analysis is used to facilitate decision-making for requalification of a controlled environment or for maintenance and sanitization schedules.

REFERENCES

Agalloco J, Akers J. Risk analysis for aseptic processing: The Akers-Agalloco method. *Pharm Technol.* 2005; 29(11):74–88.

Agalloco J, Akers J. The simplified Akers-Agalloco method for aseptic processing risk analysis. *Pharm Technol.* 2006; 31(7)60–72.

Akers, J. The proper role of environmental monitoring in aseptic processing. *Am Pharm Rev.* 2006; 9(4):24–28.

CDC, Healthcare Infection Control Advisory Committee. Guidelines for environmental control in healthcare facilities. *MMWR* 2003; 52(No. RR-10):1–42.

Favero MS, Puleo JR, Marshall JH, Oxborrow GS. Microbiological sampling of surfaces. *J Appl Bacteriol.* 1968; 31:336–346.

Hussong D, Madsen R. Analysis of environmental microbiology data from clean room samples. *Pharm Technol.* 2004; Aseptic Processing Suppl:10–15.

International Organization for Standardization (ISO). 14644-1, Clean rooms and associated environments, part 1: classification of air cleanliness. Geneva: ISO; 1999.

International Organization for Standardization (ISO). 14644-2, Clean rooms and associated environments, part 2: specifications for testing and monitoring to prove continued compliance with 14644 part 1. Geneva: ISO; 2000.

Jensen PA, Todd WF, Davis GN, Scarpino PV. Evaluation of eight bioaerosol samplers challenged with aerosol of free bacteria. *Am Ind Hyg Assoc J.* 1992; 53:660–667.

Ljungqvist B. Active sampling of airborne viable particulate in controlled environments: a comparative study of common instruments. *Eur J Parenter Sci.* 1998; 3:59–62.

Ljungqvist B, Reinmüller B. Interaction between air movements and the dispersion of contaminants: clean zones with unidirectional air flow. *J Parenter Sci Technol.* 1993; 47(2):60–69.

Ljungqvist B, Reinmüller B. Airborne viable particles and total number of airborne particles: comparative studies of active air sampling. *PDA J Sci Technol.* 2000; 54:112–116.

Maruyama M, Matsuoka T, Deguchi M, Akers J. The application of robotics to aseptic surface monitoring. *Pharm Technol.* 2007; 32(7):40–44.

Process simulation testing for sterile bulk pharmaceutical chemicals. PDA Technical Report No. 28. *J Parenter Sci Technol.* 1998; 52 S3.

Reinmüller B. Dispersion and risk assessment of airborne contaminants in pharmaceutical cleanrooms. *Building Serv Eng Bull* (Sweden). 2001; Bulletin No. 56.

Stewart SL, Grinshpun SA, Willeke K, Terzieva S, Ulevicius V, Donnelly J. Effect of impact stress on microbial recovery on an agar surface. *Appl Environ Micro.* 1995; 61:1232–1239.

Whyte W. Reduction of microbial dispersion by clothing. *J Parenter Sci Technol.* 1985; 39(1):51–60.▲*USP35*

⟨1117⟩ MICROBIOLOGICAL BEST LABORATORY PRACTICES

INTRODUCTION

Good laboratory practices in a microbiology laboratory consist of activities that depend on several principles: aseptic technique, control of media, control of test strains, operation and control of equipment, diligent recording and evaluation of data, and training of the laboratory staff. Because of the inherent risk of variability in microbiology data, reliability and reproducibility are dependent on the use of accepted methods and adherence to good laboratory practices.

MEDIA PREPARATION AND QUALITY CONTROL

Media Preparation

Culture media are the basis for most microbiological tests. Safeguarding the quality of the media is therefore critical to the success of the microbiology laboratory. Media preparation, proper storage, and quality control testing can ensure a consistent supply of high-quality media.

It is important to choose the correct media or components in making media based on the use of accepted sources or references for formulas. The manufacturer's formula and instructions for preparation routinely accompany dehydrated media and ready-made media. Because different media types may have different preparation requirements (e.g., heating, additives, and pH adjustment), it is important to follow these instructions to ensure preparation of acceptable media quality. A certificate of analysis describing expiration dating and recommended storage conditions accompanies ready-made media, as well as the quality control organisms used in growth-promotion and selectivity testing of that media.

