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Standard Practice for Aseptic Sampling of Biological Materials[1]

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1. Scope

1.1 This practice presents the principles, state-of-the-art concepts and generally accepted methods for aseptic sampling of materials involved with or produced by biotechnical processes where contamination of either the sample or the source of the sample cannot be accepted. These processes could involve living organisms such as virus, bacteria, yeasts, and mammalian cells or biologically active constituents, such as enzymes and biochemicals that must exist in a noncontaminated state.

1.2 This practice also applies to the products from these bioprocesses that can be for human consumption, sterile or parental drug applications, which also require aseptic sampling to meet regulatory, current good manufacturing practices, or other quality control requirements.

1.3 **Warning**—Since some biotechnical processes could produce flammable products, this sampling practice should be applied only after taking into account all of the factors that are pertinent to an assessment of the fire hazard of a particular end use.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1356 Terminology Relating to Atmospheric Sampling and Analysis[2]

D 4177 Practice for Automatic Sampling of Petroleum and Petroleum Products[3]

E 884 Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities[4]

3. Terminology

3.1 The definitions covered in Terminology D 1356, Practice D 4177, and Practice E 884 are applicable to this practice.

3.2 *Definitions:*

3.2.1 *aseptic sampling*—sampling process in which no extraneous microorganisms or substances are introduced into the sample or its original bulk material as a result of the sampling system and activity.

3.2.2 *current good manufacturing practices (CGMP)*—current regulations published by the United States Food and Drug Administration (FDA) regarding manufacturing, processing, packaging and storing of drug and biological products.

3.2.3 *dead leg*—any inactive, trapped or stagnant zone of a biological fluid that is to be sampled aseptically where this liquid zone would not be representative of the bulk fluid that is to be sampled. This ''dead leg'' zone could deviate from the bulk system in oxygen content, nutrients levels, material composition, temperature, bacterial contamination, and other process variables that would prevent any sample drawn through this system from representating the bulk fluid quality to be tested.

3.2.3.1 *Discussion*—This definition may be more restrictive than the FDA definition which is any unused pipe greater in length than six of its internal diameters. Since valve designs and presence of other devices in the sampling system must be considered in this aseptic sampling procedure, the entire sampling system from bulk fluid to sample container should be validated by using proper biological challenges to show that the intended sterility and sample quality objective can be met and reproduced within the prescribed limits of the specific process.

3.2.4 *pathogenic*—disease causing.

3.2.5 *sterile*—free of any living organism.

3.2.6 *validation*—the quality assurance evaluation of an item of equipment or overall process wherein the equipment or process, or both, is challenged to perform under the "worst case" conditions of process variables and applicable microorganism contamination to meet preestablished acceptance criteria.

4. Summary of Practice

4.1 A general description of aseptic sampling-system design guidelines is included either to remove a representative sample of the bulk fluid for external testing, or to directly measure the fluid properties in-situ. Validation of sampling equipment and methods is also described. Suggested sample system designs are presented for consideration and application as appropriate to specific processes. Where possible, the advantages and

[1] This practice is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.03 on Unit Processes and Their Control.

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[2] *Annual Book of ASTM Standards*, Vol 11.03.

[3] *Annual Book of ASTM Standards*, Vol 05.02.

[4] *Annual Book of ASTM Standards*, Vol 11.05.

disadvantages of different sample removal designs are presented. Fabrication and maintenance considerations are also discussed. The Appendix includes general guidelines for sterilization of aseptic sampling devices.

5. Significance and Use

5.1 This practice should be used for removing samples from biological processes in the laboratory or commercial manufacturing facilities where the sample system removes the sample from the process for use in external testing.

5.2 This practice also addresses the sampling procedures required for in-situ measurements wherein the sample is not removed from the process but must represent the process material being tested. Generally, the in-situ measurement device is either sterilized separately from the process equipment and then inserted into the sterilized equipment, or the in-situ device is permanently mounted in the equipment and then sterilized together with the equipment.

5.3 Levels of contamination are not specified in this practice since each biological system and bioprocess can differ as to the amount and types of micro-organism, bacteria, virus, and other contaminants that can be allowed in the sample and process materials for acceptable operations under CGMP or similar requirements. With the properly designed micro-organism challenges to the sterile system, then the sample system can be tested and validated.

