Certified Specialist Programme in Cell Culture

Aseptic Technique in Cell Culture

Aseptic technique is the cornerstone of successful cell culture, and a thorough understanding of its terminology is essential for any specialist. The following explanation defines the most important terms, provides examples of their use in the laboratory, and highlights common challenges that may arise during implementation. Mastery of this vocabulary will enable practitioners to communicate precisely, troubleshoot effectively, and maintain the highest standards of sterility.

Aseptic technique refers to the set of practices designed to prevent contamination of cell cultures by microorganisms, particulates, or other unwanted agents. It encompasses the use of sterilized equipment, controlled environments, and disciplined behaviours that together create a barrier against external contaminants. The term is often used interchangeably with “sterile technique,” but in the context of cell culture it specifically emphasizes the maintenance of a contaminant-free environment throughout all manipulations.

Sterile is an adjective describing an object, surface, or medium that has been rendered free of viable microorganisms. Sterilization methods include autoclaving, dry heat, filtration, and chemical disinfection. For example, a glass flask that has been autoclaved at 121 °C for 20 minutes is considered sterile and can be safely used to house a new cell line. A common challenge is ensuring that the sterilization cycle is adequate for the material being processed; some plastics may deform under high heat, requiring alternative methods such as gamma irradiation.

Aseptic workstation or laminar flow hood is a piece of equipment that provides a unidirectional airflow of filtered air across the work surface, creating a protected zone where manipulations can be performed without exposure to ambient air. The airflow is typically HEPA-filtered, removing particles down to 0.3 Mm with an efficiency of 99.99 Percent. Within the hood, the user should maintain a “clean area” by arranging tools and supplies in a logical order, keeping the work surface uncluttered, and minimizing movements that could disturb the airflow. Failure to maintain proper technique can lead to turbulence, which may draw contaminants into the sterile field.

HEPA filter stands for high-efficiency particulate air filter. These filters are capable of capturing bacterial spores, fungal hyphae, and most viruses. Regular monitoring of filter integrity is essential; most facilities use a leak-test or a pressure decay test to confirm that the filter remains functional. Replacement schedules vary, but a common practice is to replace filters annually or after a defined number of operating hours, whichever occurs first. A compromised filter is a major source of contamination and can invalidate an entire batch of cultures.

Biosafety cabinet (BSC) is a type of enclosure that provides both product protection and personal protection

for the operator. While a laminar flow hood protects only the product, a BSC also protects the user from exposure to potentially hazardous agents. The most common type is a class II BSC, which recirculates filtered air while maintaining negative pressure relative to the laboratory. Understanding the difference between a laminar flow hood and a BSC is important because each has specific operational procedures and certification requirements.

Disinfection is the process of applying a chemical agent to reduce the microbial load on a surface to a level that is considered safe. Disinfectants used in cell culture laboratories include 70 % ethanol, isopropanol, and quaternary ammonium compounds. For example, wiping the exterior of a culture flask with 70 % ethanol before placing it in the hood helps to eliminate any microorganisms that may have settled on the glass during transport. Over-use of disinfectants can cause corrosion of metal components, and some agents may leave residues that are toxic to cells, so it is important to follow manufacturer guidelines for concentration and contact time.

Antiseptic is a substance that reduces the number of microorganisms on living tissue. In the context of cell culture, antiseptics are rarely applied directly to cell lines, but they are used for hand hygiene before entering the sterile area. Common antiseptics include chlorhexidine gluconate and povidone-iodine. Proper hand washing followed by the application of an antiseptic reduces the risk of transferring skin flora to the culture environment.

Contamination refers to the unintended presence of microorganisms, fungi, mycoplasma, or other foreign agents in a cell culture. Contamination can be overt, such as visible turbidity or fungal growth, or covert, such as mycoplasma infection that does not alter the appearance of the culture but can affect experimental outcomes. Early detection is critical; routine microscopic inspection and periodic testing for mycoplasma are standard practices. Once contamination is identified, the affected cultures must be discarded, and the source of contamination must be investigated and eliminated.

