The Importance of a Strong SOP System in the QC Microbiology Lab

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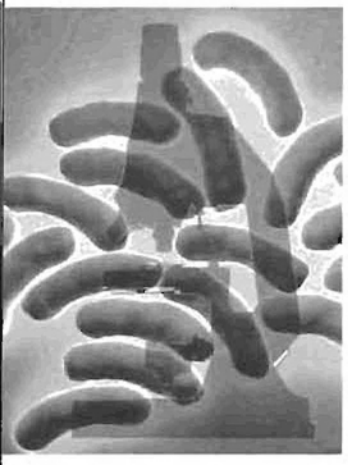

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"Microbiology Topics" discusses various topics in microbiology of practical use in validation and compliance. We intend this column to be a useful resource for daily work applications.

Reader comments, questions, and suggestions are needed to help us fulfill our objective for this column. Please send your comments and suggestions to column coordinator Scott Sutton at scott.sutton@microbiol.org or journal coordinating editor Susan Haigney at shaigney@advanstar.com.

KEY POINTS

The following key points are addressed in this article:

- Quality control microbiology test data are subject to significant variability, both avoidable and unavoidable
- Good microbiological procedures, backed by sound microbiological practices, can serve to do the following:
  - Minimize avoidable variability
  - Serve as a training structural framework
  - Assist in investigations
  - Provide assurance of good manufacturing practice (GMP) compliance
- The lab's standard operating procedure (SOP) system is a powerful tool to describe and document compliance with good practice
- The lab should determine critical areas of coverage for the SOP system to ensure a comprehensive program
- Training for the members of the lab should be tightly tied to the SOP system
- The lab SOP should describe critical parameters of the test and meet the criteria of regulatory requirements and guidance for that procedure. The documentation of compliance with these requirements is both a legitimate GMP audit concern and a useful source of information for investigations.

INTRODUCTION

Microbiology in the quality control (QC) laboratory is subject to variability. This variability can be evident in the test results. It may be caused by how the samples are taken, the manner in which they are taken (with severe limitations in sample size contributing to the problem) and the innate variability of a process heavily dependent on human interaction using a biological test system. This variability is an inescapable aspect both of the science and of the manner in which we do the science. For example, let's look at a relatively simple and basic operation such as plating. Jarvis (1) details a variety of errors (errors in the statistical sense of variability) involved in this operation (see Table).

The errors in this example might be divided into two main types—some that might be considered avoidable error (plating error, calculation error) and unavoidable error (sampling error, dilution error, distribution error). We cannot eliminate either type of error in the lab, but the general category of "unavoidable errors" is not amenable to correction by training or proper lab technique. In fact, some of the current practices of the lab, adopted for business purposes, may actually increase the effects of this type of variability. Among these unavoidable errors might be included in the following:

TABLE: Variety of errors (errors in the statistical sense of variability).

Source of error	Includes errors due to
Sampling error	Weighing Pipette volumes
Dilution error	Diluent volumes Pipette volumes
Plating error	Pipetting error Culture medium faults Incubation faults
Distribution error	Non-randomness of CFU Counting errors Recording errors
Calculation error	Manual calculations Software errors

- Insufficient sample numbers
- Insufficient number of replicate plates (2, 3, 4)
- Difficulties in using living systems (which react to treatment and growth conditions).

This last point deserves some additional discussion. It is a great temptation to writers of regulations to view microorganisms as particles. This leads to the mistake of writing specifications that are unreasonably small, well below the limit of quantification for the test method. This unrealistic expectation, coupled with the lack of attention paid to inoculum preparation, frequently leads to an unrealistic level of variability in the assay results. Simple "fixes" for variability (i.e., larger sample size, more replicate measures of each sample) are out of consideration for budgetary reasons.

Because we are not likely to be able to test large sample sizes, plate large numbers of replicate plates to increase the precision of plate counts, or do much to minimize the "unavoidable errors" in our lab, we are left with the avoidable errors. Fortunately, these can be affected fairly easily by training and solid lab leadership. This discussion will be an attempt to guide the reader to how to think about controlling the lab environment so that the results from microbiological studies are less variable.

THE SOP SYSTEM

The key to consistent work in the microbiology lab is a solid SOP system with adequate documentation. This seems obvious, but the affects of this requirement are not always so obvious.

