Conduct a Risk Assessment for Non-Sterile Facilities

IVT’s Cleaning Validation, Aseptic Processing and Environmental Monitoring

Philadelphia, August 2016
Ziva Abraham is the President and Founder of Microrite, Inc., a California based consulting firm providing consulting and training services to pharmaceuticals, biotechnology, medical devices and in vitro diagnostics in the areas of quality assurance, quality control, microbiology, and validation. Ziva has over 25 years of academic, research, clinical and industrial experience in microbiology, and quality assurance. Ziva has received her Master’s Degree in microbiology and has worked on developing microbial insecticides using entomogenous bacteria and fungi for her doctoral research. Her career also includes founding and managing clinical laboratories for Maccabi Medical in Israel. She has trained personnel from various industries in microbiology techniques and methods. She uses her extensive experience to teach why assessing risk of microbial contamination should be in the forefront of any company that has products for human/veterinary use. Her experience in clinical laboratories has provided her with the framework to understand the effects of microbial contamination in products from a patient safety perspective.
The Company

Microrite is a San Jose, CA based Consulting Company helping Pharmaceuticals, Biotechnology, Medical Devices, In-Vitro Diagnostics and Combination products in the areas of Quality Assurance, Microbiology, Process Development, Process Validation, Facility and Utility Validation.

Microrite also conducts seminars, webinars and onsite trainings

Microrite’s goal is to bring practical solutions through consulting and training services and enable life science companies to make their products safer

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Non-sterile, Low bioburden

In microbiological terms, pharmaceutical products can be divided into two groups: sterile and non-sterile.

Non-sterile drugs must satisfy the appropriate microbiological purity criteria which are included in pharmacopoeial monographs.

Nutritional and dietary supplements are non-sterile.

Certain drug device combinational products may be non-sterile. In-vitro diagnostics may be non-sterile.

So what is most important in all the above cases?

The holder of a manufacturing authorization must ensure that they are fit for their intended use and do not place patients at risk due to inadequate safety, quality or efficacy.
Define non-sterile

Is the non-sterile drug?
- Microbiology controlled
- Microbiologically uncontrolled

All systems from facility design to product testing depends on the answer!

What is the route of administration of drug?

Depending upon the route of administration, certain organisms may be objectionable
Are these organisms:
- Soil borne
- Water borne
- Human borne
Define non-sterile

How is the final product saved from microbial proliferation?
Controlled by:
• pH
• Antimicrobials
• Preservatives

Note: The organisms used to test for MLT or antimicrobial/preservative efficacy are not objectionable in each and every non-sterile product

There could be other organisms that can circumvent antimicrobial/preservative efficacy
Define non-sterile

How else can contamination affect the product?
• Microbiological assessment of non-sterile products is particularly pertinent in view of the fact that microbial contamination can:
  • Reduce or even eliminate the therapeutic effect of drugs
  • Cause drug-induced infections
  • Change the chemical and physical properties of the drugs
  • Change the contents of active ingredients
  • Can convert drugs to toxic products due to microbial metabolites

• For IVD products, microbial contamination can lead to erroneous diagnostic results
Route of Administration-Specified Microorganism(s)
Nonaqueous preparations for oral use
• Absence of *Escherichia coli*
Aqueous preparations for oral use
• Absence of *Escherichia coli*
Rectal use
• .........................
Oromucosal use
• Absence of *Staphylococcus aureus*
• Absence of *Pseudomonas aeruginosa*
Gingival use
• Absence of *Staphylococcus aureus*
• Absence of *Pseudomonas aeruginosa*
Cutaneous use
• Absence of *Staphylococcus aureus*
• Absence of *Pseudomonas aeruginosa*
Nasal use
• Absence of *Staphylococcus aureus*
• Absence of *Pseudomonas aeruginosa*
USP <1111>

Route of Administration-Specified Microorganism(s)

Auricular use
• Absence of *Staphylococcus aureus*
• Absence of *Pseudomonas aeruginosa*

Vaginal use
• Absence of *Pseudomonas aeruginosa*
• Absence of *Staphylococcus aureus*
• Absence of *Candida albicans*

Transdermal patches (limits for one patch including adhesive layer and backing)
• Absence of *Staphylococcus aureus*
• Absence of *Pseudomonas aeruginosa*

Inhalation use (special requirements apply to liquid preparations for nebulization)
• Absence of *Staphylococcus aureus*
• Absence of *Pseudomonas aeruginosa*
• Absence of bile-tolerant Gram-negative bacteria
Stomach and Intestinal Infections-Aerobic Bacteria

Aerobes
- Escherichia coli
- *Shigella* spp
- *Salmonella* spp
- *Vibrio* spp
- *Campylobacter* spp
- Yersinia enterocolitica
- *Aeromonas* spp
- *Bacillus* spp- *B. cereus* produces a number of toxins which may contribute to the development of diarrhea
- *Listeria* spp

Peritonitis or intra abdominal sepsis and liver abscesses are common result of gut infections
Anaerobes

Three main anaerobic species that are commonly associated with human disease: *Clostridium difficile*, *Clostridium perfringens*, and *Clostridium botulinum*. *C. difficile* can colonize the colon and induce diarrhea following the production of toxins (Polymicrobial Diseases).

*C. perfringens* is a common cause of food poisoning; these bacteria can secrete a toxin during the process of sporulation within the intestine.

