maintained in a pressurized state or otherwise sealed to prevent contamination from microbes; valves should be steam sterilizable in some applications. However, some products cannot be sterilized prior to filling, and certain process steps must be undertaken in closed or class 100 cleanroom environments (this means there are no more than 100 particles 0.5 micron and larger in a cubic foot of air), also called “Critical Areas.”

The Fill/Finish Process Overview

Here we reach the most critical steps of the process as it relates to maintaining sterility in a typical application. Design must be accomplished such that it is robust enough to minimize problems that lead to contamination. As well, operational aspects are crucial. At the point of entry into the Aseptic fill room, the product must be and remain sterile.

Means will be required to monitor environmental conditions on an on-going basis. (Remote particle monitoring for nonviabiles is a preferred solution in addition to settling plates for viables.) Further, viable testing can include surfaces, such as room finishes, equipment, and especially sterile product contact items, containers, and closures. Such monitoring should cover all shifts. However, no amount of monitoring will guarantee sterility. Instead, the operation will rely on Validated procedures to keep the product from risk.⁵ Obviously, time limits should be established for each processing phase.

There are several finished forms of Aseptic produced products. One thing you might have noticed when getting that dreaded shot is that the containers are translucent. That isn't a fashion statement – it is so a visual examination can confirm the liquid is colorless and sufficiently transparent.

What are the typical finished forms? (See Figure 2). The most common are glass vials (single and multi-dose; if multi-dose, it should contain a preservative to permit multiple use), made of type I glass for SVP's (Small Volume Parenterals). You are probably familiar with these, which are commonly used when receiving a vaccination (inserting a syringe needle in the top stopper, extracting the product.) Other forms include pre-filled syringes, ampoules (a sealed glass container with a long neck that must be broken off), and LVP's (Large Volume Parenterals, typically holding 100 ml or more) in bags or bottles (type II glass).



Figure 2 – Typical Parenteral Final Containers

The form of the final product can be powder or liquid. Also included are ointments and creams. Powder can be produced by a sterile crystallization process prior to filling the vials. However, this tends to have a less accurate fill than liquids, as well as offer other material handling challenges. A final liquid form is often created by adding WFI (Water for Injection) to the compound and then filtered. Filtering reduces microbiological concentration of the product supply solution rendering it sterile as discussed previously. The vials can be filled with liquid, which becomes the finished form. Sterile nitrogen is used to reduce the concentration of oxygen during the filling operation. The important thing to remember is that during the fill process (while the product is exposed) the immediate environment must be a class 100 Cleanroom,² a *Critical Area*. Once the stopper is installed, the over seal (arguably), labeling, and cartoning can be in a lower grade environment. Another promising technology is filling sterile liquid into vials with needles that are pre-sterilized/pre-sealed. The puncture is quickly sealed, maintaining Aseptic integrity. Also, disposable filling equipment is available.

To add additional stability to products when required, liquid can be freeze-dried after being placed in the vials but prior to complete stoppering. Often, biological materials require freeze-drying to better stabilize them. Certain products, such as proteins, don't react well to heat, eliminating the possibility of terminal sterilization. Freeze drying is often used for vaccines,

² Definition of Cleanroom: "Room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room, and in which other relevant parameters, e.g. temperature, humidity, and pressure, are controlled as necessary. ISO 14644-1, ISO 14644-3, ISO 14698-1, ISO 14698-2;" or, "The maximum number of particles greater than or equal to 0.5µm in diameter that may be present in a cubic foot of room air." (ISPE's online glossary, <http://www.ispe.org/glossary/definitionbyterm.cfm?term=Autoclave>)

pharmaceuticals, and blood products. A medical provider will reconstitute the product with a suitable solvent (usually WFI) prior to use. Freeze drying is called Lyophilization. Here is how it occurs. During the fill process, the vial is partially closed. Therefore, it must be maintained in an Aseptic Class 100 environment until lyophilized and finally sealed. This can present a challenge that must be thought through when designing an operation. Lyophilization consists of three distinct processes – freezing, sublimation, and desorption. Sublimation involves vaporizing a solid and condensing it without its having passed through a liquid state. Desorption involves “the release of adsorbed molecules, particles, or cells into the surrounding medium.”³

