contract research expertise

XENOTECH
A BioIVT Company

TECHNICAL PAPER

GLP vs. non-GLP:
A Practical Applicaton

Comparing required elements in a GLP-compliant and non-GLP study

1101 W Cambridge Cir Dr | Kansas City, KS 66103 | *p* (877) 588-7530 / +913.438.7450 | *f* (913) 227-7100 | www.xenotech.com | info@xenotechllc.com

GLP vs. non-GLP: A Practical Application

Abstract

Regulatory agencies in the United States, Europe and Japan do not require compliance with Good Laboratory Practice (GLP) regulations for *in vitro* drug metabolism and drug interaction studies. Published industry guidance on the design of drug interaction studies emphasizes these studies be conducted with high standards of quality ensuring reproducibility of data. XenoTech offers enzyme inhibition, enzyme induction, drug transport and drug metabolism studies as either non-GLP studies or as studies in compliance with US FDA GLP regulations, US EPA GLP regulations, Japan MHLW GLP regulations, or OECD GLP guidance requirements. This paper describes XenoTech's application of FDA GLP regulations to enzyme inhibition, enzyme induction, drug transport and drug metabolism studies in *in vitro* and *ex vivo* test systems and provides a comparison of specific FDA GLP-required elements between an FDA GLP-compliant study and a non-GLP study conducted at XenoTech.

Introduction

XenoTech conducts *in vitro* and *ex vivo* drug metabolism and drug interaction studies to characterize the safety of existing drugs or drug candidates and to assess the risk of toxicity and adverse drug-drug reactions *in vivo* from a chemical entity. Regulatory agencies in the United States, Europe and Japan do not require compliance with Good Laboratory Practice (GLP) regulations for the conduct of these non-clinical laboratory studies. However, regulatory agencies and the pharmaceutical industry both recognize the importance of drug interaction data (e.g. CYP inhibition or induction data) and reaction phenotyping data (which can identify the potential for genetically determined pharmaco-kenetic variability) on assessing drug safety and efficacy (Tucker, Houston, Huang, 2001 and Bjornsson et al., 2003). Published industry guidance on the design of drug interaction studies states that these studies must "be performed with high quality and consistency, particularly when the studies ultimately influence the design of clinical trials" (Bjornsson et al., 2003). The US FDA has indicated that these studies should be conducted in the "spirit of GLP," which they define as "the investigator taking necessary steps to assure the quality and integrity of the data" (Tucker et al., 2001). Sponsors often seek to assure the same level of data integrity and validity as non-clinical safety studies accordingly and they often request GLP-compliant studies when outsourcing studies to XenoTech.

The FDA promulgated the GLP regulations in 1978 in response to evidence suggesting that certain regulatory submissions were based on invalid or fraudulent data. The FDA designed the GLP regulations to ensure the validity, integrity and reliability of nonclinical, safety data submitted for FDA evaluation and approval. At the time, industry primarily collected safety data from *in vivo* animal test systems based on well-documented standard procedures. Since the publication of the FDA GLP regulations, industry and academia have continued to research and develop methods of assessing drug safety based on the use of alternative test systems, including *in vitro* and *ex vivo* test systems.

This paper details XenoTech's approach to applying the FDA GLP regulations to *in vitro* and *ex vivo* studies, and describes the differences between an FDA GLP-compliant and a non-GLP study conducted at XenoTech.

Applicaton of GLP Regulations

Although many elements of the GLP regulations may be applied directly regardless of test system, specific elements require consideration of intent and applicability when conducting an *in vitro* study. XenoTech assessed these elements and based on our interpretation of intent, we have determined their applicability to *in vitro* drug metabolism and drug interaction studies conducted at XenoTech.

Table 1 summarizes the results of this assessment. The table lists the specific GLP regulations from 21 CFR Part 58, 21 CFR Part 11 and the 2001 FDA bioanalytical guidance document that apply to XenoTech studies and it compares and contrasts the regulations as applied to an FDA GLP-compliant study versus a non-GLP study conducted at XenoTech.

Definitions

Selected terms defined in the GLP regulations are clarified below for their application to studies offered and performed by XenoTech.

In vitro non-clinical laboratory study means a study in which the test article is applied to tissue or tissue-derived material (such as subcellular fractions) in a test tube, culture disc or the like. Examples of *in vitro* studies include enzyme induction studies in cultured hepatocytes, enzyme inhibition studies with liver microsomes or recombinant enzymes, and reaction Phenotyping (enzyme mapping) with hepatocytes, microsomes and recombinant enzymes.