Water is the universal diluent for microbiological media. Purified Water is most often used for media preparation, but in certain cases the use of deionized or distilled water may be appropriate. Water of lesser quality should not be used for microbiological media preparation. The volume of the water used should be recorded.

Consistent preparation of media requires accurate weighing of dehydrated media or media constituents. A calibrated balance with the appropriate weight range for the ingredients should be used (See *Weighing on an Analytical Balance* ⟨1251⟩). Clean weighing containers and tools (such as spatulas) should be used to prevent foreign substances from entering the formulation. The weight of the components should be recorded.

Dehydrated media should be thoroughly dissolved in water before dispensing and sterilization. If heating is necessary to help dissolve the media, care should be taken not to overheat media, because all culture media, to a greater or lesser extent, are heat-sensitive. Equipment used in the preparation of media should be appropriate to allow for controlled heating, constant agitation, and mixing of the media. Darkening of media (Maillard-type reaction or nonenzymatic browning) is a general indication of overheating. When adding required supplements to media, adequate mixing of the medium after adding the supplement should be performed.

Preparation of media in poorly cleaned glassware can allow inhibitory substances to enter the media. Inhibitory substances can come from detergent residue after cleaning

glassware or from prior materials used in the glassware. Be sure that the cleaning process removes debris and foreign matter, and that the detergent is thoroughly rinsed out with Purified Water. See *Cleaning Glass Apparatus* ⟨1051⟩ for additional guidance.

Sterilization of media should be performed within the parameters provided by the manufacturer or validated by the user. Commercially prepared media should provide documentation of the sterilization method used. Autoclaving by moist heat is the preferred sterilization technique, except in instances when boiling is required in order to avoid deterioration of heat-labile components of the media. Sterilization by filtration may also be appropriate for some formulations.

The effects of the sterilization method and conditions on the media should be validated by sterility and growth-promotion testing of the media. In addition, if sterilized by moist heat, the autoclave cycle should be validated to ensure proper heat distribution for selected loads and volumes. Typically, manufacturers recommend using an autoclave cycle of 121° for 15 minutes using a validated autoclave. These conditions apply to time at temperature of the media. As container size and the load configuration of the autoclave will influence the rate of heating, longer cycles may be required for larger loads. However, the sterilization time will be dependent on the media volume and autoclave load. Sterilization cycles in which the autoclave is slow to come up to temperature may result in overheating of the media. Therefore, care must be taken to validate a sterilization cycle, balancing the need for sterile media against the tendency of the media to degrade under excessive heating. Storage of the media in the autoclave after the liquid cycle is completed is not recommended after cooling, as it may damage the media. Improper heating or sterilizing conditions—for commercially prepared or internally prepared media—may result in a difference in color change, loss of clarity, altered gel strength, or pH drift from the manufacturer's recommended range, as well as reduced growth-promotion activity and/or selectivity.

The pH of each batch of medium should be confirmed after it has cooled to room temperature (20°–25°) by aseptically withdrawing a sample for testing. Refrigerated purchased media should be allowed to warm up to ambient room temperature if it is to be checked for pH confirmation. A flat pH probe is recommended for agar surfaces, and an immersion probe is recommended for liquids. See *pH* ⟨791⟩ for guidance with pH measurement and instrument calibration. The pH of media should be in a range of ±0.2 of the value indicated by the manufacturer, unless a wider range is acceptable by the validated method.

Prepared media should be checked by appropriate inspection of plates and tubes for the following:

- Cracked containers or lids
- Unequal filling of containers
- Dehydration resulting in cracks or dimpled surfaces on solid medium
- Hemolysis
- Excessive darkening or color change
- Crystal formation from possible freezing
- Excessive number of bubbles
- Microbial contamination
- Status of redox indicators (if appropriate)
- Lot number and expiration date checked and recorded
- Sterility of the media
- Cleanliness of plates (lid should not stick to dish)

Media Storage

It is prudent to consider how the manufacturer or supplier transports and stores media before distribution to the end user. Manufacturers of media should use transport and storage conditions that minimize the loss of moisture, control the temperature, prevent microbial contamination, and provide mechanical protection to the prepared media.

