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Fundamentals of an Environmental Monitoring Program

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PDA TECHNICAL REPORT NO. 13
REVISED
FUNDAMENTALS OF AN ENVIRONMENTAL MONITORING PROGRAM

Table of Contents

1.0 INTRODUCTION

The purpose of this document is to identify microbiological and particulate control concepts and principles as they relate to the manufacture of sterile pharmaceutical products. It expands substantially upon the first edition of Technical Report No. 13, Fundamentals of a Microbiological Environmental Monitoring Program, published by PDA in 1990. While this publication cannot possibly supplant the wealth of information published on this subject, it provides summary information and appropriate references for the reader to consult, if necessary. The objective was to contemporize the first edition through the utilization of current definitions, recognition of improved environmental monitoring procedures, and equipment.

This document should be considered as guidance; it is not intended to establish any mandatory or implied standard.

The task force consisted of members representing global companies, to ensure that the methods, terminology, and practices reflect the procedures utilized globally. Technical reviews were performed by some of the more prominent environmental monitoring scientists in the world today.

This document serves as a source on clean room environmental test methods, and although some non-viable particulate and endotoxin testing data are included, its primary focus is microbiological control. The concepts for sterile product manufacturing are the most stringent application, but these concepts can also be applied to non-sterile product manufacture. The focus is environmental monitoring as it relates to facility control and compliance. This document was compiled to aid in setting up a program that is meaningful, manageable, and defendable.

In order to ensure a consistently acceptable production environment, a comprehensive environmental control program should be supported by: (a) sound facility design and maintenance, (b) documentation systems, (c) validated/qualified sanitization/disinfection procedures, (d) reliable process controls, (e) good housekeeping practices, (f) effective area access controls, (g) effective training, certification/qualification and evaluation programs and (h) quality assurance of materials and equipment.

Environmental surveillance is a tool utilized to evaluate the effect of controls on the manufacturing environment. A process to assess the clean room and other controlled environments of a pharmaceutical facility can serve as an adjunct to the sterility assurance program for the microbial quality of drugs. The items addressed in this document include definitions, standards, surveillance support systems, system surveillance, validation systems, appendices of definitions and typical frequencies and levels, and a bibliography.

2.0 ENVIRONMENTAL CLASSIFICATIONS

The environmental monitoring program should be designed and implemented based on sound scientific principles, the need for and the utility of the collected data, and in conformance with the regulatory requirements of the government agencies regulating the manufacturing site. Personnel administering environmental monitoring programs should be familiar with a variety of regulatory schemes if they are to be successful in serving the United States and International product markets. Efforts at harmonization are underway, and it is possible that many of the differences in the requirements for monitoring programs may disappear as the countries and organizations involved come to some agreement on the overall approach to be taken. Therefore, it is important to keep up to date on the requirements for the different countries in which the product will be sold.

This will ensure that the established program meets the monitoring requirements of each country. If the intent is to serve both the United States and the International markets, the most stringent requirements should be evaluated as the basis of an environmental monitoring program.

This section compares published environmental classifications for environmental monitoring in the United States and the European Union. Although these publications are similar in many respects, there are important differences among them in terms of the information each provides.

Federal Standard 209E establishes airborne particulate cleanliness classes categorized as Class M 1 through M 7 (SI names). All of the classifications can be applied to particles ≥ 0.5 µm, while other particle sizes, e.g., 0.1, 0.2, 0.3 and 5 µm, utilize only some of the classifications. In the United States, the pharmaceutical industry classifies production areas as Class 100, 10,000 and 100,000 (M 3.5, M 5.5 and M 6.5, respectively) based on particles ≥ 0.5 µm, the classification reflecting the number of particles per cubic foot. It should be noted that the Institute for Environmental Sciences and Technology (IEST) has recommended that Federal Standard 209E be retired by the end of 2001 as a result of the publication of the ISO 14644-1 and 14644-2 documents.

FDA's 1987 "Guideline on Sterile Drug Products Produced by Aseptic Processing" discusses environmental requirements for critical areas (Class 100), in which sterile drugs are exposed to the environment. This document also includes specifications for viable airborne monitoring for Class 10,000 and Class 100,000 areas. Viable and non-viable guidance is provided.

USP general information chapter <1116> "Microbial Evaluation and Classification of Clean Rooms and Other Controlled Environments" proposes limits for clean room levels, including air, surfaces, and personnel working within the clean area. The chapter includes three classifications that would supplement the current categories based on non-viable particulate limits.

In the European Union, *The Rules Governing Medicinal Products in the European Union, (Vol. IV: Good manufacturing practice for medicinal products)* include an air classification system in Annex 1 under the heading "Manufacture of Sterile Medicinal Products." Air quality is classified alphabetically as Grade(s) A through D, with Grade A being the cleanest. Associated with each respective grade is the maximum allowable number of particles per cubic meter.

In addition to these publications, additional guidance is available through the International Organization for Standardization (ISO) which is a world-wide federation of national standard bodies. The work of preparing international standards is normally carried out through ISO technical committees. ISO/TC 198 provides *Guidance for Sterilization of Health Care Products* and ISO/TC 209 provides *Guidance for the Classification of Airborne Particulate for Clean Rooms and Associated Controlled Environments.* Copies of these documents can be obtained from American National Standards Institute (ANSI).

It should be noted that all classifications have a direct counterpart in the documents prepared by other international groups. Tables 1 through 3 summarize and compare these specifications.

Table 1: Class 100 Monitoring Table (Max. values are given).

COUNTRY DOCUMENT	U.S. FS 209E	U.S. USP <1116>	EU (at rest, static)	EU (operational, dynamic)	EU (operational, dynamic)	ISO 14644-1
CLASSIFICATION	M 3.5 (100)	M 3.5	A and B	A	B	5
FREQUENCY	Not stated	Each Operating Shift	Not stated	Frequent, using a variety of methods	Frequent, using a variety of methods	Not stated
TOTAL PARTICULATE COUNT	3,500/m^3 (> 0.5 µm) 100/cu. ft.	100/cu. ft. (> 0.5 µm)	3,500/m^3 (equal to or above 0.5 µm) 0/m^3 (> 5 µm)	3,500/m^3 (equal to or above 0.5 µm) 0/m^3 (> 5 µm)	350,000/m^3 (equal to or above 0.5 µm) 2,000/m^3 (> 5 µm)	3,520/m^3 (equal to or above 0.5 µm) 29/m^3 (5.0 µm)
AIRBORNE VIABLES	Not stated	0.1 CFU per cu. ft.	Not stated	<1 CFU/m^3 Settle plate 90 mm <1 CFU/4 hours	<10 CFU/m^3 Settle plate 90 mm 5 CFU/4 hours	Not stated
SURFACE VIABLES (except floors)	Not stated	3 CFU per contact plate*	Not stated	<1 CFU per contact plate (no distinction for floors and walls)	5 CFU per contact plate (no distinction for floors and walls)	Not stated
SURFACE VIABLES (floors)	Not stated	3 CFU per contact plate	Not stated	<1 CFU per contact plate (no distinction for floors and walls)	5 CFU per contact plate (no distinction for floors and walls)	Not stated
PERSONNEL GOWN	Not stated	5 CFU per contact plate	Not stated	Not stated	Not stated	Not stated
PERSONNEL GLOVES	Not stated	3 CFU per contact plate	Not stated	Glove print 5 fingers <1 CFU per glove	Glove print 5 fingers 5 CFU per glove	Not stated
AIR VELOCITY UNIDIRECTIONAL	Not stated	Not stated	0.45 m/s ± 20%	0.45 m/s ± 20%	Not appropriate	Not stated
FREQUENCY OF ΔP MONITORING	Not stated	Each shift	Not stated	Continuous	Continuous	Not stated

ΔP= Differential pressure *Contact plate areas vary from 24–30 cm^2

Comment: Fed-Std-209E indicates that SI names and units are preferred for naming and describing the classes, but that English (U.S. customary) units may be used. With the publication of ISO 14644-1 and 14644-2, it is expected that Fed-Std-209E will be retired by the end of 2001.

Grade A **Terminally sterilised:** Filling of terminally sterilised products, when unusually at risk.
Aseptically prepared: Aseptic preparation and filling. Handling of sterile starting material and components. Transfer of partially closed containers in open trays.

Grade B Background for grade A. Transfer of partially closed containers in sealed trays.

Table 2: Class 10,000 Monitoring Table (Max. values are given).

COUNTRY DOCUMENT	U.S. FS 209E	U.S. USP <1116>	EU (at rest, static)	EU (operational, dynamic)	ISO 14644-1
CLASSIFICATION	M 5.5 (10,000)	M 5.5	C	C	7
FREQUENCY	Not stated	Each Operating Shift	Not stated	Not stated	Not stated
TOTAL PARTICULATE COUNT	353,000/m^3 (≥ 0.5 µm) 10,000/cu. ft.	10,000/cu. ft. (≥ 0.5 µm)	350,000/m^3 (equal to or above 0.5 µm) 2,000/m^3 (>5 µm)	3,500,000/m^3 (equal to or above 0.5 µm) 20,000/m^3 (>5 µm)	352,000/m^3 (equal to or above 0.5 µm) 2930/m^3 (>5 µm)
AIRBORNE VIABLES	Not stated	0.5 CFU per cu. ft.	Not stated	100 CFU/m^3 Settle plate 90 mm 50 CFU/4 hours	Not stated
SURFACE VIABLES (except floors)	Not stated	5 CFU per contact plate*	Not stated	25 CFU per contact plate	Not stated
SURFACE VIABLES (floors)	Not stated	10 CFU per contact plate	Not stated	Not stated	Not stated
PERSONNEL GOWN	Not stated	20 CFU per contact plate	Not stated	Not stated	Not stated
PERSONNEL GLOVES	Not stated	10 CFU per contact plate	Not stated	Not stated	Not stated
FREQUENCY OF ΔP MONITORING	Not stated	Each shift[1] 2x/week[2]	Not stated	Not stated	Not stated

ΔP= Differential pressure *Contact plate areas vary from 24–30 cm^2

Comment: Fed-Std-209E indicates that SI names and units are preferred for naming and describing the classes, but that English (U.S. customary) units may be used. With the publication of ISO 14644-1 and 14644-2, it is expected that Fed-Std-209E will be retired by the end of 2001.

