

# Successful Microbiological Investigations

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## Background

In talking with colleagues at various conferences and on the PMFList, it is clear that we all have a remarkably similar experience reporting an OOS (Out of Specification) result from the microbiology lab. The very first reaction to this news is a challenge to find out where the lab erred in the test. Frequently, if we cannot identify the "root cause" quickly enough there are Vice Presidents sending special envoys to help. It is without question an entertaining and educational experience, and one we seem to repeat on a regular basis. Even when a clear "root cause" cannot be determined, the lab is frequently assigned the blame for the unfortunate results and a technician is chosen for "retraining" as the corrective action.

It should be stated explicitly that the Agency is not amused by this behavior. Based on conversation, presentation, published 483 observations and Warning Letters, it seems that the FDA (US Food and Drug Administration) is curious when it becomes apparent that the company is willing to believe all the favorable data without hesitation, however declares that the lab must be in error when presented with unfavorable data. In other words, the only way to invalidate a negative laboratory result is by conclusively proving the result is incorrect or invalid. Absence of definitive data supports the lab result. It also should be mentioned that 483 observations and commentary in warning letters both support an increased interest in investigations by compliance officials.

In this article, we will discuss the investigation of microbiological laboratory findings (a.k.a. microbiological data deviations - MDD). Two main components contributing to the difficulties of these investigations will receive special attention - the components of Time and Laboratory Error. Following this discussion and the suggested approach to be used to minimize their affects, we will discuss the investigation itself. This will be a general discussion of investigations as a concern for the QC microbiology laboratory. Investigations for particular tests are important, but beyond the scope of this article (refer to Sutton 2010 for more information). We will consider the need to place each investigation in a larger framework of laboratory operations, most particularly the information that can be gleaned from trending investigations to look for deeper root causes. Following the discussion of the investigation, we will close on consideration of the relationship between the client and the contract testing lab, and how this affects an effective investigation.



## Time

Microbiology takes time. The result of this is that when something goes wrong, we do not learn of it for days or weeks. One seemingly obvious answer to this concern might be "Rapid Microbiological Methods" (RMM). While we seem to be on the cusp of implementing RMM in the pharmaceutical, medical device and biotechnology industries, we have been on this cusp for a couple of decades. In addition, many of the "rapid" methods require an amplification stage, and so are "rapid" only in reducing the test from weeks to days [1]. The slow pace of acceptance in the regulated industries is unexpected but undeniable. While acknowledging the possibility of RMM having an impact on investigations let's take a look at how we can improve our chances for successful investigations in today's QC environment.

The answer is a simple one, but expensive in terms of labor. Since we have no opportunity to determine which test or study will become problematic, we must as a matter of course be prepared to investigate all of them. Fortunately the best way to do this is already required by the GMP - proactive documentation. The basic idea of proactive documentation is to document all required aspects of the study as it progresses to allow later review and audit.

The next concern is to identify the required components of the study. One good place to start is by review of the compendial test, and review of the in-house SOP used to perform that test. Anytime a temperature, duration, identity (medium, equipment or microorganism), or number is specified this should be confirmable in the study documentation. The next step would be to refer to informational or guidance documents such as USP <1117> [11] or the PIC/S Guide to Inspections of QC Lab [7]. If the documentation can meet these requirements, you are well on the way to having a system in place that will provide sufficient documentation for the investigation.

As mentioned earlier, this approach is expensive in that the bulk of the investigation is performed before the problem manifests. The good news is that this approach is mandated by GMP in any event, and if your system falls short of the target it should be corrected immediately solely from a GMP perspective. Improving your investigations is really only a happy coincidence.

We should not leave this topic without a consideration of "retain" testing. All plates should be kept for further investigation until the conclusion of the test. Frequently useful information can be gleaned from the identification of the microorganism involved, but this identification should be done in a manner that allows strain-level differentiation. "Retain" samples are a bit more of a problem. Remember our test system is alive, and responds to external stimuli. This response may be to die, to grow, or just sit there. The only thing we know for sure is that the sample is not the same as it was when originally tested days earlier. If your investigation procedure allows retest of new samples or "retains", there should be a solid rationale for the practice.

