GLP-Related FDA Warning Letters—The Seven Deadly Sins

Jeff Morgan

“GLP Topics” addresses topics associated with good laboratory practice requirements. We intend this column to be a useful resource for daily work applications. The key objective for this column: Useful information.

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 Cindy Green at cindynwrs@seanet.com or to journal coordinating editor Susan Haigney at shaigney@advanstar.com.

SUMMARY
Warning letters issued to a facility inspected by the US Food and Drug Administration for non-compliance issues are not uncommon. Many relate to violations by drug and device manufacturers or Internet sites violating FDA regulations. Some clearly fall into the category of objectionable practices related to 21 CFR 58, Good Laboratory Practice for Nonclinical Laboratory Studies, commonly known as GLPs. This article points out seven of the most commonly cited GLP observations found in FDA-issued warning letters and provides commentary on how to improve compliance with the regulations.

INTRODUCTION
You’re driving down the interstate when you notice a car following you a bit too closely. Cautiously, you slow down, at which time an array of flashing, strobing lights fill your rear-view mirror. Instantly, your heart sinks and your head begins to ache. Welcome to the world of highway enforcement.

The feeling is the same when your facility receives the envelope containing the document from the Department of Health and Human Services bearing bold letters beneath the stylized eagle emblem reading WARNING LETTER. You had hoped that your responses to Form FDA-483 were adequate, but the consensus of the agency leads to your receipt of the next step in which your objectionable practices or deviations from the GLP
glp topics

regulations (1) are spelled out in agonizing detail for anyone with access to the internet to peruse.

to make matters worse, as you read your glp-related infractions you say, “why didn’t i think about that?” you are not alone. there are recurring themes of observations documented in warning letters that relate directly to laboratory and study management. the goal of this article is to characterize seven of the most common warning letter observations and suggest how you can avoid them. fda warning letters can be obtained from fda’s website, http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/default.htm.

number 1—the quality assurance unit drops the ball

one common finding involves the failure of the quality assurance unit (qau) to fulfill the requirements of the glp regulation. the following example illustrates a common theme found within warning letters:

failure of quality assurance unit to monitor each study to assure management that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with applicable regulations [21 CFR 58.35(a), 58.35(b)(1), 58.35(b)(3), and 58.35(b)(5)].

items used as specifics include lack of validation for laboratory methods, failure of the qau to perform annual validation reviews as stated in standard operating procedures (sops), environmental monitoring not performed according to established schedules, failure to determine whether any deviations from approved protocols or sops had the proper authorization and documentation as required by sops, and a general lack of ensuring that the stipulated and often implied requirements of the glp regulation are addressed satisfactorily.

fda scrutinizes the quality of final reports with regard to statements or assertions that are not supported by facts or data. it is not uncommon for a warning letter to state that final reports failed to include a description of all circumstances that may have affected the quality or integrity of the data [21 CFR § 58.185(a)(9)] (1). test and control article stability statements must be accompanied by the basis for the conclusion either by stability testing or alternative documentation regarding why the test or control article is stable for the stated period and under established storage conditions. also overlooked by the qau is the requirement for each of the individual scientists or other professionals involved in the study to sign and date their reports. finally, the lack of pharmacokinetic data and analyses and the scientist or other professionals involved in that portion of the study is a qau responsibility, which will lead to an observation.

the qau can circumvent problems and prevent issues of these types by creating a pre-study checklist such as the example presented in table i. the checklist should include all required elements for the study and will take substantial time to prepare, but it is worth the investment.

another way to ensure that activities are complete is simply by having someone in the qau that has not developed the report perform an in-depth review of the report and list areas where questions or deficiencies exist. finally, the qau may contract a third party to perform an audit that includes the entire scope of the study, including test and control articles, facilities and equipment, laboratory and clinical aspects, and a sampling of the data and reports. the audit should occur early in the process and not be put off until the study is completed to ensure that bad practices do not continue.

number 2—equipment mismanagement

when equipment is involved in a glp study, one cannot simply assume that the equipment plugs in and works as expected forever without some type of management. human intervention is always required, even if it involves only elementary cleaning. these activities must be documented in a procedure and the actions recorded. otherwise you can expect the following comment from fda:

failure to adequately inspect, clean, and maintain equipment [21 CFR § 58.63].

warning letters include multiple observations of objectionable or absent practices when it comes to ensuring that equipment used in the study operates in a manner that produces accurate and reliable results. the examples provided in table ii are not
all-inclusive because each study will involve its own equipment requirements.

The message here is if equipment is used in a GLP study, read the manual, develop written procedures, establish calibration and maintenance schedules, document daily and periodic activities, and perform reviews to ensure that things are in a state of good repair and control. Develop methods to investigate and remediate problems when deviations in operability occur and train individuals in these procedures.