Grade C **Terminally sterilised:** Preparation of solutions, when unusually at risk. Filling of products.
Aseptically prepared: Preparation of solutions to be sterile filtered.

[1] Adjacent to Class 100
[2] Support Areas - Product

Table 3: Class 100,000 Monitoring Table (Max. values are given).

COUNTRY DOCUMENT	U.S. FS 209E	U.S. USP <1116>	EU (at rest, static)	EU (operational, dynamic)	ISO 14644-1
CLASSIFICATION	M 6.5 (100,000)	M 6.5	D	D	8
FREQUENCY	Not stated	Twice/week	Not stated	Not stated	Not stated
TOTAL PARTICULATE COUNT	3,530,000/m^3 (≥ 0.5 μm) 100,000/cu. ft.	100,000/cu. ft. (≥ 0.5 μm)	3,500,000/m^3 (equal to or above 0.5 μm) 20,000/m^3 (>5 μm)	Not defined	3,520,000/m^3 (equal to or above 0.5 μm) 29,300/m^3 (>5 μm)
AIRBORNE VIABLES	Not stated	2.5 CFU per cu. ft.	Not stated	200 CFU/m^3 Settle plate 90 mm 100 CFU/4 hours	Not stated
SURFACE VIABLES (except floors)	Not stated	Not stated	Not stated	50 CFU per contact plate	Not stated
SURFACE VIABLES (floors)	Not stated	Not stated	Not stated	Not stated	Not stated
FREQUENCY OF ΔP MONITORING	Not stated	Weekly	Not stated	Not stated	Not stated

ΔP= Differential pressure *Contact plate areas vary from 24–30 cm^2

Comment: Fed-Std-209E indicates that SI names and units are preferred for naming and describing the classes, but that English (U.S. customary) units may be used. With the publication of ISO 14644-1 and 14644-2, it is expected that Fed-Std-209E will be retired by the end of 2001.

Grade D **Terminally sterilised:** Preparation of solutions and components for subsequent filling.
Aseptically prepared: Handling of components after washing.

3.0 SURVEILLANCE SUPPORT

The data should be collected in a manner that is in conformance with Current Good Manufacturing Practices (CGMP). CGMP states that the personnel supervising the environmental monitoring program should be competent in the scientific discipline and have appropriate training and authority. Equipment used should be calibrated, systems should be appropriately validated, media should be properly prepared, and all operational procedures should be written and followed.

Procedures should include appropriate controls to support their use. Cleaning, sanitization/disinfection, site selection, and frequency of testing are key components to a good environmental monitoring program. Alert and action levels should be based on individual sample sites, but one may also choose to specify alert level and action levels based on the number of excursions in one area/system for one sampling period. Establishment of appropriate alert and/or action levels and a system for monitoring implies that data obtained are subject to continual review and that alert and action decisions are made by designated, authorized personnel qualified to make such decisions. To effectively execute microbiological surveillance support systems, there should be a documented system in place for identifying excursions; in addition, there should be a feedback mechanism for verification of any action taken in response to data. All data should be documented and trended.

3.1 Cleaning and Sanitization/Disinfection

Implementation of cleaning and sanitization procedures is a critical component of overall facility control. Environmental monitoring data are used in determining the effectiveness of these procedures. It is common knowledge that the ideal sanitizer does not exist. Sanitizers that are effective against vegetative cells may be ineffective against spores. Sanitizers or disinfectants that are effective against spores are usually corrosive to equipment (e.g., acidified bleach on stainless steel) and should be used sparingly on an as-needed basis. Selection of sanitizers may include evaluation of required contact time, type of microorganisms that are to be eliminated, confirmation of efficacy, type of surface to be treated, toxicity, residue, and means of application. Validation of established cleaning and sanitization procedures should demonstrate microbial reduction. The procedures also ensure the effectiveness of removal of product and detergent residue. The goal is to demonstrate that routine sanitization procedures, performed by trained cleaning personnel, consistently result in a level of microbial control suitable for the intended use of the area. Sanitization procedures are verified for the effectiveness of microbial reduction. It is a sound practice to perform challenge testing of the selected sanitizers/disinfectants with isolates routinely recovered by the environmental monitoring program. This establishes the practical effectiveness of the disinfectants.

3.2 Sample Site Selection

Suitable sample sites vary widely depending on the clean room design and manufacturing process. Each process should be carefully evaluated when selecting sampling sites. The primary purpose of sampling should be to provide meaningful interpretable data that can help identify actual or potential contamination problems associated with specific procedures, equipment, materials, and processes. One should be able to sample those sites most likely to result in product contamination if they become contaminated; however, it may be prudent to identify indicator sites that are near, but not in contact with product.

Factors to consider in selecting sites for routine surveillance are:

1. At which sites would microbial contamination most likely have an adverse effect on product quality?
2. What sites would most likely demonstrate heaviest microbial proliferation during actual production?
3. Should site selection involve a statistical design (e.g., following the calculations in Federal Standard 209E) or should site selection be made on the basis of grid profiling? Should some sites for routine monitoring be rotated?
4. What sites would represent the most inaccessible or difficult areas to clean, sanitize, or disinfect?
5. What activities in the area contribute to the spread of contamination?
6. Would the act of sampling at a given site disturb the environment sufficiently to cause erroneous data to be collected or contaminate product? Should sampling only be performed at the end of the shift?

Note: There are some considerations applicable to specific types of monitoring; they are described in the individual monitoring sections of this document.

To establish routine sample sites, action and alert levels, and testing frequency, one should take into consideration the extent of contact or exposure that each element of the manufacturing environment has with the product. Sites having greater opportunity for contributing bioburden to the product should be sampled and monitored. Product contact sources may include compressed gases, room air, manufacturing equipment, tools, critical surfaces, storage containers, conveyors, gloved hands of personnel, and water. Examples of non-product contact sources may include walls, floors, ceilings, doors, benches, chairs, test instruments, and pass-throughs.

It must be recognized, however, that it may not always be practical to select a site at the most critical location. One should consider whether critical site monitoring would actually increase probability of product contamination. Additionally, critical sites may not be monitored if there is a low probability of contamination during processing (e.g., sterilized components which are not manipulated).

As pointed out in other sections of this document, there are many considerations in establishing an appropriate site for sampling (e.g., facility design, line configurations, validation data, process, historical data, test methodology, etc.). The sites listed in this section may or may not be applicable to a particular manufacturing process; factors pertaining to site selection are likely to be unique to individual facilities.

3.3 Sampling Frequency

Monitoring requirements may vary widely in the industry depending on several factors including, but not limited to, type of manufacturing process or product, facility/process design, amount of human intervention, use of subsequent terminal sterilization (including sterility test release versus parametric release), and historical profiles of the microbiological environmental data. No single sampling scheme is appropriate for all environments. In addition, changes in sampling frequency, whether temporary or permanent, may be required based on changes in practices, compendial requirements, development of significant microbiological trends, acquisition of new equipment, or nearby construction of rooms or utilities. The key is to select monitoring frequencies that can identify potential system deficiencies.

Examples of sampling sites.

System	Site
• Environmental air (filling line)	• Near open and/or filled containers
• Room air	• Proximal to work area
• Water	• Point of use
• Surface (facility)	• Floor, door handles, walls, curtains
• Surface (equipment)	• Filling line, control panels, stopper bowl
• Compressed air	• Site farthest from compressor
• Sterility test manifold	• Port closest to vacuum source
• Operator on filling line	• Finger impressions, at a minimum
• Laminar air flow (e.g., hood)	• Near high activity areas

The test frequency per site may be less frequent than the system or area frequency (e.g., one may choose to rotate sample sites). Test frequencies for batch-related, in-process monitoring may differ from those for routine area monitoring. In many cases, monitoring performed in conjunction with batch production may fulfill the requirements for routine area monitoring.

Prior to implementing any reduction in frequency, a summary of historical data, along with current and proposed sampling frequencies, should be reviewed and approved by the appropriate Quality Assurance personnel. After reduction, data should be reviewed periodically to determine if the reduced sampling frequency is still appropriate.

3.4 Alert and Action Levels

Environmental monitoring programs may have action levels established based on applicable guidelines and review of historical data. They frequently recommend that alert levels also be established. Some companies also choose to set levels for individual clean rooms or sample sites. Typically, the action levels will be driven by the regulatory or industry guidelines while the alert levels may be driven by historical analysis of the environmental monitoring data. The application of alert and/or action levels should follow written procedures and be employed in a consistent, non-arbitrary manner. To create consistency in treatment of alert and/or action levels, logical investigatory and/or corrective action steps should be pre-specified. Records should show that any excursion was recognized and that appropriate follow-up occurred.