Ex vivo non-clinical laboratory study means a study in which the test article is administered to a laboratory animal *in vivo*, after which organs or tissues are removed and analyzed *in vitro*. An example of an *ex vivo* study is an enzyme induction study in mice, rats, dogs monkeys, which are often conducted as part of a 14-day toxicity study in these same species.

Control article means any article other than test article that is administered to the test system for the purpose of establishing a basis for comparison with the test article. Positive and negative control articles used to provide evidence that the test system is responsive under the actual conditions of the assay are not categorized as control articles per GLP regulations.

Specimen means any material derived from a test system for examination. For example, microsomes isolated from cultured hepatocytes treated with test article or control article are considered specimens.

FOR MORE INFORMATION, PLEASE CONTACT:

Quality Assurance
XenoTech, LLC.
913.438.7450
info@xenotechllc.com

Organization and Personnel

The responsibilities of personnel outlined in the FDA GLPs are generally applied to a non-clinical study and are not specific to a particular type of study or test system. XenoTech assigns specific responsibilities—including all those listed in the GLP regulations—for the roles of study personnel, study director, facility management, quality assurance unit (QAU) and archivist. These responsibilities are outlined in an SOP, and the designated roles of study director, facility management and archivist are applied equally to both GLP-compliant and non-GLP studies.

XenoTech management ensures adequate facilities; equipment, training and resources are available for the conduct of *in vitro* and *ex vivo* studies. In addition, management ensures corrective actions taken to protocol, SOP and GLP deviations are appropriate and documented. For both GLP-compliant and non-GLP studies, one study director is assigned to each study and charged with overall responsibility for the conduct of the study. Study directors function as a single point of control for the study. As required by GLP regulations, XenoTech maintains a quality assurance unit that is responsible for monitoring a study and reporting to management and the study director any findings from that study. The quality assurance unit does not inspect the conduct of non-GLP studies or records generated specifically from non-GLP studies; however the QAU does perform facility and process-based inspections of all facility operations assuring no SOP or GLP deviations were made without proper documentation and authorization. The QAU does maintain copies of all GLP-compliant and audited protocols and a master study schedule of all GLP-compliant and audited studies conducted at the facility.

Facilities

The GLP regulations require that the facilities are adequate to conduct the procedures required of each study. Of particular importance for *in vitro* studies is the prevention of contamination. XenoTech maintains custom-designed laboratory areas specific for procedures including test article and control article receipt and storage, solution preparation, microsome preparation and LC-MS-MS analysis, sterile and aseptic procedures and biohazard procedures.

XenoTech does maintain on-site facility archives, but not specimen archives. Specimens generated during the conduct of a study may be shipped to the sponsor or another location designated by the sponsor for storage at the close of the study. Alternatively, at the close of the study the specimens are disposed of per the sponsor's request. XenoTech applies the same procedure for both GLP-compliant and non-GLP studies.

Standard Operating Procedures

SOPs covering laboratory operations listed in the GLP regulations have been written and maintained to cover both *in vitro* and *ex vivo* studies. Additional SOPs have been written to cover experimental methods and procedures specific to drug metabolism, and drug interaction studies. Management approves all new and revised SOPs. Applicable hardcopy SOPs are available in laboratories and electronic SOPs are available at all workstations. With few exceptions, the same SOPs support the conduct of both GLP-compliant and non-GLP studies.

Equipment

The GLP regulations for maintenance, calibration, testing and documentation of activities associated with laboratory equipment are applied to both GLP-compliant and non-GLP studies. Appropriate equipment is maintained for procedures to be conducted during the conduct of *in vitro* and *ex vivo* studies. XenoTech's Department of Maintenance & Metrology is a dedicated department responsible for equipment inspection, cleaning and maintenance, as detailed in XenoTech's equipment SOPs. Verification and calibration is conducted in-house by study personnel or maintenance and metrology personnel or they are contracted out to equipment vendors or specialized contractors as necessary. All equipment use is documented in instrument logbooks. Records of equipment inspection, maintenance, testing, calibration and standardization are archived and retained. Study personnel use the same laboratories and equipment for all contracted studies. Thus maintenance, calibration, testing and record-keeping procedures are applied equally to both GLP-compliant and non-GLP studies in order to maintain the equipment in proper regulatory compliance.

Test and Control Articles

The sponsor or manufacturer usually supplies test and control article characterization information. For a GLP-compliant study, if this information is not received from the sponsor, then the testing facility will conduct appropriate tests to obtain the required information. Full characterization of test and control articles is not required for non-GLP studies. XenoTech does not retain test and control article retention samples, as the in-life portion of studies (dosing to observation) is typically less than 4 weeks duration. Test and control articles remaining after the close of the study are returned or destroyed per the sponsor's request. Test and control articles are assigned internal tracking numbers. Information on test article receipt and distribution is maintained with study records.