Careful consideration must be given to all Aseptic equipment. Filling equipment must be designed to be cleanable. CIP/SIP is sometimes used (Clean in place/Sterilize in place). Moist heat is common for sterilization. (Note: Sterilize is different from Sanitize. Sterilize means to destroy viable organisms and spores, whereas Sanitize reduces viable organisms to an acceptable level.) CIP can be problematic in the Aseptic area, so proceed with caution. Endotoxins on equipment surfaces can be inactivated by heat, and removed by cleaning procedures; however, autoclaving is preferred for product contact parts.⁴ The key to controlling bioburden is to adequately clean, dry, and store equipment. Therefore, it is essential that the design of such equipment facilitate this by being easy to be assembled/disassembled, cleaned, and sanitized/sterilized.

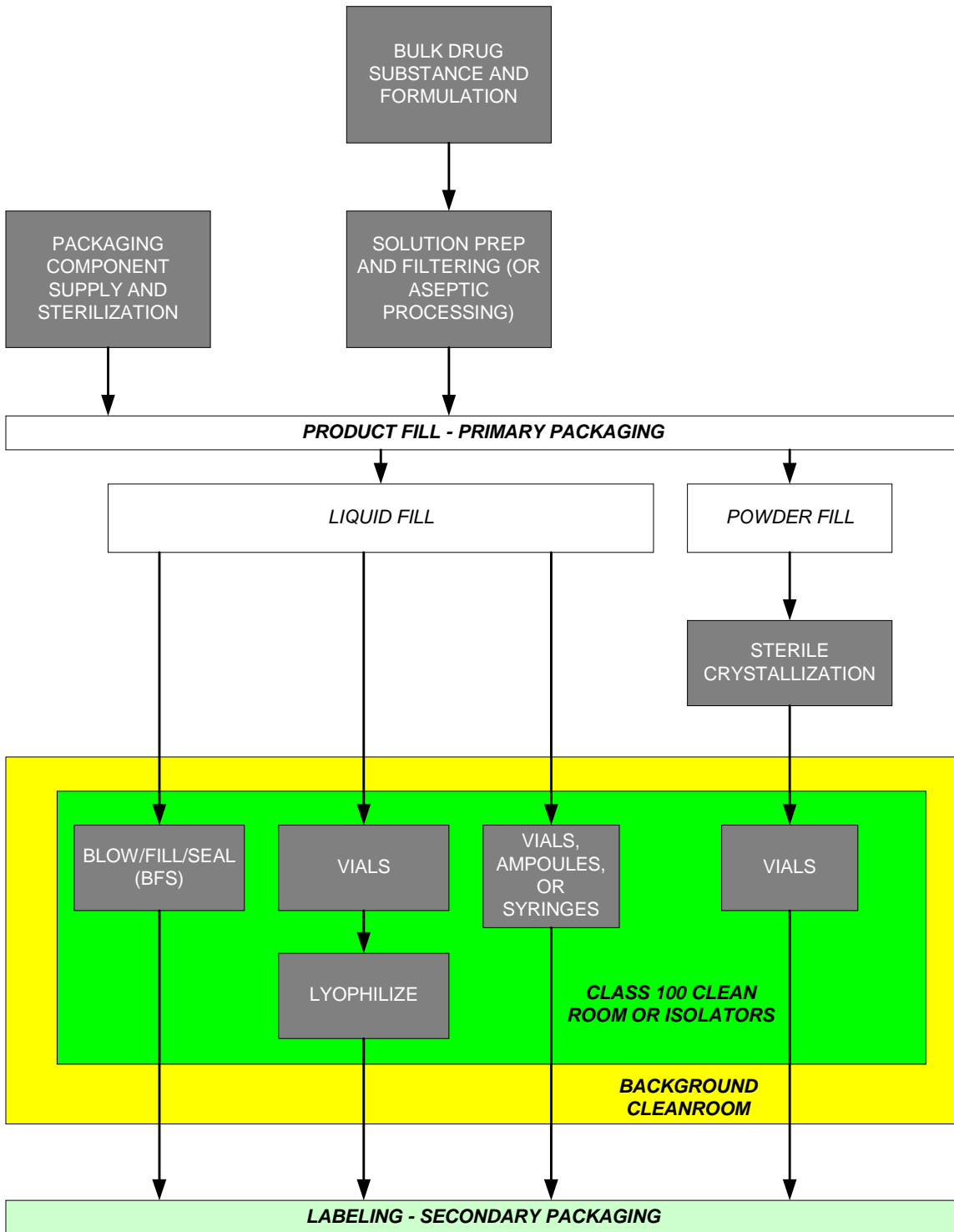
Another finish form/technology is BFS (Blow/Fill/Seal). This involves forming a parison (a tubular form) from a plastic polymer resin, inflating it, filling it, and sealing it in a single operation. However, at the present this method cannot accommodate Lyophilization.