You can break the organization of a logical SOP system down several ways. One way is operational, as follows:

- Quality requirements
- Media
- Cultures
- Equipment
- Training
- Sample handling
- Lab operations
- Testing methodology
- Data handling/reporting/archiving
- Investigations.

You will note that this method does not correlate to the US Code of Federal Regulations (CFR) organization, the medical device International Organization for Standardization (ISO) organization, Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S), *United States Pharmacopeia* (*USP*) chapter <1117>, nor the European Union (EU) GMP organizational scheme. Each of these general schemes is designed to fit a wide variety of processes and operations. In fact, this scheme may not be best for each lab either. We need to focus on a system specific to the microbiology lab as this environment has unique requirements, and each lab exists within a larger corporate culture that affects how it conducts its business. One of the simplest mistakes for a new manager to commit is to come into a new facility and impose the "correct" procedures on the workers without learning how the lab works in that environment.

Having considered the particular requirements of a lab, there are aspects of the microbiology lab operation that are critical to its success (i.e., control of cultures, media, sample handling, etc.) which may not even play a role in other types of work. These common and required microbiology-specific operations should serve as the basis for the SOP system. Although this lab system probably will not follow an external structure in lockstep, it is important to be able to correlate the system to the preferred method of the organization auditing you at that moment. This article does not go into the various organizational schemes; however, it is strongly recommended that the reader become fluent in at least the US 21 CFR 211 (5), *USP* chapter <1117> (6), and the PIC/S audit guide (7) to serve as a basis for the structure of your microbiology lab.

In general, I prefer a slight variation on the operational organization scheme listed previously. This scheme has the advantage, in my mind, of being amenable to use as a training organizational tool as well as a framework for SOP organization. In brief, the lab SOPs are broken into four main areas with several subsections, as follows:

- Testing methodologies
  - Specific test methods
  - Validation of test methods
  - Investigations
- Documentation and SOP structure
- Environmental monitoring (EM) and support
  - Viable air
  - Non-viable air
  - Surface sampling
  - Personnel monitoring
  - Media fill support
  - Qualification of facility after shutdown
  - Gowning (may share with manufacturing)
- Laboratory support activities
  - Media
  - Cultures
  - Equipment
  - Safety
  - Operations.

WHY IS THIS SCHEME USEFUL?

There are three advantages to this scheme: ease of training documentation, investigations, and GMP audit preparedness.

Primary Advantage—Ease of Training Documentation

Training is a very difficult area for the QC laboratory. Aside from the questions surrounding proficiency testing (which will not be discussed here) there are real logistical issues with determining who should be trained in what SOP and how to maintain training as SOPs are revised.

The work in a microbiology lab is performed almost entirely by technicians at the bench. This work is complex with some studies lasting only hours while others may last more than a month. Throughout, the bench technician is handling the material and the cultures as a normal part of the job—a job that is notoriously operator-dependent in nature.

Having said this, how does this organizational image of a microbiology lab SOP system help in training? A new hire will need to be immediately trained in all the SOPs concerned with documentation, lab hygiene, and lab safety. The next group of SOPs will depend on their job function. For example, a techni-

cian performing sterility tests will need additional training in the following (partial list):

- Test methods
  - Test methods
  - Relevant equipment (operation and maintenance [O&M])
  - Aseptic technique
  - Media (i.e., quarantine, handling, and expiry)
  - Biohazard disposal
  - Recognition of microbial growth
- Validation
  - Method
  - Preparation of inocula.

This structured layout of tasks and job skills also encourages different functional specialization. For example, there is no need for the technician working in the media kitchen to be trained in how to perform an antimicrobial efficacy test. Nor is it particularly efficient for the bench worker to be running back and forth to the kitchen to check on media. By separating the jobs, the flow of work in the lab is simplified. Major support functions such as media preparation and release, stock culture maintenance, and equipment tracking can each be handled by a suitably trained manager with backup.

Secondary Advantage—Investigations

Microbial data deviations (MDD) are notoriously difficult to resolve. Part of the reason is the time between the event of concern and the recognition that the data are suspect and an investigation is required. The interval may be days or weeks, leaving the investigator trying to reconstruct what happened in the past using only available records.