5.4 Since biological process samples can vary widely in sterility requirements, sample size, material composition, (liquid, vapor, slurry, etc.), stability, and other characteristics, the practices described herein are general and are to be applied as appropriate to each specific situation. These practices are limited to aseptic sampling conditions and are not intended to apply to containment of highly toxic or hazardous materials that require additional precautions to avoid exposure of the sample contents to the environment, or workers, where health and other safety considerations could require more stringent practices. Sample applications can include the following:

5.4.1 *External Testing of Removed Samples:* Liquid chromatography, spectroscopy (ultraviolet, infrared, and fluorescence), fiber optics, mass spectroscopy,

5.4.2 *Direct Measurement of In-Situ Sample:* Temperature, pressure, pH, etc.

6. Procedure for Aseptically Removing Samples from a Biological System

6.1 *General Criteria for Designing Sampling Systems*:

6.1.1 Sample removal devices should access the bulk material at the desired location:

6.1.1.1 If the sample is to be representative of the average quality of the bulk material, then sufficient agitation of the bulk material is required to ensure uniformity within the equipment. With a homogeneous, single-phase bulk material this uniformity can be achieved and validated using sufficient agitation. Caution must be exercised to evaluate the agitation in all locations of the bulk material since localized areas of insufficient agitation could affect accurate sampling.

6.1.1.2 With a heterogenous or multiple phase material that is encountered frequently with fermenter slurries of liquids and solids (such as living organisms), then a representative sampling method must be designed considering a uniform slurry from which a sample is withdrawn.

6.1.2 General criteria to be considered when removing the sample from the bulk material could include:

6.1.2.1 Obtain a fresh sample and avoid a “dead leg” of older material that could compromise the quality of the desired sample.

6.1.2.2 Consider the kinetics of the reactions that may require the sample to be treated with freezing, neutralization, filtration, or other appropriate processes, immediately after collection, to maintain the sample quality at the actual time of sampling. This consideration would be specific to each biological process.

6.1.2.3 Consider the change of process conditions, (temperature, pressure, mixing efficiency, component concentration, etc.) from the bulk material to the sampling device and container. These changes should not affect the sample quality. Again, the sample should represent the bulk material source in all desired respects. If pH is the desired analytical measurement, then separation of some solids from the slurry in the sample container may be acceptable, where it would not be acceptable for a solids content determination. A rapid pressure drop or temperature change could adversely affect the living organisms if a live cell count is desired.

6.1.2.4 Consider the removal of solids by filtration or other means, to permit collecting a single phase sample for analysis where solids are not tolerated or could affect the quality of sample as it is prepared for analytical testing. If the solids are yeast or nutrients that could continue to react and change the time-value of the sample, then the effect of these components must be negated to have a reliable sample.

6.1.2.5 Validate these procedures and facilities using appropriate system challenges to document the variations in the sample results. This validation is performed after determining the specific sampling criteria for the process, the sampling method documented, the sampling apparatus constructed, the sample handling techniques documented, and the personnel trained in these procedures. Movement of the sampling device to other locations in the bulk material container, such as closer to mixing devices, etc., could be used to verify the optimum sampling device method and location for the specific process.

6.1.2.6 Consider particle size when designing the sample removing device, the sample container, and the method for removing the sample from the container, for the analytical testing. Plugging, separation of solids, and agglomeration, are some of the potential problems to be considered in the sampling system.

6.1.2.7 Reusing the sample device during the same batch requires the consideration of:

(a) (*a*) Does carry over or residual material from the previous sample adversely affect the quality of the next sample? If flushing the sample device prior to collecting the next sample is adequate, then multiple samples can be taken through the sampler. For pH determination this procedure may be accurate, but may not be acceptable for live cell counts, pyrogen determinations, etc., where previous sample residue may be a detriment.

(b) (*b*) Can the sampling device be sterilized between

sample collections to purge previous sample residue? Some sampling devices are designed with methods to steam sterilize portions of the sample flow channel. If complete sterilization is required then careful attention is needed to ensure that all sample device components are sterilized that are in contact with the sample. Normally, a ball valve, rising stem valve, or similar close tolerance device, is in contact with the bulk material being sampled. Portions of this valve are very difficult to sterilize without the sampler being autoclaved or sterilized when the bulk container is being sterilized. Possibly a combined sterilization and thorough flush prior to collecting the next sample can achieve the sampling objectives. If not, then a multiple sampling device system (one for each sample), may be required to effectively and economically ensure that an aseptic sampling device is used. When the sampling device is qualified and the aseptic system validated, then the sampling protocol would be used in routine operations.