## Laboratory Error

Microbiology has a well-deserved reputation for variability. This aspect of the data is both an outcome of the science itself and the manner in which we perform the work [9]. 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. For example, let's look at a relatively simple and basic operation such as plating. Jarvis [4] detailed a variety of errors (errors in the statistical sense of variability) involved in this operation:

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	

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" are 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:

1. Insufficient sample numbers - Due to the expense involved, virtually all microbiological tests are performed with a sample size that is completely insufficient in terms of a statistical sample plan
2. Insufficient number of replicate plates [2,8,3] - While the research recommends a minimum of three replicates (the better to estimate the mean of the population and to recognize an outlier), industry practice is to use duplicate plating, and it is not uncommon to see labs only using single plates.
3. 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 frequently paid to inoculum preparation, frequently leads to an unrealistic level of variability in the assay results. Simple "fixes" for variability (larger sample size, more replicate measures of each sample) are out of consideration for budgetary reasons.



Since 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 effected 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.

## Recommendation: The SOP System - Procedures and Well-Designed Data Sheets

The key to consistent work in the microbiology lab is a solid SOP system with adequate documentation. This seems obvious, but the benefits of this practice, done correctly are not always plain.

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

1. Quality Requirements
2. Media
3. Cultures
4. Equipment
5. Training
6. Sample Handling
7. Lab Operations
8. Testing Methodology
9. Data Handling/reporting/archiving
10. Investigations

You will note that this method does not correlate to either U.S. or EU organizational schemes, the Medical Device ISO organization, PIC/S, nor USP <1117> [7,11]. Each of these general approaches is designed to fit a wide variety of processes and operations. In fact, I would argue that the outlined 10 point scheme may not be best for your laboratory 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.

There are aspects of the microbiology lab operation that are critical to its success (control of cultures, media, sample handling etc.) which may not even play a role in other disciplines of laboratory work. These common and required microbiology-specific operations should serve as the basis for the SOP system. We will not go into the various organizational schemes here other than to strongly recommend that you become fluent in at least the US 21 CFR 211, USP chapter <1117> and the PIC/S audit guide to serve as a basis for the structure of your microbiology lab [11,7]. This will be important during an audit when you must be able to explain your laboratory organization to the auditor based on his background and preferences.

In general, I prefer a slight variation on the operational organization scheme listed above. This scheme is shown on the outline below. 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:

1. Testing Methodologies
  - a. Specific Test Methods
  - b. Validation of Test Methods
  - c. Investigations
2. Documentation and SOP Structure
  - a. Data Entry
  - b. Record Retention
  - c. Control of Contract Lab Work
3. Environmental Monitoring (EM) and EM Support
  - a. Viable air (microbial monitoring)
  - b. Non-viable Air (particulate monitoring)

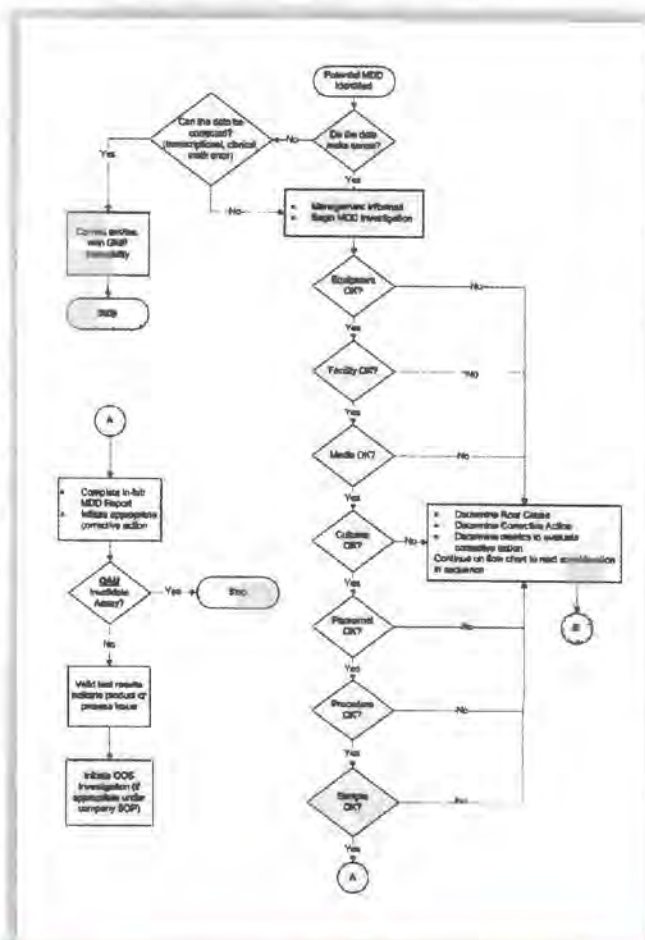


Figure 1. Plot of Turbidity Response Over Time for Nominal Suspension Concentrations