For a comparative overview/PFD (Process Flow Diagram) for typical approaches, see Figure 3.

³ (ISPE’s online glossary, <http://www.ispe.org/glossary/definitionbyterm>)

⁴ Definition of Autoclave: “An apparatus into which moist heat (steam) under pressure is introduced to sterilize or decontaminate materials placed within (e.g. filter assemblies, glassware, etc.). Steam pressure is maintained for pre-specified times and then allowed to exhaust. There are two types of autoclaves: 1. Gravity displacement autoclave: this type of autoclave operates at 121°C. Steam enters at the top of the loaded inner chamber, displacing the air below through a discharge outlet. 2. Vacuum autoclave: this type of autoclave can operate with a reduced sterilization cycle time. The air is pumped out of the loaded chamber before it is filled with steam” (ISPE’s online glossary, <http://www.ispe.org/glossary/definitionbyterm.cfm?term=Autoclave>)

FIGURE 3: TYPICAL ASEPTIC PARENTERAL OPERATIONS/OPTIONS for Liquids and Powders



The Fill Environment and Operational Requirements

As discussed previously, the manufacturing operation classification depends on whether the product will be sterilized prior to fill, or must be maintained in an Aseptic environment (to be avoided if possible.) The focus on this section of the course will be to understand the fill environment and associated operations specifically. The common approach to ensure the environment remains appropriate is to “cascade” cleanrooms from cleanest to unclassified. For example, in the fill room the environment in the immediate vicinity of the exposed product must be class 100. Air must be unidirectional (verified by smoke tests) to ensure air is not being pulled from the lower grade background environment. This background environment consists of the remainder parts of the room in which the Class 100 area resides. Usually, the entire room is not class 100, but has a class 10,000 environment away from the vicinity of the exposed product.

People and product/component flow is crucial. Workers and materials enter the fill suite through distinct airlocks. Employee entry gowning areas cascade up to the environment of the fill suite and the final “gowning” entry airlock should match the classification of the entering room (for example, the final airlock is class 10,000). The employees go through more restrictive gowning layers until the airlock just prior to the fill suite, where they also add sterile garments. A typical gowning exercise could be as follows. Workers initially prepare by changing into dedicated shoes and non-sterile garments, apply head cover, wash hands/sanitize, and put on 1st sterile gloves. Then, they move into the higher class gowning room and apply sterile attire, starting at the top and working down, consisting of a sterilized hood, body garment, facemasks, goggles, and final sterile gloves.

Materials and product entering the fill suite must be sterilized prior to entering. Generally, particulate is reduced by filtration; sterilization and autoclaving reduce microbes; elevated temperatures or chemicals remove endotoxins. A Sterility Assurance Level (SAL) of 10^{-6} or better can be achieved with heat sterilization. (SAL is the probability of sterility. 10^{-6} means that there is a probability of one in one million that a single viable microorganism will be present after sterilization. Another way of saying this is that there is a 6-log reduction. A 1-log reduction means to decrease by a factor of 10 the micro population.) Vials are washed (the final rinse with WFI), and then depyrogenized (destroy or remove pyrogens) via dry heat. Heat is the preferred method of sterilization. Rubber materials are also washed, and sterilized with moist heat. Plastic

containers can be sterilized with gas (such as Ethylene Oxide, or EtO), which should be the last resort, or irradiation (ultraviolet irradiation is not normally acceptable). Once sterilized, care must be taken that the components remain in a sterile state, and introduction into the Aseptic area does not promote contamination. Items should be introduced unidirectionally (such as a double door autoclave, oven, etc.).

