Microbiologic Monitoring of Controlled Processes

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INTRODUCTION

Microbiologic monitoring of controlled pharmaceutical and medical device manufacturing, and pharmacy compounding processes, is mandated in numerous standards and guidelines,[1–3] although procedures, limits, and frequencies are not well defined.[4] Because many characteristics of microbiologic sampling limit its value as a monitoring method,[5] efforts to detect contamination in controlled environments require carefully developed and executed sampling plans to produce reliable data that confirm the acceptability of operating conditions.

Monitoring of any controlled process is a component of an outcome-producing, closed-loop system for assuring continued operation of critical processes in accordance with validated design conditions. To achieve this goal, a monitoring plan must be developed, conducted, and evaluated within the context of a Validation and Monitoring protocol. All results must be related to the original validated process, either as evidence that it continues to operate within acceptable limits, or as a means of detecting shifts in the process that might impinge on product quality. Ideally, monitoring results will also provide information that will be useful in determining the cause of such shifts.

The objectives of the monitoring plan within the validation and monitoring system for quality management must be clearly defined so that the information collected will be relevant to system goals. The limitations of sampling equipment and methods must be taken into consideration when developing the sampling plan and interpreting results. The underlying causes for shifts in various monitoring results must be understood in order to facilitate development of effective corrective action plans.

VALIDATION AND MONITORING RATIONALE

The regulatory requirements for validation of pharmaceutical aseptic processes are clear.[6] Generally accepted quality assurance principles require initial demonstration of the efficacy of any process (*validation*), followed by regular, periodic observation to demonstrate that the process continues to operate in accordance with validation conditions (*monitoring*).

Validation usually consists of a series of "worst-case" process simulations, wherein a sterile growth medium is substituted for product to demonstrate that processing consistently yields products of acceptable quality.[6] During this Process Qualification (PQ) phase, variable conditions that might effect product quality are carefully defined, controlled, monitored, and documented, and the assumption is reasonably made that the process will then yield the same product quality achieved during the PQ, so long as all variable factors are controlled to duplicate validation conditions. This assumption is based upon the results of monitoring data obtained from a variety of sources. The validity of the assumption of acceptable quality is, therefore, dependent upon the reliability of the monitoring data as a measure of control of process variables.

Validation Protocol

The validation protocol should define the manufacturing or compounding process, its purpose in terms of the desired positive impact on product quality, and how that impact will be demonstrated. The protocol should include the following components:

1. A description of the product, and applicable release criteria including AOQL/ROQL;
2. The facility design rationale for maintaining process integrity, including identification and elimination of inaccessible areas that may be difficult to decontaminate, enumeration of the clean-space engineering controls, and how these controls will be applied, tested, and monitored;
3. A schematic description of the aseptic process and the critical work surfaces, work zones, and support areas, including the designation of particulate cleanliness class,[7] microbial target

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values[8,9] (Table 1), and engineering control equipment validation methods[a];[10]

4. The selection and justification of gowning and barrier techniques to ensure adequate isolation of personnel, based upon industry standards[3] and process requirements;
5. A definition of the aseptic techniques and work practices of operative personnel, and a report of findings based upon videotaped observation of the actual work stream during prequalification runs for identification and elimination of personnel-generated contamination sources, identification of susceptible areas including critical sites and steps, and indicator sites;
6. A description of sanitizing methods and sanitizing compound validation;
7. A definition of the equipment and methods to be used in assuring reliable test data; and
8. All test data, including instrument calibrations, testing and certification reports, and statistical justification.

Monitoring Plan

Following evaluation of all environmental monitoring data collected during the PQ, a monitoring plan[11] defining ongoing monitoring procedures, locations, and frequency should be implemented. The PQ data from product testing should be compared to environmental and process monitoring results to determine the monitoring sites and methods that best correlate with shifts in product quality. The plan should

1. Assure specified, periodic monitoring of critical manufacturing or compounding process parameters at critical points during periods of peak activity, and establish the circumstances and frequency with which monitoring is to be carried out to assure a reliable basis for claiming process control.
2. Provide for standardized, quantitative microbiologic sampling of process air, environmental surfaces, and personnel barriers, as well as sampling of other, related parameters.
3. Include sampling location maps, sample sizes, probe heights, methods, equipment, and frequency during manufacturing operations, and a method for statistical justification of results.
4. Include alert and action limit criteria for acting upon ongoing monitoring information.
5. Include a system for evaluating and modifying the monitoring plan to assure collection of reliable, useful data, and
6. Include a corrective action plan, and methods of verifying the efficacy of any corrective actions taken.

[a]Validation testing of HEPA filters requires an exacting aerosol challenge of 100% of the filtration media, frame, and locking device in accordance with Secs. 40 and 50 F.S. 209b.[18] Successful testing in this manner establishes control of the ‘‘first air’’ emanating directly from the filter, as it approaches the entrance plane within the unidirectional slipstream, to better-than-Class I conditions. Monitoring of HEPA filters in accordance with F.S. 209e[7] involves an average of DPC readings derived from a number of representative locations to assure Class 10 or 100 conditions at the entrance plane of the unidirectional slipstream.[10]

Limitations of Microbiologic Monitoring

The minimum media-fill validation requirement of not more than one sterility failure per thousand units, representing the minimum sterility assurance level of 10^{-3} (>99.9%) is the only microbiologic limit in the validation and monitoring scheme that is based upon demonstrated product quality. Achievement of this sterility assurance level represents the aggregate impact of all process design and control factors, including sampling and attendant laboratory procedures. (This limit, however, probably does not reflect the true integrity of a valid aseptic process.)[12] All other limits are indices, which are used indirectly to demonstrate that the process is under control as validated. Because all environmental monitoring is necessarily performed at some point downstream and apart from the product, no absolute evaluation of product quality is obtainable through monitoring procedures, however intensive. In addition, testing and monitoring methods do not always parallel or identify the pathways through which contaminants are introduced into the product.

Difficulty in validating microbiologic monitoring methods results from a lack of comprehensive testing standards, reliable test equipment, and reliable methods for correlating sample data to predictions of product quality. Several characteristics and qualities of both contamination events and sampling methods limit the usefulness of microbiologic monitoring as a method of determining the acceptability of a specific product batch:

1. Microbiologic contamination events in controlled facilities are usually not randomly distributed in time, space, or by type of organism;
2. No single sampling method repeatedly recovers a known and consistent percentage of all types of organisms;
3. For most types of contamination detected, there are usually many possible sources, not the least of which are the sampling personnel, equipment, and lab processing; and
4. An extended interval is required for development of results.

