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.