For example, if the temperature of a refrigerator is found to be outside the allowable limit, don’t ignore it. Determine what is in the refrigerator that relates to the study, assess its stability, determine the impact on the study, document corrective action and, by all means, document both the out-of-specification temperature and the temperature after remediation. Don’t forget to “tag out” nonconforming equipment as out of service if it fails to perform. Finally, apply these rules across the board. Remember, you are responsible for your own house and the study sites, so make sure that all equipment receives this measure of scrutiny, regardless of its location.

NUMBER 3—BAD LABORATORY PRACTICES

The lab is a world of its own. Often misunderstood in its capabilities, duties, regulatory requirements, and throughput, the laboratory and the professionals working in it are required to respond to both reasonable and unreasonable or impossible tasks to support the GLP study. It is important to understand that just because an array of equipment fills a room, not all laboratory workers comprehend the criticality of their work in a study.

I have seen the best of the best and the worst of the worst, and while the GLP regulations are intended to impart a state of control, the details are sparse. It is not practical for a study director to ask a laboratory to perform a test that is not routinely performed and to have an analytical system in place the next day. Likewise, it is naïve of laboratory personnel to assume that they can read a procedure from a scientific paper or even the United States Pharmacopeia (USP) and produce quality results without a systematic validation.

Numerous citations in FDA warning letters relate to the lack of the use of validated laboratory methods. Without even minimum validation, there is no assurance that the results of an analytical method reflect the true status or concentration of the analyte in the sample submitted for analysis. Methods for performing analytical method validation are beyond the scope of this article, but the resources listed in the references section will provide sufficient detail (2, 3, 4).

Overlooking the laboratory and the counsel of quality lab workers can lead to a train wreck with the FDA, such as the following example:

The protocol required an analysis for CBC with differential to be performed with regard to the test system,
sheep. Instead of conducting this analysis as required by the protocol, you employed a contract facility, [redacted], that was not capable of performing this analysis on sheep blood and instead attempted to run the test on equipment calibrated for humans, generating results you knew to be invalid. Even though you were aware of this problem, you did not change to a laboratory capable of running the tests required by the protocol and did not submit a protocol amendment to eliminate the requirement to conduct these hematology analyses.

This observation by FDA is a multi-layer failure that could have been avoided if communication, method validation status, and lab capabilities had been considered. The lesson here is that attention to detail is critical when choosing a contract lab.

It is acknowledged that not all studies can be performed in an in-house laboratory. Methods may require expensive equipment, specialized training, expensive materials, or even specialized environments. It may be a requirement to refer samples. But not all contract labs are created equal. The following are some points to consider:

- **Inspect the facility**—especially relating to its portion of the study. Ask questions. Ask to see equipment maintenance logs, SOPs, contingency plans, storage capabilities, receipt, storage, and traceability procedures for samples.
- **Insist on GLP**—Ensure that the contract laboratory is capable of operating under the GLP regulations.
- **Review quality control**—Good labs perform a strict regimen of quality control (QC) that accompanies the assays they perform. There should be a comprehensive quality control procedure that details how results of quality control samples are interpreted and remedial activities when QC results fall out of specification. Review this remedial action and ask yourself if you would be satisfied with the documentation, approach, and strategy.
- **Prepare a contract**—A contract with an outside lab should not only include the scope of testing, but should also define the means of communication that is required (i.e., problems, remediation, changes in personnel, changes in methodology, and the impact on the study), and the timing, format, and content of interim and final reports.
- **Ensure the lab is capable**—If the GLP testing includes rodents, primates, or other species, ensure that the laboratory has the proper experience with these animals. Additionally, request that normal limits for clinical pathology, blood, urine, and other sample values be provided.

Even if an in-house lab is used, the points to consider should be applied to an assessment of internal capabilities, practices, and attention to quality. It all pays off when the inspector visits.

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**TABLE II: Equipment related GLP observations listed in warning letters.**

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<tr>
<th>Observation</th>
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<tr>
<td>Maintenance logs or daily quality control records are incomplete</td>
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<tr>
<td>Lack of adherence to maintenance schedules</td>
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<tr>
<td>Failure to provide procedures for autoclaves, centrifuges, analyzers, etc.</td>
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<tr>
<td>Failure to record maintenance or performance checks</td>
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<tr>
<td>Failure to write and follow adequate procedures for maintenance and cleaning</td>
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<tr>
<td>The final report containing the incorrect temperature</td>
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<tr>
<td>Failure to provide written procedures for documenting deviations in temperatures of refrigerators or freezers, and for remedial action in response to the deviations. Failure to document omissions in temperature records, and remedial actions taken after temperature deviations.</td>
</tr>
<tr>
<td>No written documentation of the inspection, cleaning, maintenance, testing, calibration, or standardization of the equipment in which the test article for the study was stored</td>
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NUMBER 4—SOPS ARE MIA

The following excerpt from a GLP-related warning letter says it all:

The testing facility management failed to establish standard operating procedures (SOPs) adequate to ensure the quality and integrity of the data generated during the course of a study, to limit unauthorized and undocumented procedural deviations, and to establish controls to ensure accountability of SOPs [21 CFR 58.63(b), 58.81(a), 58.81(b), 58.81(d), 58.83, 58.90(i), and 58.107].