Table 1 Process monitoring targets/frequency

Function/Process	Cleanliness class[a,b]						Monitoring frequency				C.F.U.[c] Target									
	Class 100[a]	Class 10,000[a]	Class 100,000[a]	M 3.5	M 5.5	M 6.5	Each shift	Daily	Twice weekly	Weekly	<0.1 Per Ft.[d]	<0.5 Per M[d]	<2.5 Per Ft.[d]	<3.0 Per M[d]	<20 Per M[d]	<100 Per Ft.[d]	3 Per Contact plate	5 Per Contact plate	10 Per Contact plate	20 Per Contact plate
Critical worksurface[e]	•			•			•				A			A			S			
Support areas[e]		•			•		•					A			A			S		
Other support areas[e]			•			•			•				A			A				
Other potential prod./container contact[e]		•			•				•				A			A				
Other non-prod./container contact[e]			•			•				•			A			A				
Laminar airflow hoods	•			•			•				A			A			S[f]			
Biological safety cabs	•			•			•				A			A			S[f]			
Host-cell culture	•			•			•				A			A			S[f]			
Immediate Processing	•[g]		•[h]	•[i]		•[h]	•[g]			•[h]	A[i]		A[h]	A[g]		A[h]	S[f,i]		S[h,i]	
Formulation		•			•		•					A			A			S		
Final Production	•			•			•				A			A			S[f]			
Equipment[e]	•			•			•										S[f]			
Equipment[e]		•			•			•										S		
Floor[e]	•			•			•										S			
Floor[e]		•			•		•											S		
Floor[e]			•			•			•										S[j]	
Personnel gloves[e]	•			•			•										S			
Personnel gloves[k]		•			•		•[g]		•[k]										S	
Personnel barriers[e]	•			•			•											S		
Personnel barriers[e]		•			•		•[k]		•[g]											S

[a]Federal Standard 209e U.S. Customary.
[b]SI.
[c]Colony-forming units.
[d]Cubic valve.
[e]U.S.P. ⟨1116⟩.
[f]Including floor.
[g]Other support areas.
[h]Closed validated systems.
[i]Open systems.
[j]Recommended.
[k]Immediately adjacent to Class 100.
A: Aerobiologic; S: Surface.
Table courtesy of Lab Safety Corp., Des Plaines, II.

Perspectives

These considerations underscore recent concerns that regulatory groups may require that unreliable environmental monitoring data be used as release criteria.[15] Current industry standards and regulatory guidelines do not, and should not be interpreted to condone the rejection of batches on the basis of absolute environmental counts alone. Microbiologic monitoring is employed for practical reasons, not because it is ideal or unique in detecting shifts in process conditions.

Regulatory agencies and auditors understandably seek easy-to-interpret data as a basis for decisions regarding product acceptability, and are becoming increasingly hesitant to accept product release in the absence of demonstrable levels of microbiologic control. Conversely, industry is justifiably reluctant to set microbiologic monitoring limits because regulators may misinterpret their meaning in a quality assurance (QA) context. The failure to meet process control limits is quite different from the failure to meet product specifications. Failure to meet a monitoring limit means only that monitoring data can no longer demonstrate validation conditions, and product quality *may* be adversely affected. Enhanced product testing or other corrective actions may be indicated, but batch rejection should not be extrapolated from QA monitoring results, alone.

Setting Limits

In the QA context, limits are established to trigger specific actions, or outcomes. The alert (warning) limit is the point at which the operator should become alerted to the possibility of a deteriorating trend. When an action limit is exceeded, the operator must take action to identify and correct the condition(s) that are causing a verified trend before a ‘‘fail’’ limit is reached and the data fail to indicate process control and support continued production. In a well-designed and executed process, however, such a fail limit should never be exceeded, except in the event of a sudden and catastrophic breakdown of a critical process control component.

Akers noted that values presented in the current U.S.P. ⟨1116⟩[8] are target values.[13] Given this designation, it is reasonable to consider these values to be *operational target levels,* rather than *product quality control limits.* There are several models for setting alert, action and fail limits, although many only establish alertand action limits[14] (other terminology may be used). Extending one current model,[14] the alert limit might be considered to be the 95th percentile. Analysis and trending of actual data allow the calculation of this limit, as well as the 97th percentile for the action limit, and the 99th percentile as the fail limit. Regardless of the model used initially to set limits, they should be based upon both historical data, and an evaluation of correlations between monitoring results and product quality. Data analysis should include a mechanism for evaluation and modification of the monitoring program and limits.

It is expected that results will fall within normally anticipated operating levels[8,9] (Table 1) with 95% confidence, if randomness in critical environments and operations is sufficiently controlled. If data from successful PQ runs (when the process is demonstrated to be under control) do not meet this criterion, the monitoring methods may not measure a phenomenon that relates directly to process control, may not be sufficiently reproducible to provide useful information, or may have been incorrectly conducted. Every effort should be made to develop monitoring methods that comply with this performance expectation so that data will be useful.

Initial limits may be calculated and compared to results of any unsuccessful trials. These limits should eventually be adjusted based on historical data (Fig. 1). When evaluating data to adjust limits, Wilson[14] noted, ‘‘Including data taken from a period of unusually high counts, where the process was out of control, will lead to inappropriately high alert/action limits.’’

Conduct of Sampling

Quality management and sampling personnel require both an in-depth understanding of the environmental sampling rationale, and a complete understanding of commonly available equipment, materials, sampling techniques, and development methods. Reporting forms should be carefully designed to convey all relevant information including identification of the technician, sample location (from a standardized sample map), date and time, media (including lot, expiration, and validation date), method, duration of sampling, and equipment (including calibration date and serial number). In addition, information such as the product batch, number and names of personnel, line throughput rate, number and nature of line interventions, and other available monitoring data such as room pressure and other engineering control status readings should be recorded. Any observed deviations from standard operating procedures (SOPs) should be noted and communicated to the individuals responsible for training and management of operative personnel. It is essential to repeat samples when such deviations occur in order to evaluate the impact they may have on results.

Sampling and laboratory personnel must be highly competent on both philosophical and functional levels,