Mycoplasma is a class of bacteria lacking a cell wall, which makes them resistant to many antibiotics that target cell-wall synthesis. Mycoplasma infections are a frequent problem in cell culture laboratories because the organisms can pass through 0.2 Mm filters and are not removed by standard sterilization procedures. Regular PCR-based screening or fluorescent staining can detect mycoplasma at low levels. Preventive measures include using antibiotics that are effective against mycoplasma, maintaining strict aseptic technique, and avoiding the reuse of consumables that have not been validated as sterile.

Cross-contamination describes the accidental transfer of cells from one culture to another, leading to mixed or misidentified cell lines. This can occur when tools such as pipette tips or loops are reused without proper sterilization, or when the same media bottle is used for multiple cell lines without adequate segregation. To prevent cross-contamination, many laboratories adopt a “one-person-one-cell-line” approach, where each researcher works with a dedicated set of consumables and equipment for each cell line. Documentation of cell line provenance and regular authentication by short tandem repeat (STR) profiling are additional safeguards.

Authentication is the process of confirming the identity of a cell line, typically by genetic profiling methods such as STR analysis or DNA barcoding. Misidentification of cell lines is a widespread issue that can compromise research findings. Authentication should be performed upon receipt of a new line, after a certain number of passages, and before publication of data. The term also encompasses verification of species origin, as some cell lines may be contaminated with murine or other non-human cells.

Passage (or subculture) refers to the process of transferring a portion of a growing cell population into fresh medium to maintain optimal growth conditions. Each passage is assigned a passage number, which is an important piece of information for reproducibility. Over-passaging can lead to genetic drift, senescence, or phenotypic changes, while under-passaging can cause nutrient depletion and waste accumulation. A typical practice is to limit the number of passages for a given experiment and to document the passage number in all records.

Culture medium is a liquid or semi-solid formulation that provides nutrients, growth factors, and buffering capacity required for cell survival and proliferation. Media can be serum-based, such as Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum, or serum-free, containing defined growth factors. The preparation of medium must be performed under aseptic conditions; sterilization is commonly achieved by filtration through a 0.22 Mm membrane. The term "media" is often used in the plural to refer to multiple formulations or the collection of all reagents used in a culture.

Serum is a complex mixture of proteins, hormones, and attachment factors derived from animal blood, most commonly fetal bovine serum (FBS). Serum provides essential nutrients and promotes cell attachment, but it also introduces variability because its composition can differ between batches. Many laboratories perform a "lot testing" procedure to identify serum batches that support optimal growth for a particular cell line. Serum-free media are increasingly used to reduce variability and eliminate the risk of viral contaminants inherent to animal-derived products.

Antibiotic-antimycotic solution is a cocktail added to culture medium to suppress bacterial, fungal, and mycoplasma growth. Typical components include penicillin, streptomycin, and amphotericin B. While antibiotics can help prevent overt contamination, reliance on them may mask low-level infections and promote the development of resistant strains. Consequently, many experts recommend limiting antibiotic use to short-term rescue situations rather than incorporating them into routine culture protocols.

Incubator is a controlled environment that maintains optimal temperature, humidity, and gas composition for cell growth. Most mammalian cell cultures require a temperature of 37 °C, 5 % carbon dioxide, and high humidity to prevent evaporation of the medium. Incubator doors should be opened minimally, and the interior should be cleaned regularly to avoid the accumulation of dust or microbial spores. An incubator alarm system that alerts users to temperature deviations is a valuable safety feature.

CO_2 incubator is a specific type of incubator that supplies a regulated concentration of carbon dioxide, which equilibrates with the bicarbonate buffer system in the medium to maintain physiological pH. The pH

of the medium is critical because many cellular processes are pH-sensitive; a shift from 7.4 To 7.2 Can alter enzyme activity and affect experimental outcomes. Calibration of the CO_2 sensor should be performed monthly using a calibrated gas mixture.