Media should be labeled properly with batch or lot numbers, preparation and expiration dates, and media identification. Media should be stored according to the manufacturer's instructions. Media prepared in house should be stored under validated conditions. Do not store agar at or below 0°, as freezing could damage the gel structure. Protect stored media from exposure to light and excessive temperature. Before prolonged storage, agar plates should be placed into a sealed package or container to retard moisture loss.

Remelting of an original container of solid media should be performed only once to avoid media whose quality is compromised by overheating or potential contamination. It is recommended that remelting be performed in a heated water bath or by using free-flowing steam. The use of microwave ovens and heating plates is common, but care should be taken to avoid damaging media by overheating and to avoid the potential injury to laboratory personnel from glass breakage and burns. The molten agar medium should be held in a monitored water bath at a temperature of 45° to 50° for not more than 8 hours. Caution should be taken when pouring the media from a container immersed in a water bath to prevent water from the bath commingling with the poured sterile media. Wiping the exterior of the container dry before pouring may be advisable.

Disposal of used cultured media (as well as expired media) should follow local biological hazard safety procedures.

Quality Control Testing

Although growth media can be prepared in a laboratory from individual components, many laboratories, for ease of use, use dehydrated media or purchase commercially prepared media in plastic plates or glass containers. Manufacturers of media attempt to standardize raw materials from biological sources, but must constantly deal with unavoidable differences in raw materials obtained from natural sources, and therefore, lot-to-lot variability of media must be considered. In addition, the performance of media prepared in a laboratory or by a manufacturer is highly dependent on preparation and storage conditions. Improper media preparation can cause unsatisfactory conditions for microbial growth or recovery and unreliable results.

Therefore, quality control tests should be performed on all prepared media, including media associated with swabs or media in strips and other nontraditional formats. Tests routinely performed on in-house prepared media should include pH, growth promotion, inhibition, and indicative properties (as appropriate), and periodic stability checks to confirm the expiration dating.

When in-house prepared microbiological media are properly prepared and sterilized using a validated method, the growth-promotion testing may be limited to each incoming lot of dehydrated media, unless otherwise instructed by the relevant compendial method. If the media preparation procedure was not validated, then every batch of media should be subjected to growth-promotion testing. Test organisms may be selected from the appropriate compendial test chapter. In addition, microorganisms used in growth-promotion testing may be based on the manufacturer's recommendation for a particular medium, or may include representative environmental isolates (but these latter are not to be construed as compendial requirements).

Expiration dates on media should have supporting growth-promotion testing to indicate that the performance of the media still meets acceptance criteria up to and including the expiration date. The length of shelf life of a batch of media will depend on the stability of the ingredients and formulation under specified conditions, as well as the type of container and closure.

When a batch of media does not meet the requirements of growth-promotion testing, an investigation should be initiated to identify the cause. This investigation should include a corrective action plan to prevent the recurrence of the problem. Any batch of media that fails growth-promotion

testing is unsuitable for use. [NOTE—Failed growth-promotion test results may not be used to negate positive test results.]

Some reagents are used for diagnostic purposes to help support identification of microbial organisms, e.g., Gram stain and oxidase test reagents. These may have attributes that can be quality control tested similar to microbiological media. Select the correct quality control standard microorganisms, following the manufacturer's instructions, and perform the testing before unknown sample diagnostic testing. All relevant diagnostic reagents should be subjected to incoming quality confirmation before use.

Special care should be taken with media that is used in sterility tests (see *Sterility Tests* ⟨71⟩ for requirements) and in environmental monitoring studies. Media used for environmental monitoring of critical areas should preferably be double-wrapped and terminally sterilized. If terminal sterilization is not performed, media should be subjected to 100% pre-incubation and inspection before use within a critical area. [NOTE—Growth-promotion testing for this media must be performed after the pre-incubation stage.] This will prevent extraneous contamination from being carried into controlled environments and will prevent false-positive results. A raised agar level for surface contact plates should be verified.