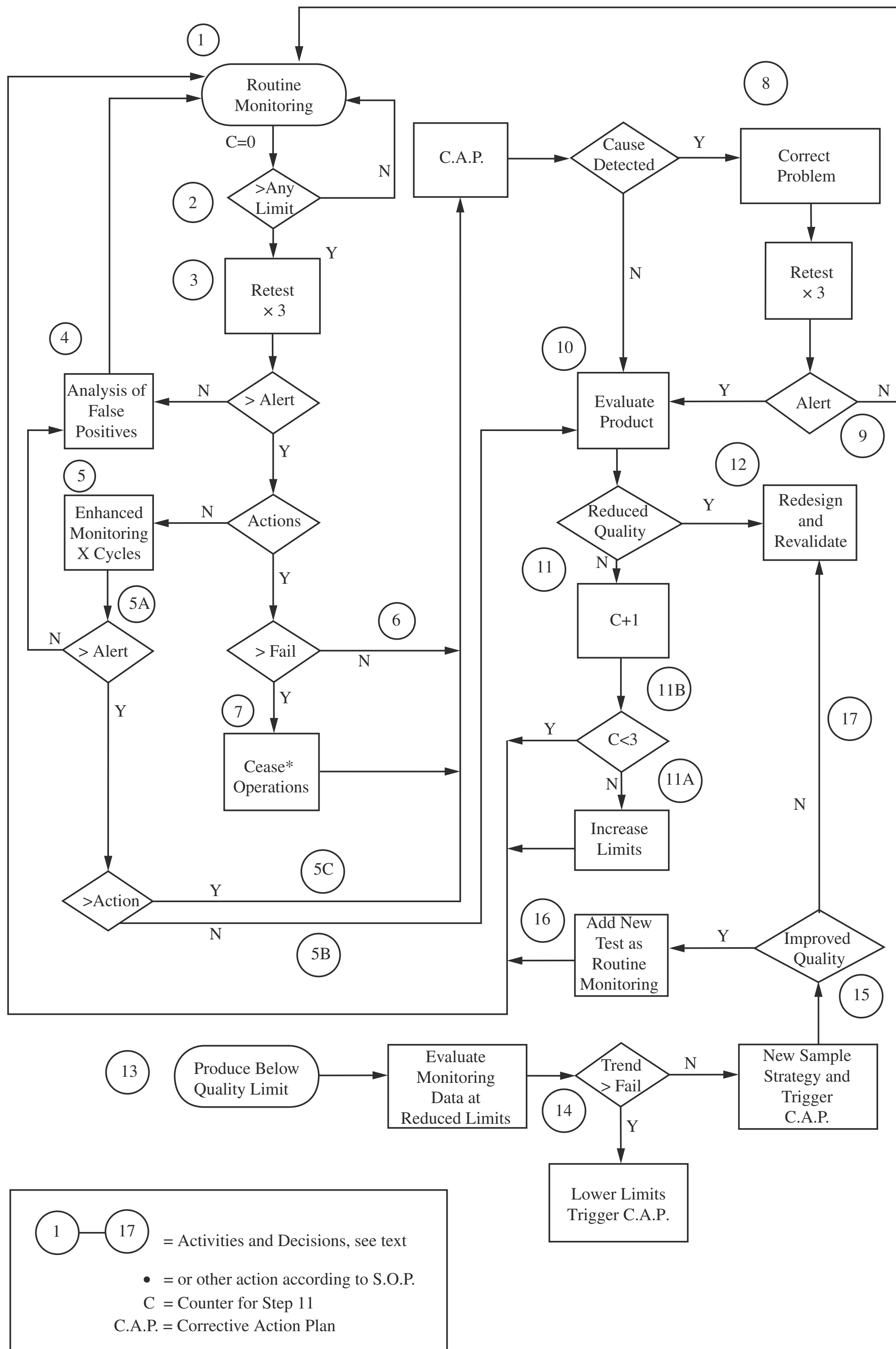

Fig. 1 Evaluation of monitoring plan and limits. (Courtesy of Lab Safety Corp., Des Plaines, IL.)

and must develop and exercise *perfect* aseptic technique.[13] A training program and operating procedures should be established defining all monitoring steps, including gowning, preparation of samplers, aseptic sampling techniques, sample recovery, handling and transport, and laboratory techniques for aseptic sample development. A laboratory QA program should assure that monitoring personnel conform to operating procedures and that technician skills are periodically tested and validated for high competence and flawless technique.[13]

Sample Handling

Sampling, sample transport, and sample development should be conducted in a way that does not affect results. For example, if agar plates are improperly transported, condensate may form on the lid and drip onto the agar surface, redistributing microorganisms over the surface and around the edges of the plate, causing false readings. Agar plates should, therefore, be kept inverted and oriented horizontally during storage and transport. They should be handled gently, and transferred to the incubator as quickly as possible after exposure. With sieve impactors, false positives can usually be identified as colony forming units (CFUs) that fall outside the star pattern of jet indentations in the agar surface below the holes. Counts >20 CFUs may also be statistically corrected for increased accuracy by using the positive-hole correction table.[15]

It is recommended that colony counts be made at several points in the incubation process, with separate tallies for bacterial and fungal colonies that tend to merge at a critical point during incubation, when fungal colonies may overgrow and obscure bacterial colonies. For this reason, any bacterial subcultures should be made prior to the onset of rapid fungal growth. Whenever possible, optical electronic colony counters with sufficient backlighting and magnification to enhance contrast and enumeration should be employed to increase accuracy. In the presence of known or potentially high counts, the microscope enumeration method should be used to closely differentiate and count microcolonies in impact areas on sample plates following a short incubation period.

DEVELOPING A MONITORING PLAN

Site Selection

A critical site is a point at which the product is exposed to the environment, when something is added to the product or product pathway, or a point at which unprotected product is manipulated. Any intervention into the process line increases the potential for contamination. (Examples of line interventions include the introduction, removal, or manipulation of materials and product, equipment adjustments, and sampling activities.) Particular attention should be given to these sites and events in the development of the monitoring plans.[3]

Analysis of a videotape of repetitive prequalification should be studied for behavior and practices that may produce or harbor environmental contamination, leading to the refinement and optimization of work practices, and development of the formalized process to be instituted for the PQ validation run. The videotape may be used for identifying indicator sites, which should be incorporated into the monitoring plans, and intensively sampled during the validation run. These tapes should be retained and edited for both training and informational purposes.

For critical processes, it is important to select noninvasive sampling methods that have high collection efficiency for a broad range of organisms. To select the most suitable monitoring methods and equipment, the probable route of contamination for each critical site or process should be identified. For example, when the most likely route of potential contamination is touch, select surface sampling techniques for personnel barriers. When the most likely route is transfer from contaminated work surfaces, sampling of these surfaces is most useful. At sites where unprotected product is exposed to the environment, aerobiological monitoring is indicated, and, in unidirectional airflow, must be carried out isokinetically and isoaxially[b] in the manner of non-viable particle-count testing. Some processing steps may require multiple sampling methods.

Controlled support areas adjacent to critical areas are the essential interfaces in the transition from the general environment to the aseptic processing core. These areas should be adequately pressurized, facilitating a gradient flow of contaminants from cleaner to dirtier areas.[10] Controlled staging, support, material storage areas, and work practices should be examined and indicator sites identified. Controlled areas should be maintained and monitored in accordance with guidelines and industry standards (Table 1).

[b]Isoaxial: A condition of sampling in which the direction (axis) of the airflow into the sampling probe inlet is the same as that of the unidirectional airflow being sampled.[7] Isokinetic sampling: The condition of isoaxial sampling in which the mean velocity of the air entering the probe inlet is the same as that of the unidirectional airflow being sampled.[7]

Personnel, Equipment, and Facility

Validation and monitoring of a process are normally divided into three main areas of concern: personnel, equipment, and facility.

The human factor is the greatest potential variable in any process. Uncontrolled variation in personal health and hygiene, barrier techniques, and aseptic technique may cause wide variation in contamination of controlled support areas and process materials during staging and preparation, as well as adventitious contamination of the aseptic process core and product. A suitable aseptic process, defining appropriate and standardized personal hygiene expectations, scrubbing and preparation techniques, barrier techniques, and operator techniques should be developed and challenged intensively during the PQ exercise. Personnel should periodically take both written and media-fill skill tests.[3]

Ongoing monitoring for compliance with pertinent SOPs should then be conducted. Sampling of personnel barriers, such as gloves, shoe covers, hair cover, and gowns facilitates detection of potential ''fallout'' contaminants shed from personnel for evaluation of both barrier and aseptic techniques. This information may be useful in establishing required garb-change intervals, based upon measured garb-penetration times by endogenous contaminants. All accumulated data should be used periodically to develop a facility trend analysis which, in turn, modifies training and work practices as necessary.

All equipment used in controlled manufacturing or compounding processes should be designed, staged, and sanitized in a manner that facilitates unvarying routine operation, with minimal human intervention. This reduces the potential for random cross-contamination by operative personnel. Improperly sanitized or sterilized equipment or components are also a possible source of contamination.[c] Monitoring of representative surfaces of process equipment should be carried out and documented.

Facility sampling should be carried out under both as-built and at-rest[7] conditions during initial installation qualification (IQ) and operational qualification (OQ) of the facility, in order to baseline and ''bracket'' performance of the engineering controls, and to identify the normal background flora present in the manufacturing environment. Sampling should then be conducted in-process under operational conditions[7] during the PQ, to identify the impact of the process and personnel on the product and environment. It is important to monitor the validation process during all shifts and throughout the shift. Sites should be standardized and selected by statistical models or grid profiling,[16] based upon testing and monitoring requirements appropriate to the specific process (Table 1).