Air pressure in the various rooms is important to prevent airborne migration of contamination. The Cleanrooms have positive pressures in relation to lower rated areas and into airlocks, typically 0.04" to 0.06" water gauge (10-15 Pascals). This is to keep objectionable particulate from migrating into the space.

Barrier Isolators are also a good application in some cases in lieu of open Class 100 areas. Barrier Isolators totally contain the product in a protective state consistent with Class 100 requirements. It should be obvious by now that the goal is to protect the product from contamination (Level I Isolators provide this protection). But what about protection from workers when the product is potent/toxic? Not only must the product be protected in this case, but the worker must be protected when there are hazards of cancer, mutation, or developmental/reproductive problems resulting from product exposure. Barrier isolators (Level II) are especially helpful in this application, and avoid the use of pressurized suits. Barrier isolators also can simplify/minimize the requirement for cleanrooms, which avoids first-cost as well as the complexity and expense of operating and working in more restrictive cleanroom environments. Background environment requirements are relaxed. In addition, Barrier Isolators can address an OSHA preference to rely less on PPE (Personal Protective Equipment). However, there are many challenges with this technology, both to initially design and install, as well as on-going operations. Some of the challenges that you need to consider when designing and developing operational requirements for Barrier Isolators are as follows:

1. Issues associated with transfer methods
2. Leak integrity design and testing
3. Maintain Aseptic (Class 100) environment
4. Cleaning and sterilizing (Hydrogen Peroxide Vapor or Chlorine gas are common methods, but the workers must be protected.)
5. Run speeds are often lower in Barrier Isolators, such as 100 vials/minute or lower.
6. How to handle potent products while simultaneously protecting the product

7. High first-costs
8. Ergonomic problems/access
9. Difficulty of maintenance access

Another method of having better control in the Aseptic environment is to provide a Restricted Access Barrier System (RABS). This simply separates the operator from the Aseptic environment to minimize the risk of introducing operator contamination. However, this is not a self-contained barrier isolation system, and Aseptic conditions must be maintained by other means.

Practical Design Considerations

This section will focus on some practical design considerations for various elements of the facility. Obviously, these are not all-inclusive, but represent many of the typical considerations.

First, let's look at the essential utility, HVAC. It is essential that HVAC systems be designed to produce the required air quality, as well as "flush out" particulate from the space. Rooms need to recover, including after fumigation. The cleanroom designations are usually in a dynamic mode (i.e. people there and production underway). The air is filtered via HEPA (High Efficiency Particulate Air) filters, which filter out particles down to the 0.3 micron size at 99.97% or better efficiency. The better the cleanroom rating, the higher the air change rate, or ACPH (Air Changes Per Hour). Cleanrooms typically start at about 20 ACPH for class 100,000, for example. Careful attention should be given during design to enable pressures to be maintained, and effective air currents (remember unidirectional for class 100 zones.) Terminal HEPA filters are necessary for higher-level cleanrooms, although remote filters have been effective for class 100,000. Remember to consider the dewpoint to avoid condensation on the vials. Also remember to design to significantly cooler ambient conditions at the fill area since the workers will have layers of gowning. The monitoring system must be Validated, and report/record/trend critical parameters such as Humidity, Temperature, and Differential Pressure.

Architectural considerations are also essential. A few Architectural considerations may include the following (see Figure 4 for an example of an Aseptic Fill/Finish concept layout):

1. With any project, the process drives the layout. Be sure you fully understand the process, material, and people flow. These should flow in a logical order. Consider flow of components as well as the completed product.