*C. botulinum* is also an important because of food poisoning. This bacterium can multiply and produce a neurotoxin in the intestine.

Yeast and Mold

Aerobes
Recently, it has been recognized that oral infection, especially periodontitis, may affect the course and pathogenesis of a number of systemic diseases, such as cardiovascular disease, bacterial pneumonia, diabetes mellitus, and low birth weight (*Clin. Microbiol. Rev. October 2000 vol. 13 no. 4 547-558*)

Gum infections, peridontal diseases may also lead to subdural Empyma (dark area between brain and skull): Staphylococcus - 17%; Anaerobes - 35%, Streptococci 12% and facultative gram negative bacteria are also seen (Pubmed)

Oral infection by *Staphylococcus aureus* in patients affected by White Sponge Nevus (Pubmed)

Recalls
*Related to Pseudomonas aeruginosa and* Burkholderia infections caused by contaminated mouth swabs.
Yeast

- *Candida albicans* and *parapsilosis* account for 80-90% of infections

- Other species responsible for oral infections have also been identified including *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dublieniensis*, *C. tropicalis*, *C. kefyr* and *C. guilliermondii*, *C. inconspicua*, *C. lusitaniae*, *C. norvegensis* and *C. rugosa*

Fungal

- Noncandidal oral infections include aspergillosis, cryptococcosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, and zygomycosis (mucormycosis). These could be opportunistic

- Although these noncandidal fungal infections are considerably less common than oral candidiasis, they commonly produce subclinical infection, especially pulmonary infections
Oromucosal Infections - Anaerobes

Obligate and Facultative Anaerobes

Orofacial and Odontogenic infections also include *Staphylococci*, *Streptococci* and anaerobes such as *Bacteriodes, Fusobacterium, Peptostreptococcus* and *Actinomycetes*. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* appear to be particularly likely to cause aggressive periodontal disease.

Other bacteria associated with periodontal disease are *Treponema denticola, T. socranskii*, and *P. intermedia*.

For Vincent’s Angina, *Fusobacterium* is the key pathogen. Ludwig’s angina or neck space infections are caused by dental infections, tonsillar infections.
Cutaneous Infections-Bacterial

Aerobes

Most bacterial infections of the skin are caused by *Staphylococcus aureus*, *Pseudomonas* and a form of *Streptococcus*

Biofilm

Biofilm formation by *Pseudomonas aeruginosa* has been implicated in the pathology of chronic wounds (Antimicrob Agents Chemother. 2013 Jan 14)

These biofilms can be single or multiple species (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* sp. And *Micrococcus* sp.)
Cutaneous Infections - Anaerobic Bacteria

Anaerobes
Skin and soft tissue infections can occur due to anaerobes such as:
• *Clostridium*
• *Bacteroides fragilis* and other *Bacteroides*
• *Peptostreptococcus*
Often such infections involve multiple microorganism both aerobic as well as anaerobic bacteria

Fungi
Dermatophytes are fungi that cause skin, hair, and nail infections. Infections caused by these fungi are also sometimes known as "ringworm" or "tinea."
These anamorphic (asexual or imperfect fungi) genera are: *Microsporum, Epidermophyton* and *Trichophyton*

Yeast
In humans, *C. neoformans* may cause wound or cutaneous cryptococcosis
Scytalidium causes Tinea pedis like infection
Geotrichum candidum causes Tinea barbae like infection

Other fungi that cause superficial infections include:

- *Exophiala werneckii* (*Tinea nigra*) - black spots
- *Malassezia furfur* (dimorphic)
- *Pityriasis versicolor*
- *Piedraia hortae* (*black nodules on scalp and hair*)

Subcutaneous fungal infections

- *Sporothrix schenckii* (*Dimorphic*)
- *Cladosporium sp.*
- *Fossecaea sp. Phialophora sp.*
- *Madurella sp.*
- *Pseudallescheria sp.*
Nasal

Aerobes
Chronic sinusitis is cased by *Haemophilus*, *S. aureus*, pneumococci and even enterobacteriaceae
*Moraxella catarrhalis* often isolated from sinusitis

Anaerobes
Anaerobes such as *Peptostreptococcus*, *Bacteriodes*, *Fusobacterium*, *Eubacterium* and *Actinomyces* also are causative microorganisms

Fungi
Mucor species are among the fungi causing infections referred to as *Zygomycosis* and may cause many infections including pulmonary infections

*Conidiobolus* spp. is involved in subcutaneous infection involving nasal cavity causing paranasal cavity infections. This may be acquired via inhalation of spores

*Bipolaris* is one of the causative agents of phaeohyphomycosis causing chronic invasive sinusitis and may lead to like other organisms to bronchopulmonary disease

*Fonsecaea* is the etiological agent of nasal chromoblastomycosis

There are more fungal genera isolated from nasal infections
Nasal Infections

Yeast and Mold

- It is important to note that Aspergillosis has become a major cause of pulmonary infections of immune compromised patients along with Pneumocystis cariini which is a yeast

- Nasal infections may lead to many other invasive infections such as ear, pulmonary, and even systemic infections

- Pneumonia - necrotizing, bronchopneumonia in immunocompromised, cystic fibrosis patients is rapidly fatal
Inhalation-Bacterial

Aerobes

*Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Staphylococcus aureus* often colonize the nasopharynx.