In any MDD evaluation, the first question must be to determine if the lab was at fault and the test results invalid. This is very difficult to do; clear evidence of lab error is required to meet the GMP "burden of proof" that assumes the validity of the data. In other words, if you cannot prove that the data are invalid, they are valid (even, or perhaps especially, if they jeopardize your batch release). By organizing the SOP system into manageable pieces, it encourages and assists the lab in designing data recording forms that capture all necessary information about the test, the equipment used, the personnel, the samples, etc. This information is invaluable in the investigation even if the lab is not at fault and the investigation proceeds to an out-of-specification (OOS) investigation.

Tertiary Advantage—GMP Audit Preparedness

Having a logically designed and organized SOP system allows for easy analysis of gaps in GMP compliance. While this is not necessarily the main reason to design a lab SOP system in a particular manner, it certainly is a useful benefit in the establishment of such a system.

I would propose a general structure for the lab SOP system consisting of four main topics with all operations organized in this structure. The topics would be as follows:

- Testing methodologies
- Documentation and SOP structure
- Environmental monitoring and support
- Laboratory support activities.

The following sections describe each of the functions in more detail.

TESTING

Each major type of test performed will have an associated SOP. This SOP should list critical pieces of equipment (and training will necessitate familiarity with the "Operation and Maintenance" SOP for each critical piece of equipment). The test will also list specific organisms to be used (if appropriate), necessitating training in relevant culture SOPs. Finally, each SOP will list required media, necessitating training in release and expiry requirements for the relevant media (how do you determine which media can be used for your test?). Finally, the test may require training in the department's SOP on how to count colony forming unit (CFU) on plates, and on the lab's methods of handling basic math operations (e.g., rounding, significant figures, $\log_{10}$ conversions, etc.). If data forms are used by the lab, these forms should be under document control and cited in the SOP. The form should be specific

enough so that all measures (i.e., temperature, time, etc.), cultures, media and reagents, as well as individual pieces of equipment used should be explicitly documented. If forms are not used, this data must be captured in some other GMP compliant manner.

In addition, each test method SOP should be accompanied by an SOP on how to "validate" the method. This usually consists of demonstrating suitable microbial recovery from samples spiked into the sample or into a neutralizing broth (see *USP* chapter <1227>). Specific tests may have additional validation requirements depending on the region. Sterility tests, for example, have additional requirements by PIC/S over those recommended in *USP* <1227> and required in the harmonized sterility test.

It will be prudent to develop an SOP on how to handle failing or questionable results. This may be test-specific (and should be for some tests with strong regulatory guidance such as the sterility tests) or generalized, but should detail what level of evidence is acceptable to invalidate a test and state clearly that if the test cannot be invalidated it is valid. Development of the "Lab Investigation" SOP is important, as the company's out-of-specification/corrective and preventative action (OOS/CAPA) procedures will almost certainly be directed at the analytical chemistry group or manufacturing group, and be completely inappropriate to the microbiology lab. Further, an investigation into a putative sterility test failure will be fundamentally different from that of a putative failure of the antimicrobial effectiveness test. There will, of course, be elements in common, but they are defined by their differences.

A separate category of testing involves microbial identification. This group should include basic tests (e.g., Gram's stain, spore stains, biochemical reactions, and the use of selective/differential media) as well as more advanced methods such as the operation and maintenance of proprietary identification technologies as equipment, and their use in microbial identification. As so much of any potential investigation may require accurate identification, the following controls on the different stages of identification might be prudent:

- Streaking the sample for pure, monoclonal microbial culture
- Controls on the gram stain to ensure accurate result
- Controls on the identification run to ensure accuracy.

DOCUMENTATION ISSUES AND SOP

It is frequently useful to have an SOP on what a good test method SOP should include. Each data sheet for the test method should include sufficient information to determine the culture used (tracing back to the initial receipt from the national stock culture), all critical pieces of equipment used, all buffer and media lots used, time and date of activities and who performed them, and date all information was reviewed. This is in addition to the actual data for the test (e.g., dilution factor and CFU/plate for plate count methods).