Surface sampling is useful in verifying the effectiveness of housekeeping and sanitizing procedures. It may also provide an alert to poor materials preparation prior to introduction into the controlled environment, or to lapses in personnel technique or barrier use. Aerobiologic sampling is most useful when conducted in conjunction with a complete program for testing of the engineering control system.[10] Recommended tests include the following:

1. Facility pressurization, which should be routinely monitored at recommended intervals;[20]
2. High efficiency particulate air (HEPA) filter velocity anduniformity testing for laminar airflow,[21] and volume in cubic ft/min (CFM) for conventional flow, including a determination of room installation air changes;[10]
3. HEPA filter leak-integrity testing;[18]
4. Non-viable particulate cleanliness testing;[7] and
5. Smoke-tracer visualization for establishing the integrity of unidirectional-flow areas.[10]

Periodic retesting of challenges 1–4 is required by some regulatory groups, with the interval determined by the nature of the process and product in a given area.[17] Repeating Test 5 may be useful in evaluating failures and can be an extremely valuable training tool. Concomitant particle count testing may be useful in identifying contamination indicator sites.

Monitoring of laminar airflow workstations (LAFWs) requires a complete understanding of HEPA filtration system performance, and is frequently conducted in ways that do not yield useful information. When properly validated in accordance with Federal Standard 209b [Appendix A, para. 40 and 50[a]], LAFWs provide air at the entrance plane which is far cleaner than Class 100.[10] Testing to this cleanliness level would permit particulate contamination levels two orders of magnitude greater than during filter OQ validation testing. More important, the use of any apparatus that samples discrete locations in a unidirectional slip stream is unlikely to detect filter leakage because isoaxial and isokinetic sampling at the exact point of leakage would be required. Therefore, placement of a sampling probe upstream from the product is unreliable and an unnecessary threat

[c]The sterilization process for any equipment or supplies that are sterilized prior to introduction into the controlled environment must be validated, with sterilization records and verifications included in all product batch histories. Validation of sterilization equipment, alone, is not sufficient to assure sterility. Because the types of materials being sterilized, and the arrangement of articles within the sterilizer can effect results, standardized load configurations must be developed and validated.

to sterility. The only practical, in-process use of these instruments is to detect shifts in the amount of particles and microbiologic contaminants caused by the process at some point adjacent to or downstream from the product. Such a shift might signal a lapse in personnel technique, barrier use, or prestaging material preparation, or be caused by HEPA filter loading, which reduces airflow velocity.

Avoiding Sampling-Induced False Positives

Line interventions for sampling purposes must be balanced carefully against the total number of interventions necessary for production purposes. Sampling should present the minimum risk of contamination, which is theoretically the same for every line intervention. Because sampling-induced positives should not exceed 10% of total positives ($10^{-1}N_p$),[17] the number of sampling interventions should be significantly lower than the number of production line interventions. In isolators or other isolated critical processes, where no line interventions occur during production, not more than one, carefully controlled, aseptic sampling intervention is recommended.

Surface sampling the exterior of finished products, as indicator sites, assembled from purportedly sterile components as they exit the process while still under aseptic conditions, may be a more efficacious method of estimating microbiologic contamination potential than invading the critical production site. This method allows sampling the most critical site adjacen to the product, and more sites may be non-invasively sampled over a longer interval. In addition, this method may substantially reduce the incidence of sampling-induced contamination.

Monitoring Frequency

The frequency of monitoring should be determined by the maximum interval acceptable for an over-limit condition to remain undetected.[19] This depends upon the critical nature of the process within the monitored area. In general, the minimum frequency should be consistent with applicable regulatory guidelines (Table 1). Although it has been suggested that monitoring frequency can be reduced if no over-limit condition is detected within a predetermined number of monitoring cycles, this practice is inconsistent with basic monitoring rationale. Monitoring is conducted to detect a breakdown in process controls, which may occur at any time. Even if no control component has failed for a prolonged period, it must be assumed that a failure will occur eventually and must be detected within the predetermined interval. In addition, lack of over-limit test results may be due to the fact that monitoring method(s) are not sufficiently sensitive, or that limits are too high.

EVALUATION OF THE MONITORING PLAN AND LIMITS

Most discussions of microbiologic monitoring recommend that the monitoring plan and limits be based on historical data, but offer little guidance on how this can be accomplished. Fig. 1 provides a guide for evaluation and revision of the monitoring plan and limits. An in-depth evaluation may be triggered by over-limit results from monitoring (Entry Point 1) or by adverse product testing results without detection of any over-limit condition through routine monitoring (Entry Point 2).

Entry Point 1:

1. Conduct routine monitoring. A counter (C) is used for Step 11. $C = 0$ at the beginning of the routine monitoring program.
2. If the results do not exceed any limit, then continue routine monitoring.
3. If the results exceed any limit, then perform retesting in triplicate to verify the accuracy of results. Retest under the same conditions noted on the sampling form (i.e., same time of day, same location and operator, same type of production).
4. If triplicate retest results are not over-limit, it is assumed that the original over-limit result was due to a non-assignable cause (*NAC*). Determine the probable cause of the over-limit count (i.e., unusual activities noted on test documentation, sampling, lab error, etc.). A record of positive NACs should be kept and analyzed to determine ways to improve affected processes and sampling procedures. Return to routine monitoring.
5. If results are over an alert limit, but not over the action limit, then enhance monitoring frequency for X cycles. (X is determined by the critical level of the area and process where the over-limit event occurred, but should provide an adequate interval to assure detection of a continued deterioration of process control.)
   a. If the alert limit is not exceeded again within X cycles, then return to Step 4.
   b. If the alert limit is exceeded but the action limit is not, then proceed to Step 10.
   c. If the action limit is exceeded, then go to the corrective action plan (CAP).[5]
6. If results following the triplicate retesting are over the action limit, but not the fail limit, then go to the CAP.

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7. If the results following the triplicate resting are over the fail limit, traditional QA protocols usually require that operations cease. However, the appropriate action taken should depend on the critical nature of the monitored step and other conditions. An alternative to operation shut down may be to segregate and hold the product for enhanced testing for adverse effect; go to the CAP.
8. If implementation of the CAP results in the determination of the cause of the over-limit condition, then correct the condition, and retest in triplicate to verify that the problem was corrected. If no cause was found, then proceed to Step 10.
9. If test results following corrective action are within limits, then return to routine monitoring.
10. If test results following corrective action are still over-limit, or if no cause of the over-limit condition can be identified, then evaluate the product for adverse effects.
11. If no adverse impact on product quality can be detected, add 1 to the counter (C). The result may indicate that limits are too low, but one event is not sufficient to support a decision to increase limits.
    a. If $C = 3$, then the limits are too sensitive, and should be increased.
    b. If $C = <3$, return to routine monitoring, Because the results are over-limit at this point, a repeat investigation of the cause of over-limit results will be triggered. Limits should be increased judiciously, and it is important to be thorough in attempting to resolve any cause of over-limit testing with reasonable certainty before increasing limits. For example, if the cause of the over-limit result is sampling mistakes or lab error, there will be no detectable cause in the production facility, the process or engineering control evaluations, and probably no adverse effect on product quality. This should not, however, be interpreted to mean that limits are too sensitive.
12. If product is adversely affected, and no cause can be detected following implementation of the CAP, the monitoring plan and/or the process should be redesigned and revalidated.

Entry Point 2:

13. If product quality is below limits, but monitoring data did not detect the shift, then reevaluate monitoring data using lower limits to determine whether or not the process shift could have been detected. If the data have been graphically represented, this should be quite simple; increasing the amplitude of the graph may be useful.
14. If lower limits would have detected the shift, then lower the limits and institute the CAP.
15. If lower limits would not have detected the shift, then evaluate the cause of the failure, and develop a new sampling strategy for the key step(s) where failure occurred. Institute the CAP and verify that corrective actions taken were effective in improving product quality.
16. If product quality improves, then add the new sampling method to the routine monitoring program.
17. If it does not, return to Step 12.