Humidity control in the incubator prevents medium evaporation, which can concentrate salts and alter osmolarity. Many incubators include a water pan or a humidification system that must be refilled regularly with sterile distilled water. Failure to maintain humidity can lead to increased osmolality, causing cell shrinkage or detachment.

Cryopreservation is the process of storing cells at ultra-low temperatures, typically in liquid nitrogen at –196 °C, to halt all metabolic activity and preserve viability for future use. Cells are suspended in a cryoprotective agent, most commonly dimethyl sulfoxide (DMSO), at a concentration of 10 % before being cooled at a controlled rate of approximately –1 °C per minute. Rapid cooling or warming can cause ice crystal formation, leading to cell membrane rupture. Proper aseptic technique during the addition of cryoprotectant is essential to avoid introducing contaminants that could proliferate during thawing.

Thawing is the reverse process of cryopreservation, where frozen vials are rapidly warmed, usually in a 37 °C water bath, to minimize the time cells spend at sub-optimal temperatures. After thawing, cells are transferred to pre-warmed medium and centrifuged briefly to remove excess DMSO, which can be toxic if left in the culture. The entire procedure must be performed quickly and under aseptic conditions to prevent contamination while the cells are most vulnerable.

Laminar flow is a term describing the smooth, parallel movement of air within a laminar flow hood or BSC. The flow pattern is designed to sweep particles away from the work surface and prevent turbulent eddies that could carry contaminants into the sterile field. Disruption of laminar flow can be caused by improper placement of equipment, rapid arm movements, or opening the sash too far. Users should be trained to move deliberately and keep objects within the defined “safe zone” of the hood.

Sash is the movable glass barrier on a laminar flow hood that separates the user from the sterile workspace. The sash can be raised or lowered to provide access while maintaining the integrity of the airflow. Most hoods have a recommended operating height, typically 4–5 inches above the work surface; exceeding this height can significantly reduce protection. The sash should be closed when the hood is not in use to preserve the sterile environment and reduce energy consumption.

Air change rate (ACH) is a measure of how many times the air within a room or enclosure is replaced per hour. For a cell culture laboratory, an ACH of 15–20 is generally recommended to dilute airborne contaminants and maintain a low particulate load. The ACH is calculated based on the room volume and the flow rate of the ventilation system. Inadequate ACH can lead to accumulation of spores and increase the risk of contamination.

Positive pressure room is a space where the internal air pressure is higher than the surrounding areas, causing airflow to move outward when doors are opened. This design prevents external contaminants from

entering the room, making it suitable for housing laminar flow hoods and BSCs. Positive pressure is achieved by supplying filtered air at a rate slightly greater than the exhaust flow. The system must be regularly inspected for leaks and pressure differentials to ensure effectiveness.

Negative pressure room, by contrast, maintains a lower internal pressure relative to adjacent spaces, drawing air inward. This configuration is used for handling hazardous biological agents, where containment of airborne pathogens is critical. While a negative pressure environment is not typical for routine cell culture, understanding the distinction is important for labs that work with both safe and potentially dangerous cell lines.

Sterile field is the area within a laminar flow hood or BSC where all objects are considered free of contaminating microorganisms. The field is established by turning on the hood, allowing the HEPA filter to purge the air, and confirming that the airflow is stable. Any item introduced into the sterile field must be disinfected prior to entry, and the user must avoid reaching over the field to prevent turbulence. Maintaining a clean sterile field is essential for preventing both microbial and particulate contamination.

Disinfectant wipe is a pre-moistened cloth impregnated with a chemical agent, such as 70 % ethanol, used for rapid surface decontamination. Wipes are convenient for cleaning the exterior of equipment, the sash, and other non-critical surfaces. However, wipes should not be used on delicate optical components or plastic surfaces that could be degraded by the disinfectant. The correct technique involves wiping in a unidirectional motion, allowing the solution to remain wet for the recommended contact time before the surface dries.