6.1.2.8 Consider available quantity of sample required for purging and retained sample. Small reactors could be adversely affected by the quantity of material removed thereby upsetting the reaction kinetics and equilibrium. The procedure must consider the quantity of material purged around the sample container and the quantity diverted into the container.

6.1.2.9 Consider disposal requirements of purged material and exposure of this material to the environment and other living systems. Provide proper containment devices and disposal procedures that are consistent with regulatory rules such as CGMP and Good Laboratory Practice (GLP), operating procedures, and other regulations or guidelines.

6.1.2.10 Develop proper sample handling procedures from the sampling device through the analytical testing to disposal or retained sample storage. This includes proper sample identification, stable storage, repetitive uniform sample retrieval from the container for analytical tests, sample security, proper disposal if sample is no longer needed, and proper sterilization of the sample container if it is to be reused.

6.1.2.11 Design sample containers to withstand both the bulk process and the sample handling design conditions of temperature, pressure, composition, corrosivity, toxicity, flammability, other hazardous properties, slurry removal, and cleaning and sterilization procedures. If these containers are to be reused then develop and qualify a container testing procedure and schedule.

6.1.2.12 Consider disposable, nonreusable containers for these samples as appropriate to achieve the sampling objectives and design considerations listed in 6.1.

6.2 *Typical Sample Device Design Considerations for Externally Removed Samples*:

NOTE 1—The following general descriptions of frequently used aseptic sampling devices are presented with the caution that actual applications to a specific process must be tailored appropriately and validated as required to ensure that accurate samples are obtained and that the sampling objectives are attained that meet or exceed regulatory or other requirements.

6.2.1 *Flush-Mounted Sample Valves*—The following considerations apply to selecting the proper valve to remove samples aseptically.

6.2.1.1 Sample valve should minimize holdup of sample within the valve especially if subsequent samples are to be taken under aseptic conditions. Diaphraghm and ball valves generally have less holdup than gate or globe valves. Diaphragm valves also are normally sterilized easier than other standard design valves. Specially designed rising or lowering stem valves can reduce holdup further. Close tolerances between moving parts should minimize trapped sample materials.

6.2.1.2 Sample valves should have minimum lengths of piping connection on suction and discharge ports to avoid trapping sample material that cannot be removed and sterilized between samples.

6.2.1.3 If a sample valve is to be used for consecutive aseptic samples then sterilize the valve between samples. These and criteria are described in 6.1.

6.2.1.4 Make sure that bulk fluids composition at valve inlet is representative of entire material to be sampled.

6.2.2 *Recycle Loop for Aseptic Sample*—Primary application is for homogeneous, single phase fluids.

6.2.2.1 Make sure that source of material entering sample tubing is representative of bulk of material being sampled.

6.2.2.2 Isokinetic sampling considerations should be followed where appropriate. Make sure that velocity of material entering and within the tubing or piping is adequate to maintain uniform composition throughout entire recycle loop especially at the location of the sample removal. If material is a slurry then the fluid design velocity, piping layout and fittings design, should maintain a uniform sample quality throughout the recycle loop. Avoid sample removal near wall of recycle pipe where velocity is lowest or near abrupt fluid direction changes that could distort the solids distribution in the slurry. Generally, turbulent flows will enhance uniform distribution within the material being sampled.

6.2.2.3 Specifically design sterilization procedures for the recycle loop and its sample removal system to ensure that full sterilization is achieved. Document sampling procedures for the aseptic sampling system validation.

6.2.3 *Draw Tube or Siphon Tube*—These devices are extremely difficult to use and to ensure that a fresh representative sample is obtained on a uniform basis. Primary application of this device applies to homogeneous, single phase fluids. If slurries are sampled then its composition could vary in the tube that is normally stagnant and not representative of the bulk material being sampled. Cellular or viscous materials could stick to the wall of the tubes and cause sampling errors. Excessive purging and proper velocity control may be needed on a repetitive basis to obtain a representative sample.