9 Emerg Infect Dis. 2008 October; 14(10): 1584-1591

*Haemophilus influenzae* has been very frequent followed by *Klebsiella pneumoniae* *Streptococcus pneumoniae, Staphylococcus aureus* and *Streptococcus*.

*Mycoplasma pneumoniae, Legionella pneumophila* and *Bordetella pertussis* though rarer have been isolated from respiratory tract infections.

*S. aureus* colonizes the upper airways in about 20% of healthy persons - causes serious sinus or nasal disease due to Wegener’s granulomatosis develop secondary infections, in which S. aureus is predominantly involved.
Inhalation-Fungal

Some of the fungi related to upper and lower respiratory tract infections include:

- *Candida pneumonia*
- *Cryptococcus neoformans*
- *Blastocchizomyces*
- *Aspergillus*
- *Coccidioides*
- *Mucor*
- *Stachybotrys*
- *Paracoccioides*
- *Cunninghamelaa*
- *Rhizopus*
- *Sporothrix*
- *Histoplasma*
- *Paecilomyces*
- *Emmonsia*
- *Bipolaris*
- *Pseudoallescheria*
- *Fusarium*
- *Geotricum*
- *Scedosporium*
- *Aureobasidium*
- *Rhizomucor*
Anaerobes in respiratory tract infections

Pleuropulmonary Infections (aspiration pneumonia, lung abscess, empyema, etc) can be caused by anaerobes such as:

- *Bacteroides oris*
- *B. buccae*
- *B. oralis*
- *B. ureolyticus group*
- *B. fragilis*
- *Fusobacterium*
- *Peptostreptococcus sp.*
- *Streptococcus viridans*
The most common bacteria that cause ear infections include:

- *Haemophilus influenzae* (which is associated with respiratory infections in children)
- *Streptococcus pneumoniae* (also called pneumococcus)
- *Moraxella (Branhamella) catarrhalis*
- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*

*P. aeruginosa* can cause infections in the external ear canal—so-called "swimmer's ear"—that usually disappear without treatment. The bacterium can cause a more serious ear infection in elderly patients, possibly leading to hearing problems, facial paralysis, or even death.

Biofilms are hypothesised to cause chronic suppurative otitis media, and to explain the condition’s resistance to antibiotic treatment.
Ear Infections-Anaerobes

Anaerobic bacteria, *Peptostreptococcus intermedius* and *Propionibacterium acnes*, were found in mixed culture specimens from four to ten tested cases of chronic secretory otitis media.

Common fungi related to Otitis Externa

- *Aspergillus niger* (80 to 90% of cases) with black exudate
- *Candida albicans* (second most common cause) with cheesy white exudate
- *Actinomyces*
- *Trichophyton*

Mycotic otitis media can also be caused by many fungal species such as:

- *Bipolaris*
- *Paecilomyces*
- *A. flavus*
- *Wangiella*
Bacteria that dominate the vaginal flora during infection include *Gardnerella vaginalis* or *Mobiluncus*, although other bacteria, such as *Escherichia coli* from the rectum have also been shown to cause the disease.

Menstrual toxic shock syndrome (TSS) is a serious illness that afflicts women of pre-menopausal age worldwide, and arises from vaginal infection by *Staphylococcus aureus* *Streptococcus agalactiae*, a Group B streptococcus, in pregnant women.

Anaerobes that have been isolated from vaginal infections include:

- *Bacteroides*
- *Peptostreptococcus*
- *Candida albicans* is the main causative agent of vaginitis/vaginosis
- *Candida kefyr* and *Candida glabrata*
- *Sachharomyces* is an emerging causative agent of opportunistic mycosis
Eye Infections

• Bacterial conjunctivitis is an infection caused by bacteria, such as Staphylococci, Streptococci or Haemophilus

• A particular feature of bacterial keratitis is its rapid progression; corneal destruction may be complete in 24-48 hours with some of the more virulent bacteria

• *Pseudomonas* is one of the most common bacteria causing ulcerative keratitis in persons wearing contact lenses

• Other Gram negatives such as Proteus have also been isolated from eye infections

• Some highly virulent bacteria; *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Corynebacterium diphtheriae* can invade an intact corneal epithelium
Eye Infections

Anaerobes

• Most common anaerobe is *Peptostreptococcus* spp., isolated alone or mixed with other bacteria

• Other anaerobes are *Bacteroides fragilis*, pigmented *Prevotella* and *Porphyromonas*, fusobacteria, and bifidobacteria
Penicillium species has been isolated from patients with keratitis. Keratitis or corneal ulcers have been attributed to other fungi such as:

- *Curvularia*
- *Exserohilium*
- *Lasiodiplodia*
- *Paeciliomyces*
- *Alternaria*
- *Fonseceae*
- *Fusarium*
- *Scopulariopsis*
- *Pseudallescheria*
- *Bipolaris*
- *Phialophora*
- *Trichosporon*
- *Wangiella and more*
Guidances and Standards

There is no clear guidance for:


• Maintenance of a non-sterile facility-Does ISO 14644 need to be followed and to what extent?

• SAL requirement for gowns-When and where to use sterile and non-sterile gown?

• Cleaning validation/verification-To what extent, depends on risk to product

• Water quality and testing-quality and limits
Guidances and Standards

(1115) BIOBURDEN CONTROL OF NONSTERILE DRUG SUBSTANCES AND PRODUCTS

“Microbial content in nonsterile products is controlled to a level consistent with patient safety. Use of excessive controls that would add complexity or cost without a commensurate safety benefit is not advantageous in terms of added value to either the patient or the manufacturer.