While study protocols, contract agreements, professional affiliations, and stacks of impressive resumes are rarely overlooked during a GLP study, SOPs often are overlooked. Again, the details matter. Evenlowly SOPs such as operation of a pH meter are important if pH measurement comes into play in a study. Table III demonstrates a sampling from warning letters regarding the importance of having a complete suite of SOPs to support the study.

Note that it is not sufficient to have SOPs, but to have effective and directive SOPs that state how something is accomplished or performed. If there is a risk of failure when the task is performed, the procedure should direct the operator in specific steps to take. Every stage of the study where activity is required must be defined in writing that is complete, reviewed, controlled, and approved. The responsibility for this ultimately falls in the study director's lap. However, it is really driven by the QAU, who must assess the requirements for and provide guidance to the development, training, and use of the procedures related to the study. The take-away here is to assess, develop, and operate under a documented system.

NUMBER 5—THE CASE OF THE MISSING SPECIMENS AND DATA

You must take care of and account for specimens received and used in the GLP study. This requirement involves means of identifying, preserving, handling, and storing clinical and biological samples and the data that result from laboratory studies or clinical examinations. Failure to properly store specimens and data violates 21 CFR 58.51. The following are a few examples taken from warning letters that should have been no-brainers, but occurred:

- Raw study data are archived and maintained on open shelves in an unused restroom
- There is no individual who is identified as being responsible for maintaining the archived data
- Access to the archive area is based on an honor system since all study personnel are issued keys to the room
- The study director failed to assure that all raw data, documentation, protocols, specimens, and final reports were transferred to the archives during or at the close of the study
- The animal organs directed for collection in study were not transferred to the archives at the close of the study
- The protocol for study and resulting data were not transferred to the archives at the close of the study
- Neither final report references the location of reserve samples, as required by 21 CFR 58.185(a)(13).

A good GLP study will provide up-front thought to ensure that adequate plans are made to account for the samples and documentation. Fail to do this and a warning letter will appear in your mailbox.

NUMBER 6—ANIMALS DON’T PLAN, YOU DO

Several volumes could be filled with examples of warning letter observations related to animals used in studies. These include animal mistreatment, failure to comply with the 1966 Animal Welfare Act, and generally showing a lack of caring. Looking at this topic from a GLP compliance standpoint, there are prime examples where warning letter contents point to basics that should have been addressed during conception of the study, but were somehow overlooked. That is, until FDA arrived and stated the following:

- You failed to establish procedures for animal care [21 CFR 58.90]
- You lacked standard operating procedures for the housing, feeding, handling, and care of animals [21 CFR 58.90(a)]. Specifically, you lacked procedures for dosing study animals, for monitoring animal care; for sacrificing study animals, and for evaluating the health status of newly acquired animals in accordance with acceptable veterinary medical practice.
- For warm-blooded animals used in laboratory procedures that require manipulations and observations over an extended period of time or in studies that require the animals to be removed from and returned
to their home cages for any reason, the regulations require appropriate identification. All information needed to specifically identify each animal within an animal housing unit is required to appear on the outside of that unit [21 CFR 58.90(d)]. Your studies fall within this requirement. You did not have cage tags for animals used in Study [redacted].

• The cage tags used for Study [redacted] did not identify the study and animals within the cage.
• Protocols [redacted], [redacted], and [redacted] do not contain a description and identification of the diet used in the nonclinical laboratory studies.

Careful planning and development of procedures would have avoided these observations. Too often individuals involved in a study have their focus solely on the center of the target and fail to see the concentric circles that surround the preparation for and execution of the study. Or the details are left to someone not conversant with GLP requirements. Or worse yet, the QAU and/or study director is not familiar enough with this aspect of the study—such that questions are never formulated.

Several years ago, a client was using mice in a study. It was nearing the end of the year and the contract lab used had a problem obtaining mice of the acceptable age for the study due to supply problems. Besides, there was no lower limit on size stipulated in the documentation sent to the lab. So the lab used what was available, which resulted in very young, inoculated mice that could squeeze through their cage wire, escaping to all corners of the vivarium and compromising that portion of the study. It's all in the details.

TABLE III: Warning letter examples related to SOPs.

