The goal of this white paper is to increase basic microbiology and aseptic technique knowledge and comprehension, explain common microbiological terminology, explain the role of cleaning and sanitizing in preventing product contamination, and to explain how disinfectants work and the most common mistakes made when using disinfectants.

**SOME BASIC MICROBIOLOGY TERMINOLOGY**

**TRANSIENT FLORA VS. RESIDENT FLORA**

**TRANSIENT FLORA**

Transient flora are microorganisms that are acquired through interaction (contact) with people and the environment. Transient flora can be removed by proper sanitization.

**RESIDENT (NORMAL) FLORA**

Resident (normal) flora are microorganisms that are normally found living and multiplying in or on the human body. Their existence and maintenance in and on our body is an important factor in resisting colonization by transient flora. Resident flora is much more difficult to remove and reduce in large numbers. Even if resident flora is reduced, in a relatively short amount of time their numbers return to normal.

**ANTISEPTIC**

An antiseptic is a chemical that is used to reduce the number of viable transient and resident flora on human tissue. It should be relatively nontoxic and nonirritating, e.g., Purell. Other terms: antisepsis.

**DISINFECTANT (SANITIZER)**

A disinfectant is a chemical that is used to reduce the number of viable transient and resident microorganisms on inanimate objects. Disinfectants are generally harsher, and made of stronger concentrations of chemicals than antiseptics. They may actually contain the same chemicals found in an antiseptic, but at a higher concentration, e.g., bleach, Lysol, LpH and Vesphe. Other terms: disinfection, sanitization
TYPES OF DISINFECTANTS

There are many fast and effective disinfectants on the market. The active ingredients in disinfectants vary from product to product. It is important to know what is (are) the active ingredient(s) in the products you are using. It is important to obtain literature about products you are using, including information on the antimicrobial spectrum and killing time. It is important to know the shelf-life of your disinfectant and the proper use dilution. Microorganisms can develop resistancy to disinfectants over time, especially if they are not used as directed. Therefore, it is generally recommended to rotate disinfectants on a monthly basis.

It is difficult to say whether one active ingredient is better than another. The key point is that the product must work when used as directed and the company must prove (validate and document) that the product works.

CIDAL VS. STATIC (INHIBITION)

CIDAL

Some chemicals exert a cidal (killing) effect on microorganisms. This is obviously preferred.

STATIC (INHIBITORY EFFECT)

Some chemicals exert a static or inhibitory effect on microorganisms. This means the microorganism cannot multiply (divide) in the presence of the chemical.

WHAT ARE PARTICLES? HOW DO THEY GET INTO THE ENVIRONMENT?

Particles are microscopic contaminants that are released into the environment (including air) all the time. The magic size for particle contamination is 0.5 microns and larger. Most of these particles cannot be seen with the naked eye and are floating in the air, carried by drafts, movements, wind, heat and air conditioning from place to place. They may settle out by gravity.

The more particles in the air, the more microorganisms in the air. Microorganisms stick (adhere) to particles. The more particles in a cleanroom, the more likelyl a company's product will be contaminated by microorganisms.

Particles come from dust, dirt, shoes, clothing, hair, skin, the mouth, make-up, walking (running increases particles), smoking, eating, cardboard boxes, cloth, paper towels and many other sources.

SOME TYPES OF ACTIVE INGREDIENTS IN DISINFECTANTS

- Phenols
- Glutaraldehydes
- Chlorine compounds (bleach, chlorine dioxide)
- Alcohols (e.g., isopropyl alcohol)
- Quaternary ammonium compounds (benzalkonium chloride)

OTHER IMPORTANT TERMS

- Sterilization - is complete destruction of living organisms
- Residual effect - Some antiseptics and disinfectants leave a residual effect on tissues or surfaces. They continue to kill or inhibit microorganisms after they have been washed away or removed.
Your job is to limit the number of particles you bring into and release into an aseptic processing area. For example, the human body gives off 100,000 particles each minute while standing perfectly still. Normal walking gives off in excess of 10 million per minute.

The number of particles in an environment can be measured (counted) using an instrument called a particle counter.