A critical consideration in ensuring product quality is to prevent conditions within the manufacturing facility or manufacturing process that favor the proliferation or ingress of microorganisms. Microbial growth in excipients, components, and drug substances is a concern because it creates the possibility that viable microbial content could reach unacceptable levels.

What is Excessive?
What level of control?
# Microbial Contamination Risk Assessment

<table>
<thead>
<tr>
<th>Dosage Form</th>
<th>Water Activity</th>
<th>Single or Multiple Use</th>
<th>Potential support of Microbial growth</th>
<th>Invasive of the Route of Administration</th>
<th>Potential for patient Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenteral products</td>
<td>0.99</td>
<td>Pre-filled-single Vials-multiple</td>
<td>Moderate to High</td>
<td>Extremely High</td>
<td>Low due to process</td>
</tr>
<tr>
<td>Ophthalmic products</td>
<td>Liquids, creams, ointments 0.97 to 0.55</td>
<td>Single-if form filled sealed Contact lens solutions-multiple</td>
<td>Moderate to High</td>
<td>High</td>
<td>Low if controls are good</td>
</tr>
<tr>
<td>Inhalation Solutions</td>
<td>0.99</td>
<td>Multiple</td>
<td>High</td>
<td>High</td>
<td>High due to mode of administration</td>
</tr>
<tr>
<td>Aerosol Inhalants</td>
<td>0.25</td>
<td>Multiple</td>
<td>None</td>
<td>High</td>
<td>Moderate due to water activity</td>
</tr>
<tr>
<td>Nasal Sprays</td>
<td>0.99</td>
<td>Multiple</td>
<td>Moderate to High</td>
<td>Moderate</td>
<td>Moderate to low</td>
</tr>
<tr>
<td>Vaginal Suppositories</td>
<td>0.30</td>
<td>Single</td>
<td>Low</td>
<td>Moderate to low</td>
<td>Moderate to low</td>
</tr>
<tr>
<td>Topicals</td>
<td>Lotions, creams and ointments 0.97-0.55</td>
<td>Multiple</td>
<td>Low to Moderate</td>
<td>Low to moderate</td>
<td>Moderate to low</td>
</tr>
</tbody>
</table>
# Microbial Contamination Risk Assessment

<table>
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<th>Potential for patient infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Liquids Aqueous</td>
<td>0.90</td>
<td>Multiple</td>
<td>Moderate to High</td>
<td>Moderate to High</td>
<td>Moderate to High due to water</td>
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<tr>
<td>Oral Liquids Non-Aqueous</td>
<td>0.60</td>
<td>Multiple</td>
<td>Low</td>
<td>Low</td>
<td>Low - due to water activity</td>
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<tr>
<td>Rectal Suppositories</td>
<td>0.30</td>
<td>Single</td>
<td>Very low</td>
<td>Very low</td>
<td>Very low Most organism are not objectionable</td>
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<tr>
<td>Liquid-filled capsules</td>
<td>0.30</td>
<td>Single</td>
<td>Very low</td>
<td>Very low</td>
<td>Low</td>
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<td>Compressed tablets</td>
<td>0.36</td>
<td>Single</td>
<td>Very low</td>
<td>Very low</td>
<td>Very low</td>
</tr>
<tr>
<td>Powder filled capsules</td>
<td>0.35</td>
<td>Single</td>
<td>Very low</td>
<td>Very low</td>
<td>Very low</td>
</tr>
</tbody>
</table>
Sources of microbial contamination

Contamination may originate from:

- Raw materials, and excipients used in manufacture of reagents and product
- Cell banks used in the manufacture of immunodiagnostic or other bulk products
- Components and containers used for manufacture of product due to improper cleaning and inadequate sterilization to reduce microbial load
- Equipment used in the manufacturing process due to improper cleaning or sterilization for microbial reduction
- Water used for making of reagents and cleaning
- Compressed air and compressed gases used directly in contact with product
Sources of microbial contamination

Contamination may originate from:

- Manufacturing environment and surrounding areas
- Due to inadequate protective gear during manufacturing
- Personnel and Material flows
- Inadequate cleaning and disinfection
- Inadequate cleaning and sanitization of manufacturing facility and equipment surfaces
- Inadequate microbiology testing laboratory design and cleaning
Type of contamination

• Cross contamination (of a product/material with another product/material)
• Cross contamination of product with cell lines when equipment is shared
• Non-microbial particulate contamination (non-viable particles)
• Biological/microbiological contamination (viable particles/microorganisms)
• Process microorganisms in environment
• Objectionable organisms (microorganism that may affect the efficacy of product over its shelf life)
Factors Influencing Microbial Contamination

To achieve appropriate microbial control in the relevant non-sterile manufacturing process the following factors should be scrutinized:

• Microbial quality of raw materials
• Purity of master cell banks and working cell banks as starting materials
• Validated process to ensure consistency in bioburden levels in microbiologically controlled product
• Methods to maintain low level of microbial contamination in in-process materials through process control such as:
  o In-process filtration for bioburden reduction
  o Microbial load reduction in containers/closures using sterilization methods
• In-process bioburden monitoring
• Assessing bioburden during equipment cleaning: manual cleaning; cleaning equipment out of place; and clean in place (CIP) of stationary equipment
Factors influencing Microbial Contamination