6.2.3.1 *Vertical Sample, Tube Design*, where fluid is pressured or pumped upward in the tubing should be designed to ensure that a fresh, not contaminated, representative sample is withdrawn using minimal purging. Velocity considerations are important to avoid settling of solids and flashing of volatile liquids if the pressure drop in the piping system causes vaporization and two phases of products. Avoid sample removal from side wall of tube as described in 6.2.2.2. Inert, sterile gas could be used for blowback of the sample tube to minimize stale sample accumulations. Other general sampling

pipe systems, automatic sampling guidelines, and other information for consideration are presented in Practice D 4177, where appropriate.

6.2.3.2 *Vertical Sample Tube Design*, where fluid is pressured or pumped downward for removal should avoid designs where solids could accumulate and fill the tube while in the passive mode. This stratification and solids accumulation problem can be minimized by using a horizontal section at the tube inlet and sterile gas blowback as appropriate. Other considerations in 6.2.3 apply to this sampling method.

6.2.3.3 *Sample Tube Extensions*, to valves described in 6.2.1 also should follow the same guidelines as described in 6.2.3. These extensions permit withdrawing representative samples near sources of fluid agitation near mixers and away from the bulk container wall.

6.2.4 *Insertable Sterile Sample Probe or Draw Tube*—Several devices are included in this category such as (1) sterile syringe and membrane barrier, and (2) insertable sterile probe into system to withdraw sample.

6.2.4.1 *Sterile syringe*, can be used to penetrate a membrane at the sample port, extend the sample needle into the bulk of the material, remove the sample, and use the syringe as a sample container where applicable. With small diameter syringe needles, this sampling technique is usually limited to homogeneous, single phase system.

6.2.4.2 Inserting a sterile sample probe aseptically into the bulk container usually requires special sterilization techniques for the membrane or probe port entry device. Special sterilization fluids or steam would be needed to ensure that the sampling system is sterile.

6.2.5 *Containment of Sample Collection Emission*—When any sample is collected aseptically, design considerations should include containment of vapor purged when the sample container is filled, collection and disposal of any purged liquids, excess samples, and byproducts from resterilization. Glove boxes and other containment systems can be designed into the process and validated.

6.2.6 *Sample System Fabrication and Maintenance Considerations*:

6.2.6.1 Use all welded construction where permitted. Threaded connections are more difficult to sterilize.

6.2.6.2 Minimize “dead legs” in piping system.

6.2.6.3 Select valves that minimize dead space for bacteria growth.

6.2.6.4 Avoid rough surfaces where microorganisms can grow uncontrolled. Grind welds smooth. Use top quality welding procedures.

6.2.6.5 Select proper materials of construction.

6.3 *Typical Sample Device Design Considerations for In-Situ Measurement Devices*:

6.3.1 Design in-situ measurement devices that are permanently attached to be sterilized with the overall system.

6.3.1.1 Design consideration for in-situ measurement devices such as pressure gages, thermocouples, pH probes should meet the same design conditions as for the bulk container sterilization.

6.3.1.2 Locate measurement device at the desired sample position. Ease of removal for maintenance is also an important consideration.

6.3.2 Design in-situ measurement devices that can be removed for sterilization with proper containment precautions for the specific material being sampled, while maintaining aseptic conditions during reinstallation. Each system would be designed to the specific process conditions. Proper sealing devices are needed to avoid leakage from the bulk material vessel or systems to the environment.

APPENDIX

(Nonmandatory Information)

X1. STERILIZATION GUIDELINES

X1.1 Know the organisms, and infectious strains that need to be removed by sterilization. Adjust the sterilization method and materials to effectively remove these undesirable components.

X1.2 Normally, saturated steam is used for sterilization since bacterial spores are more effectively destroyed. Superheated steam requires cooling to reach saturation quality for maximum effectiveness. Wet steam has a lower heat content and adds more water to the system that must be removed.

X1.3 Remove air from the sample system to avoid steam dilution and air pockets that prevent full sterilization. The saturated steam is the sterilizing medium. Air removal is quired to avoid reducing the steam partial pressure which would be equivalent to superheating the steam that reduces its sterilization effectiveness.

X1.4 If parts of the sample system can be detached from the vessel, pipe or equipment item, then they can be sterilized separately in an autoclave or similar device.

X1.5 Sample device sterilization temperatures using saturated steam must be maintained accurately. External appendages of the sample system can increase heat transfer and cool faster than the main vessel that could prevent effective sterilization of the sampling system.