Test article dosing solutions are analyzed for concentration and stability for GLP-compliant studies; this analysis is not required for non-GLP studies. The sponsor may provide information of the stability of test solutions; however, if stability is unknown and XenoTech does not conduct appropriate tests to determine, solutions are prepared fresh daily. Control article dosing solutions are not analyzed for GLP-compliant nor non-GLP studies. Solubility information is provided by the sponsor and assessed at XenoTech in the specified test system.

Protocol and Study Conduct

XenoTech conducts contract studies according to the applicable GLP regulations and the protocol. XenoTech uses preprinted forms with selected data that are verified by the analyst at the time of study conduct. The same documentation requirements are applied to both GLP-compliant and non-GLP studies.

For more information on XenoTech's Products and Services, please visit www.xenotech.com or call 913.438.7450

Reporting

XenoTech prepares a final report summarizing the study methods and results of each study including applicable components listed in the GLP regulations. In a report for a GLP-compliant study, XenoTech includes a compliance statement stipulating the regulations followed in the conduct of the study and any GLP deviations that occurred during its conduct. Protocol deviations are reported for both GLP-compliant and non-GLP studies. Corrections or changes to a final report are only made through amendment to the final report as described in the GLP regulations, with prior approval from the sponsor. XenoTech offers alternatives to preparing a complete final report for a non-GLP study. A data summary or other simplified versions of a summary report may be prepared based on the needs and requests from the sponsor.

Records Storage, Retrieval and Retention

XenoTech archives and maintains all study records in a facility archive. The records are indexed and stored either at XenoTech's facility or transferred to an offsite commercial archive facility. XenoTech stores records from GLP-compliant studies in fire-resistant cabinets within its restricted access archive room. Access to the archives is controlled, and all access to the archival records is documented and logged. Records from non-GLP studies are stored in the same archive room with controlled access, but they are not necessarily stored in fire-resistant cabinets.

The record retention requirements set forth in the GLP regulations use time frames from the point of application for a permit or submittal to the FDA by the sponsor. Tracking these dates often becomes difficult following completion of the drug metabolism or drug interaction study. XenoTech established a standard record retention policy and retains records generated from any contract study for a minimum of five years following completion of the study. Sponsors may request an alternative retention period for study records or may request that records be transferred to them after a designated period of time.

Electronic Record and Electronic Signatures

FDA 21 CFR Part 11 regulations for electronic records and signatures generated by XenoTech instruments, software and networked environment are applied differently in a GLP-compliant versus a non-GLP study. Computerized systems used in GLP- compliant studies must meet all Part 11 requirements including validation and electronic signatures. Computerized systems used in non-GLP studies may not be validated or include electronic signatures.

XenoTech maintains a Computerized System Master List which includes the identification of all systems and their validation status. Individual system SOPs include procedures for the use of electronic signatures and the maintenance of electronic records.

Bioanalytical Method Validation

The application of FDA guidelines for the validation of bioanalytical methods is defined in XenoTech SOPs. All methods used in GLP-compliant studies must be validated. Methods are tested for (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5), reproducibility, and (6) stability. Routine sample analyses are conducted with QCs used to accept and reject runs. Non-GLP studies may use methods that have not been validated. Typically, the same methods validated for GLP-compliant studies are used for non-GLP studies; however QCs are not used to determine acceptability of the batch.

Table 1: Summary of a GLP-compliant vs. a non-GLP study at XenoTech

CFR citation / reference	Explanation and application at XenoTech	Non-GLP study	GLP-compliant study
21 CFR 58			
Subpart B-Organization and Personnel			
58.29 Personnel	Training program includes on-the-job, scientific, GLP and safety training. Training records and job descriptions for all study personnel are maintained.	Yes	Yes
58.31 Test facility management	Testing facility management with defined responsibilities. Designates a study director for each study.	Yes	Yes
	Assures corrective action to GLP deviations are taken and documented.	No	Yes
	Assures corrective action to SOP and Protocol deviations are taken and documented.	Yes	Yes
58.33 Study Director	One study director designated per protocol with overall responsibility for the study.	Yes	Yes
	All SOP and Protocol deviations are recorded, discussed and acknowledged by the study director.	Yes	Yes
	All GLP deviations are recorded, discussed and acknowledged by the study director.	No	Yes
58.35 Quality assurance unit	A separate QA department reporting to management. QA maintains the MSS and copies of all GLP study protocols. For each study QA conducts a protocol audit, data audits, critical phase audits and final report audit. QA inspections are reported to the study director and management. All QA records are maintained and archived at the close of the study.	No	Yes
	For all operations conducts facility and process-based inspections determining that no SOP or GLP deviations were made without proper authorization and documentation Inspections reported to Management.	Yes	Yes
Subpart C-Facilities			
58.43 Animal Care, 58.45 Animal supply facilities	XenoTech does not house or use live animals in contract studies. Test systems for studies are *in vitro* systems typically liver microsomes or hepatocytes.	NA	NA
58.47 Facilities for handling test and control articles	Separate receipt, preparation and storage areas for test and control articles. Test articles are kept in locked storage at appropriate storage conditions.	Yes	Yes
58.49 Laboratory operation areas	Custom-designed laboratory areas. Designated laboratory areas for solution preparation, microsome preparation, LC-MS-MS analysis, aseptic procedures and biohazard procedures.	Yes	Yes
58.51 Specimen and data storage facilities	Secure archive room for the storage and retrieval of study materials from completed studies. XenoTech does not have a specimen archive.	Yes	Yes