- Bioburden reduction of stationary equipment using Steam in Place (SIP)
- Assessing risk of bulk product hold before filling
- Air quality requirements pertaining to each unit operations
- Certification, testing and maintenance of HVAC systems
- Personnel flows to minimize bioburden in product
- Material flows to avoid contamination
- Monitoring air quality and bioburden in rooms where each unit operation is performed
- Qualification and maintenance of product contact gases and air systems
- Monitoring of product contact gas and air systems
- Disinfection and cleaning of production areas
- Qualification of systems used to generate water used for cleaning of parts and equipment and use in the manufacturing process
- Gowning requirements to reduce bioburden in room and product
Factors influencing Microbial Contamination

- Microbiology laboratory facility requirements to reduce contamination of product and laboratory errors
- Test methods used for monitoring bioburden in excipients and product
- Quality control of incoming media and buffers used for bioburden testing
- Assessing out of specification results occurring during microbiological testing
- Training requirements for Microbiology laboratory personnel
- Microbiological stability of product over its shelf life
- Trend analysis to assess microbiological quality of product
Facility
Determining an appropriate room classification/control should be dependent upon the types of actions to be performed in the room.

The differential pressures between rooms should be balanced to designated levels and to ensure that airflow is moving in the desired directions.

Case Study: OSD facility shuts down HVAC every night.
Factors influencing Microbial Contamination

Certification of HVAC System
HVAC may not be certified per ISO Standard 14644 standards, however, the HVAC system should be maintained to yield relevant air quality required for each processing area per design and operational classification.

Maintenance
HVAC systems providing HEPA filtered air to controlled environments should be maintained and tested on a regular interval to confirm proper operation and control.

This interval should be based on environmental trends; may include differential pressure, temperature, humidity, and non-viable and/or viable particulates.

Facilities processing powders may need to examine ducts.
Personnel Flow

Personnel flow within the facility should be designed to minimize microbial contamination of process/product and cross contamination when multiple products are manufactured in the same facility.

A personnel flow plan should include the following aspects:

- Gowning
- Access restriction
- Sequence of activities
- Spatial separation of activities

In critical rooms, gowning and degowning activities for controlled rooms should be kept separate to avoid cross contamination (where cell banks are made).

For other less controlled areas, this may be accomplished spatially by use of separate entrance and egress airlocks or procedurally by restricting activities to only gowning or degowning at a time within the same airlock.
Material Flow

Materials flow should be designed to avoid contamination of process and product by controlling the movement of such materials into the controlled area.

Movement into and controlled areas

Materials entering controlled areas should be cleaned and sanitized in an airlock prior to introduction to the area. These “wipe down” activities must be kept separate from gowning activities either spatially or procedurally as described above. “Clean” and “Dirty” areas within the airlock should be clearly designated and used for staging of materials pre and post wipe down.

Staging and Storage

Staging and storage areas should be designated to allow clear segregation of materials to avoid cross contamination between materials and to protect materials from the environment or other contaminants.
Waste Path

The movement of waste within and out of controlled areas should be designed to prevent contamination of:

- Environment
- Incoming materials
- Process
- Personnel

Physical separation must be maintained between waste materials and the manufacturing environment

Staging of waste materials should be restricted to the area of waste generation or dedicated holding area isolated from in process material

The length of time waste material is held within controlled areas should be limited
Monitoring

During nonsterile product manufacturing, the air sampling methods used for environmental monitoring may be active or passive.

Personnel monitoring typically is not required in nonsterile product manufacturing except when near-aseptic gowning materials are employed, especially when cells banks are manufactured.

Sampling locations should be selected based on risk evaluation followed by a general hygienic survey of the environment.

There are no standardized environmental sampling methods and because monitoring is intended as a qualitative evaluation of general facility hygiene, product contamination risk should be considered during developing an EM program.
Sampling

The frequency of monitoring should reflect the potential risk associated with the dosage form.

Additionally, some products may have innate antimicrobial activity because of their attributes such as low water activity or inclusion of an antimicrobial preservative or an active ingredient that is itself an antimicrobial agent.

Products that are resistant to microbial growth or have microbiocidal or microbiostatic characteristics require little or no microbiological monitoring.

In general, environments for tablet and powder- and liquid-filled capsule manufacturing should require no monitoring or infrequent monitoring.

Monitoring programs should be risk based, and the frequency and number of sampling sites should reflect the risk level.
Monitoring

Manufacturing areas for higher-risk dosage forms such as inhalant products require more frequent monitoring and typically are manufactured in rooms classified to a particulate air quality level.

Contaminated nasal and inhalant products have greater clinical implications.