This then brings up the question of proactive documentation. If you read the compendial tests, you will note that there are several parameters spelled out. These parameters might include temperatures of incubation, time of incubation, handling, proficiency requirements, etc. At a minimum the test documentation should serve witness that all the parameters detailed in the test method were met. This includes documentation that the technician was trained, the equipment was in repair and calibrated, and all conditions of the test were met. The reader should understand that although this discussion of proactive documentation is occurring in one particular section, it is a general requirement of GMP—you must be able to document that the test was performed correctly.

A benefit of this practice is that it will cut down on the most egregious source of variability in the microbiology lab—technician creativity. Microorganisms are living creatures and respond to stimuli. If Technician A handles them in one fashion and Technician B in another, it should not be surprising that the technicians will frequently record data that are not consistent. This is not a problem with the microorganisms; it is a problem with the lab leadership.

This can also be an extremely useful tool for self audits. One way to approach this task might be to take a particular activity that has been problematic and pull out all relevant documents (i.e., SOP, regulatory requirements, regulatory guidance, industry group technical reports). With this information, create a chart of critical parameters for that test as described by the SOP; these might include temperatures, incubation times, or any other specific instructions in the SOP. Repeat that exercise for the task using each relevant supporting document you have. Now you are ready to answer the following two questions:

- Does your SOP satisfy critical parameters for that test as described in *USP*, FDA guidance, or other supporting documentation? It is a good idea to write this up as a white paper in preparation for being asked this question during an audit.
- Does your documentation capture sufficient detail to provide witness that you performed the test in a manner that met all critical parameters identified? This question is critical for GMP and in preparation for any potential investigation.

Investigations in the microbiology lab are extremely difficult, not only due to the detective work involved but also because very few labs adequately document the work performed. If a problem is not identified until days or weeks after the event there is very little actual material to investigate. The most useful tool in an investigation is the GMP documentation if it is up to the task. This is also the area where most labs are the weakest, as the documentation is not up to the task. Frequently, in these cases, we will end up with an inconclusive determination of a root cause for a particular problem. This will be a frequent event in any case due to the nature of the discipline, but more comprehensive documentation will allow for better investigations into events that happened days or weeks earlier.

ENVIRONMENTAL MONITORING AND SUPPORT

Environmental monitoring and support is set aside as its own group only because of the complexity. In addition to the obvious issues of sampling (and the equipment used for that sampling), gowning, and aseptic technique, this area will also have to be concerned with trending of the environmental monitoring data, media fill support, and disinfectant qualification.

This area is so complex that many organizations split off the EM group from microbiology altogether. This is, in my opinion, a mistake. It clearly is a huge role for a microbiology department, but a competent, technically qualified manager should be able to take care of the range of requirements (see reference 6 for a discussion of the qualifications of the lab manager). The fragmentation of the EM group from the microbiology group serves only to separate the sample acquisition and data analysis functions from the incubation and plate reading/data recording functions. This sets up a situation that encourages avoidance of responsibility for unwelcome results. In addition, it limits the opportunity of the lab head to shift resources to areas of great, if temporary, need. If the analysts cannot perform EM sampling, they cannot be used if needed. If the EM technicians are not part of microbiology, they cannot help out in the lab. Splitting the functions into two departments requires the company to hire two competent microbiologists with experience to lead the groups. As this is extremely unlikely to happen, one group inevitably is weaker than the other and discrepancies in microbiological technique creep into the procedures of the two groups leading to conflict or apathy. Finally, the temptation will be strong for each group to use its own SOPs for common tasks.

SOPs unique to this area might include not only sampling techniques for air, surface, and personnel, but also sample handling and transport, incubation, and whatever trending and data handling procedures are needed. In addition, specific consideration might be given to media fill support activities, qualification of the facility after shutdown, and gowning procedures and qualification (these may be shared with manufacturing).

LABORATORY SUPPORT ACTIVITIES

The laboratory support activities are probably the most misunderstood part of the microbiology lab, especially among those in management. Part of the problem, of course, is that this is all overhead to the lab and so it is a very tempting target when the

lab management is instructed to reduce spending. However, this is probably one of the worst places to tighten the budgetary belt as all work depends on these functions being performed properly.