**TYPES OF MICROORGANISMS**

**BACTERIA (GRAM POSITIVE AND GRAM NEGATIVE)**

The word "gram" refers to a staining technique used to classify all bacteria called the gram stain. Gram positive bacteria stain violet (purple). Gram negative bacteria stain red (pink). Whether a bacteria stains gram positive (purple) or gram negative (pink) depends on the chemical makeup of its cell wall. Gram positive bacteria have fewer lipids in the cell wall; gram negative bacterial have more lipids in the cell wall. Transient flora may be gram positive, gram negative, yeast, molds or viruses.

**GRAM POSITIVE BACTERIA**

Most gram positive bacteria are coccus shaped (round, spherical). Most of the resident bacteria on the skin are gram positive.

**GRAM NEGATIVE BACTERIA**

Most gram negative bacteria are rod shaped. Most of the resident flora in the intestines is gram negative.

**FUNGI**

**MOLDS**

Molds are not gram positive or gram negative. Molds can be distinguished from bacteria because they produce spores (various colors) and they produce hyphae ("fuzzy structures" - think of how mold grows on bread).

**YEAST**

Yeast are not bacteria, but they are microscopic. They are not gram positive or gram negative. Yeast can be distinguished from bacteria by microscopic examination - think of yeast used to make bread, or ones that cause yeast infections.
VIRUSES

Viruses are microorganisms that are intracellular parasites of humans, animals, plants, and bacteria. Viruses are not bacteria, molds, or yeast! People who are infected with viruses release viruses into the environment from their nose, mouth and/or other orifices of the body. Viruses can be killed by disinfectants. Some are more difficult to kill than others.

PRINCIPLES OF MICROBIAL DISINFECTION

HOW CHEMICALS KILL MICROORGANISMS

Chemicals (active ingredients) must penetrate into the microorganism and then react with (destroy) some essential part of the microorganism. Essential parts of bacteria are the cell wall, cell membrane, ribosome, nucleus, enzymes.

FACTORS INVOLVED IN KILLING MICROORGANISMS

+ Contact time - how long the chemical is allowed to have contact with the microorganism. Some are more difficult to kill than others - think of how mold grows on bread.
+ Number of bacterial present
+ Amount of water present
+ Amount of inorganic matter present - dirt, blood, mucous, etc.
+ Types of microorganism present - spore-formers, Pseudomonas, Tuberculosis and MRSA
+ Resistance - it must be noted that some organisms are notoriously more resistant to chemicals than others
+ Inactivation by anionic detergents - some detergents can inactivate the active ingredient(s) of a disinfectant

MOST COMMON MISTAKES MADE WHEN CLEANING AND SANITIZING

CROSS CONTAMINATION

Cross contamination is when microorganisms from one source (water, wipes) are spread to (contaminate) another place (site) because of faulty technique or disinfection.

THE MOST COMMON WAYS OF CROSS CONTAMINATING A SURFACE DURING CLEANING

• Using soiled or dirty wipes from one area to clean another area
• Not spraying gloves with 70% IPA frequently or not changing gloves frequently
• Water - only use water for injection (WFI) in preparation/processing and change the water frequently
• Mop heads - Always use new mop heads, and change mop heads frequently. Never use the same mop head you used to clean an outside floor and then use it in a cleanroom (that’s cross contamination)
REMOVING DIRT
If a surface contains a great deal of dirt or soil, it may need to be disinfected with several washings, or be washed first to remove dirt and then disinfected. If you don’t remove the dirt first, the disinfectant may not work.

DILUTION OF THE DISINFECTANT
Never over-dilute a disinfectant. The disinfectant must be prepared as directed on the label. The amount of disinfectant added and the amount of water used must be measured.

DISPENSING OF DISINFECTANT
Disinfectants should be properly dispensed. Try to avoid pouring the disinfectant directly onto the floor because it is hard to measure and/or quantify the amount being used. It is also difficult to be consistent when pouring out by hand. The mop head should be dipped into the solution until it is damp, then applied to the area being cleaned. Some mop buckets are mechanized and will consistently dispense a measured amount of disinfectant onto the mop head each time.