MAINTENANCE OF MICROBIOLOGICAL CULTURES

Biological specimens can be the most delicate standards to handle because their viability and characteristics are dependent on adequate handling and storage. Standardizing the handling and storage of cultures by the user laboratory should be done in a way that will minimize the opportunity for contamination or alteration of growth characteristics. The careful and consistent treatment of stock cultures is critically important to the consistency of microbiological test results. Cultures for use in compendial tests should be acquired from a national culture collection or a qualified secondary supplier. They can be acquired frozen, freeze-dried, on slants, or in ready-to-use forms. Confirmation of the purity of the culture and the identity of the culture should be performed before its use in quality control testing. Ready-to-use cultures should be subjected to incoming testing for purity and identity before use. The confirmation of identity for commonly used laboratory strains should ideally be done at the level of genus and species.

Preparation and resuscitation of cultures should follow the instructions of the supplier or a validated, established method. The "Seed-Lot" technique is recommended for storage of stock cultures.

The original sample from the national culture collection or a qualified secondary supplier is resuscitated and grown in an appropriate medium. Aliquots of this stock culture (the first transfer or passage) are suspended in a cryoprotective medium, transferred to vials, and frozen at –30° or below, until use. If stored at –70°, or in lyophilized form, strains may be kept indefinitely. These frozen stocks can then be used to inoculate monthly or weekly working cultures. Once opened, do not refreeze unused cell suspensions after culturing a working suspension. The unused portion should be discarded to minimize the risk of loss of viability and contamination of the stock.

The number of transfers of working control cultures should be tracked to prevent excessive subculturing that increases the risk of phenotypic alteration or mutation. The number of transfers allowable for specific compendial tests may be specified in that test. One passage is defined as the transfer of organisms from a viable culture to a fresh medium with growth of the microorganisms. Any form of subculturing is considered to be a transfer/passage.

LABORATORY EQUIPMENT

Most equipment (incubators, water baths, and autoclaves) is subject to standard validation practices of incoming qualification, operational qualification, and performance qualification. Additionally, periodic calibration (generally annually) is commonly required. New equipment, critical to the operation of the laboratory, should be qualified according to a protocol approved by the quality assurance unit (QAU). In addition, regular cleaning and sanitization of equipment such as incubators, refrigerators, and water baths should be performed to minimize the potential for contamination in the laboratory. Door seals of incubators and refrigerators should be cleaned and checked for state of repair.

Instruments (pH meters and spectrophotometers) used in a microbiology laboratory should be calibrated on a regular schedule and tested to verify performance on a routine basis. The frequency of calibration and performance verification will vary based on the type of instrument and the importance of that equipment to the generation of data in the laboratory.

Equipment that is difficult to sanitize (such as refrigerators and incubators) should be dedicated to aseptic operations (such as storage of media for testing and incubation of sterility test samples) and live culture operations to minimize the potential for inadvertent contamination of the tests.

Autoclaves are central to the operation of the laboratory and must have proper validation in place to demonstrate adequate sterilization for a variety of operations. Autoclave resources must be available (and validated) to sterilize waste media (if performed in that laboratory) as well as the media prepared in that laboratory. The choice of one or several autoclaves is not driven by a need to separate aseptic and live operations (everything in the properly maintained autoclave is sterile after the cycle) but rather driven by resource considerations (see below).

LABORATORY LAYOUT AND OPERATIONS

Laboratory layout and design should carefully consider the requirements of good microbiological practices and laboratory safety. It is essential that cross-contamination of microbial cultures be minimized to the greatest extent possible, and it is also important that microbiological samples be handled in an environment that makes contamination highly unlikely.

In general, a laboratory should be divided into clean or aseptic areas and live culture areas. Areas in which environmental or sterile product samples are handled and incubated should be maintained completely free of live cultures, if possible. If complete separation of live and clean culture zones cannot be accomplished, then other barriers and aseptic practices should be employed to reduce the likelihood of accidental contamination. These barriers include protective clothing, sanitization and disinfection procedures, and biological safety cabinets designated for clean or aseptic operations only. Procedures for handling spills or mishaps with live cultures should be in place, and all relevant technical personnel should be trained regarding these methods.