CFR citation / reference	Explanation and application at XenoTech	Non-GLP study	GLP-compliant study
21 CFR 58			
Subpart D-Equipment			
58.63 Maintenance and calibration of equipment	Equipment is inspected, cleaned and maintained per equipment-specific SOPs. Equipment use is documented in logbooks and inspections, maintenance, testing, and calibration is documented.	Yes	Yes
Subpart E-Testing Facilities Operation			
58.81 SOPs	Management approved SOPs for all laboratory operations; operations specific to animal care and handling do not apply. SOPs are immediately available in laboratories and electronic SOPs are available at all workstations.	Yes	Yes
58.83 Reagents and solutions	Reagents and solutions are labeled with identify, titer, storage and expiration date and are not used past their expiration date.	Yes	Yes
Subpart F-Test and Control Articles			
58.105 Test and control article characterization	Test and control article identity, strength, purity and stability information is supplied by the sponsor or manufacturer. If not received, then XenoTech will conduct appropriate tests to determine. Stability and purity information is not required in order to conduct a non-GLP study.	No	Yes
	All test and control article storage containers are labeled to indicate name, batch number, expiration date, in any and storage conditions and containers assigned for the duration of the study.	Yes	Yes
	XenoTech studies are typically, less than 4 weeks duration (from dosing to observations); therefore, reserve samples from each batch are not required. Test and control articles remaining after the close of the study are returned or destroyed per sponsor request. General use control articles from marketed products are stored up to their expiration date.	Yes	Yes
58.107 Test and control article handling	Test and control articles are assigned internal tracking numbers. Receipt and distribution are documented.	Yes	Yes
58.113 Mixtures of articles with carriers	Test article dosing solutions are analyzed for concentration. Stability of test and control article solutions is either provided by the sponsor/vendor or determined by XenoTech. If stability is unknown, solutions are prepared fresh daily. Dose concentration analysis is not conducted for non-GLP studies.	No	Yes
	Control article dosing solutions are only analyzed for concentration if requested by the sponsor	No	No

CFR citation / reference	Explanation and application at XenoTech	Non-GLP study	GLP-compliant study
21 CFR 58			
Subpart G-Protocol for and Conduct of a Nonclinical Laboratory Study			
58.120 Protocol	Each study has a sponsor approved protocol, signed and dated by the study director. The protocol details the objectives and all methods for the conduct of the study.	Yes	Yes
58.130 Conduct of a nonclinical laboratory study	Studies are conducted per approved protocol and amendments. Any deviations are noted in the final report Automated and manual data recording and correction practices are per GLP requirements	Yes	Yes
Subpart J-Records and Reports			
58.185 Reporting	A final report is prepared for each study. For non-GLP studies, the sponsor may choose a final report or alternative data summary.	No	Yes
	Final reports include the test system, methods, study results and conclusions. For multi-site studies, principal investigators provide report of phase conducted. The final report is approved by the study director. Management and sponsor also typically review and sign the report.	Yes	Yes
	A compliance statement is included reporting all GLP and protocol deviations. GLP deviations are not reported for non-GLP studies; however protocol deviations are. A QAU statement of study inspections and reporting included.	No	Yes
58.190 Storage and retrieval of records and data	Hard copy and electronic archived records are stored in onsite archive or offsite commercial archive. One individual is designated as archivist. Only designated personnel may access the archives and an entry log to the archives is maintained.	Yes	Yes
	Records are stored in fire resistant cabinets when onsite.	No	Yes
58.195 Retention of records	Study records are retained a minimum of five years unless other arrangements are requested by the sponsor.	Yes	Yes
	XenoTech does generate specimens during the conduct of induction studies when extracting microsomes from treated hepatocytes. XenoTech does not maintain a specimen archive. Specimens are transferred to the sponsor or disposed if requested.	No	No
	QA records, facility inspection records, MSS, organization chart, floor plan, training records of former employees and equipment records are retained.	Yes	Yes