Media

The activities that need SOP coverage here include the receipt and acceptance of incoming dehydrated and prepared media, its quarantine, growth promotion confirmation (which may require training in cultures and preparation of inocula), and media release for use. In addition, the mixing and sterilization of in-house media, establishment of its expiry dating, and labeling of all media are important. All lab workers who perform testing that involves microbial growth media will require relevant training in how to identify usable media, even if they are not trained in its preparation.

In addition to the direct SOPs on media receipt, preparation, and release there are supporting SOPs on relevant equipment O&M procedures, with particular attention to the autoclaves (sterilizers) and their validated cycles and load configuration.

Cultures

The integrity of the culture collection is critical to the QC microbiology lab. This begins with receipt of the culture from the national stock collection and procedures in place to confirm the identity and purity of the sample. SOPs should be in place to govern receipt, quarantine, quality check, release, and seed lot technique. Many of these functions can be combined into the seed lot technique method (8).

In addition to the seed lot technique out to the working cultures, a specific SOP may need to be in place for preparation of the inocula for the various tests (although this might be included in the test method SOPs).

It is frequently found to be useful to have two or three individuals in the lab responsible for maintenance of the culture collection. This relieves others of trying to keep up with the procedures and allows the specialists to trade off responsibilities in a rotation schedule.

Equipment

I have found equipment to be sufficiently involved to require a dedicated worker (and backup) for the same reasons cited for media and cultures. Someone needs to maintain the equipment master files (containing vendor qualifications, manuals, certifications, etc.), track preventative maintenance (PM) schedules for critical equipment, review performance logs, and ensure autoclave cycle records are maintained.

Each critical piece of equipment should be qualified as "suitable for its intended use" (9) and should have an "Operation and Maintenance" SOP that is sufficiently detailed so that test procedure SOPs will not need to describe how to use the equipment, merely reference the appropriate O&M SOP. Obviously, qualification to perform a particular test would require proficiency in all relevant equipment SOPs.

Finally, many pieces of equipment in the microbiology lab have additional requirements beyond the standard PM scheduled work. Equipment designed to maintain temperature (e.g., incubators, refrigerators/cold rooms, and water baths) must be monitored to document compliance. In addition, equipment that is used to house "dirty" samples (e.g., incubators, refrigerators, water baths, etc.) must be cleaned regularly to minimize the potential for contamination. The method and frequency of this cleaning should be described by the SOP and documented.

Lab Safety

Many companies have lab safety requirements. These might involve the requirement to maintain available material safety data sheets (MSDS), what to do in case of spills, fires, earthquake, tornado, or other natural disaster. They may also cover acids, bases, flammables, toxins, equipment, etc. In terms of equipment there is a real need to address the use of autoclaves and compressed gasses in the microbiology lab.

An additional, and somewhat unique, requirement for the microbiology lab is to have a biosafety manual prepared and ready to handle at least risk level 2 microorganisms. This is not too difficult as most requirements are met by basic good practices (i.e., no mouth pipetting, lab coats, use of containment hoods for operations lead-

ing to aerosols, etc.), but it is important to formalize the requirements to avoid misunderstandings.

Lab Operations

This is somewhat a catch-all category of SOPs. It isn't that the activities are unimportant, but rather that they are so basic to the operation of the lab that all parties are involved.

Control of Incoming Samples and Materials. The lab should have an SOP governing how to log incoming samples for testing, and beyond that how to track date-on-test, date-off-test, and report date. In addition, the lab should have a general procedure on acquisition and acceptance of perishable consumables.

Documentation Concerns. These can range from version control on data sheets to data entry into lab notebooks and the laboratory information management system and on to record retention for different documents. All documentation concerns should be described by an SOP.

Training and Proficiency Requirements. An SOP should exist for all job functions in the lab. This should describe the job function in a manner that allows easy categorization of the SOP to meet the job requirements (as an aside, if job responsibilities exist for which there is no SOP, write the SOP). This allows SOP training to be assigned by job function, and allows easy identification of technicians who need retraining when an SOP is revised.

A system should be in place to demonstrate the technician's proficiency in activities critical to their job. This system is, of course, described by the SOP. The system should identify critical skills needed, recertification periods, and methods of initial certification and recertification.

Laboratory Hygiene. SOPs should be in place to describe the cleaning and sanitization of the laboratory benches at the beginning and end of each day, the general state of the lab, and expectations of the lab environmental monitoring program (if one is in place). The preparation and expiry dating of sanitizers should be part of this procedure.