Special attention should be paid to objectionable microorganisms recovered, especially mold.
<table>
<thead>
<tr>
<th>ISO Classification Number (N)</th>
<th>Maximum allowable concentrations (particles / m³) for particles equal to and greater than the considered sizes shown below</th>
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<td>ISO Class 8</td>
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<td>ISO Class 9</td>
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Table from ISO 14644-1:1999
Cells in Yellow Reflect the omitted values in 2014 Version
Action Levels

EU GMP Annex 1- Basic Elements Clean Room Classification

<table>
<thead>
<tr>
<th>Grade</th>
<th>At rest</th>
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<th>In operation</th>
<th></th>
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<td>0.5 μm</td>
<td>5.0μm</td>
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<tr>
<td>A</td>
<td>3 520</td>
<td>20</td>
<td>3 520</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>3 520</td>
<td>29</td>
<td>3 520 000</td>
<td>2 900</td>
</tr>
<tr>
<td>C</td>
<td>352 000</td>
<td>2 900</td>
<td>3 520 000</td>
<td>29 000</td>
</tr>
<tr>
<td>D</td>
<td>3 520 000</td>
<td>29 000</td>
<td>Not defined</td>
<td>Not defined</td>
</tr>
</tbody>
</table>

**Recommended limits for microbial contamination (a)**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Air sample cfu/m^3</th>
<th>Settle plates (diameter 90 mm) cfu/4 hours (b)</th>
<th>Contact plates (diameter 55 mm) cfu/plate</th>
<th>Glove print 5 fingers cfu/glove</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>
Microbial Identification

Gram reaction, and simple diagnostic testing are sufficient to understand baseline flora in the facility

It is beneficial to characterize when some organisms are recurrent and are predominant

If predominant organisms are objectionable, they may pose risk to product

Knowing the type of bioburden to some extent help in improving upon cleaning and disinfection procedures

Microorganisms that may cause spoilage of product over its shelf life should be identified

Organisms objectionable to the product can be established by trending microbiology related product failures, recalls, and returned product
Added Gases

Process gas vendors should be qualified for quality control, however all product contact process gases and product compressed air should be tested once a quarter at a minimum.

Depending upon the risk, gases may be tested for non-viable, and viable particles, moisture and hydrocarbon content.

Note: Classes mentioned in ISO 8573 are not the same as ISO 14644.
Contamination Control

Critical factors for the prevention of microbiological contamination during nonsterile product manufacturing are the control of the microbiological quality of ingredients and water, along with the development of proper cleaning and sanitization procedures.

Cleaning and disinfection program should consider:

- Physical removal of powders and debris
- Removal of bioburden brought in through raw materials and personnel

Microbiological monitoring does not mitigate risk, but it may serve as added assurance.
Disinfectant Facts

**Phenolics**
Do not kill spores, fungal or bacterial, bactericidal and virucidal claims, leaves residue. Examples: Vesphene, LpHse

**QUATS**
Do not kill spores, fungal or bacterial, bactericidal and virucidal claims, leaves residue. Example: Intercept

**Bleach**
Kills spores, very unstable, available chlorine may vary depending upon time on shelf

**H202**
3% kills vegetative bacteria, greater than 6% sporicidal, safe enough to use for food industry
Mixture of peracetic acid and H2O2 forms a potent sporicidal agent

Formaldehyde and Gluteraldehyde potent sporicides, highly carcinogenic

Sanitizer, 30% water required to seep through cell wall barrier and disrupt cell contents, some bacteria may use it as carbon source (pseudomonads)
Material Compatibility

Cellulose and Bleach interaction
Cellulose and H$_2$O$_2$ interaction

- Bleach (sodium hypochlorite) definitely reacts with cellulose
- It was the first treatment used to bleach wood and paper to make it turn white
- The problem with this is the efficacy against organisms will be used quickly and lost, bleach with a higher pH will react slower but also takes longer to kill microbes
- This happens with H$_2$O$_2$ as well, but with the addition of peracetic acid the ability to power through organics remains high
Material Compatibility

Bleach and QAC interaction - Chemical Reactions from Mixing Bleach and Ammonia

• Mixing bleach and ammonia is extremely dangerous, since toxic vapors will be produced

• The primary toxic chemical formed by the reaction is chloramine vapor, with a potential for hydrazine formation
**Microbial Quality of Water**

Water quality such as de-ionized water, purified, water, and water for injection may be used as excipients, for cleaning equipment, facilities per product requirements. Other water types can be generated by using different systems. Specifications can be set per guidance provided in USP or EP or using routine monitoring trends. Specifications should consider the application and use of the water types for non-sterile low bioburden products.

**Potable Water**

- May be used for initial wash or dirty equipment
- May be used for making media if media is sterilized
- De-ionized water (DI)
- May be used for: making media
- as makeup purified water or WFI
- facility cleaning and for final rinse of equipment which is subsequently sterilized
Microbial Quality of Water

Purified water

• Used for as an excipient in the production of non-sterile products
• For cleaning of parts and equipment
• As source water for SIP and Clean steam
• For making media
• Use in Autoclave
• For CIP

Water for Injection

• May be used for cleaning of equipment and for diluting disinfectants used in most critical areas
• May be used to generate pure steam
Microbial Quality of Water

Distilled water
This water is produced by vaporizing liquid water and condensing it in a purer state. Should be used primarily as a solvent for reagent preparation, rinsing of analytes as calibration standard or analytical blank and for test apparatus cleaning.

De-ionized water
This water is produced by ion-exchange process. Water meeting the requirements of Purified water that is derived by other means of purification could be equally suitable where De-ionized water is specified.
Microbial Quality of Water

Qualification and Maintenance of Water Systems

- Initially water systems should be qualified during
- Length of qualification should include scientific rationale and risk to product
- Requalification should be considered when excessive excursions are observed in bioburden, conductivity and TOC
- System should be checked for biofilm formation
- Sanitizing should be routinely performed and the system components should be maintained
Gowning

- Gowns used should be evaluated for the operation
- When choosing laundered gowns, minimally pore size, fray rate and the number of laundering cycles gowns can withstand should be considered
- Laundered gowns should be sized correctly for the personnel, as large gowns can cause a bellowing effect introducing skin particles into the environment and tight fitting gowns may be prone to tearing during operations
- For single use disposable gowns pore size, and particle generation should be evaluated
- Gown change should reflect risk
- Sterile gowns are not necessary, however managing gowns is necessary
Intrinsic factors that contaminate raw materials should be considered when establishing microbial specification and testing requirements, e.g. moisture content, pH and acidity, biological structure, naturally occurring and added antimicrobials, types of packaging/atmospheres, storage/holding conditions, product history and traditional use.