Some samples will demonstrate microbial growth and require further laboratory analysis to identify the contaminants. When growth is detected, the sample should be taken from the clean section of the laboratory to the live culture section without undue delay. Subculturing, staining, microbial identification, or other investigational operations should be undertaken in the live culture section of the laboratory. If possible, any sample found to contain growing colonies should not be opened in the clean zone of the laboratory. Careful segregation of contaminated samples and materials will reduce false-positive results.

Staff engaged in sampling activities should not enter or work in the live culture handling section of a laboratory unless special precautions are taken, including wearing protective clothing and gloves and careful sanitizing of hands

upon exiting. Ideally, staff assigned to sampling activities, particularly those in support of aseptic processing, should not work in the vicinity of live culture laboratory operations.

It is important to consider that microbial contamination of samples, which leads to false-positive results, is always possible unless careful aseptic precautions are taken. Facilities should be designed so that raw material and excipient sampling can be done under controlled conditions, including proper gowning and sterilized sampling equipment. It may not always be possible to sample utility systems, such as water systems, under full aseptic conditions; however, it should be noted that when samples are not taken aseptically, their reliability is inevitably compromised.

Environmental sampling methods should require minimal aseptic handling in loading and unloading sampling instruments. Whenever possible, sampling equipment should be loaded with its microbiological recovery media in the environment that is to be sampled.

All testing in laboratories used for critical testing procedures, such as sterility testing of final dosage forms, bulk product, seed cultures for biological production, or cell cultures used in biological production, should be performed under controlled conditions. Isolator technology is also appropriate for critical, sterile microbiological testing. Isolators have been shown to have lower levels of environmental contamination than manned clean rooms, and therefore, are generally less likely to produce false-positive results. Proper validation of isolators is critical both to ensure environmental integrity and to prevent the possibility of false-negative results as a result of chemical disinfection of materials brought into or used within isolators (see *Sterility Testing—Validation of Isolator Systems* ⟨1208⟩).

SAMPLE HANDLING

Viable microorganisms in most microbiology samples, particularly water, environmental monitoring and bioburden samples, are sensitive to handling and storage conditions. Critical parameters in these conditions include product (or sample) composition, container composition, time of storage, and temperature of storage. Therefore, it is important to minimize the amount of time between the sampling event and the initiation of testing and to control, as much as possible, the conditions of storage. If the sample is to be transported to a distant location for testing, then the conditions of transport (time, temperature, etc.) should be qualified as suitable for that test and sample. Guidance for water testing in this regard can be found in *Water for Pharmaceutical Purposes* ⟨1231⟩. Product mixing before sampling may need to be evaluated and applied in order to ensure microbial dispersement and representation in the sample aliquot.

All microbiological samples should be taken using aseptic techniques, including those taken in support of nonsterile products. If possible, all microbiological samples should be taken under full aseptic conditions in specialized sampling areas. The areas should be as close to the point of use as possible to minimize contamination during transit.

Samples submitted to the microbiology laboratory should be accompanied by documentation detailing source of the sample, date the sample was taken, date of sample submission, person or department responsible for the submission, and any potentially hazardous materials associated with the sample. The testing department should acknowledge receipt of the sample and reconcile the identity and number of samples as part of this sample documentation.

MICROBIOLOGICAL MEDIA INCUBATION TIMES

Incubation times for microbiological tests of less than 3 days' duration should be expressed in hours: e.g., "Incubate at 30° to 35° for 18 to 72 hours". Tests longer than 72 hours' duration should be expressed in days: e.g., "Incubate at 30° to 35° for 3 to 5 days". For incubation times expressed in hours, incubate for the minimum specified time, and exercise good microbiological judgment when exceeding the incubation time. For incubation times expressed in days, incubations started in the morning or afternoon should generally be concluded at that same time of day.

TRAINING OF PERSONNEL

Each person engaged in each phase of pharmaceutical manufacture should have the education, training, and experience to do his or her job. The demands of microbiological testing require that the core educational background of the staff, supervisors, and managers be in microbiology or a closely related biological science. They should be assigned responsibilities in keeping with their level of skill and experience.