SELECTION OF MONITORING METHODS, MATERIALS, AND EQUIPMENT

Effective microbiologic monitoring of controlled processes usually includes sampling of process air for aerobiologic contamination, and facility, equipment, and operative personnel barriers for surface contamination. Equipment and methods used in monitoring procedures must be carefully considered for attributes and limitations and must be matched to sampling objectives to ensure that methods and techniques are non-invasive, and to facilitate development of well-organized sampling plans, techniques, data, and data trending analysis.

Surface Sampling

Surface sampling may be performed at the conclusion of critical operations to minimize disruption of these processes[8] and prior to sanitizing procedures[20] to estimate cumulative, inprocess contaminant burden.[4] In addition, presanitization surface sampling is beneficial in detecting operations-induced bioburden and cross-contamination between environmental and equipment surfaces. Postsanitization surface sampling is useful for evaluating sanitizing methods and in retrieving sanitization-resistant isolates for identification and trend analysis in demonstrating sanitizing compound efficacy. The two most common types of surface sampling are swab-sampling, and surface contact sampling.

Swab-sampling

Swab-sampling is normally used for flat or irregular, non-absorbent surfaces with qualitative development by inoculation of the swab matrix directly into nutrient broth, observed for growth/no growth.

Quantitative development is also possible.[5] The main advantage of the swab method is accessibility to difficult-to-reach equipment surfaces and areas of the production environment. Limitations are excessive time consumption, increased potential for adventitious contamination due to the cumbersome nature of the procedure, and failure of enumeration processes to correlate to full recovery of organisms.

Contact plates

Surface contact plates are normally used for sampling flat or irregular, absorbent or non-absorbent surfaces. The surface contact plate consists of a clear plastic base housing a convex protrusion of nutrient agar with a plastic cover. Sampling is accomplished by pressing the agar against the site.

The covered plate is then incubated for development, and the CFUs per square centimeter enumerated.[21] Advantages of surface contact plates are reproducibility, speed, simplicity of collection mechanism, and minimized potential for adventitious contamination; collection and correlation to recovery of organisms are superior to swab-sampling.

Aerobiologic Sampling

Aerobiologic sampling is conducted in critical and controlled areas to detect airborne viable contaminants present during manufacturing operations. Aerobiologic sampling procedures, frequency, and limits should be established based upon environmental conditions required to maintain product quality, and established for each processing step (Table 1).[4] Aerobiologic sampling employs two basic methodologies:

1. The gravity settle plate, which provides passive measurement of microorganisms likely to deposit by sedimentation at critical and controlled sites within a given period, and
2. The volumetric air sampler, which provides active measurement of viable contaminants by mechanical aspiration and dynamic inoculation of process air.

Gravity settle plates

The gravity settle plate measures microorganisms settling from the air onto a known surface area in a known time. Settle plates may be positioned within the critical area at indicator sites where the product may become exposed to airborne contamination, and in controlled areas at locations identified as likely sources or areas of ‘‘fallout’’ aerobiologic contamination. Settle plates are not appropriate aerobiologic sampling method for monitoring the efficiency of unidirectional (laminar) airflow or other air-cleaning devices. This is based upon studies,[22,23] and the general assumption that ‘‘. . . the settling velocity of contaminants (in unidirectional airflow) is negligible, which implies that gravitation plays an inferior role. With the assumption of a constant value of the diffusion coefficient, the diffusion equation in a velocity field within rectangular coordinates becomes

$$\frac{\partial_c}{\partial_t} + v_x \frac{\partial_c}{\partial_X} + v_y \frac{\partial_c}{\partial_Y} + v_z \frac{\partial_c}{\partial_Z} = D\left(\frac{\partial_c^2}{\partial_X^2} + \frac{\partial_c^2}{\partial_Y^2} + \frac{\partial_c^2}{\partial_Z^2}\right) \quad (1)$$

where c is concentration: v_x, v_y, v_z are velocities in the x, y, and z directions: and D is diffusion coefficient.

This gives the simplest possible mathematical model which describes a system with regard to transport of contaminants emitted in a source of an arbitrary position . . . ,’’[23] demonstrating that particle dispersion in undisturbed streamlines is primarily a function of streamline uniformity and velocity. Disruptions of the parallel (laminar) airflow streamlines caused by equipment, personnel movement, and product result in turbulent flow, creating small and temporary vortices and eddies. It is only turbulent diffusion within the vortex that causes removal of entrained contaminants.[23] Therefore settle plates, strategically placed, are reported to provide a superior method of predicting potential product contamination by mimicking the deposition of microbe-carrying particles (MCPs) into or onto the product[d].[24] They are inexpensive, may be used to continuously monitor the entire production interval, are less invasive of aseptic operations, and may usually be placed closer to exposed products than volumetric air samplers.

Settle plates cannot be used for quantitative measurement of airborne microorganisms because the

[d]Regardless of placement of an aerobiological sampler in a laminar airflow work zone, it can at best measure the effect of the process at some point downstream from the product. For example, the mouth of a flask may be situated in ‘‘first air’’ issuing from the HEPA filter, while air impinging on the surface of a plate adjacent to it will be affected by disruptions of the airstream caused by the flask. Contamination found on the plate then results from a different set of conditions than those to which the product is subjected and does not exactly parallel the product contamination mechanism. Only a media-fill process simulation can fulfill this function. Aerobiologic sampling immediately downstream of the critical orifice can, however, detect downward shifts in the overall cleanliness of the critical process air, which in turn may indicate increased contamination potential near the product.

sample volume of sedimentation air samples cannot be measured. Air turbulence around an open plate may also effect collection results, and smaller particles may not settle at all.[22] In addition, extended exposure times may result in some desiccation of the nutrient agar, resulting in poor microbial growth.[25]

Volumetric air samplers

As an active sampling method, the volumetric air sampler aspirates a known volume of process air, capturing microorganisms into or onto a nutrient agar medium, a liquid, or a filter. Microorganisms are developed and quantified as an estimate of CFUs present in the sampled environment per cubic foot of air (or other volumetric measurement).[4] The quantitative principles of volumetric (active) air sampling may be expressed by

$$S\,(R_t)\,C \;=\; R_f \qquad (2)$$

where S is source intensity, R_t is transport rate, C is correction factor, and R_f is failure rate.

Volumetric air sampling is accomplished by a number of different methodologies, including impingement, impaction through single or multiple orifices, centrifugal impaction, and filtration. Each method has inherent advantages and disadvantages that affect the value of the data collected relative to the specific application. Tables 2A and B presents a comparison of popular samplers based upon relative cost, difficulty of use, appropriate applications, and other factors.

Impingement: In an impinger, a known volume of air is drawn through fluid in a glass vessel.[20,30] Particles separate from the airstream by impinging at the flask bottom, where they are stopped and retained by the liquid as the air continues to flow out through the pump system. High air velocities passing through the impinger effectively break up bacterial/particulate aggregates, resulting in microbial counts, which more closely reflect the actual number of microorganisms, leading to recommendations that impingers be used as the standard reference method for monitoring aerobiologic contamination.[26,28] However, impingers may require the addition of antifoam agents and replacement of fluid, due to agitation and evaporation loss during longer sampling procedures.