CFR citation / reference	Explanation and application at XenoTech	Non-GLP study	GLP-compliant study
21 CFR 11			
11.10 Controls for closed systems	Computer systems identified for use in GLP-compliant studies are validated. Computer systems identified for use only in non-GLP studies may not be validated.	No	Yes
	Computer systems are configured with individual logins requiring unique user name and password for applicable staff.	Yes	Yes
	Computer systems configured with audit trails if available. Controls used for systems not capable of producing an audit trail. Electronic signatures as part of an audit trail may be disabled on select systems for non-GLP studies.	No	Yes
11.50 Signature manifestations	Electronic signatures are used indicating signer, date/time and meaning. Hand-written signatures applied to printed data when electronic signatures not available.	Yes	Yes
11.100 General requirements	XenoTech has provided certification to the FDA indicating use of electronic signatures.	Yes	Yes

CFR citation / reference	Explanation and application at XenoTech	Non-GLP study	GLP-compliant study
FDA Bioanalytical Method Validation Guidance, 2001			
II Background	LC-MS-MS methods are validated using the fundamental parameters (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5), reproducibility, and (6) stability. Routine sample analyses are conducted with QCs used to accept and reject runs. Methods for non-GLP studies may not be validated per guidance.	No	Yes
III Reference Standard	Authentic reference standards are used for metabolites for enzymatic activity assays	No	Yes
IV Method Development	Method development includes determining (1) selectivity, (2) accuracy, precision, recovery, (3) calibration curve and (4) stability of analyte in spiked samples. Freeze thaw and IS stability not determined. Typically, the same methods developed for GLP-compliant studies are used for non-GLP studies.	Yes	Yes
VI Application to Routine Analysis	Sample analyses performed in batches. Each batch contains study samples and calibration curve samples, blanks and QC samples. Analytical acceptability of the batch is determined based on standards and QCs. In non-GLP studies, QCs are not used to determine acceptability of the batch.	No	Yes
VII Documentation	Validation report issued of results and SOP summarizing the method. All supportive documents and records are archived.	Yes	Yes

Conclusion

Although regulatory agencies in the United States, Europe and Japan do not require compliance with Good Laboratory Practice (GLP) regulations for *in vitro* drug metabolism and drug interaction studies, published industry guidance on the design of drug interaction studies emphasizes these studies be conducted with high standards of quality ensuring reproducibility of data. For this reason XenoTech offers GLP-compliant and non-GLP studies. A comparison of applicable FDA GLP regulations between GLP-compliant and non-GLP studies conducted at XenoTech is shown in **Table 1**. This comparison demonstrates many similar procedures including laboratory space, equipment, personnel roles, personnel functions and laboratory procedures and processes. Nevertheless, there are some notable differences between a GLP-compliant and a non-GLP study including, in the latter case, no QAU inspection of the study (unless requested by sponsor), no strict adherence to selected GLP regulations for computer system validation and electronic signatures, final reports, test and control article/mixture characterization, archived records storage and FDA bioanalytical guidance for method validation and data acceptance.

References

Tucker, Geoffrey T., J. Brian Houston, and Shiew-Mei Huang, Optimizing Drug Development: Strategies to Assess Drug Metabolism/Transporter Interaction Potential-Toward a Consensus Pharmaceutical Research, p.1071-1080, 2001.

Bjornsson et al., The Conduct of *in vitro* Drug-Drug Interaction Studies: A Pharmaceutical Research and Manufacturers of America (PhRMA) Perspective, p815-831, 2003.

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 14, Advisory Document of the Working Group on Good Laboratory Practice, The Application of the Principles of GLP to *in vitro* Studies, ENV/MJ/MONO (2004) 26, 30-Nov-2004.

Guidance for Industry, Bioanalytical Method Validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). May, 2001.

21 CFR Part 58; The Food and Drug Administration's Good Laboratory Practice for Nonclinical Laboratory Studies.

21 CFR Part 11; The Food and Drug Administration's Good Laboratory Practice for Nonclinical Laboratory Studies.

Abbreviations

FDA: Food and Drug Administration, USA

OECD: Organization for Economic Co-operation and Development, Europe

MHLW: Ministry of Health, Labour and Welfare, Japan

CFR: Code of federal regulations

SOP: Standard operating procedure

QC: Quality control

QA: Quality assurance