Autoclave is an apparatus that uses saturated steam under pressure to achieve sterilization. The standard cycle for most laboratory glassware is 121 °C for 15–30 minutes, followed by a drying period. Items that cannot withstand high temperatures, such as certain plastics, must be sterilized by alternative methods. Validation of autoclave performance includes monitoring temperature, pressure, and time, often using biological indicators such as spore strips.

Dry heat sterilizer is an oven that sterilizes equipment by exposing it to high temperatures, typically 160–180 °C for several hours. Dry heat is suitable for metal instruments, glassware, and powders that are heat-stable. Unlike autoclaving, dry heat does not involve moisture, which can be advantageous for materials that are damaged by steam. The sterilization cycle must be calibrated to ensure uniform heat distribution throughout the chamber.

Filtration sterilization is a method that removes microorganisms by passing a solution through a membrane filter with a pore size of 0.22 Mm or smaller. This technique is commonly used for heat-sensitive media, antibiotics, and other reagents. After filtration, the filtrate is collected in a sterile container under aseptic conditions. Filters should be pre-wet with the solution to be filtered to improve flow rate and reduce the risk of tearing.

Laminar flow hood certification is the process by which a qualified technician verifies that the hood’s airflow,

filter integrity, and environmental controls meet specified standards. Certification includes measuring face velocity, performing a smoke test to visualize airflow patterns, and conducting a filter leak test. Certification must be performed annually, or after any major repair or relocation of the hood, to ensure continued protection.

Biosafety level (BSL) designates the containment practices required for work with organisms of varying pathogenicity. Cell culture typically occurs at BSL-1 or BSL-2, depending on the nature of the cell line and any associated pathogens. BSL-2 laboratories require additional protective equipment, such as face shields and double gloves, and may mandate the use of a class II BSC for all manipulations. Understanding the appropriate BSL for a given project is essential for compliance with institutional and regulatory guidelines.

Personal protective equipment (PPE) includes items such as lab coats, gloves, goggles, and face shields that protect the user from exposure to hazardous agents. In the context of aseptic technique, gloves are the most critical component of PPE because they prevent direct hand contact with sterile surfaces and reduce the risk of transferring skin flora. Gloves should be changed frequently, especially after handling contaminated material, and should be inspected for tears before each use.

Glove box is a sealed container that provides an isolated environment for handling highly sensitive or hazardous materials. The interior is filled with filtered gas, and manipulations are performed through built-in gloves attached to the walls of the box. While glove boxes are not commonly used for routine cell culture, they are valuable for work with highly infectious agents or for processes that require an absolute exclusion of oxygen, such as anaerobic cultures.

Incubator shaker combines the functions of an incubator with a shaking platform, allowing suspension cultures to be kept in motion while maintaining temperature, CO_2, and humidity control. Agitation promotes uniform nutrient distribution and prevents cell clumping. Shakers must be placed inside the incubator to avoid temperature gradients, and the speed should be calibrated to avoid excessive shear stress that could damage delicate cell types.

Shear stress is the mechanical force exerted on cells by fluid movement, such as stirring or pipetting. Certain cell types, particularly primary neurons or delicate stem cells, are highly sensitive to shear and may undergo apoptosis if subjected to vigorous mixing. To minimize shear stress, researchers can use low-speed orbital shakers, gentle pipetting techniques, or specialized low-shear bioreactors. Recognizing the impact of shear stress is important when designing protocols for cell expansion.

Bioreactor is a closed system that provides a controlled environment for large-scale cell culture, often incorporating automated monitoring of pH, dissolved oxygen, and temperature. Bioreactors can be operated in batch, fed-batch, or continuous modes, each offering different advantages for cell growth and product yield. Maintaining aseptic conditions in a bioreactor requires strict sterilization of all components, including tubing, sensors, and sampling ports, often achieved by steam or gamma sterilization.