2. Design the layout to ensure there will not be mix-ups in product, components, or raw materials.
3. Aseptic area finishes should be nonshedding, nonabsorptive, cleanable, and nonreactive to sterilizing agents. Finishes should be smooth with coved corners at floors, walls, and ceilings. The room must be periodically sterilized, and specified finishes must be robust against sterilant attack. Room sterilization is accomplished using liquid sterilants or other agents. Fumigation may be useful, especially in hard-to-reach places.
4. Ledges/horizontal surfaces should be minimized, and surface mounted items should be avoided.
5. Keep layouts simple with minimal equipment in Aseptic areas especially.
6. Except where building codes preclude, swing doors in the direction of the pressure flow - otherwise, you will have a hard time keeping them closed.
7. Do not have sinks and drains in the Aseptic areas (avoid sinks or drains in classifications more stringent than Class 100,000).
8. If robotics are used, you may be required to construct super-flat floors.
9. Remember to keep material and personnel access separate to Aseptic areas, as well as have separate gowning and degowning areas (preferred).
10. As much as possible, locate utility support outside rooms. Enable replacement of lights, etc., outside Aseptic areas. Consider walkable ceilings to aid in accessing items above the Aseptic area.
11. Make certain the space is well lit. Place switches outside Aseptic areas.
12. Consider telecommunications equipment to avoid requiring personnel from moving in and out of fill rooms excessively. Video monitoring is also helpful.
13. Investigate whether the facility should be dedicated/self-contained. This is required for some sensitizing materials, possibly in the case of certain antibiotics, hormones, cytotoxics, or highly active drugs. Facilities that handle *Bacillus anthracis*, *Clostridium botulinum*, and *Clostridium* should be dedicated until the organisms are inactivated.
14. Plan for staging outside the Aseptic area. Cardboard, wood, and other materials that could shed fibers should not be introduced to open product areas.
15. Airlocks need to have their doors interlocked to prevent both doors from being opened concurrently. Remember to include override in the event of an emergency.

16. Don't forget those other important support areas, such as
 - a. Laboratory support (must be separated from production areas.)
 - b. Offices
 - c. Cafeteria and other employee support
 - d. Central Utility Building
 - e. Conference areas
 - f. Warehousing and storage (with defined segregation)
 - g. Washing areas
 - h. Pre-weigh