Note: USP may not have monographs for some raw materials that can promote microbial growth. It is up to the company to perform risk assessment.
## Raw Materials

<table>
<thead>
<tr>
<th>Raw Material</th>
<th>Microbial Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic</strong></td>
<td>Capable of promoting microbial growth. Tested at adequate frequency for bioburden assessment. Stored under conditions that restrict microbial proliferation.</td>
</tr>
<tr>
<td><strong>Inorganic</strong></td>
<td>May be accepted per vendor C of A. Vendor to supply raw material of consistent microbiological quality. Microbial load documented on C of A for each lot. (some inorganic materials may promote growth such as mold)</td>
</tr>
<tr>
<td><strong>Inert materials</strong></td>
<td>May be accepted per vendor C of A with no testing (inert materials do not promote microbial growth)</td>
</tr>
</tbody>
</table>
Prospective validation is also used for microbiologically controlled and for microbiologically uncontrolled products because of the limitations of statistical sampling.

Retrospective validation is the examination and evaluation of historical data for the process and the product.

Retrospective validation is not used to justify a bad system or a bad product.

It is intended to be used to examine a system objectively and determine whether it is acceptable, whether changes need to be made, or whether the entire process needs to be replaced.
In-Process Microbial Control

In-process filtration

In process filtration should be performed where there is a chance of microbial contamination and proliferation during process steps. Examples of such process conditions include open processing, processing in an uncontrolled environment, lengthy process or hold steps and elevated temperatures. Filtration should be performed to remove particulates both viable and non-viable, in order to reduce microbial load.

Filter Types
Filter type and pore size should be chosen to be most compatible with the process step. For final filtration prior to filling or any process step where significant microbial reduction is essential, a 0.2 µ membrane filter should be used. In applications where there is a large particulate (viable or non-viable) load, a larger pore size pre-filter may be used to extend the life of the 0.2 µ terminal filter.
In-Process Microbial Control

Filter Integrity Testing
At a minimum, filters used in the final filtration step, must tested for integrity post-use

In other in process steps a risk assessment should be performed to determine the need for integrity testing
In-Process Microbial Control

Container Closures
- Minimally container closures must be washed using a validated wash cycle or a validated automated washer
- Microbial load reduction for Container closures may be accomplished using a validated sterilization cycle
- The cleaning process for container closures may be used as the sole bioburden reduction step if supported by appropriate validation

Cleaning
- De-ionized water or water of better quality should be used for the final rinse
- Final rinse water should be tested for bioburden at an adequate frequency
In-Process Microbial Control

Clean hold times for container closures
Water type used: Storage conditions of cleaned container closures should be defined and controlled and the maximum storage time should be determined during validation

• CIP cycles should be validated for microbial load reduction

• Validation testing should include bioburden to measure the microbial load and removal of product and bioburden

Dirty and Clean Hold Times

Dirty and Clean Hold Time studies should be performed and tested as part of cleaning validation to define worst case cleaning conditions, no matter method of cleaning is used
Equipment Cleaning

Equipment shared by multiple products, must have cleaning validated and routine testing for a multiproduct environment to ensure there is no cross contamination

Manual Cleaning or COP

De-ionized water or water of better quality should be used for the final rinse
Bioburden Reduction

• Media tolerant to steam sterilization may be SIPed for bioburden reduction as an alternative to sterile filtration when required

• All SIP processes should be validated

• Validation should include temperature mapping of vessels and lines within and up to the sterile boundary

• This applies to SIP cycles performed for bioburden reduction as well
Bulk Product Hold Time

Hold Time Conditions

• If the non-sterile purified bulk is held for a long period of time, it should be frozen
• The length of time the bulk is held unfrozen should be evaluated for microbial proliferation
• This also applies to Oral Dosage Forms

Validation

• A validation study should be performed to determine the maximum hold time at chosen conditions evaluating product stability and microbial quality
Final Product

- Filling operations should be performed in an ISO 7 or better environment if the bioburden needs to be tightly controlled
- ISO 8 environment or equivalent environment may suffice if the product has low water activity

Validation of Filling Process
- The filling process should be validated to ensure repeatability of the process in terms of microbial control and consistency in final product bioburden

Final Product Testing
- Non-sterile products do not require media fills
- Bioburden and other tests such as AET should be validated

Note: At times these could be contamination with objectionable organisms which are not used in AET testing
Common Microbiology Laboratory Errors

Cold rooms a prominent source of contamination
  • No defined frequency for cleaning
  • Cardboard boxes placed in the moist environment
  • Opened media packets

Refrigerators another source of contamination
  • New and media with growth stored in the same refrigerator
  • Drain pans rarely cleaned
  • Growth promoting materials kept in refrigerator
  • Tubing full of biofilms
  • Only manufacturing cleaning instructions followed
  • You should not have to walk down the hallway to get your cell line
Common Microbiology Laboratory Errors