Once alert and/or action levels have been established, they should be periodically reviewed as part of routine trend analysis. They may also be revised to reflect improvements, advances in technology, changes in use patterns, or other changes.

When no regulatory or industry guidelines are provided, alert and/or action levels may be derived statistically from historical data. An occasional excursion from these levels is to be expected at frequencies characteristic for the specific mathematical model utilized in their derivation. In some situations, only one level may be employed, with any excursions triggering action. In other instances, a level may be used with a single excursion eliciting an alert/action level response and multiple or sequential deviations requiring action.

These levels are conservative measures designed to signal potential or actual drift from historical or design performance characteristics. They are not extensions of product specifications, but are intended to flag changes so that corrective action may be taken before product quality is adversely affected. Not all situations require use of both alert and action levels.

Since there is no consensus as to the best mechanism to use for setting these levels, the following are approaches that have been used successfully within the pharmaceutical industry. Where compendial requirements exist, they supersede the methods used in the following examples.

a. Cut-off Value Approach

All the test data for a particular site are arranged in a histogram and the alert and action levels are set at values whose monitoring results are respectively 1% and 5% higher than the level selected. Other percentiles may be used in establishing levels. A variation is to take the last 100 monitoring results and use the 95th and 99th percentile values as the alert and action levels.

b. Normal Distribution Approach

This approach is best used for high counts only (a Poisson distribution is used for low counts). The mean and standard deviation of the data are calculated and

the alert and action levels are set at the mean plus two and three times the standard deviation, respectively.

c. Non-parametric Tolerance Limits Approach

In this approach, alert and action limits are set using non-parametric (distribution free) methods. This is valuable for environmental monitoring data that typically is not normally distributed, i.e., exhibits high levels of skewness towards zero counts. For the alert limit, the tolerance limit was set at a level of $\gamma = 0.95$ and $P = 0.95$. The action limit resulted from a tolerance limit set at $\gamma = 0.95$ and $P = 0.99$. These limits allow us to assert with confidence at least 95% that 100(P) or 99% of a population lies below the value, depicted by the stated limits for the respective data. For a discussion of this non-parametric procedure, see "Practical Nonparametric Statistics," 3rd edition, by W. J. Conover, page 150.

Other models based on negative binomial, Poisson, Weibull, or exponential distributions are possible. It may be appropriate to determine the model that best fits the data and use that model to set the levels. Typically, contamination in strictly controlled environments does not fall within a normal distribution. Environmental monitoring data may be evaluated to determine the suitability of the approaches to level setting.

3.5 Data Management (Data Collection, Analysis, Approach, and Interpretation)

Routine review and analysis of environmental monitoring data is essential to aid in the interpretation of process stability and assess overall control performance. Management should be kept abreast of trends and the subsequent state of operations within their facilities.

Based on the large number of samples tested by a given facility, a computer-based data tracking system is recommended. Prior to implementation, all database applications used should be validated/qualified for specific software applications.

3.5.1 Data Collection

Routine data may be pooled into a designated database in a consistent record format. The record format should include (at a minimum): monitoring date, specific sampling locations, sampling methods, colony forming units (CFU) or non-viable count results, identification performed, product lot information, and current action level. A manual data entry or image scanner system with advantages of speed and accuracy can be used to populate tables. Data integrity must be verified prior to analysis.

3.5.2 Data Analysis

Trends are often difficult to obtain and recognize, given the low colony forming unit (CFU) result usually obtained with viable environmental monitoring data. Histograms, defined as pictorial graphs characterized by a number of data points that fall within a common frequency, are a valuable tool. Different room classifications with definite requirements will produce different histograms. The CFU spread obtained across a Class 100,000 data set will not be observed in a data set from a Class 100 area. Therefore, each area (or area type) and accompanying data set must be viewed as distinct. A mathematical model could be applied not only with the objective in mind, but also the type of data to be analyzed.

Moreover, data collected in Class 10,000 or 100,000 areas tend to assume distributions. A Class 10,000 facility may lend itself to an exponential distribution where the majority of data points can be observed below the mean and thus appear not normally distributed; and a class 100,000 or non-classified area often demonstrates greater variability around the mean with a normal distribution. A Class 100 area distribution may be less obvious where an unsystematic approach, although less powerful, may work best.

The following table provides some examples of different analysis objectives and the associated descriptions of what the analysis may include.

3.5.3 Data Approach

The following approach describes a generalized method for data to assess the environmental control:

a. Determine objective of analysis (e.g., site location alert/action, action level review, management update).

b. Specify data set to be analyzed.

c. Apply data plots such as histograms or pictorial plots to access the basic data and to determine the nature of the distribution, if any. Such data plots can also be used to locate peculiarities such as outliers or patterns.

d. Observe the distribution and proceed with the appropriate mathematical model that best fits the overall objective. If data conform to a specific distribution, a parametric mathematical model may be applied. If the data are not consistent with a particular distribution, then a non-parametric approach may be applicable.

e. Typically, an action level at the 99th percentile is employed. Consistent with the action level at the 99th percentile are the following mathematical models. Models can only be applied if the character of the data assumes a definite distribution.

Action level estimate for a data set reflecting an exponential or non-normal distribution = 4.6 x (mean CFU)

Action level estimate for a data set reflecting a normal distribution = 2.33 σ + (mean CFU)

Note: When the action level is determined at the 99th percentile, an occasional excursion is expected due to the model applied.

f. Regardless of the statistical model chosen, the analytical method should be consistent with the data and documented in the data summary along with results.

Examples of possible analysis objectives and possible report descriptions.

Analysis Objective	Report Description
Using alert/action results to determine "corrective action"	Plot data over time to observe trends and process variation. Process control charts can be a useful tool. Modify cleaning, process or equipment.
Determine appropriateness of current alert/action levels	Calculate action level from historical data and compare to current. Action level derivations may be applied to adjust for more reasonable levels that are achievable with current operating procedures. (This may not always be possible if regulatory requirements are present.)
Management update, with periodic reporting. Annual report to comprise data summaries as well as process action level reviews	Routine report may include all monitored facilities/personnel data summaries with a list of current action levels, list of outliers and clusters or patterns, identifications, result ranges, sample totals, new action level derivations, and description of statistical method used for any calculations applied. Characterizations should also be included. Process capability and process control charts are often useful in assessing control/variation.
Determine process capability	Perform a quality study to determine specifications. Calculate action levels based on historical data. Histograms and process capability charts are useful tools.

3.5.4 Data Interpretation

Data generated should be summarized and evaluated to determine whether the production environment is in a state of control. Statistical process control is one method of performing this evaluation.

Trends may show a gradual increase or decrease in the overall counts observed over time, or a change in flora or counts on several plates of a particular area on a given day. Interpretation of the impact of a significant fluctuation in counts or a change in flora should be based on the experienced judgment of a qualified person.

Some considerations for assessing process state of control are listed below:

a. In assessing environmental monitoring process reliability, derived action levels reflecting higher values than those currently imposed may be indicative of a process specification that is no longer appropriate. A review of the process may be needed.

b. Several consecutive points or drifts may be considered to be a pattern or cluster formation that, if above the alert level, signals a trend that requires an investigation.

c. Significant fluctuations or jumps in the values for the process are also significant where recurring cycles may point to seasonal variations.

d. One or more values markedly higher or lower than the majority of the data may or may not be process outliers.

Understanding the potential impact of the results generated during environmental monitoring is critical to a successful environmental monitoring program.

3.6 Characterization of Isolates

Characterizing microorganisms recovered from environmental and personnel monitoring is an important part of surveillance programs. The characterization system selected by the laboratory should be defined in writing, including the frequency of characterization and the standard procedures for the methods.

Initially, many isolates may be characterized to establish a database of the microorganisms found in the area. Characterization may include any of the following examples: morphology, Gram stain, automated or manual identification systems. See Appendix B for additional information on identification systems.

Not all isolates need to be speciated, but they should be characterized sufficiently to develop a database. Once a database is established, the number of isolates characterized may decrease, but routine characterization should continue to determine whether isolates are part of the normal microbial flora or represent something different.

Characterization of isolates also may be useful in investigating situations such as positive sterility test results, positive media fill results, alert and action level excursions, or introduction of a common organism that may signal a developing resistance to a sanitizing agent. A change in the microbial flora or the introduction of a previously undetected species might signify a change in a system that should be investigated. Characterizations can be useful clues as to the possible source of isolates. For example, *Staphylococcus* species are commonly found on skin and the former *Pseudomonas* species are usually associated with water. (Many of these species have been re-classified, e.g., *Ralstonia pickettei*, *Buckholderia cepacia*, *Sterotrophomonas maltophilia*.)

The characterization of microorganisms is qualitative and relies on scientific training and good judgment. Microorganisms recovered from production environments may be highly stressed due to physical factors such as limited nutrients, contact with chemicals, or thermal stress. It may be difficult to obtain genus/species matches in identification system databases. The databases for commercial test kits and identification systems were designed originally for clinical isolates and may be incomplete with regard to industrial isolates; this may lead to misidentification of species or unidentifiable isolates. This area is continuing to be developed and enhanced.

3.7 Investigations/Corrective Actions

When excursions occur, there may be a drift from the baseline. An investigation is needed to determine what happened and what should be done to prevent a recurrence. Records should show that the excursions were recognized and appropriate follow-up occurred.