CONTACT TIME
For a disinfectant to kill microorganisms, it requires contact time. This is the time it takes for the chemical agent(s) to penetrate into the microorganisms and cause death. It takes longer periods (10-30 minutes is not unusual) of time to kill certain types of microorganisms — e.g., spore-formers, molds, Tuberculosis, some viruses.

Always follow the SOP, which should identify the contact time. Never put down a disinfectant and immediately wipe it back up because that doesn’t allow enough contact time to kill the microorganisms.

A very common mistake is to put on the disinfectant and then wash the floor with water to remove the disinfectant (unless directed to do this in the SOP). We recommend allowing the disinfectant to air dry on the floor (if possible) to allow maximum contact time.

LOSING CONSISTENCY
When cleaning multiple times due to abnormal circumstances, such as a room shutting down or part of a corrective action, it may seem redundant or even unnecessary to perform the full cleaning each time, especially if the room has not been used in between. However, it is imperative for each cleaning to be performed the exact way each time to ensure the best possible results for the area being cleaned. If certain steps are left out, it could result in a deviation, or worse.
A cleanroom environment is designed to have controlled temperature, controlled humidity, control of small particle contamination. A cleanroom may be a place where sterile products are made and exposed to the environment for a short period of time.

CLASSIFICATION OF CLEANROOMS

CLASS 100 (ISO CLASS 5)

+ Class 100 is the “cleanest” of the rooms. The number refers to the number of particles allowed (0.5 microns and larger) per cubic foot (or liter) of air.
+ All air coming into the room must pass through a HEPA (High Efficient Particle Air) filter. This removes most of the particles in the air coming into the room.
+ Particles that enter the room carried by people and objects must be controlled and kept to a minimum. That’s why you must “gown up” before entering a Class 100 environment.
+ Most cleanrooms are under positive pressure, which means the pressure inside the cleanroom is higher than the pressure outside the cleanroom. Therefore, when you open the door to the cleanroom, air flows out, helping to prevent particles and microorganisms from entering the cleanroom.
+ A Class 100 cleanroom can have a maximum of only 100 particles (0.5 microns and larger) per cubic foot of air.

CLASS 10K (10,000) ROOM

A Class 10K room can have a maximum of 10,000 particles (>0.5 microns and larger) per cubic foot of air.

CLASS 100K (100,000) ROOM

A Class 100K room can have a maximum of 100,000 particles (>0.5 microns and larger) per cubic foot of air.

GOWNING AND ASEPTIC PROCESSING

Before processing, a person must “gown up.” Always follow the SOP for gowing. Each person must be qualified in propoer gowing procedure.

Remember, gowing acts as a filter for particles that are released by the human body and the clothing we wear. Gowing garments include items such as booties to cover shoes, hair covering to cover the head, masks to cover the nose and mouth, gloves to cover the hands, and clean lab coats to cover the clothing.
HOW CONTAMINATION OF THE ASEPTIC PROCESSING ENVIRONMENT OCCURS

MICROBES IN THE ENVIRONMENT AND ON THE HUMAN BODY

Microorganisms are everywhere. The human body is covered with microbes. The clothes we wear and our shoes pick up and drop off microbes in the environment all day. It is important to remember that we are constantly releasing microbes and particles all the time, especially when a person is working in an aseptic processing environment.

SOURCES OF CONTAMINATION OF THE CLEANROOM ENVIRONMENT

+ People - clothing, shoes, skin, hair, mouth, nose, cosmetics, food, smoking
+ Air - heating, ventilation and air conditioning systems, wind, open windows, dust, dirt, raw materials
+ Water - using contaminated water to clean floors, tables, surfaces (cross contamination)
+ Equipment and supplies - wheels on carts, chairs, furniture, wastebaskets, supplies (cardboard boxes), wipes, centrifuge bottles, aspiration flasks and cold room mixers

HOW THE ASEPTIC PROCESSING ENVIRONMENT IS SAMPLED (ENVIRONMENTAL MONITORING)