A coherent system of standard operating procedures (SOPs) is necessary to run the microbiology laboratory. These procedures serve two purposes in a training program. Firstly, these SOPs describe the methodology that the microbiologist will follow to obtain accurate and reproducible results, and so serve as the basis for training. Secondly, by tracking the procedures in which a particular microbiologist has demonstrated proficiency, the procedure number or title also serves to identify what training the microbiologist has received specific to his or her job function.

Training curricula should be established for each laboratory staff member specific to his or her job function. He or she should not independently conduct a microbial test until qualified to run the test. Training records should be current, documenting the microbiologist's training in the current revision to the particular SOP.

Periodic performance assessment is a wise investment in data quality. This performance testing should provide evidence of competency in core activities of the microbiology laboratory such as hygiene, plating, aseptic technique, documentation, and others as suggested by the microbiologist's job function.

Microbiologists with supervisory or managerial responsibilities should have appropriate education and in-house training in supervisory skills, laboratory safety, scheduling, budgeting, investigational skills, technical report writing, relevant SOPs, and other critical aspects of the company's processes as suggested in their role of directing a laboratory function.

Competency may be demonstrated by specific course work, relevant experience, and routinely engaging in relevant continuing education. Achieving certification through an accredited body is also a desirable credential. Further, it is expected that laboratory supervisors and managers have a demonstrated level of competence in microbiology at least as high as those they supervise. Expertise in microbiology can be achieved by a variety of routes in addition to academic course work and accreditation. Each company is expected to evaluate the credentials of those responsible for designing, implementing, and operating the microbiology program. Companies can thus ensure that those responsible for the program understand the basic principles of microbiology, can interpret guidelines and regulations based on good science, and have access to individuals with theoretical and practical knowledge in microbiology to provide assistance in areas in which the persons responsible for the program may not have adequate knowledge and understanding. It should be noted that microbiology is a scientifically based discipline that deals with biological principles substantially different from those of analytical chemistry and engineering disciplines. Many times it is difficult for individuals without specific microbiological training to make the transition.

LABORATORY RESOURCES

The laboratory management is responsible for ensuring that the laboratory has sufficient resources to meet the existing testing requirements. This requires some proficiency in budget management and in determining appropriate measures of laboratory performance. A measure of laboratory performance is the number of investigations performed on tests conducted by the laboratory, but this measure alone is not sufficient. In addition to tracking investigations, the period of time between sample submission and initiation of testing should be tracked, as well as the period of time between end of test and report release (or test closure). Significant delays in these measures are also indications of an under-resourced laboratory staff.

The laboratory management should have sufficient budget to meet testing requirements. Particular measures of budgetary requirements will be specific to the given laboratory, but budgetary considerations related directly to the need of the laboratory for sufficient resources must be addressed to ensure reliable testing results.

DOCUMENTATION

Documentation should be sufficient to demonstrate that the testing was performed in a laboratory and by methods that were under control. This includes, but is not limited to, documentation of the following:

- Microbiologist training and verification of proficiency
- Equipment validation, calibration, and maintenance
- Equipment performance during test (e.g., 24-hour/7-day chart recorders)
- Media preparation, sterility checks, and growth-promotion and selectivity capabilities
- Media inventory and control testing
- Critical aspects of test conducted as specified by a procedure
- Data and calculations verification
- Reports reviewed by QAU or a qualified responsible manager
- Investigation of data deviations (when required)

MAINTENANCE OF LABORATORY RECORDS

Proper recording of data and studies is critical to the success of the microbiology laboratory. The over-riding principle is that the test should be performed as written in the SOP, the SOP should be written to reflect how the test is actually performed, and the laboratory notebook should provide a record of all critical details needed to reconstruct the details of the testing and confirm the integrity of the data. At a minimum, the laboratory write-up should include the following:

- Date
- Material tested
- Microbiologist's name
- Procedure number
- Document test results
- Deviations (if any)
- Documented parameters (equipment used, microbial stock cultures used, media lots used)
- Management/Second review signature

Every critical piece of equipment should be noted in the write-up, and all should be on a calibration schedule documented by SOP and maintenance records. Where appropriate, logbooks or forms should be available and supportive of the laboratory notebook records. Equipment temperatures (water baths, incubators, autoclaves) should be recorded and traceable.