The overall purpose of the investigative action is to establish, to the greatest degree possible, a cause-effect relationship between the observed level of environmental quality and causes for the excursions (i.e., sources of contamination).

To create consistency in the treatment of excursions, investigative and/or corrective action steps should be pre-specified in a written plan. A progression of investigative/corrective actions or responses may be used in which sequential or multiple excursions require greater consideration than single or widely separate excursions. Likewise, excursions that occur in areas which are critical to the manufacturing process may require a more rigorous investigation and corrective action than those occurring in areas that are judged less critical to the integrity of the manufacturing process.

When an alert/action level is exceeded, the following actions may be appropriate:

- Notify the appropriate management.
  - Initiate an investigation to determine the causes and consequences of the excursion from the specified operating parameters.
- Perform corrective actions to address the problem, as needed. (A table of typical corrective actions follows.)
- Follow-up review to assess effectiveness of corrective action.

The previous listing is not all-inclusive, as these recommendations are only intended to suggest investigative activities and corrective actions when sampling and laboratory failures have been ruled out. Appropriate corrective actions are dependent upon the individual facility's design and process designs.

The reviewer may exert scientific judgment to postpone any corrective action until the result is confirmed and/or an investigation has been completed. It may also be appropriate to provide management with a routine summary of action level excursions for review. All corrective actions listed include an evaluation of the action for effect on the product.

3.8 Documentation

The following list includes items to be considered for documentation records:

a. Date and time of test
b. Test method/procedure reference
c. Activity level at site during test
d Equipment identification
e. Location
f. Area classification
g. Schematics of areas showing sample site locations
h. Sample site (critical or non-critical)
i. Test results
j. Evaluator of results
k. Date results read
l. Alert and/or action level
m. Temperature and duration of incubation
n. Control test results
o. Certification date, validation date, and expiration date of media used
p. Characterization of contaminants
q. Name of reviewer
r. Reporting of data
s. Review of historical data
t. Change control system
u. Calibration date on instrumentation
v. Methodology, analysis used to specify action/alert levels
w. System for documenting investigative/corrective action:
   (1) Description of deficiency
   (2) Possible cause(s) of problem
   (3) Identification of persons responsible for relevant corrective action
   (4) Description of action steps and their schedule for implementation
   (5) Evaluation of effectiveness of action steps

Typical corrective actions for different systems.

• **Compressed Gas System**	• Repeat test immediately. • Integrity test the filter. • Check and, if necessary, replace filter if excursion confirmed on retest. • Evaluate impact upon processed component and/or product.
• **Room Air/HVAC**	• Review level of personnel activity. • Review/perform air flow patterns/smoke tests. • Review aseptic technique of personnel. • Review gowning requirements for area. • Inspect incoming air filters for leaks in filter and pressure differential across filter. • Review room disinfection/sanitization procedures, sanitization intervals, and disinfectant efficacy. • Check area pressure differentials, particularly with respect to the last sanitization. • Evaluate mechanical equipment in area as possible source of contamination. • Evaluate integrity of the room (e.g., peeling paint, cracks in ceiling, walls, and floor). • Review risk to product.
• **Facility Surfaces**	• Perform investigation for possible sources of contamination. • Evaluate sanitization/disinfection practices review cleaning records. • Review possible unusual events during manufacturing operation. • Examine areas during usage. • Verify that controls were not circumvented. • Review risk of product contact. • Determine sensitivity of isolate to disinfectants being used. • Review isolates for occurrence in other types of tests.
• **High Purity Water Systems (WFI, clean steam, purified water)**	• Examine endotoxin and water chemistry data for system. • Examine bioburden data for other samples or sites in system - port contamination vs. system contamination. • Review efficacy of sanitization procedure and schedule. • Inspect system preventive maintenance records. • Verify integrity of sample collection and use procedures. • Inspect system for dead-legs, proper sloping, proper sample port design and location. • Evaluate impact upon processed component and/or product.
• **Personnel Gowning (gowning and gloves)**	• Evaluate possible operator impact upon product. • Review sterility test data. • Review other environmental monitoring data for area. • Review preparation and expiry dates for disinfectants used on gloves. • Identify all morphologically unique isolates (human vs. environmental). • Evaluate training of operator. • Interview operator for potential causes. • Retrain/requalify operator.

4.0 SYSTEM SURVEILLANCE

4.1 Introduction

4.1.1 Terminal Sterilization

The terminal sterilization environmental control program is concerned with microbial flora that contributes to the bioburden and endotoxin content of the product prior to sterilization. This includes distilled water, sterilizer cooling water, treated water and city water. Air, surfaces, and microbial levels of containers and closures are also routinely monitored. While control of the environment in which the products are prepared is important, the most critical aspect of the program is the bioburden of the filled product to be sterilized. Controlling this aspect of the manufacturing process ensures that the spore (heat resistant) bioburden levels presented to the product sterilization cycle do not exceed the validated capabilities of the process and that the desired sterility assurance levels are achieved.

4.1.2 Aseptic Filling

The aseptic environmental control program is specifically designed to determine the number and type of microorganisms associated with direct assembly or preparation of product prior to sealing of the filled containers. The number of sample sites and frequency of monitoring are generally greater than that monitored for established terminal sterilization processes. Air, water, personnel, compressed gases, floors, walls, machinery, and other surfaces within the filling room are routinely monitored. Adequate environmental control is an integral part of the aseptic manufacturing process and a critical factor in contributing to sterility assurance. A review of the routine environmental control data should be included in the manufacturing documentation for aseptically filled products.

4.1.3 Isolation Technology

The environmental control program for aseptic filling isolator systems may be similar to that used for a conventional aseptic filling operation with the exception of surface and personnel monitoring. After sufficient data is collected, routine surface and air monitoring may not be warranted if a validated sanitization cycle exists for the interior surfaces of the isolator. However, particulate air sampling might be performed routinely if the product might be adversely affected by higher than normal environmental particulate levels. Surface monitoring may be used during initial validation runs to support the effectiveness of the sanitization cycle and maintenance of clean isolator surfaces between sanitization cycles. If surface monitoring is performed, it should be done after the completion of filling so as to not introduce any extraneous contamination or residual growth media during the filling operation. Monitoring of personnel is not required for isolator systems, however, monitoring of isolator gloves/half-suits should be considered.

4.2 Water Monitoring

Water is a widely used substance, raw material, or ingredient in the production, processing, and formulation of many pharmaceutical products. Control of the microbial quality of water is of great importance in the pharmaceutical manufacturing facility since it may be used for formulating product, as well as for various washing and rinsing processes. Once a water system is validated and shown to be in a state of control, appropriate samples should be taken from the holding and distribution system to assess the microbiological quality of the water for its intended use. As pointed out in other sections of this report, there are many considerations in establishing an appropriate site for sampling (e.g., facility design, line configurations, validation data, process, historical data, test methodology, etc.). For additional information, see the Appendix C.

In the United States, the source or feed water should meet the requirements of the National Primary Drinking Water Regulations (NPDWR) (40 CFR 141) issued by the Environmental Protection Agency (EPA). There is a corresponding EU drinking water standard. These requirements ensure the absence of coliforms.

Note: the plate count methodologies described below were obtained from the Standard Methods for the Examination of Water and Wastewater, 19th edition.

It is recognized, however, that other combinations of media, time, and temperature of incubation can be appropriate. Recommended methodologies from “Water for Pharmaceutical Purposes” general information chapter <1231> of USP 24 are described below.

Drinking Water (City Water and Potable Water)

Residual chlorine in the potable water needs to be neutralized with sodium thiosulfate.

Sampling - Collect samples in a manner consistent with manufacturing practices. For example, if use points are routinely flushed prior to use, it is appropriate for samples to be collected with the same flush cycle. On the other hand, if use points are not normally flushed, there should be no flush prior to sample collection. It is also recommended to sample through hoses and not directly from the tap if manufacturing practices require the use of hoses. Do not sample from leaking taps (leaking taps should be repaired prior to use for processing and testing). Carefully choose distribution system sample locations to demonstrate microbiological quality throughout the distribution system. Start microbiological examination of water promptly after collection. If immediate processing is not possible, refrigerate samples at 2° - 8°C upon receipt in the laboratory. Time elapsing between collection and examination generally should not exceed 24 hours.

Similarly, purified water and water for injection systems should be monitored at sufficient points and with sufficient frequency to ensure appropriate microbiological quality is maintained throughout the system and at all points of use.

4.3 Compressed Gas Monitoring

The use of compressed air and compressed gas in aseptic environments may adversely affect the environmental conditions if appropriate precautions, routine testing and critical controls are not designed into the system. The following points should be considered:

- Compressed gases used to pressurize or blanket product in sterile holding tanks should be introduced via hydrophobic vent filters and monitored at a frequency that assures that the gas does not challenge the bacterial retention of the filter.
- Compressed air/gas that is used in aseptic environments should be filtered through sterilizing-grade filters and tested on a frequency that assures that the air/gas does not adversely effect the environment.
- All compressed air connections which do not affect the air to the workspace should be monitored with less frequency, however, any connection which introduces air to the environment should be monitored on a frequency as to assure the conditions of the environment class.
- A medium used for evaluation and incubation and rendering evaluations should follow the standard practice as is done for normal monitoring sites.