Companies will sample (check) their aseptic processing environments to make sure they meet the desired/required standards. This is called environmental monitoring. Areas that are sampled may include:

+ Air - The air in a cleanroom is checked on a regular basis (e.g., daily, weekly) for particle counts and viable counts (the number of living microorganisms in the air).
+ Surfaces (including floors, walls, tables, etc.) - Surfaces are checked on a regular basis for viable counts.
HOW THE AIR IS SAMPLED IN THE ASEPTIC PROCESSING AREA

SETTLING (GRAVITY PLATES)
Petri dishes containing sterile growth media are exposed (opened) to the environment. Viable microorganisms that settle onto the media surface will grow after the plates are incubated. Plates are usually exposed 30-60 minutes.

AIR SAMPLERS
There are several types of air samplers. Most draw an exact amount of air into the device whereby the company can determine the number of viable microorganisms per cubic foot or liter of air.

HOW SURFACES ARE SAMPLED IN THE ASEPTIC PROCESSING AREA

CONTACT (RODAC) PLATES
Rodac plates are special petri dishes that contain sterile growth medium (agar), prepared in a manner that results in the surface of the media protruding above the sides of the plate.

The contact plate is pressed against any flat surface that needs to be sampled. Any viable microorganisms on the surface will stick to the agar surface and will grow upon proper incubation. This technique estimates the number of viable microorganisms on a surface. These counts should be very low.

SWABS
Swabs are like sterile “Q-tips” that are immersed (dipped) in a suitable sterile liquid. The swabs are then rubbed over the test surface. The microbiologist can then determine the number of microorganisms on the swab.

Swabs are good for surfaces that are not flat, like certain pieces of equipment. The number of viable microorganisms on the swab should be very low in a cleanroom.

ENVIRONMENTAL MONITORING
Environmental monitoring is observing the level of microorganisms that provide an early warning of a potential problem from normal operating conditions. Exceeding the alert level usually causes the company to start an investigation to discover why microbial counts are on the increase.
**ALERT LEVEL**

An alert level is established based on historical data generated from the environmental monitoring results. It is used as an early warning of an upward trend in microbial counts.

**ACTION LEVELS (CORRECTIVE ACTION) - COMPANIES ESTABLISH**

If the number of viable microorganisms in the air or on surfaces is above the set acceptable limits (usually established by guidance documents such as the USP), then a planned sequence of correct actions occurs. The purpose of these corrective actions is to return the facility to acceptable limits of viable microorganisms and, if possible, determine what caused the viable counts to be above the acceptable limits. Be sure that they will look at the cleaning procedures as the first possible source.

**ASEPTIC TECHNIQUE**

- Always wash hands
- Always glove hands and change gloves on frequent basis
- Sanitize gloved hands frequently, especially if touching dirty surfaces
- Spray surfaces, equipment and supplies with 70% IPA on a frequent basis
- Keep movement and personnel to a minimum
- Clean and sanitize common equipment used within the cleanroom
- Use multiple mop heads when cleaning a cleanroom facility; change out as often as required
- Use accurate measuring devices when preparing the disinfectant solutions
- Clean and sanitize any spillage immediately
- Maintain a consistent sanitization pattern
  - Clean from the top down
  - Clean from the furthest point from exit to the exit to avoid walking back over the cleaned areas
  - Use overlapping mop strokes to ensure complete coverage of the surface with the disinfectant solution
REFERENCES

3. VWR, Non-published information.

ABOUT ANALYTICAL LAB GROUP

ALG is an industry leader in the specialty contract lab space and comprises FDA- and EPA-focused laboratories across the United States, with facilities in San Francisco, Minneapolis and Boston. Our ALG team is focused on Helping Protect Life™ through infection prevention, with the most comprehensive testing solutions in the antimicrobial, pharmaceutical, medical device, biotech and healthcare spaces. Our facilities are GLP/GMP compliant, FDA registered, DEA licensed, ISO/IEC 17025:2017 accredited and A2LA accredited #3383.01 & 3383.02.

Our client-focused scientific, quality assurance, technical staff has built an unparalleled reputation for technical expertise, regulatory compliance knowledge, and superior client service.