In Microbiology Labs Incubators are a major source of contamination

• Inadequate cleaning
• Humidity is high
• Water in the pans does not contain antimicrobial
• Nothing stops contamination from proliferating RH injection
• HEPA Filters not maintained
• HEPA filters not checked
• HEPA filters along with humidity become a source of contamination
• Proximity to other heat generating equipmet
• Placed in high traffic areas
Common Microbiology Laboratory Errors

• Media often placed in water baths for stabilizing temperature
• Contaminated water baths will contaminate flasks and tubes
• Poured media could be contaminated and often contamination not detected if sterility check is not performed
• Microbiological contamination is not evenly distributed hence the portion of the medium or negative control may not show contamination while part of the batch of medium may be contaminated
• Water baths or notorious for biofilms
• If water baths are not drained after use and dried (including the tubing) contamination may not be removed
• Disinfection of water baths should be adequate
Common Microbiology Laboratory Errors

- Janitorial staff used for cleaning labs.
- Disinfectants not known
- String mops, cotton or otherwise
- Sponge mops used very long
- Hoods cleaned with IPA only
- Cleaning under the grill uncommon routine practice
- Alcohol wipe packets can be a source of contamination
- Lack of expiry for laboratory cleaning agents
- Lack of active agent due to long hold times
Common Microbiology Laboratory Errors

- The pH of each batch of medium should be confirmed
- Refrigerated purchased media should be allowed to warm up to ambient room temperature before use
- In-house or purchased media should be checked by appropriate inspection of plates and tubes for the following:
  - Cracked containers or lids
  - Unequal filling of containers
  - Dehydration resulting in cracks or dimpled surfaces on solid medium
  - Hemolysis
  - Excessive darkening or color change
  - Crystal formation from possible freezing
  - Excessive number of bubbles
  - Microbial contamination
  - Status of redox indicators (if appropriate)
  - Lot number and expiration date checked and recorded
  - Sterility of the media
  - Cleanliness of plates (lid should not stick to dish)
Common Microbiology Laboratory Errors

Maintenance of Cultures

- Standardizing the handling and storage of cultures to minimize contamination
- Cultures for use in compendial tests should be acquired from a national culture collection or a qualified secondary supplier
- Preparation and resuscitation of cultures should follow the instructions of the supplier or a validated, established method
- The number of transfers of working control cultures should be tracked to prevent excessive sub-culturing that increases the risk of phenotypic alteration or mutation
- When cryo-preserving in house isolates a quality program should be established to test the viability and purity of cultures
- Expiry dating is critical
Common Microbiology Laboratory Errors

- Are media accepted per C of A?
- How are in-house isolates used?
- How are in-house isolates decided?
- How are in-house isolates made into stock cultures?
- How are passes calculated?
- Have objectionable organisms been identified? How?
- What are the QC procedures and thawing procedures for stock cultures?
- How long are working cultures maintained?
- How are the lots to be growth promoted decided?
Laminar Air Flow Hoods and Biological Safety Cabinets

• Should be used for critical testing where aseptic technique is required
• These hoods should be certified at a minimum once a year to establish filter efficacy and leak test
• Biological Safety Cabinets (BSC) are LAF hoods that provide product containment for personnel protection as well
• BSC should not be installed close to a room entrance and should have a minimum of twelve inch clearance
• Qualification and maintenance of BSCs and LAFs should follow direct Controlled room requirements
• Major errors due to
  o Location
  o Cleaning in and around
  o Traffic around and of course flawed aseptic techniques
Errors in Gram Staining

Fixation with excessive heat alters cell morphology and makes organisms more susceptible to over-decolorization.

Low concentrations of crystal violet make gram-positive organisms more susceptible to over decolorization.

Insufficient exposure to iodine and lack of available iodine can prevent crystal violet from bonding firmly with the cell wall, thus making gram-positive organisms more susceptible to over decolorization.
Errors in Gram Staining

Prolonged decolorization, especially with acetone, can cause gram-positive bacteria to appear gram-negative.

Insufficient decolorization can make gram-negative organisms falsely appear gram positive.

Insufficient counterstaining can fail to stain gram-negative bacteria and background material.
Errors in Gram Staining

Excessive counterstaining will leach the crystal violet-iodine complex from gram positive bacteria and stain them with safranin, thus making them falsely appear gram-negative.

Prolonged washing between any of the steps can cause over-decolorization.
Personnel and Training

- Classification
- Clinical importance as related to product
- Colony Morphology
- Staining-gram, acid fast, spore, mold
- Biochemical tests and ancillary tests
- Confirmed identification vs presumptive identification
- Semi-automated and automated technologies
- Similarity index and confidence level
Why an ID needs to be correct?

- Problems concerning the potential misidentification of bacteria when commercial identification systems are used
- Problems due to lack of expertise to review and validate IDs
- However, this concern is only the tip of an iceberg of a potentially larger problem with more important ramifications?
Why an ID needs to be correct?

The reviewer may:

- Accept the identification at face value not understanding the morphology vs. identification
- May not have the know how if the organisms may be present in the environment of the ID is erroneous
- May not understand the clinical importance of the organism

Example: Trichopyton rubrum
Neisseria gonorrhea
Haemophilus
Obligate anaerobe when the plates were incubated aerobically
Thank You!