4.4 Air Monitoring

A comprehensive environmental monitoring program should include routine monitoring of both viable and non-viable airborne particulates. Viable particulates are generally of most concern in sterile product manufacturing environments, however, non-viable particulates should also be monitored as a reliable indicator of the proper function of the environmental control systems. Viable bacteria derived from people are typically associated with skin flakes, so higher non-viable particulate counts may be indicative of increased viable counts. Current techniques for monitoring viable particulates in air are limited by: (a) the equipment available, (b) the time necessary to demonstrate the presence of microorganisms in the sample of air taken, (c) the inability to re-sample the environment in a timely fashion when results warrant, and (d) difficulties in continuously monitoring the environment due to considerations such as drying out of the culture media.

Although the use of high efficiency particulate air (HEPA) filters to remove particles from the air is a very effective way to reduce the particle load in an environment, especially under static conditions, normal activity levels of equipment and people in a room may greatly reduce their effectiveness. People are a major contributor of viable and non-viable particulates to the environment. The intent of an airborne environmental monitoring program is to determine if there are viable and/or non-viable airborne particulates in locations that would allow them to settle on product contact surfaces and thereby find their way into process intermediates or final product. FDA expects monitoring under dynamic conditions (1), however outside of the United States, static monitoring may be necessary in addition to dynamic monitoring to satisfy regulatory requirements.

(1) Roscioli, Nancy A., Carolyn A. Renshaw, Alicia A. Gilbert, Christina F. Kerry, and Peter G.Probst, "Environmental Monitoring Consideration for Biological Manufacturing," *BioPharm*, pp. 32-40, September, 1996.

For most older-model samplers, the sampling volume is less than one cubic meter. A sampling volume of ten cubic feet is considered insufficient in Europe. Many of the newer model samplers are also capable of sampling one cubic meter.

4.4.1 Non-Viable Monitoring

Monitoring of non-viable airborne particulates is a necessary component of an environmental monitoring program. Such monitoring demonstrates control of potential contaminants in the environment to which the product, during the manufacturing process, is exposed. Classification of production areas is generally made based upon the level of non-viable particulates in the air.

Federal Standard 209E describes, in detail, classification of air cleanliness for clean-rooms and clean zones based on specified concentrations of airborne particulates. It prescribes methods for verifying air cleanliness in the traditional particulate size range(s) and also with respect to ultra-fine particles. This document has been commonly referenced with respect to non-viable particulate monitoring in the pharmaceutical, biological, biotechnology, and medical device industries as well as the electronics industry. More recent publications on the classification of air cleanliness are the ISO 14644 series of standards on "Cleanrooms and associated controlled environments," and ISO 14698 series of standards on "Biocontamination in a clean room environment." Following the publication of the ISO 14644-1 and 14644-2 standards, Federal Standard 209E is expected to be retired (as a standard for conducting business with the US government) by the end of 2001.

The 1987 FDA aseptic processing guide recommends daily monitoring for non-viables during operations, and in the United States, monitoring non-viable particles equal to or larger than 0.5 μm during routine manufacturing operations is common (exceptions include aseptic powder filling operations). Although monitoring particles in different size ranges may seem prudent, particles of 0.5 μm and larger are generally recognized as indicators of environmental contamination. Requirements outside of the United States may also include monitoring 5.0 μm particles.

A commonly used monitoring method is optical particle counting. It is based on the principle of passing an aerosol through a focused light source, which results in light scattering from single particles by refraction, reflection, and diffraction. In this way, both the size, based on the intensity of the scattered light, and the number of particles can be measured simultaneously. This method provides real-time data on the environment and provides a useful tool to demonstrate that the environment remains in a state of control with respect to particulate contamination.

Selection of an optical particle counter for use in a clean room or other controlled environment is typically based on such factors as sensitivity, flow rate, particle size range, portability, data storage capability, alarm capability, construction, and sanitization compatibilities. Although there are technical differences between instruments from different manufacturers, it is generally accepted that these instruments are interchangeable. However, when switching from one manufacturer's instrument to another's, it may be prudent to assess whether a change in alert or action levels is indicated, due to differences in equipment sensitivity.

In addition to portable particle counters, systems have been developed for permanent installation in manufacturing areas to allow continuous monitoring of the manufacturing process with centralized data storage and alarm capabilities.

4.4.2 Viable Monitoring

Microbes in air are generally associated with solid or liquid particles. These particles may consist of a single unattached cell or more commonly as clumps of organisms. Organisms may adhere to a dust particle or other "raft," or, if unattached, exist as a free-floating particle suspended in the air. These particles may remain suspended in the air for extended periods of time due to the local air currents. HVAC systems in controlled environments are designed to remove these particles through frequent air changes or with unidirectional airflow in critical areas.

Although total particulate determinations can be useful in monitoring air quality in a pharmaceutical, biotech, biological, or medical device facility, viable airborne contamination is of primary importance in manufacturing environments that require control of bioburden in the final product. This is particularly true

for aseptic production processes, although it applies to all production processes requiring control of viable contaminants in the final product (including those used to manufacture terminally sterilized products).

4.4.2.1 Sites

The principles previously mentioned for site selection in Section 3.2 are applicable. However, in addition to these general considerations for sampling site selection, there are considerations more specifically aimed at airborne monitoring. A monitoring location specified for critical areas (i.e., Class 100, laminar flow) by the 1987 FDA *Guideline on Sterile Drug Products Produced by Aseptic Processing* is not more than one foot away from the work site, and upstream of the air flow, during filling/closing operations. It is important to consider air flow patterns in choosing these critical sampling locations, as well as the introduction of potential contaminants by environmental monitoring personnel, equipment, and practices. The potential for contamination of the product due to the necessity of monitoring must be considered and avoided.

Additional monitoring locations should be chosen based upon a defined rationale for the remainder of the room in which the process is occurring. This can be based upon initial validation/qualification sampling of the environment, personnel flow, and processing activity levels.

4.4.2.2 Methods

The FDA currently expects active air sampling of environments on a routine basis to demonstrate control of possible viable airborne particulates (see reference, Section 4.4). Therefore, although useful in some circumstances, passive methods such as settling plates are not generally recommended for such monitoring programs in the United States. Generally, quantitative sampling methods are required, with operating levels being defined per unit volume of air.

Presently, several countries outside the United States require the use of settling plates as well as active air sampling. Thus, an airborne monitoring program may require the use of both active and passive air sampling methods to satisfy the requirements of the countries in which the final product will be sold. Settling plates may also be useful for monitoring isolators or laminar airflow cabinets.

4.4.2.3 Equipment

A number of types of viable airborne sampling devices are currently used routinely in the industry, and others are available for particular uses such as viable particle size distribution. The most commonly used types of equipment will be presented here to attempt to provide an overview of the advantages and disadvantages associated with each instrument. These considerations are, of course, subject to individual interpretation, specialized uses, and application to traditional clean rooms or to barrier/isolation systems.

Generally, active air samplers are used for monitoring viable airborne contamination levels in production facilities. These instruments allow the measurement of known volumes of air, allowing quantification of airborne viable contaminants by unit volume of air.

The most widely used instruments are of the solid culture medium impaction type. These include the following categories and representative instruments:

1) Slit Impactors

Slit-to-Agar (STA) Air Sampler

The slit-to-agar air sampler utilizes a revolving agar plate at a precise distance from a slit-type orifice to impinge the air sample (and particles) directly onto the surface of a solid nutrient collection medium.

Advantages:

- Measures a large volume of air
- Time-concentration relationship is available
- Remote sampling probe can be used
- Can be used for sampling compressed gases

Disadvantages:

- Equipment is large and cumbersome
- Some equipment cannot be steam sterilized
- Some systems require 150 mm agar plates

2) Sieve Impactors

Surface Air Sampler

The SAS air samplers operate on the principle that air is drawn into the unit by means of an impeller, is drawn over the surface of a contact plate, and is exhausted.

Advantages:

- Convenience
- Speed
- Portability and flexibility
- Self-contained power supply
- Perforated cover plate can be steam sterilized
- Measures a large volume of air
- Uses standard contact plates
- Airflow can be calibrated

Disadvantage:

- Equipment is somewhat cumbersome

Surface Vacuum Sampler

This sampler utilizes a simple stainless steel chamber containing a Petri dish filled with nutrient collection medium. An air sample (and particles) is drawn across the surface of the plate using a vacuum source, thereby depositing the particles onto the surface of the solid medium. A centrally installed system and a portable system are also available.

Advantages:

- Small size allows relatively easy placement along filling lines and in small areas and enclosures
- Entire sampling unit can be steam sterilized
- Can be used for sampling compressed gases
- Can be remotely placed in small isolators
- Airflow can be calibrated
- Able to sample large volume of air

Disadvantage:

- Equipment is somewhat cumbersome (with vacuum source)

3) Centrifugal Impactors

Centrifugal Samplers

These air samplers operate on the principle that air is drawn into the unit by means of an impeller and the particles are deposited on the surface of a solid nutrient collection medium (strip) by centrifugal force.

Advantages:

- Convenience
- Speed
- Portability and flexibility
- Self-contained power supply
- Head assembly can be steam sterilized
- Measures a large volume of air
- Airflow can be calibrated

Disadvantages:

- Single source for media strips
- Direct calibration of sampling volume not possible
- Laboratory handling of media strips is atypical (i.e., requires more handling inserting and removing the strip into the head)
- Potential disruption of laminar airflow by turbulent input and exhaust air

4) Filtration

This method uses an air sampler which employs a vacuum source to draw air through a filter where particles are collected on the filter. The filter is aseptically removed for culturing in the laboratory on an appropriate nutrient medium.