The governing SOP and revision should be clearly noted in the write-up. Changes in the data should be crossed off with a single line and initialed. Original data should not be erased or covered over.

Test results should include the original plate counts, allowing a reviewer to recreate the calculations used to derive the final test results. Methods for data analysis should be detailed in cited SOPs. If charts or graphs are incorporated into laboratory notebooks, they should be secured with clear tape and should not be obstructing any data on the page. The chart or graph should be signed by the person adding the document, with the signature overlapping the chart and the notebook page. Lab notebooks should include page numbers, a table of contents for reference, and an intact timeline of use.

All laboratory records should be archived and protected against catastrophic loss. A formal record retention and retrieval program should be in place.

INTERPRETATION OF ASSAY RESULTS

Analytical microbiological assay results can be difficult to interpret for several important reasons: (1) Microorganisms are ubiquitous in nature, and common environmental contaminants—particularly organisms associated with humans—predominate in many types of microbiological analysis; (2) the analyst has the potential to introduce contaminating organisms during sample handling or processing in the laboratory; (3) microorganisms may not be homogeneously distributed within a sample or an environment; and (4) microbiological assays are subject to considerable variability of outcome. Therefore, apparent differences from an expected outcome may not be significant.

Because of these characteristics of microbiological analysis, laboratory studies should be conducted with the utmost care to avoid exogenous contamination as previously discussed in this chapter. Equally important, results must be interpreted from a broad microbiological perspective, considering not only the nature of the putative contaminant, but the likelihood of that organism(s) surviving in the pharmaceutical ingredient, excipient, or environment under test. In addition, the growth characteristics of the microorganism should be considered (especially in questions of the growth of filamentous fungi in liquid media).

When results are observed that do not conform to a compendial monograph or other established acceptance criteria, an investigation into the microbial data deviation (MDD) is required. There are generally two distinct reasons for the observation of microbial contamination that does not comply with a target or requirement: There may be either a laboratory error or laboratory environmental conditions that produced an invalid result, or the product contains a level of contamination or specific types of contaminants outside established levels or limits. In either case, laboratory management and, in most cases, the Quality Unit should be notified immediately.

A full and comprehensive evaluation of the laboratory situation surrounding the result should be undertaken. All microbiological conditions or factors that could bring about the observed condition should be fully considered, including the magnitude of the excursion compared to established limits or levels. In addition, an estimate of the variability of the assay may be required in order to determine whether the finding is significant.

The laboratory environment, the protective conditions in place for sampling, historical findings concerning the material under test, and the nature of the material, particularly with regard to microbial survival or proliferation in contact with the material, should be considered in the investigation. In addition, interviews with the laboratory analyst(s) may provide information regarding the actual conduct of the assay that can be valuable in determining the reliability of the result and in determining an appropriate course of action. If laboratory operations are identified as the cause of the nonconforming test outcome, then a corrective action plan should be developed to address the problem(s). Following the approval and implementation of the corrective action

plan, the situation should be carefully monitored and the adequacy of the corrective action determined.

If assay results are invalidated on the basis of the discovery of an attributable error, this action must be documented. Laboratories also should have approved procedures for confirmatory testing (retesting), and if necessary, resampling where specific regulatory or compendial guidance does not govern the conduct of an assay investigation.

⟨1118⟩ MONITORING DEVICES—TIME, TEMPERATURE, AND HUMIDITY

This chapter provides background on the science and technology of temperature and humidity monitoring. It describes the available technologies and their performance characteristics, and it provides recommendations for verification and validation of performance. The shelf life of a drug is a function of the temperature and humidity conditions under which it is stored and transported as well as the chemical and physical properties of the drug substance and preparation. For this reason, the ability to monitor those conditions is important in the shipping and storage of temperature- and humidity sensitive preparations. Historic geographic and seasonal trends may be used as a planning tool in selecting among the types of temperature and humidity monitoring devices. Meteorological forecasts are available for any pertinent location.

TEMPERATURE MEASUREMENT TECHNOLOGIES

The devices described in this section are those most commonly used to monitor temperature in the storage and distribution of drugs in North America. The measurement of temperature at extremes, such as close to absolute zero or above those reasonably expected to be experienced by drugs, is not addressed.