Sampling port is a sealed access point on a bioreactor that allows for the withdrawal of culture samples

without compromising sterility. Ports are typically equipped with sterile connectors and filters that prevent entry of contaminants. When sampling, the port should be disinfected with an appropriate agent, and a sterile syringe or sampling device should be used. Improper handling of sampling ports is a frequent source of bioreactor contamination.

Closed system is a culture approach in which the entire process, from inoculation to harvest, occurs without exposure to the external environment. Closed systems can include sealed flasks, bag cultures, or bioreactors with sterile connectors. The advantage of a closed system is a dramatically reduced risk of contamination, as there are no open manipulations. However, closed systems require specialized equipment and thorough validation of each component's sterility.

Open system, in contrast, involves manipulations that expose the culture to the ambient laboratory environment, such as transferring cells between dishes on a laminar flow hood. Open systems are more flexible and allow for easy observation and intervention but demand rigorous aseptic technique to prevent contamination. Many routine cell culture procedures, such as routine passaging, still rely on open-system methods.

Decontamination is the process of eliminating all viable microorganisms from a surface, equipment, or environment. In addition to routine disinfection, laboratories may perform periodic deep cleaning using agents such as sodium hypochlorite or peracetic acid. Decontamination of the entire laboratory may be required after a major spill of infectious material, and it typically involves fogging or vaporized hydrogen peroxide to reach all surfaces. Validation of decontamination efficacy can be done using biological indicators placed throughout the space.

Fogging is a method of distributing a disinfectant as a fine aerosol that can penetrate hard-to-reach areas. Fogging with vaporized hydrogen peroxide is a common practice for decontaminating BSCs, incubators, and other equipment after a contamination event. The process requires sealing the environment, introducing the vapor, allowing sufficient contact time, and then aerating the space to remove residual chemicals. Proper safety protocols must be followed because the chemicals used in fogging can be toxic to personnel.

Airlock is a small compartment that provides a transitional space between a sterile environment and the external laboratory. Airlocks are used in high-containment facilities to reduce the flow of contaminants when personnel or equipment move in and out of a cleanroom. The airlock typically includes interlocking doors and may be equipped with UV lamps for surface sterilization. Proper use of an airlock involves allowing time for the air to be exchanged before opening the second door.

UV-C (ultraviolet-C) irradiation is a sterilization technique that uses short-wavelength ultraviolet light (200–280 nm) to inactivate microorganisms by damaging their nucleic acids. UV-C lamps are installed in biosafety cabinets, laminar flow hoods, and some incubators to provide an additional layer of decontamination. The effectiveness of UV-C depends on exposure time, distance from the lamp, and the presence of shadows;

therefore, surfaces must be arranged to avoid occlusion. Overexposure can degrade certain plastic components, so manufacturers' guidelines should be followed.

Indicator organism is a microorganism used to assess the efficacy of a sterilization or disinfection process. For autoclave validation, Bacillus stearothermophilus spores are commonly employed because of their high resistance to heat. For chemical disinfectants, Geobacillus stearothermophilus or bacterial spores may be used. The presence or absence of growth after exposure to the sterilization process indicates whether the procedure was successful. Indicator organisms provide a reliable, quantitative measure of sterility assurance.

Sterility assurance level (SAL) is a quantitative estimate of the probability of a viable microorganism remaining after a sterilization process. An SAL of 10^{-6}, for example, means that there is a one in a million chance that a single viable organism remains. Achieving a specific SAL is required for medical devices and certain high-risk cell therapy products. Calculating SAL involves understanding the bioburden, the efficacy of the sterilization method, and the statistical distribution of microorganisms.

Bioburden is the number of viable microorganisms present on a product or surface before sterilization. Measuring bioburden helps to determine the required sterilization parameters to achieve a target SAL. In cell culture, bioburden can be assessed by plating samples on agar and counting colony-forming units (CFU). Reducing bioburden through cleaning and disinfection prior to sterilization improves the efficiency and reliability of the process.