Advantages:

- Measures a large volume of air
- Wide choice of filter media and pore sizes available
- Use of gelatin membrane filters may be useful to overcome desiccation of collected microorganisms
- Filter holder is sterilizeable

- Airflow can be calibrated
- Usable in isolators

Disadvantages:

- Membranes with collected samples must be placed on nutrient media for enumeration of viable microorganisms
- Equipment is somewhat cumbersome

5) Liquid Impingement

In this method, air is delivered through a tube whose outlet is submerged beneath a liquid collection medium. Viable particles are impacted into the liquid medium while the gas phase rises and is removed from the system.

Advantages:

- Allows samples with high viable counts since liquids can be diluted before sampling
- Allows choice of collecting medium such as Phosphate Buffered Saline (PBS) or media (media may require anti-foaming agent)
- Measures vegetative cells and spores
- Vegetative cells are more apt to survive in the liquid media
- Inexpensive

Disadvantages:

- High velocity impingement could destroy vegetative cells
- Sample handling may cause contamination
- Breakable glass components

6) Settling Plates or Liquid Media

This method involves the use of settling or fallout plates. There is a minimum and maximum time for use that must be determined/qualified. This method of air sampling utilizes a simple system of solid nutrient collection medium in a Petri dish, which is directly exposed to environmental conditions. Particles in the air settle out on the agar surface where they can be counted directly, after incubation. In general, settling plates are used in conjunction with active (volumetric) air sampling to yield a broader picture of the environment.

In the settle bottle, a liquid medium is used rather than an agar, which minimizes desiccation during extended sampling times. With the advent of isolation technology, the use of settling plates and bottles are becoming more prevalent due to their smaller size.

Advantages:

- Ease of use
- Economical
- Virtually any media can be used
- Small size allows relatively easy placement along filling lines and in small areas and enclosures such as biosafety hoods
- Allows “continuous” monitoring over prolonged periods of time by changing plates
- No power connection required
- Settle bottles are essentially impervious to poisoning by sterilizing gases used in isolators

Disadvantages:

- Generally considered semi-quantitative at best for settle plates, (+) or (-) for settle bottles
- Microbial count cannot be correlated with air volume
- Particle deposition is affected by the size of the particles, temperature, and flow/volume of air passing across its surface
- Plates can desiccate if left exposed for too long a period

4.4.3 Surface Monitoring

4.4.3.1 Introduction

In addition to conducting viable air monitoring to determine the microbial bioburden surrounding the manufacturing operations, surface monitoring is conducted to determine the microbial bioburden of surfaces within the manufacturing area as well as on equipment and product contact surfaces.

4.4.3.2 Methodology/Test Method

The method of testing should be considered when the sampling plan is established. Care should be taken to consider the limitation in accuracy and reproducibility

when choosing a method; influential factors include suitability for the surface type, criticality of the surface, and the type of information provided. The type of media used will influence the detection of representative flora from the sample site. Neutralizers may be added in the media to inactivate surfaces treated with chemical disinfectants.

The basic methods include contact plates, swabs and surface rinses. Each provides data that can be used to determine the impact (if any) on product quality. Testing methods can provide qualitative or quantitative information. Also, the accuracy of the sampling is impacted by the collection and handling of samples so proper training is essential to an effective sampling and testing program.

4.4.3.2.1 Contact Plates

Contact plates are commonly used because they are easy to use and they provide quantitative results. The plates are typically 50mm in diameter and are filled so that the media forms a dome. The media may contain a neutralizing agent, depending upon its intended use. The surface of the media is pressed against a flat surface, resulting in a sampled area of approximately 25 cm^2. The sample plate is then placed in the incubator for the required period of time. Colonies, if present, are counted at the end of the incubation. Some of the disadvantages of this method are: (a) it is not suitable for irregular surfaces, (b) if the media is wet, microorganism confluence can occur, and (c) media residue must be removed from the sample site.

4.4.3.2.2 Flexible Films

Media can be deposited on a flexible substrate which can be used in an identical manner to that employed for contact plates. These films can also provide a defined sampling area. The surface of the media is pressed against a flat surface. The exposed film is then placed in the incubator for the required period of time. Colonies, if present, are counted at the end of the incubation. Some of the disadvantages of this method are: (a) it is not suitable for irregular surfaces, (b) if media is wet, microorganism confluence can occur, and (c) media residue must be removed from the sample site.

4.4.3.2.3 Swabs

This method is employed for equipment and irregular surfaces for which contact plates are not suitable. This method can be used on flat surfaces, provided a template is used to define the sample size – usually approximately 2 inches x 2 inches (approximately 25 cm^2).

Types of swabs that can be used for this method include cotton, Dacron™, and calcium alginate materials with the appropriate diluent. The cotton and Dacron™ swabs can be used to provide qualitative results by placing the used swab into broth media. They also can be used quantitatively and allow for diluting highly contaminated samples. Calcium alginate swabs, used with transport media, allow for the dissolving of the swab fiber, thus releasing the organisms into the solution for plating. Quantitative samples can be tested by the pour plate or membrane filtration method. Some disadvantages to this method are: (a) technique and sampling can affect results, and (b) requires manipulation to culture the sample.

4.4.3.2.4 Surface Rinse Method

This method is best used for large surface areas where the interior surface bioburden needs to be determined. This includes kettles, equipment trains, and tanks. Sterile water is typically the fluid that comes in contact with the interior surfaces; it is then collected and tested by membrane filtration to yield a quantitative result. Some disadvantages are: (a) it is not suitable for many applications, (b) it requires extensive manipulations, and (c) techniques and sample processing can affect results.

Surface monitoring is a critical part of a viable environmental monitoring program that is employed to ensure the effective control of the aseptic processing area. The design of the program requires knowledge of the process in order to provide a meaningful sampling plan.

4.5 Personnel Monitoring

4.5.1 Description

Personnel are a primary source of contamination in an aseptic environment. It is therefore essential that all employees entering an aseptic environment be carefully selected and adequately trained so they can perform their required tasks in a well-disciplined manner. This training should include personal hygiene, an introduction to microbiology, aseptic techniques, and gowning. After an individual has been trained, routine microbiological monitoring of garments and finger impressions should be completed to assess the ongoing practice of aseptic technique.

4.5.2 Training/Certification of Personnel for Aseptic Manufacturing Area

Training/certification of aseptic area personnel may include but is not limited to, the following subject areas:

a. Personal hygiene/habits

- Cleanliness of hair, skin, fingernails, and clothing
- No make-up, nail polish, sculptured fingernails, glue-on nails, gum, candy
- No eating, drinking, chewing, or smoking

b. Illness

- Report all colds, flu, infections, wounds, or sunburn
- Report all disease or chronic skin conditions

c. Clothing

- Dedicated plant or area uniforms required
- No watches or protruding jewelry
- Protective clothing required

d. Introduction to microbiology

- Common sources of microorganism types

e. Introduction to aseptic techniques

f. Gowning practices

- Personnel are documented to properly gown (i.e., not add contamination) via gowning certification.
- Gowning certification may include additional sampling sites beyond those routinely monitored – the forehead, mask, neck area, back of head, garment zipper, arms, fingers.
- Routine monitoring may include garment samples from both forearms, and finger impressions from both hands. Overall profiles may also be evaluated.

g. Participation in media fills to demonstrate aseptic skill level.

All training and certification activities should be documented and kept as part of the employee file.

4.5.3 Retraining

Gowning Certification

If samples from garment or finger impressions (dabs) exceed the alert/action level, the employee should be retrained on all appropriate procedures and re-certified before entry into the aseptic area is approved.

Routine Monitoring

If samples from garment or finger impressions exceed the action level, it may require that the employee should be retrained on appropriate procedures and re-sampled at the earliest possible time. If a trend of over alert/action level occurrences develops, further corrective action, which may include complete re-certification or reassignment to new duties outside the aseptic area, may be considered.

Annual retraining and re-certification should occur for all employees required to work in an aseptic environment. In addition, all employees involved in aseptic manufacturing should participate in a process simulation test (media fill) at least annually. All retraining and re-certification activities should be documented and kept as part of the employee file.

4.6 Product or Component Bioburden

Product or component bioburden monitoring is not considered part of all environmental monitoring programs. Bioburden testing is performed on a non-sterile product to determine its microbial load. The intended use

of the product, the nature of the product (growth promoting product which is held during processing), or the manufacturing process used may dictate the establishment of acceptance levels and the exclusion of objectionable microorganisms. Listed below are some factors that may impact product or component bioburden:

- Raw material source: Bioburden may range from very high (derived from natural sources) to zero.
- Water: It is often the highest volume raw material in product formulations.
- Components: Various grade glass or plastic components can be obtained either sterile or non-sterile.
- Manufacturing environment: It should not adversely affect product quality.
- Processing of formulation: Formulations incorporating filtration steps or requiring heating for dissolution may reduce bioburden. Other manufacturing steps such as timed storage at ambient temperature may increase bioburden.
- Equipment: The equipment used and its level of cleanliness will impact final product bioburden.
- Antimicrobial activity: The presence of preservatives and the antimicrobial properties of the raw materials used will determine the formulation susceptibility to contamination.
- Water activity: Water activity (a determinant in preservative selection) is an indicator of formulation susceptibility to contamination.