- c. Surface Sampling
- d. Personnel Monitoring
- e. Media Fill Support
- f. Qualification of Facility After Shut-down
- g. Gowning (may share with manufacturing)
4. Laboratory Support Activities
  - a. Media
  - b. Cultures
  - c. Equipment
  - d. Operations
  - e. Hygiene and Monitoring
  - f. Lab Math
  - g. Colony Counting Rules
5. Data Sheets (controlled documents designed to encourage capture of all relevant information)

We are looking at the SOP system from the perspective of successful investigations. Microbial Data Deviations (MDD) are notoriously difficult to resolve. 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 OOS investigation. It also encourages complete review of the individual components of the different tasks to ensure that good microbiological practices are in place.

Although we have not spent time discussing other advantages of this approach to SOP organization, it is clear that this structure will provide advantages during audits of the lab and of individual tests, and that it will also greatly simplify tracking training if all critical activities are covered by SOP, each SOP is under revision control, and all technicians are trained under the same system.

## The Laboratory Investigation

This then brings us to the lab investigation. It should be noted that what is under discussion is a lab investigation, not an OOS investigation.

At this stage we do not have assurance that the data are reliable and we have reason to be concerned, as presumably we routinely make acceptable product, and this situation is not the norm, we are justified in conducting an investigation of this unusual occurrence.

A common mistake is to treat all microbiological laboratory investigations as unique events. It is strongly recommended that you create an SOP to deal with laboratory investigations. This SOP should address common data entry errors first:

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- Incorrect math
- Sample dilution series error
- Transcription error
- Clerical error
- Nonsensical microbial identification

Of the forgoing errors, the one most reliant on the expertise of the microbiology supervisor is the determination of a nonsensical microbial identification. This situation might arise from an unlikely organism being identified, or the uncritical acceptance of an automated identification outside the instruments validated parameters. The SOP should allow for confirmation of the identification in question by repeated identification using the same method (back to the point of streaking for single colonies followed by an accurate gram stain). In addition, a method

of completely different technology (ideally phenotypic and genotypic) should be used to confirm the initial identification. It is the responsibility of the laboratory management to resolve the identification.

If the obvious data entry errors are not an issue, the laboratory investigation should be initiated immediately. This practice of conducting a preliminary laboratory evaluation (preferably before the full OOS) should be covered by SOP and documented.

The details of the investigation will differ from company to company. However, all investigations should follow the same general progression whether OOS or laboratory investigation of MDD:

1. Identification of the issue
2. Determination of root cause
3. Determination of corrective action
4. Demonstration of effectiveness of corrective action

An example of this process is presented in Figure 1. The details of the investigation will differ depending on the company, the lab and the test under investigation. However, major lab systems should be evaluated:

- Equipment - calibrated, qualified, operated correctly?
- Facility - clean, appropriate?
- Nutrient Growth Media - released for use, correct formulation?
- Stock Cultures - confirmed correct microorganism, in correct physiological state?
- Personnel - Hygiene, training, proficiency, workload?
- Procedure - correct, validated, observed?
- Sample - taken correctly, storage, amounts?

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Should these common sources of errors not be the cause of the potential MDD, then management should be notified immediately and a formal OOS investigation initiated. As this formal investigation will be coordinated through the Quality assurance unit, the microbiology laboratory will be in a support role. In that role, the microbiology unit retains its responsibility as the Subject Matter Experts (SME) in microbiology and in the particular tests.

It was mentioned above that the investigations will have differences depending on the particular test in question. While there is a significant amount of guidance to assist in investigations of the sterility tests, others will rely on the lab management to design suitable procedures (see Sutton 2010 for review). Keeping the inherent limitations of microbial enumeration and identification in mind while designing the procedures, and conducting the investigations, is paramount to the successful investigation.

## The MDD Investigation as Part of the Larger Whole

It is always tempting to treat each investigation as an isolated incident. This is, however, a serious mistake. Every individual investigation should have a trending component, looking for any commonality that might exist between this investigation and previous ones to determine if there is an evident trend in the situations over time. In addition, the components of this event (personnel, equipment, media, cultures, etc) should be evaluated against other ongoing tests to determine if there is evidence of a deeper problem that is not evident on the surface. Only after this comparison is completed should the investigation move to the stages of closure.