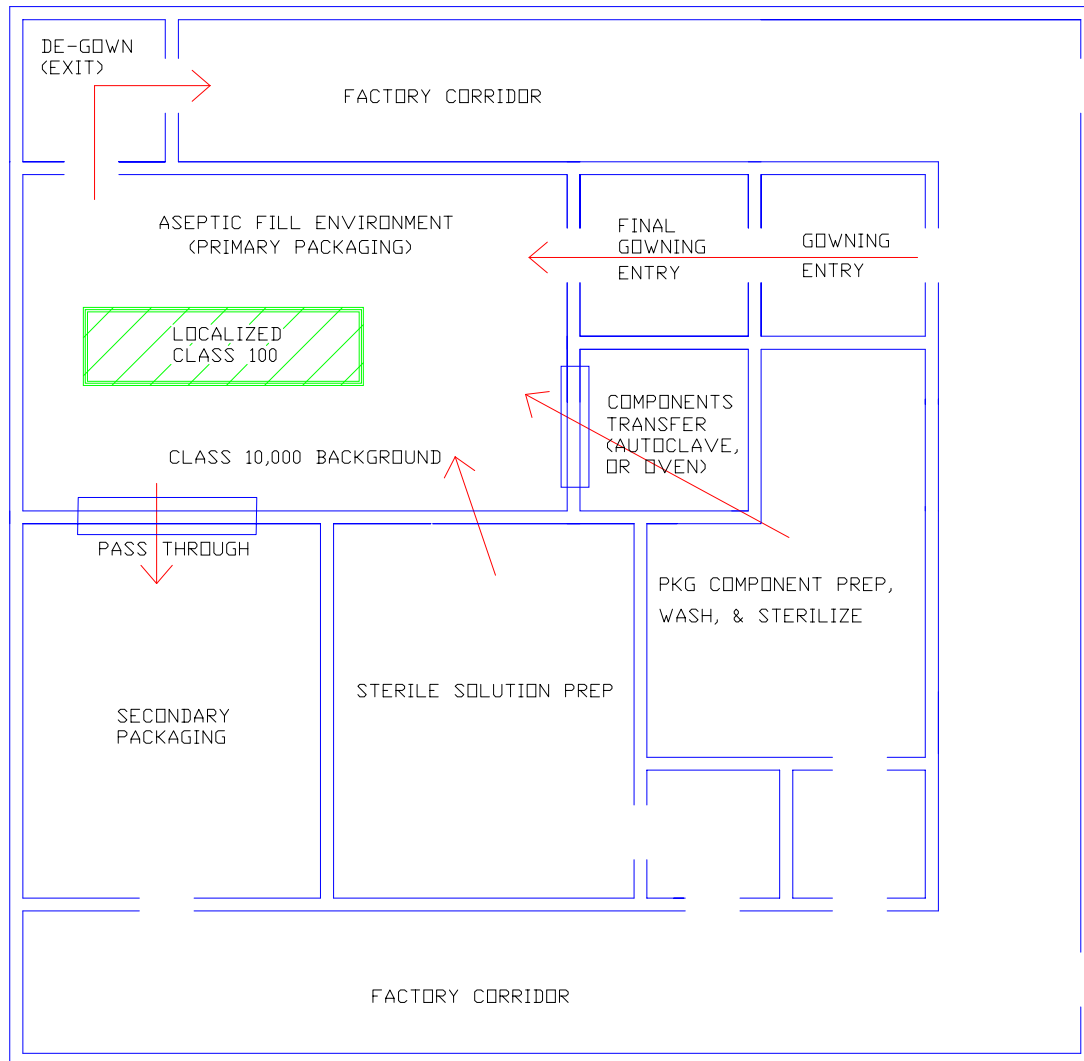


FIGURE 4 - ASEPTIC FILL/FINISH CONCEPT LAYOUT

Critical utilities (those essential to preserving Aseptic conditions) may include the following:

1. Clean steam
2. Water for Injection. This must be produced and distributed such that microbial growth is prevented. This often includes circulating above 70°C.
3. Filtered gasses, such as Nitrogen and even Compressed Air

Commissioning and Validation

The requirements for Commissioning and Validation are extensive, and are beyond the

scope of this course. However, all Direct Impact elements must be Validated/Qualified. Obviously, for an operation this critical the Commissioning exercise must be thorough and robust. In addition, the effectiveness of the process to produce sterile product must be verified. This is done via a process simulation utilizing media fill, or a nutrient medium that encourages microbial growth. These are repeated during the year, and must be done for each shift. Properly performed, this will result in an upper 95% confidence limit (Poisson variable), which will verify the ability of the facility/process to produce sterile product. There are two common media, Fluid Thioglycollate (for anaerobic simulations or for microorganisms that thrive best/only when deprived of oxygen) and Soybean-Casein Digest (for aerobic simulations or for microorganisms that require oxygen.)

The Regulatory Environment and Resources

Obviously, drug regulatory agencies are especially interested in products required to be sterile. Clearly, Aseptic filling is one of the most critical activities in the biopharmaceutical industry. As noted previously, this course intentionally avoids specific references (for the most part) due to the ever-changing regulatory environment. In addition, there are efforts underway at the moment to harmonize various countries' regulations – there are some differences. Some of the most quoted and discussed regulations are from the FDA⁶ and the EU⁷. Know where your product will be sold. If the product is to be distributed in multiple countries, you must ensure the most restrictive requirements of the various regulations govern. Also, refer to the excellent publication by ISPE (International Society of Pharmaceutical Engineers), "Volume 3 - Sterile Manufacturing Facilities." Other important resources can be found from the PDA (Parenteral Drug Association) and USP (United States Pharmacopoeia).

Remember to consider environmental, health, and safety issues as well. OSHA publishes PEL's (Permissible Exposure Limits) that must be considered. Dusts and flammable liquids can cause explosions and fires. There are limits to concentration levels permitted in the air and waste streams. There are ergonomic considerations, and many safety aspects that require attention.

Conclusion

This course provides an introduction to Aseptic Pharmaceutical Engineering. It is now up to you to carefully study the regulations of the countries in which you plan to sell your product.

As well, other industry publications are available and helpful. Equally importantly is to understand your process requirements and product sensitivities. I hope you agree, this is pretty cool stuff (at least to an engineer.)

Specific Key References

¹ “Volume 3 - Sterile Manufacturing Facilities,” ISPE, Pharmaceutical Engineering Guides for New and Renovated Facilities

² Ibid

³ FDA’s “Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice,” dated September, 2004

⁴ “Volume 3 - Sterile Manufacturing Facilities,” ISPE, Pharmaceutical Engineering Guides for New and Renovated Facilities

⁵ Ibid

⁶ At the time of the writing of this course, see FDA’s “Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice,” dated September, 2004

⁷ At the time of the writing of this course, see the European Commission’s “EC Guide to Good Manufacturing Practice – Revision to Annex 1,” dated May 30, 2003.