4.6.1 Determination of Product or Component Bioburden

Product or component bioburden levels may be determined through various test methods. Some methods are listed below:

Pour plating
Spread plating
Membrane filtration
Most Probable Number (MPN)
Automated rapid microbiology systems

The test method used will be based on the level of sensitivity necessary to: (a) meet the established acceptance criteria, and (b) neutralize any anti-microbial property that may be inherent to raw materials or as a result of added preservatives. Some automated rapid microbiology systems give higher counts than manual methods, since they may include counts of non-culturable or injured organisms.

All relevant factors must be considered when establishing acceptance criteria for product and component bioburden. An acceptable bioburden level is that which does not adversely affect product quality.

For many terminally sterilized products, bioburden counts alone do not provide sufficient information. It also may be necessary to assess the thermoresistance, or D-value, of the bioburden. Total bioburden counts that are within limits may cause a significant problem if the bioburden exceeds the thermoresistance anticipated for the sterilization model.

D-values can be determined using sophisticated equipment (thermoresistometer) with square wave heating, with heat-up and cooling times less than or equal to 10 seconds. For routine screening of bioburden, a heat shock or boiling water test can be used to rule out the presence of organisms exceeding a predetermined D-value.

4.6.2 Parametric Release and Bioburden

The acceptance of parametric release by the FDA in 1985 increased the importance of bioburden testing, characterization, and resistance of recovered microorganisms. FDA Compliance Policy Guide 7132a.13 issued in 1987 details the necessary criteria for parametric release. As defined in the policy guide, parametric release is a sterility release procedure based upon effective control, monitoring, and documentation of a validated sterilization process cycle in lieu of release based upon end-product sterility testing.

Major emphasis is placed on the resistance of recovered spore formers. Recovered spore formers with greater resistance than the indicator organism used in the cycle validation would render the batch non-sterile in terms of the guidance.

4.6.3 In-Process Testing

In-process environmental monitoring samples may be taken to evaluate:

- The ability of the equipment to perform within specified environmental quality standards
- Operator ability to maintain area cleanliness during process operations
- Effectiveness of cleaning for the facility and its equipment

This monitoring is typically performed in areas and during operations where product is potentially exposed to environmental or operator contamination, however, it is not always included for closed systems since the results may not have a correlation to product impact.

Process-related monitoring may include surface and air sites near aseptic connections or product transfer steps. The manufacturing operations monitored may occur in an open room, under laminar flow, or within a "closed" system. Sites should be chosen to demonstrate process integrity in both "open" and "closed" processes. Sample sites and levels also should be chosen to provide meaningful data about a given operation. As an example, non-viable particle counts taken during loading of powdered media into a vessel in a Class 100,000 area may not provide data that is indicative of process quality. Non-viable particle counts taken during aseptic processing operations (excluding powders) in Class 100 areas may provide more valuable information about process control.

The subsequent purification/bioburden reduction steps in a process may also impact the degree to which in-processing testing is warranted. Test frequencies for batch-related, in-process monitoring may differ from those for routine area monitoring. In many cases, environmental monitoring performed in conjunction with batch production activities may fulfill the requirements for routine area monitoring.

Surface and viable air samples that select for the host organism may be appropriate in a fermentation/recovery process area. This data may help to demonstrate process integrity and/or cleaning effectiveness during a product changeover.

The following table describes examples of different activities and possible sampling locations. The table is not meant to be all inclusive.

Process-related environmental monitoring activities and locations.

PROCESS	PROCESS ACTIVITIES TO CONSIDER MONITORING	LOCATIONS TO MONITOR
• **Fermentation/Primary Recovery**	• Inoculation of inoculum scale-up vessels • Inoculation of fermenter • Homogenization of harvest material • Product transfer operations (harvest of product)	• Connection points on transfer lines • Near seals and gasket on fermenter • Near pistons on homogenizer • Near centrifuges • Sterile additions/sampling ports
• **Purification**	• Loading of process vessels, chromatography columns • Collection of fractions • Pooling of fractions	• Air and surfaces near process activities where the product is exposed to the environment • Bench of laminar flow unit • Near fraction collection unit • Loading port of chromatography column or ultrafiltration skid
• **Formulation**	• Loading of formulation vessel • Addition of components during formulation • Sterilizing filtration process	• Opening of formulation vessel • Point of aseptic connection from formulation vessel to sterile bulk tank
• **Filling and Finishing Operations**	• Before filling (pre-fill) • Fill line set-up • During filling • Mechanical intervention on fill line • Loading of lyophilizer • After filling (post-fill)	• Fill room and adjacent support rooms which constitute the aseptic suite • Fill line at set-up during interventions • Areas of operator activity • Fill line during filling • Near container staging • At the filling nozzles • Near the stoppering mechanism • At the lyophilizer loading door • HEPA-filtered transfer carts • Fill line and aseptic suite surfaces post-fill • Operator gowns and gloves at end of shift; include janitorial staff

4.7 Environmental Monitoring During Routine Sterility Testing

Background
Sterility testing facilities should be designed and operated in an equivalent manner to aseptic processing areas. Environmental monitoring should be conducted in an active mode during each shift, with alert and action limits set that are comparable to those used in aseptic process areas in the manufacturing plant. Monitoring should be conducted to demonstrate continuous microbial contamination control, consistent technician performance and to obtain information concerning the possible source of the microorganisms associated with sterility failures.

Air Monitoring
Options include active samplers and/or settling plates. Air settling plates may be exposed on the work area during the sterility testing.

Surface Monitoring
The work surface and items that are not terminally sterilized should be routinely monitored using contact plates or surface swabs.

Personnel Monitoring
Gloves and gowns of personnel conducting the sterility tests should be routinely monitored.

Trend Analyses
In general, the recommended guidelines for Class 100 aseptic processing area can be employed as action levels. Alert levels may be set using historic monitoring data. Trend analysis should be undertaken by sterility test location and sampling site. Corrective action, in terms of review of environmental controls, sanitization, and technician training should be standardized in response to out-of-trend results. The environmental monitoring data should be compared to the first-stage sterility failures by sterility test location, product, and sterility testing technician.

5.0 VALIDATION/QUALIFICATION OF ENVIRONMENTAL MONITORING SYSTEMS

Under the scope of environmental monitoring, validation/qualification is required for classified environments and clean utilities, such as compressed gases and high purity water systems, depending upon the intended use. The specific validation requirements are specified in many regulatory and industry guidelines. For this document, validation and qualification are considered synonymous.

The validation requirements, including acceptance criteria, are typically described in procedures that are specific for each process or system being validated. An overview of some validation considerations is included in this section.

When the process or equipment design is changed or replaced, a partial or full validation may be required before the process can resume. Routine monitoring usually can continue under the same conditions as those under the original validation. Some companies choose to perform periodic revalidation or requalification, while others manage through a change control process to determine when revalidation is required.

5.1 Environment/HVAC Systems

Testing of classified environments within which the aseptic filling process is performed is divided into two basic types: static and dynamic. Environmental validation testing under static and dynamic conditions is performed to determine the ability of the system to provide an environment of acceptable quality.

The static condition provides for the monitoring of the area with all HVAC systems in operation, with all equipment in place, and with no personnel present. Performance tests executed under static conditions serve as baseline information to demonstrate that the areas can maintain a high quality environment with no personnel activity. Static testing also ensures that the environment is of acceptable quality prior to dynamic testing.

Testing under dynamic conditions provides for the monitoring of the area with all HVAC systems in operation, equipment in operation, and operational personnel present. The dynamic testing demonstrates that the area can maintain a high quality environment during routine manufacturing conditions. Prior to validating the complete environment/HVAC system, it is assumed that the

individual pieces of HVAC equipment have been validated.

Typical tests include:

a. Cleaning and sanitizing/disinfecting procedures utilize microbial surface monitoring to evaluate the effectiveness of the cleaning procedure to reduce the microbial level. (Cleaning and sanitization may be validated either separately or as part of the same protocol.)
b. Airborne non-viable particle count testing is performed to demonstrate that the manufacturing environment is maintained at a particle count level within the specified limits under both static and dynamic conditions.
c. Airborne viable particle count tests are performed to demonstrate that the airborne bioburden is within specified levels under both static and dynamic conditions.

Additional tests may be performed to verify the correct operation of the HVAC system and of the clean room.

5.2 Utilities

Utility systems are usually qualified initially and again when a substantial change has taken place. Since most companies trend the data from these systems on an ongoing basis, periodic requalification is frequently not performed. Alternatively, there are periodic reports assessing the trending data.

5.3 Validation of Aseptic Processes – Media Fills (Process Simulation Tests)

Media fills are useful in assessing the quality and process capability of aseptic process conditions and techniques in the manufacture of drug and diagnostic products by simulating aseptic processing, using microbiological growth media in place of product. Media fills are a good way to assess the total system for production and environmental monitoring.

Initial performance qualifications are conducted to validate new products, processes, or changes to filling operations. Initial process simulation tests should be performed after equipment qualification and sterilization validation is completed. Environmental monitoring data must also show that the room is functioning in the desired level of control. At least the same level of environmental monitoring performed for production should be performed during a media fill. Some regulatory agencies have specified detailed lists of environmental data to be collected during the media fill.