These additional steps increase the possibility of adventitious contamination. It has been demonstrated that the sampling efficiency of an impinger is dependent upon both system design and the particle sizes being sampled.[29] Accuracy and reproducibility of results have been reported to be difficult, and particles of $<5.0\,\mu m$ have been demonstrated to pass through the impingers tested.[30]

Impaction: In slit-to-agar (STA) or sieve impactors, a known volume of air is aspirated through a single orifice (STA), or multiple orifices (sieve), and viable particles, due to their inertia, are forced out of inlet airflow streamlines and impacted onto perpendicular, target nutrient agars as the streamlines abruptly change direction to bypass the target stage. In the centrifugal impaction sampler, high centrifugal forces created by “spinning” air through an impeller turbine at sufficient velocities to cause separation of microorganisms from sample air streamlines result in their impaction onto a nutrient agar strip placed at the inner periphery of the sampling chamber, parallel to the inlet airflow axis.

Sieve impactors are available in single-stage or multistage designs that facilitate both enumeration and sizing of aerobiological contaminants. As the sample air transits the device, sample velocities increase at each stage, resulting in gradient deposition and accurate sizing of microorganisms of smaller diameters and lower mass. Microorganisms aspirated by sieve samplers through a matrix of multiple-inlet orifices impact directly onto an agar medium for development from a single agar plate for each vertically stacked stage, with no further subculture steps required for enumeration. Advantages of sieve samplers are generally high particle deposition rates, the ability to size particles and vary sampling time and volume, and superior collection efficiencies when compared to other methods of aerobiological testing. Single- and six-stage configurations have been reported to be two of the three sampling methods of choice.[27]

Use of STA samplers in isolators and critical process zones should be accomplished using a sterile sampling hose and probe, facilitating remote location of the sampler in a non-critical area. In monitoring a unidirectional slipstream, this hose/probe configuration should be both isoaxially oriented, and isokinetic[b], in order to minimize disruption of the slipstream. Advantages of the STA include the ability to revolve the plate at varying rates so that the samples may demonstrate changes in aerobiological concentrations directly over time, and the ability to obtain multiple samples with a single petri dish.[31] STA samplers have historically been the standard against which other air samplers are assessed.[17,32] Agar plates are easily removed from the sampler for development, with contamination enumerated as CFUs per unit of air sampled.

The STA is reported to be both unsuitable for use in the presence of high concentrations of organisms[33] and cumbersome to use.[17] In addition, it has been demonstrated that a significantly higher percentage of particles sized $0.5–0.8\,\mu m$, and a significantly lower percentage of particles sized $3.0–25.0\,\mu m$, were present

Table 2A Relative cost/difficulty comparisons

Sampling method/sampler	Acquistion cost[a]	Cost of use/sample 1–6[b]	Ease of use 1–6[b]	Sampling speed 1–6[b]	Mobility 1–6[b]	Contamination potential to sample	Contamination potential to environment	Reproducibility 1–6[b]	Applications A–C	Isolators 1–6[b]
Swab sample typical	•	5	2	2	2	4	1	2	C	4
Contact plate typical	•	2	1	1	1	1	1	1	B	1
Gravity settle plate typical	•	1	1	1	1	1	1	3	A	1
SAS super 90 air sampler	5	2	2	2	2	2	2	2	A	3
STA New Brunswick	5	1	3	2	4	2	3	2	A	1[c]
Sieve impactor Andersen 1-STAGE	2	1	3	2	2	2	3	1	A	3
Centrifugal biotest RCS plus	3	3	3	2	2	4	2	3	A	6
Sieve impactor Anderson 6-STAGE	5	4	6	6	3	4	3	2	A	5
Gel membrane sartorius MD8	4	6	2	1	1	2	2	1	A	1[c]
SMA P200 impactor	6	1	2	2	2	2	3	2	A	4
Glass impinger all glass	6	2	4	3	4	4	3	2	A	4

Table 2B Relative cost/difficulty comparisons

Sampling method/sampler	Laminar airflow 1–6[b]	Critical environments	Production areas 1–6[b]	General areas 1–6[b]	Flat environmental surfaces	Irregular environ. surfaces	Personnel barriers 1–6[b]	Volumetric (SP) Y/N	Remote probe possible	External power	Sample
Swab sample typical	4	2	2	1	2	1	4	N	•	•	S
Contact plate typical	1	1	1	1	1	4	1	N	•	•	S
Gravity settle plate typical	1	1	1	1	•	•	•	N	•	•	S
SAS super 90 air sampler	2	3	2	2	•	•	•	Y	N	Y	S
STA New Brunswick	1[d]	3	1	2	•	•	•	Y	Y	Y	S
Sieve impactor Andersen 1-STAGE	2	2	1	1	•	•	•	Y	N	Y	S
Centrifugal biotest RCS plus	5	5	3	1	•	•	•	N	N	N	P
Sieve impactor Anderson 6-STAGE	5	2	2	2	•	•	•	Y	N	Y	S
Gel membrane sartorius MD8	1[d]	1	1	1	•	•	•	Y	Y	Y	P
SMA P200 impactor	3	3	2	1	•	•	•	Y	N	Y	S
Glass impinger all glass	4	3	2	2	•	•	•	Y	N	Y	S

[a]Acquisition cost in thousand dollars.
[b]Difficulty: 1–6 (easiest–hardest).
[c]With Hose/probe attachment.
[d]With Hose/isokinetic probe attachment.
A: Aerobiologic samples; B: Flat surface samples; C: Irregular surface samples; P: Proprietary media system; S: Standard Commercially-available system.
(Courtesy of Northview Biosciences Inc., Northbrook, IL.)

in sample air, which had passed through the slit of an STA, than were found in ambient air.[34] This was attributed to fragmentation of larger particles following passage through the slit of the STA.[e]

Due to dehydration of the agar reported to occur over long sampling periods, continuous sampling exceeding 30 min using an impaction sampler is not recommended. Areas of loss have been reported for sieve samplers,[39] including *inlet loss* (the effect of cross-wind at the sample inlet point), *interstage loss* (deposition of particles on internal surfaces other than the impaction agar), and *particle re-entrainment* (particles reintroduced into the airstream due to particle ''bounce,'' resulting from dehydration of the impaction agar).

Advantages of the centrifugal sampler are the capability of sampling large amounts of air (40 L/min) in a short time; it is quiet, lightweight, self-contained, and does not require cumbersome air pumps or external power for operation. Centrifugal samplers provide a good indication of environmental isolates.[17]

Centrifugal sampling cannot be carried out isokinetically,[23] and the accuracy of results is dependent upon the sizes of the particles being sampled. Since particulate sizes in the air volume being sampled are not routinely determined, the validity of the centrifugal sampler as a quantitative device has been called into question,[35] especially for quantification of small particles.[27,36] Another recent study indicates that centrifugal sampling causes air to move in a turbulent, mixing manner, introducing heavily disturbed airflow patterns around the sampler which may, in turn, impart disturbances to any unidirectional airflow patterns being sampled.[23] Reaspiration of sampled air is also a problem with earlier designs, creating difficulty in discriminating between incoming and outgoing airstreams, which is necessary to quantify microorganisms.[37] Proprietary agar medium strips are specially designed and unique to this system, and require careful technique to insert and remove aseptically.