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<tr>
<th>Warning letter examples related to SOPs.</th>
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<tr>
<td>There is no suitable SOP to track the handling of test and control articles that would preclude error in the receipt and distribution of each batch documented</td>
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<tr>
<td>There are no SOPs for the laboratory tests specifically required by study protocols</td>
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<tr>
<td>There are no SOPs to determine the acceptability of reagents and solutions</td>
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<tr>
<td>There is no suitable SOP for the collection and handling of specimens shipped to contractors for analyses</td>
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<tr>
<td>There is no suitable SOP established for the testing and maintenance of autoclave located in the surgical room</td>
</tr>
<tr>
<td>There is no suitable SOP established for the testing and maintenance of the defibrillator located in the operating room of the surgical unit</td>
</tr>
<tr>
<td>Deviations in a study from SOPs were not always authorized by the study director and documented in the raw data</td>
</tr>
<tr>
<td>A historical file of SOPs and all revisions including dates of such revisions was not maintained</td>
</tr>
<tr>
<td>SOP [redacted], does not include any steps that describe how to document and obtain approval of routine deviations from SOPs</td>
</tr>
<tr>
<td>SOPs [redacted], do not address how to track samples for the movement of test articles in and out of the laboratory while a study is in progress</td>
</tr>
<tr>
<td>SOP [redacted], does not require the QA unit to periodically provide written status reports for each study to the study director, as required by 21 CFR 58.35(b)(4).</td>
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NUMBER 7—BAD DOCUMENTATION PRACTICES

The final deadly sin is basically lack of attention to detail and common sense. While there are no written FDA guidelines that describe good documentation practices (GDP), it doesn’t require Stephen Hawking’s I.Q. to clear this hurdle. The following excerpt from an FDA warning letter is both informative and entertaining in that the investigator demonstrates the increasing frustration over multiple GDP failures:

• Your nonclinical site failed to directly, promptly, and legibly record data generated, as required by 21 CFR 58.130(c). For example:
• Study # [redacted] did not have a record of the study director’s findings related to the isolate failing
to grow as required by the test protocol. There were lab notes for the incubation period of the isolates on [redacted], however, there were no further records indicating the progress of the study.  

- There was no record of the study director’s findings for Study # [redacted] regarding test results not being reproducible and that the study would be discontinued.  

- Your corrective action appears to be adequate; however, your preventative action is inadequate, in that the [redacted] review for the greater than [redacted] day inspections and the random selection by the [redacted] has not prevented the failure of recording data. Please provide in your response detailed steps of how you plan to ensure that data is reported for ongoing and future studies.

Poorly documented records are increasingly growing in intolerance by investigators and are an unfortunate finding, because the concept is elementary; good, clear documentation is the easiest of all quality practices to perform. GDP relates to forms, reports, changes, inspection and receiving records, and all data used in generation of a study report. Recording information clearly and legibly in a timely manner, using ink and following a documentation control SOP if a mistake is found is the key to compliance of this GLP requirement.

Making major changes in a document or data requires an audit trail to determine the basis for the change. Minor errors should be made by drawing a single line through the incorrect information, initialing and dating the changes, and including a brief explanation regarding the change. If a change is made after approval, have original approvers initial and date their approval. Never use temporary stick-on notes, obliterate original entries, use whiteout, use pencil, or water-soluble ink.

Train all individuals in GDP who have anything to do with the study, its equipment, test performance, data generation, and information recording. Inspect recorded information early in the process and perform periodic sample GDP audits to ensure that this often overlooked aspect stays on point. You’ll be glad you did.

**CONCLUSIONS**

There is a need for personnel to understand GLP requirements; especially the testing facility management. This familiarity helps to ensure that the study personnel are aware of the requirements set forth in the regulation that may alleviate violations. If the testing facility decides to conduct a nonclinical laboratory study subject to GLP requirements, then the management of the testing facility must assure that the nonclinical laboratory study complies with the requirements.

Not only understanding the GLP regulations, but planning, attention to detail, training, and good old common sense will help keep your mailbox free of the dreaded, and expensive, warning letter.

**REFERENCES**

1. 21 CFR Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies, Title 21—Food And Drugs Chapter I—Food And Drug Administration Department Of Health And Human Services, Subchapter A—General, Part 58, Good Laboratory Practice For Nonclinical Laboratory Studies, 43 FR 60013, Dec. 22, 1978.

2. USP, United States Pharmacopeia, “General Information <1225> Validation of Compendial Methods.”


4. ICH, Q2B Guideline Validation of Analytical Procedures Methodology, U.S. Department of Health and Human Services, Food and Drug Administration, November 1996. GXP

**ARTICLE ACRONYM LISTING**

- FDA: US Food and Drug Administration
- GDPs: Good Documentation Practices
- GLPs: Good Laboratory Practices
- QAU: Quality Assurance Unit
- QC: Quality Control
- SOPs: Standard Operating Procedures

**ABOUT THE AUTHOR**

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