The hygiene expectations of the workers should also be addressed. Requirements for clean clothes and bodies, closed-toed shoes, clean lab coats, gloves, and other personal protective equipment (PPE) as required should be part of the stated expectations as should the proper use of hand-washing equipment.

Biohazardous Waste Disposal. There should be a procedure or procedures for decontamination and disposal of biohazardous waste.

Plate Count Procedures and Basic Math. This type of SOP is designed to standardize common practices in the lab. The plate count SOP seems silly until you realize that the CFU/plate recorded by the technician is really only her estimation of the CFU, and that estimate is immediately interpreted further (10). It is important to establish some consistency in this most basic function in the lab.

The basic math SOP is also critical. This should address at least the topics of rounding issues, significant figures, $\log_{10}$ conversions and the deduction of CFU/mL from the dilution and the CFU/plate. This might also be a good place to define what the lab means when an SOP states a 5-day incubation period vs. a 120-hour incubation. A good source for some basic math practices can be in found in the "General Notices" section of *USP*.

CONCLUSIONS

This article has attempted to describe an SOP system for the QC microbiology lab in a regulated industry. This is not presented as the only SOP system possible, or even that it will be sufficient to your particular needs. It should, however, serve as a starting point or to help with benchmarking your system. A good SOP system should serve as guidance to regulatory compliance, assist in investigations, and be useful as a framework for training.

By looking at the SOP system from a functional perspective we can easily group media, stock culture, equipment, and documentation requirements to test activity, making the creation of "job skills" relatively straightforward. This, in turn, simplifies the assignment of SOPs to individuals based on their job functions and simplifies tracking of individuals effected by SOP revisions.

The importance of controlling variability (also referred to as minimizing "avoidable error") in the

microbiology lab cannot be overstated. Microbiology as a discipline is inherently variable, with a business culture that results in increasing some aspects of this variability (usually in an effort to minimize overhead and labor costs). In addition, microbiology in particular is exquisitely sensitive to operator effects. A strong and coherent SOP system coupled with aggressive training and enforcement will minimize at least the avoidable variability in data from the lab for testing and validation work.

REFERENCES

1. Jarvis, B., "Statistical Aspects of the Microbiological Examination of Foods, *Progress in Indust. Microbiol*, 21, Elsevier Scientific Publishers B.V. Amsterdam, 1989.
2. Eisenhart, C. and P. Wilson, "Statistical Methods and Control in Bacteriology," *Bact Rev.* 7:57-137, 1943.
3. Stearman, RL, "Statistical Concepts in Microbiology," *Bact Rev.* 19:160-215, 1955.
4. Ilstrup, D., "Statistical Methods in Microbiology," *Clin Microbiol Rev* 3(3):219-226, 1990.
5. FDA, "Amendments to the Current Good Manufacturing Practice Regulations for Finished Pharmaceuticals/21 CFR Parts 210 and 211," *Federal Register* 73(174):51919–51933, 2008.
6. USP, "Chapter <1117> Microbiological Best Laboratory Practices (in-process revision)," *Pharm Forum* 35(4):945-951, 2009.
7. PIC/S. PI 023-2 Aide-Memoire: Inspection of Pharmaceutical Quality Control Laboratories, 2007.
8. Pharmaceutical Microbiology Forum, *PMF Newsletter*, Volume 13, #11, MicrobiologyForum.org, November 2007.
9. USP, "Chapter <1058> Analytical Instrument Qualification," *United States Pharmacopeia* 32, pp 508-514, 2009.
10. Pharmaceutical Microbiology Forum, *PMF Newsletter*, Volume 12, #9, MicrobiologyForum.org, September 2006.

GXP

ARTICLE ACRONYM LISTING

CAPA	Corrective Action and Preventive Action
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
EM	Environmental Monitoring
EU	European Union
GMP	Good Manufacturing Practice
ISO	International Organization for Standardization
MDD	Microbial Data Deviations
MSDS	Material Safety Data Sheets
O&M	Operation and Maintenance
OOS	Out of Specification
PIC/S	Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme
PPE	Personal Protective Equipment
QC	Quality Control
SOP	Standard Operating Procedures
USP	United States Pharmacopeia

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