Membrane filtration: Membrane filtration (MF) sampling is accomplished by capturing aerobiological contamination as it passes through a cellulose membrane filter (CMF) or gelatin membrane filter (GMF). The mechanisms of MF particle removal are inertial impaction, diffusional interception, and direct interception. Following collection, the GMF may be plated aseptically onto an agar petri dish to dissolve, allowing microorganisms to grow directly on the nutrient medium. Dissolution of the membrane into a sterile solution is also possible.[31]

[e]Interestingly, this attribute was reported by investigators to be an advantage of the all-glass Impinger.[31,32]

While MF sampling has been demonstrated to be the most effective means of retaining aerobiological contamination, CMF sampling exhibits a lower recovery rate than an impinger when tested against stress-sensitive microorganisms, such as *Serratia marcescens*[38] or*Escherichia coli*[27] due to desiccation on the CMF surface. Studies have indicated that gelatin foam filters incorporated into GMF gave significantly higher recovery rates than CMF over the same sampling period.[39,40] Recent comparisons of sampling systems indicate that GMF is equally as effective as the STA sampler, irrespective of particle size, and is significantly more effective than centrifugal sampling in the collection of microorganisms with sizes $<5.0\,\mu m$.[31] A recent study comparing the GMF system with centrifugal, sieve, and STA systems in sampling the unidirectional airflow slipstream in the presence of visual tracers indicates the GMF sampler to be the only sampling method capable of isokinetic and isoaxial samplng with novisual disturbance to the laminar airflow pattern.[31] However, in this study, the STA was tested without the remote hose-isokinetic probe device.

Limitations of the GMF are an additional aseptic subculture step, which increases the probability of adventitious contamination, and a proprietary membrane filter, which results in a per-sample cost currently exceeding 12 times that of the one-stage sieve, SAS, STA, SMA, and glass impinger systems, and four times that of the centrifugal sampler.

Growth Media

Growth and collection media used in microbiologic monitoring should be selected on the basis of the target organisms, areas and surfaces sampled, and inhibitory residues that may remain on the sampled surfaces. Media commonly used for environmental monitoring are listed in Table 3. Under certain circumstances (e.g., when obligate anaerobes are recovered from the product), additional, specific media and methods should be selected by a qualified microbiologist.[17]

Comparison of Aerobiologic Samplers

The different characteristics and operating principles of aerobiological samplers do not facilitate direct and simple comparisons. The user should, therefore, carefully evaluate the numerous advantages and disadvantages of each method in selecting a sampler for the intended application (Tables 2A and B).[41] Two studies that provide basic comparisons of aerobiological sampling systems may offer useful information:

A study comparing eight bioaerosol samplers was carried out by Jensen et al. in 1992.[27] Results indicated

Table 3 Media commonly used for environmental monitoring

Medium	Selective for	Sample application
Tryptic soy agar (TSA)[a,b]	Aerobes and facultative anerobes	Air and surface
Letheen agar[c]	Aerobes and facultative anerobes	Surface
DE neutralizing agar[d]	Aerobes and facultative anerobes	Surface
Sabouraud dextrose agar	Yeast and molds	Air and surface
Rose bengal agar	Yeast and molds	Air and surface
Buffer solution[e]		Surface

[a]Tryptic soy agar is also known as soybean casein digest agar.
[b]Unmodified general purpose medium use for culturing bacteria and/or fungi.
[c]Contains additives used to neutralize residuals of halogen-based disinfectants, such as sodium hypochlorite (bleach).
[d]Contains additives used to neutralize residuals of halogen and quaternary ammonium chloride-based disinfectants.
[e]Samples collected using sterile swabs and buffer solution must be transferred to media for culturing and enumeration.
(Courtesy of Northview Biosciences, Inc., Northbrook, IL.)

that the Andersen 6-STG, I-STG, and Ace Glass AGI 30 samplers were the samplers of choice for recovering aerosols of free bacteria (i.e., mostly single cells of *E. coli* and *B. subtilis*, $d_{ae} \geq 2\,\mu m$) under the controlled conditions of the study.[42] Another study, comparing seven samplers commonly used in controlled environments, was conducted by Ljungqvist and Reinmiiller in 1998.[43] This study indicated widely varying results for the impaction samplers tested. The limited number of parallel tests performed prevented an evaluation of comparative collection efficiencies based upon statistical considerations. The salient recommendations of this study are that results should be seen more "... as an indication of a [contamination] level and not be taken as a true absolute value,'' and that aerobiological samplers be selected carefully, based on practicalities of using different types for different locations or situations. Furthermore, this study recommends the simultaneous use of a discrete particle counter (DPC) to measure the total number of airborne particles present in the area sampled.[f]

[f]Because it is impossible to derive instantaneous results from microbiologic testing, the authors agree that such data should, where possible, be correlated with a DPC as an instantaneous data source, in developing useful historical data. Although "... no universal relationship has been established between the total concentration of airborne particles and the concentration of viable airborne particles...'',[7] such a correlation may be possible under controlled operational conditions within a specific area or facility (determined by Ljungqvist and Reinmüller in two facilities) to be approximately 10^{-4} (10,000 : 1). Such a correlation would facilitate a "viability index'' as a rational means of correlating shifts in instantly available particle count values with probable corresponding shifts in aerobiological contamination. This technique would be very useful in the instantaneous identification of contamination indicator sites. Similar correlations have been established on a facility-specific basis by the Lab Safety Corp. during the course of regular, periodic aerobiological sampling of three bone marrow transplant complexes over a period of several years. In all cases, a correlation of the total population of aerobiological contaminants to instantaneous DPC data (termed the "viability index'') was used to trace the distribution and probable presence of the life-threatening organism *A. niger* in immunocompromised patient populations. Although it was found that in these highly controlled facilities (e.g., Class 1000 or better) the correlation was one to two orders of magnitude higher than that described by Ljungqvist and Reinmüller,[41,43] the correlations were consistent, allowing facility managers to reliably detect possible life-threatening deteriorations of the critical patient environment through the use of instantaneous DPC data as an aerobiological contamination indicator. The data analyzed indicate that aerobiological contaminants appear to increase in proportion to non-viable contaminants as the cleanliness of a facility increases. This is probably due to the fact that, as general environmental contamination is eliminated, human activity becomes the principal source of contamination.

ANALYSIS AND INTERPRETATION OF MONITORING RESULTS

Effective interpretation of data from microbiologic monitoring of the environment can be the most difficult aspect of the monitoring process. Several factors complicate this process, including the inherently nonrandom distribution of most microbial contamination events, errors in sample handling, variation of sampling technique from one monitoring event to the next, and seasonal shifts in the type and level of contaminants likely to be present in the general environment.

The purpose of statistical evaluation of sample data is to extrapolate from a collection of individual events (e.g., 30 min of process time) to the entire population of events (e.g., 8-h shift). Because microbial monitoring data usually measure the impact of human activity, which is not reproducible exactly from one event to the next, results usually do not fit standard statistical models for normal distributions. In spite of this limitation, it is necessary to summarize the data for comparison to limits. The best statistical methods of evaluation are determined by the nature of the data.[14] Wilson suggests that microbial monitoring data histograms generally resemble Poisson or negative

exponential distributions,[14] whereas Akers points out that Poisson distributions may only be appropriate for systems with minimal human intervention.[12] The formula for the Poisson distribution[44] is given by

$$P(C) = \frac{(np_0)^c}{C!} e^{-} np_0 \quad (3)$$

where C is individual sample count, np_0 is average count, and $e = 2.718281$.

Trend analysis of results at individual sample locations may be more useful than statistical analysis of data summaries because each sampling location probably reflects a unique situation. Non-traditional groupings of data may also be valuable. For example, grouping all locations where a specific activity was noted on the sample collection form, grouping all data collected during a specific time frame (i.e., just after lunch, or near the end of a production cycle), or grouping all data for each operator may reveal specific problem areas.

The example control charts presented by Besterfield in Fig. 2 demonstrate four major types of out-of-control patterns.[44] A fifth pattern is due to mistakes, which will usually show up as isolated, out-of-control points. All apply equally to production and sampling operations. All patterns may be observed on both range (R) charts and standard (or reference) process average charts but are usually more common to charts.