Colony-forming unit (CFU) is a unit used to estimate the number of viable bacteria or fungi in a sample. One CFU corresponds to a single organism that can grow into a visible colony on agar. CFU counts are essential for monitoring environmental contamination, testing the effectiveness of cleaning protocols, and validating sterilization processes. Accurate CFU enumeration requires proper dilution, plating technique, and incubation conditions.

Environmental monitoring is the systematic sampling of air, surfaces, and water within a cell culture facility to detect microbial contamination. Routine monitoring may include settle plates, air samplers, and surface swabs. Results are compared against predefined acceptance criteria; for example, a maximum of two colonies per 100 cm^2 of work surface. Deviations trigger corrective actions, such as deep cleaning or equipment maintenance. Effective environmental monitoring is a proactive measure that helps maintain a sterile environment.

Settle plate is a petri dish containing growth medium that is left uncovered for a defined period to collect airborne microorganisms. After exposure, the plate is incubated, and colonies are counted. Settle plates provide a simple method for assessing the overall microbial load in a laboratory, but they do not differentiate between specific sources of contamination. Placement of settle plates near high-traffic areas or equipment can help identify hotspots of aerosol generation.

Air sampler is a device that actively draws a known volume of air through a filter or onto agar, capturing microorganisms for subsequent incubation. Air samplers provide quantitative data on airborne

contamination levels and can detect transient spikes that settle plates might miss. Calibration of the sampler is essential to ensure accurate volume measurement, and the sampling duration should be standardized for reproducibility.

Surface swab is a tool used to collect microorganisms from a defined area of a work surface, equipment, or container. Swabs are moistened with a neutralizing buffer to improve recovery, then streaked onto agar or placed into enrichment broth. Surface swabs are valuable for pinpointing specific contamination sites, such as the rim of a flask or the inside of a pipette tip box. Proper technique includes using a consistent swabbing pattern and applying uniform pressure.

Enrichment broth is a liquid medium that supports the growth of low-level contaminants, allowing them to become detectable. Samples collected from surfaces or air can be incubated in enrichment broth before plating to increase the sensitivity of detection. Common enrichment media include tryptic soy broth and brain-heart infusion broth. Over-enrichment can lead to overgrowth, which may mask the presence of slower-growing organisms, so incubation times must be controlled.

Quality control (QC) in cell culture refers to the systematic procedures used to verify that cultures, reagents, and equipment meet defined standards. QC activities include testing for mycoplasma, verifying cell line identity, checking media pH, and confirming sterility of reagents. A robust QC program reduces variability and ensures that experimental results are reliable. Documentation of QC results is required for regulatory compliance in many jurisdictions.

Standard operating procedure (SOP) is a written document that outlines the step-by-step instructions for performing a specific laboratory task. SOPs for aseptic technique cover topics such as hood decontamination, glove changes, media preparation, and waste disposal. Consistent adherence to SOPs minimizes operator-dependent variability and promotes reproducibility across different personnel and laboratories. SOPs should be reviewed regularly and updated when new equipment or methods are introduced.

Waste disposal is the process of discarding hazardous or contaminated materials in a manner that prevents environmental release and exposure to personnel. In the cell culture laboratory, waste includes used pipette tips, disposable flasks, culture plates, and liquid waste containing biological agents. Sharps should be placed in puncture-resistant containers, and liquid waste may be decontaminated by autoclaving or chemical treatment before disposal. Proper labeling and segregation of waste streams are essential for compliance with biosafety regulations.

Biosafety cabinet certification, decontamination procedures, and regular maintenance of equipment are all integral components of an effective aseptic technique program. By mastering the terminology described above, specialists can develop a shared language that supports clear communication, efficient troubleshooting, and continuous improvement of sterile practices. The precise use of these terms in documentation, training, and daily operations ensures that the highest standards of cell culture are

maintained, safeguarding both experimental integrity and personnel safety.