## Closing the Investigation

The MDD investigation must be formally closed, as are all investigations. This requires

1. Identification of the possible root cause.

This determination must be driven by data. If no clear root cause is demonstrated, the lab analysis must be determined to be accurate. It is at this point that the effort will proceed to a formal OOS investigation as the product (or test sample) has been shown to be "Out-of-Specification". This closes the MDD Investigation.

However, if a root cause can be determined, it is then the responsibility of the Quality

Assurance Unit to determine if the lab error invalidates the test. An invalid test does not affect product disposition. Although the invalid test and investigation should be part of the batch analysis records, product disposition is based only upon valid analytical test results. Whatever the QAU disposition of the product, the microbiology lab is left with a clearly identified problem and the root cause must be corrected.

2. Determination of the Corrective Action/Preventative Action (CAPA)

This is self evident.

3. Follow-up study to document the adequacy of the CAPA

This final step is frequently omitted in the press of business. However it is arguably the most important of the closing activities for the investigation. Data must be generated to prove that the problem will not recur.

4. Close out the Investigation.

This step requires QAU sign-off as an independent review of the investigation and corrective action.

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## The Contract Lab

The proper use of a contract lab partner can be beneficial to both parties. However, the manufacturer has to remember that it is his data that the lab is generating. I commonly find contract lab reports at client sites that consist of little more than an executive summary from the lab consisting of the results of the test (no raw data) and a statement that the test was conducted under GMP (or GLP) conditions. This report, even if covered by an internal summary and entered into the company's document control system, is useless and even dangerous. It is useless as it does not contain even the bare minimum of information required by GMP (21 CFR211.194) or the expectations of USP [11]. In addition, without the requisite information there is no ability to perform an investigation. This is usually not a problem as the contract facility will almost certainly offer to conduct the investigation on their study themselves.

The problem for the manufacturer in this arrangement is that as far as FDA is concerned, any work done under contract at a different facility is considered work performed by that manufacturer. Therefore, you are responsible for the quality of the work as if your own QC labs had conducted it. This introduces complexity into any potential lab investigation involving work done at that contract lab. This consideration is not a large concern, however, if the client is willing to accept these external lab results uncritically [5,6].

A far better arrangement would be to have a technical audit of the contract lab performed in conjunction with the Quality audit normally performed. This technical audit should be conducted by a subject matter expert (SME) and there should be a contractual understanding that all data are included in the reports. In addition, the client should have complete access to all data and records at the testing site in the event of an investigation.

## Conclusions

The MDD investigation offers several challenges. As the event took place days or weeks earlier, it can be argued that the sample no longer exists and that all investigation is driven from documentation. The direct approach to this is to ensure that the test documentation is complete. This is assured through a comprehensive approach to the structure of the laboratory SOP system, and the data sheets for each test to ensure complete, proactive documentation. The use of contract testing labs does not lessen these requirements.

Time spent developing a complete SOP system will pay off not only in improved quality of the MDD investigation, but in smoother audits and in simplifying training record requirements.

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## Author Biography

**Dr. Scott Sutton** earned his B.S. in Genetics from the University of California at Davis, and his Masters and Ph.D. in Microbiology from the University of Rochester (Rochester, NY). After an NIH postdoctoral fellowship at the Medical College of Virginia (Richmond, VA), he went to work for Bausch and Lomb (Rochester, NY) until 1994 when he accepted a position at Alcon Laboratories (Fort Worth, TX). Dr. Sutton left Alcon Laboratories in 2004 as a Director in the R&D division to accept a position as Pharma Consultant (Microbiology) with Vectech Pharmaceutical Consultants, Inc which he left in 2009 as Sr. Director, Microbiology Services.

Scott Sutton is the Principal of Microbiology Network, Inc, a company he started in 1996 as a means to encourage training and communications within the microbiological community. He is a recognized consultant and trainer with emphasis in GMP, investigations, Environmental Monitoring and contamination control (both Aseptic manufacturing and non-sterile production facilities) and microbiology laboratory audits and operations. The Microbiology Network supplies consulting, training, webinars and Email discussion groups (PMFList, PSDGList and C-CEList) Dr. Sutton is an active author and speaker for the industry, supports PDA and has served with the USP Analytical Microbiology Committee of Experts since 1993.