Alcohol or Mercury Thermometers—These devices are based on the change in volume of a liquid as a function of temperature. Mercury thermometers are typically used in the ranges from 0° to 50° with a precision of about 0.1°. [NOTE—Some local regulations apply to mercury-based thermometers. Alcohol thermometers may have a precision as good as 0.01°, but they must be quite large to measure temperatures in ranges of more than a few degrees. Both types of thermometers may be designed to indicate the maximum and minimum temperatures measured. See *Thermometers* ⟨21⟩.]

Chemical Device—This is a device based on a phase change or chemical reaction that occurs as a function of temperature. Examples include liquid crystals, waxes, and lacquers that change phase, and thereby their appearance, as a function of temperature. Such materials represent the least expensive form of temperature measurement, but they may be difficult to interpret.

Other types of chemical sensors include systems in which a reaction rate or diffusion process is used to deduce a temperature equivalent integrated over time rather than the temperature at a specific moment in time such as a spike or critical threshold, for which a separate device may be preferred. Thus, chemical sensors provide a measure of accumulated heat rather than instantaneous temperature. It should be noted that these devices are generally irreversible; once a color change or diffusion process has taken place, exposure to low temperatures will not restore the device to its original state. Accuracy and precision vary widely among different types, to differentiate often limited by their ability or their ability to visually interpret diffusion distances.

Infrared Device—This is a device based on measuring the IR radiation from the article whose temperature is being determined; the IR radiation varies as a function of the object's temperature. The advantage of the device is that the article may be at some distance from the IR sensor. However, IR devices are expensive compared to other temperature sensors.

Resistance Temperature Detector (RTD)—This is a device based on the change in electrical resistance of a material as a function of temperature. Precision and accuracy depend on the quality of the electronics used to measure the resistance. Therefore, although RTDs are among the most stable and accurate temperature sensors, their accuracy may change with the age and temperature of the device as its electronic components are affected. A particular type of RTD uses platinum or platinum alloy wire as the sensor. These are referred to as platinum resistance temperature detectors (PRT or PRTD).

Solid State Device—This is a device based on the effect of temperature on either an integrated circuit (see *Thermistor* below) or a micromechanical or microelectrical system. These devices can attain the highest precision available and also have the advantage of producing a digital output. Their accuracy is typically limited by the accuracy of the calibrating system employed.

Thermistor—This is a semiconductor device whose resistance varies with temperature. Thermistors are able to detect very small changes in temperature. They are accurate over a broad range of temperatures.

Thermocouple—This is a device based on the change in the junction potential of two dissimilar metals as a function of temperature. Many metal pairs may be used, with each pair providing a unique range, accuracy, and precision. Precision and accuracy depend on the quality of the electronics used to measure the voltage and the type of temperature reference used. Accuracy may be a function of temperature reference used. Thermocouples have relatively poor stability and low sensitivity, but are simple and cover a wide temperature range.

Thermomechanical Device—This is a device based on the change in volume of a solid material as a function of temperature. For example, a mechanical spring, which expands or contracts as a function of temperature, thus opening and closing an electrical circuit or moving a chart pen, is such a device. Precision may be as good as 0.05°, but in practice it is rarely better than 0.5°. Accuracy is often in the range of ±1.0°, but it may change with the age and temperature of the device.

TIME–TEMPERATURE INTEGRATORS

Time–temperature integrators, commonly referred to as TTIs, change color or physical appearance as a result of exposure to a temperature above a specific threshold for a specific time duration, and thus accumulate heat. TTIs are typically single use, disposable devices that react irreversibly. Once the color changes, it will not revert to the original one even if the temperature returns to the acceptable, normal range. The four basic types of chemical-based TTIs are described below.

Table 1 lists the four types of chemical TTIs presently in use. The closer the activation energy of the TTI's color change to the activation energy of the degradation process of the drug being monitored, the more accurately the TTI will reflect the status of the drug. In actual practice, the activation energy for degradation of a particular drug is not known precisely enough to enable selection of a particular type of TTI. The range of possible activation energies of a