Likely causes for each type of pattern can be identified, and a checklist of assignable causes applicable to the particular process should be developed through cause and effect (C&E) analysis.[44] Examples of likely causes for these patterns are:

a. A change or jump in pattern caused by an inexperienced operator, a change in raw materials, or a failure of an equipment part;
b. A trend or steady change in level due to a gradual change in the production environment, a gradual change in equipment performance (e.g. HEPA filter loading), or a gradual tendency toward lax observation of SOPs;
c. Two populations may be due to more than one process line or piece of critical equipment on the same chart, more than one operator on the same chart, or different samplers or sampling techniques; and
d. Recurring cycles may be caused by periodic operator rotation, operator fatigue and rejuvenation cycles, sanitizing and cleaning cycles, and seasonal shifts.

Recurring cycles may be missed if sampling intervals happen to coincide with the cycle frequency, in which case only the low or high range of the cycle may be

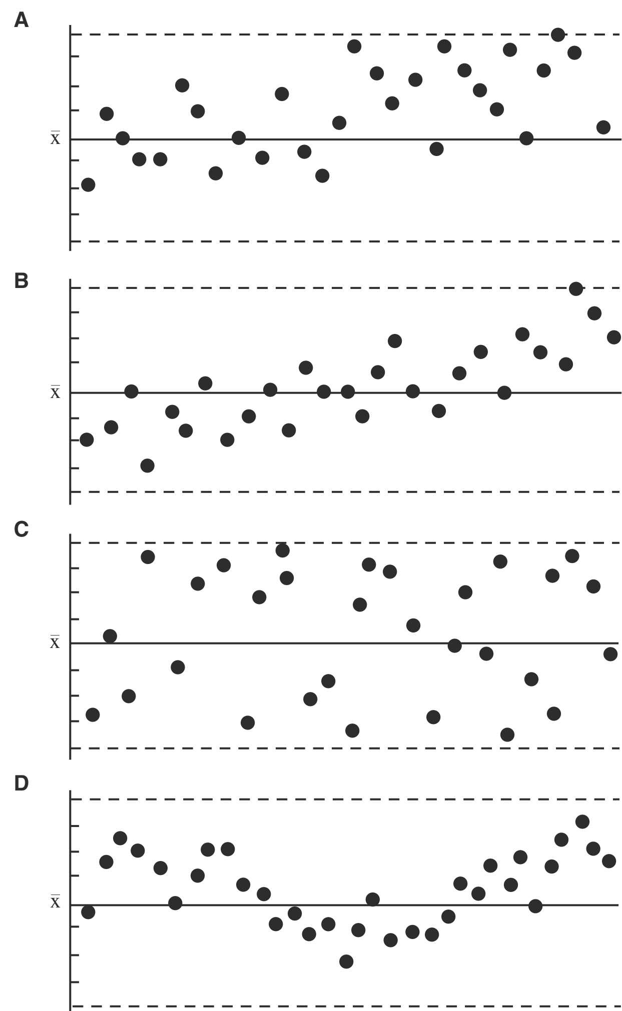

Fig. 2 Out-of-control pattern recognition. (A) Change or jump in level; (B) Trend or steady change in level; (C) Two populations; (D) Recurring cycles. (Chart courtesy of Prentice Hall, Inc., Upper Saddle River, NJ.)

detected. Out-of-limit trends near the lower limits of the R chart represent superior performance and should be analyzed to identify methods of maintaining these process levels.[44] Whatever statistical methods are employed for summarizing data, graphic representations, such as histograms and process control charts can be extremely useful for detecting trends or cyclic patterns in test results.

There are two types of over-limit results: *Random* results are due to chance (unassignable) causes, whereas *non-random* results are due to assignable causes. For a controlled process and facility, the objective is to differentiate between individual data points that are assignable and those that are not. If the individual over-limit event is not repeated during subsequent, multiple retests, it is not assignable and does not represent a deteriorating trend. All statistical evaluation methods include mechanisms for ''discarding''

spurious data. There is, however, a cause for any unassignable result, and efforts should be made to identify and understand it. All data have meaning, and may be useful for improving the process or testing procedures.

Speciation

Speciation of microorganisms is indicated when product testing results detect the presence of a specific organism, when evaluating the efficacy of sanitizing compounds and routines, and when monitoring results trigger the corrective action plan. Speciation should be carried out and analyzed by a qualified microbiologist familiar with the sampling equipment, sampling methods employed, and the origins of organisms commonly found in cleanrooms.[17,45] Speciation should also be conducted periodically to identify isolates normally recovered when the process is operating within limits, and may be useful in identifying the probable cause(s) of any out-of-limit condition. During the initial phase of the corrective action plan, an analysis of probable contamination sources and routes should be made for all organisms identified.[17] Information obtained by speciation may immediately indicate the most likely source. This information may also indicate less common contamination sources, such as perverted cleaning solutions.

Periodic re-evaluation of the monitoring plan should be carried out, and seasonal effects considered in trend analysis. Many sampling methods do not collect all organisms with equal efficiency, and organisms likely to be present may vary seasonally. Any seasonal shift (up or down) should be investigated by speciation, and sampler correction factors for the predominant organisms applied.

CORRECTIVE ACTION PLAN

The CAP should clearly define and document

1. The method of data analysis;
2. Alert, action, and fail limits;
3. Corrective actions to be employed in the event of detection of a deteriorating trend or an over-limit condition; and
4. A means of confirming the effectiveness of corrective action(s).

A verified trend above the action or fail limit should immediately trigger implementation of the CAP. Because human activity is the most likely source of process control failure, the investigative process normally begins with personnel, and proceeds through the various possible causes from most to least likely. An exception to this general plan is verification of room pressurization, which is a primary indication of engineering control equipment efficiency. Although routine monitoring of pressurization should detect any out-of-limit results, the simplicity of verifying proper pressurization suggests this as a first step.

In general, the cause of any deterioration in process or environmental control can be traced to one of three principle systems: a) personnel controls; b) process controls; or c) facility (engineering) controls. Increases in detected airborne microbiologic contamination levels may result from any of several conditions, and a simple set of logical challenges can be applied to the data to determine the most likely cause.

Challenge 1, *Is the increase real and reproducible?* If it is not reproducible, it may be due to sampling error, or NACs. If it is reproducible, it may be due to an actual increase in levels, or due to enhanced collection efficiency, due to changes in methods, materials, or seasonal or other shifts in the kinds of contaminants present (different organisms have different sampling efficiencies); Challenge 2, *If the increase is real, is it due to an increase in source intensity, or to a decrease in the ability of engineering controls to maintain a clean air supply?* The easiest way to differentiate between these possibilities is to examine particle count data. There are several possible combinations of test results, each indicating a different cause for increased airborne contamination: a) If particle counts taken under operational conditions have not risen, but airborne microbiologic contamination has, it is most likely due to a breakdown in personnel discipline and/or gowning procedures; b) If operational particle counts have risen, but at-rest counts have not, it is again likely that the cause of elevated microbial contamination is personnel activity and that it represents an increase in source intensity (when human activity is eliminated, engineering controls are able to produce the same conditions that were present during the OQ validation phase); and c) If at-rest particle counts have risen, the increase is probably due to a decrease in the efficiency of the engineering controls.

Similar logical tests can be applied to increases in surface contamination levels, which may be due to increases in source intensity, or decreases in the efficiency of barrier controls or cleaning and sanitizing procedures. Flow charts illustrating the logical evaluation of data, and investigation of out-of-limit results are useful as starting points in the development of corrective action plans.[5]

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