Routine performance requalifications are required to be performed for each aseptic process and filling line as well as each container/closure system. Typically, routine media fills are performed at least every six months. All personnel who may be in an aseptic area should take part in a process simulation test at least annually. Effective aseptic processing programs need to address the following:

- Worst Case/Interventions
- Media Growth Promotion Testing
- Incubation duration, temperature, and orientation of filled units
- Documentation
- Acceptance Criteria
- Investigation and Corrective Actions

For additional details, the reader is referred to PDA Technical Report No. 22, "Process Simulation Testing for Aseptically Filled Products," PDA Technical Report No. 24, "Current Practices in the Validation of Aseptic Processing – 1996," PDA Technical Report No. 28, "Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals," the 1987 FDA *Guideline on Sterile Products Produced by Aseptic Processing*, and the 1994 FDA *Guidance for Industry for the Submission of Documentation for the Sterilization Process Validation in Applications for Human and Veterinary Drug Products*.

6.0 CONCLUSION

The Task Force believes that this document can assist the reader in establishing the fundamentals of an environmental monitoring program related to facility control and compliance. Its intent was to serve as an aid in setting up a meaningful, manageable and defendable program.

NOTES:

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8.0 APPENDICES

APPENDIX A: Definitions

Essential to understanding this document is an appreciation of key environmental monitoring concepts. Definitions have been provided to help clarify the concepts. Some of the definitions vary between companies; however, the definitions described below are consistent for uses within this document.

Action Level
A level that, when exceeded, indicates a process has drifted from its normal operating range. A response to such an excursion should involve a documented investigation and corrective action.

Alert Level
A level that, when exceeded, indicates a process may have drifted from its normal operating condition. Alert levels constitute a warning, but do not necessarily warrant corrective action.

Aseptic Filling
Part of aseptic processing where a pre-sterilized product is filled and/or packaged into sterile containers and closed.

Aseptic Processing
Handling sterile materials in a controlled environment, in which the air supply, materials, equipment and personnel are regulated to control microbial and particulate contamination to acceptable levels

Barrier
A processing system which provides for some measure of separation between the critical zone and operating personnel.

Colony Forming Unit (CFU)
A single macroscopic colony formed after the introduction of one (or more) microorganism(s) to a microbiological growth medium.

Cleaning
Chemical or physical means used to remove soil and/or microorganisms from surfaces.

Continuous Monitoring
A process of data collection where conditions are monitored continuously. In most United States applications, this definition implies "during production." For ISO applications, this means twenty-four hours per day, seven days a week.

Controlled Area
Area where unsterilized product, in-process material, and containers/closures are manufactured or prepared. Different types and levels of controlled areas exist and, depending on their function, different class designations and resulting conditions are maintained.

Corrective Action
A response taken to an excursion or failure.

Critical Area
Area where sterilized products or containers/closures are exposed to the environment.

Critical Surface
Surfaces within critical areas that may come in direct contact with sterilized products or containers/closures.

Critical Zone
The surfaces or areas within a barrier that may come in direct contact or close proximity with sterilized products or containers/closures.

Disinfection
Chemical or physical inactivation of pathogenic microorganisms on inanimate surfaces.

D-value
The time, in minutes, at a specific temperature required to reduce the microbial population by 90% (or one [1] log) in defined conditions, e.g., method of sterilization (dry heat versus steam), solute, carrier, etc. It may also be referred to as thermal death time or lethality.

Dynamic Monitoring
Monitoring of an environment during normal operations, e.g., equipment operating, personnel present, and the process or simulated process is ongoing. Per the EU and ISO documents, this is synonymous with operational condition.

Environmental Control Parameters
Conditions and corresponding measurements as associated with facilities and equipment utilized in the manufacturing process which may impact the identity, strength, quality, or purity of a product. Among such parameters are air flow rates and patterns, pressure differentials, materials and personnel flow, temperature and relative humidity, as well as non-viable and viable particulates.

Frequent Monitoring
A process of collecting data where conditions are monitored at least once per hour. In most United States applications, this means during production. In most ISO applications this means twenty-four hours per day, seven days per week.

Grid Profiling
A process of dividing areas of equivalent classifications into "grids" for the purpose of uniformly assessing contamination characteristics in that area.

Heat Shock
A process where bacterial cultures are subjected to a heat stress (usually 80° C - 100° C) which causes heat-resistant spores to form, if the culture can produce spores.

Isolator
A flexible or hard-sided system which completely encloses an aseptic area. Access to the manufacturing or aseptic area is through glove ports/half suits or by access doors uniquely designed to maintain aseptic conditions.

Manufacturing Process
Manufacturing operations including, but not limited to, materials handling, product processing, fabrication and formulation, product filling and packaging, as well as sampling, testing, and inspection support functions.

Non-Viables
A term used in reference to particulates, which are not capable of living, growing, or developing and functioning successfully; "unable to divide."

Parametric Release
A sterility release procedure based upon effective control, monitoring and documentation of a validated sterilization process cycle in lieu of release based upon end-product sterility testing.

Process Control Parameters
Conditions and corresponding measurements associated with the manufacturing process which may have a potential for impact on the identity, strength, quality, potency and purity of a product. Examples of parameters of concern include bioburden, process rate, weight, volume, temperature, and pressure.

Risk Analysis
A determination made to assess the hazards and consequences associated with an occurrence.

Sanitization
Reduction of microbial contaminants to safe levels as judged by Public Health requirements.

Spore
A bacterial form highly resistant to adverse conditions.

Static Monitoring
Monitoring of the environment in the absence of normal operations, i.e., no equipment operating and no personnel are present - at rest. For Medicines Control Agency (MCA) regulated companies, this includes the equipment operating when no personnel are present. Per the EU and ISO standards this is synonymous with at rest.

Sterilization
Destruction of all microorganisms by exposure to chemical or physical agents, or to ionizing radiation.

Terminal Sterilization
A process where the drug product is sterilized in its final container.

Trend Analysis
A review performed either routinely or in response to an alert or action condition. This review provides an analysis of specific environmental monitoring data.

Vegetative Cell
A cell type that does not have the ability to protect itself from adverse conditions.

Viable
Capable of living.

APPENDIX B: Equipment Manufacturers and Suppliers

Note: *Not intended to be all-inclusive.*

Viable Air Samplers

Biotest Diagnostics Corp.	(RCS, RCS Plus)
Veltek Associates, Inc.	(SMA)
International pbi	(SAS, SAS Super 90)
Sartorius	(MD-8)
New Brunswick Scientific	(STA)
Mattson-Garvin. Barrimundi Corp.	(STA)
Millipore	(MAIRT)

Particle Counters

Climet
Met One, Inc.
Particle Measuring Systems (PMS)
HYIAC/ROYCO
TSI
Biotest Diagnostics
LaSair

Microbial Identification Systems

BioMerieux Vitek, Inc.	(Vitek)
MIDI, Inc.	(MIDI)

Biolog PE Applied Biosystems
Qualicon™, a DuPont Subsidiary
API

TOC Analyzers

Anatel
Sievers
Shimadzu

Isolators/Barriers

La Calhene, Inc.
The Baker Company
Containment Technologies Group, Inc.
Despatch Industries
Isolation Technologies, Inc.
Kuhlman Technology
Laminar Flow, Inc.
Liberty Industries
Plas Labs, Inc.
Clestra Cleanroom
TSI
HEMCO
Machine Kinetics M Braun
Merrick

Rapid Counting Methods

Chemunex Scan RDI Celsis

Data Reporting/Trending Software

Compliance Software Solutions

APPENDIX C: Examples of current typical environmental monitoring frequencies and levels - utilities. Guidelines for utilities monitoring.

	Source (Potable) Water[1]	Purified Water[2]	Water for Injection[2]	Clean Steam	Compressed Gases
Site:	Inlet to the Plant.	Representative use points on the distribution system.	All accessible ports on the distribution system.	Sample at the generator and the distal point of use.	Post compressors, upstream and downstream of point-of-use filters.
Methods:	Pour plate minimum sample 1.0 mL.	Chemistry, Pour plate minimum sample 1.0 mL or Membrane filter 100 mL.	Chemistry, LAL, and Membrane filter 100 mL.	Chemistry and LAL.	Viable and non-viable particles, oil, and moisture.
Equipment:	Plate count agar and 30-35° C incubator or R2A agar and 20-25° C incubator.	Conductivity/TOC analyzers and Plate count agar and 30-35° C incubator or R2A agar and 20-25° C incubator.	Conductivity/TOC analyzers and Plate count agar and 30-35° C incubator or R2A agar and 20-25° C incubator, LAL supplies.	Sampling condensers, Conductivity/TOC analyzers, and LAL supplies.	Viable air sampler, particle counter, moisture analyzer, oil detector.
Frequencies:	Weekly TPC and coliforms. Annual EPA requirements. *Note:* It is important to understand source water quality due to seasonal variation.	Monitor distribution system daily when in production.	Rotate testing at all use points weekly for micro, test return loop daily for chemistry and endotoxin. Test feed water to still daily.	Monthly.	Quarterly.
Acceptance Levels:	50,000 CFU/100 mL and Absence of coliforms.	Meets Chemistry specifications and <100 CFU/mL for micro.	Meets Chemistry specifications and <10 CFU/100 mL for micro, < 0.25 EU/mL for endotoxin.	Meets WFI criteria.	<1,000 ppm, moisture <1 mg oil/m^3 Viable and non-viable counts meet room classification

[1] National Primary Drinking Water Regulations (40 CFR 141)
[2] USP 23 Fifth Supplement

NOTES:

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