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Review

Guidelines on good clinical laboratory practice: Bridging operations between research and clinical research laboratories

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Abstract

A set of Good Clinical Laboratory Practice (GCLP) standards that embraces both the research and clinical aspects of GLP were developed utilizing a variety of collected regulatory and guidance material. We describe eleven core elements that constitute the GCLP standards with the objective of filling a gap for laboratory guidance, based on IND sponsor requirements, for conducting laboratory testing using specimens from human clinical trials. These GCLP standards provide guidance on implementing GLP requirements that are critical for laboratory operations, such as performance of protocol-mandated safety assays, peripheral blood mononuclear cell processing and immunological or endpoint assays from biological interventions on IND-registered clinical trials. The expectation is that compliance with the GCLP standards, monitored annually by external audits, will allow research and development laboratories to maintain data integrity and to provide immunogenicity, safety, and product efficacy data that is repeatable, reliable, auditable and that can be easily reconstructed in a research setting.

Keywords: Good clinical laboratory practice standards; GCLP; Quality control; Verification; Review

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1. Introduction

The Good Clinical Laboratory Practices (GCLP) concept possesses a unique quality, as it embraces both the research and the clinical aspects of GLP. The development of GCLP standards encompasses applicable portions of 21 CFR part 58 (GLP) [1] and 42 CFR part 493 (Clinical Laboratory Improvement Amendments, CLIA) [2]. Due to the ambiguity of some parts of the CFR, the GCLP standards are described by merging guidance from regulatory authorities as well as other organizations and accrediting bodies, such as the College of American Pathologists (CAP), and the International Organization for Standardization 15189 (ISO) [3]. The British Association of Research Quality Assurance (BARQA) took a similar approach by combining Good Clinical Practice (GCP) and GLP in 2003 [4].

The GCLP standards were developed with the objective of providing a single, unified document that encompasses IND sponsor requirements to guide the conduct of laboratory testing for human clinical trials. Examples of these types of tests include protocol-mandated safety assays such as diagnosis of HIV-1 infection, blood processing to obtain high quality specimens routinely [5], and cellular and serological immunogenicity assays (e.g., enumeration of antigen-specific cells by ELISpot [6] or flow cytometry [7]), or enzyme-linked immunosorbent assays (ELISA) [8] to support clinical trials on a product licensure pathway. The intent of GCLP guidance is that when laboratories adhere to this process, it ensures the quality and integrity of data, allows accurate reconstruction of experiments, monitors data quality and allows comparison of test results regardless of performance location.

In this paper, we create a comprehensive description of GCLP utilizing GLP and CLIA as a foundation, augmented with specific guidance from organizations such as CAP and ISO. A comprehensive version of the GCLP standards with accompanying templates and examples is available at [http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/labs/GCLP.pdf] [9]. To illustrate the need for a single unified GCLP standards document, Table 1 compares major elements of US, UK and other international guidance documents, showing current gaps. The GCLP core elements described in this paper include: organization and personnel; laboratory equipment; testing facility operations; quality control program; verification of performance specifications; records and reports; physical facilities; specimen transport and management; personnel safety; laboratory information systems and quality management. By recognizing these standards as the minimum requirements for optimal laboratory operations, the expectation is that GCLP compliance will ensure that consistent, reproducible, auditable, and reliable laboratory results from clinical trials can be generated for clinical trials implemented

Table 1
Comparison of laboratory regulations/guidance

Topic	GLP [1]	CLIA [2]	CAP [14]	ISO (15189) [3]	BARQA GCLP [4]
Organization and personnel	No significant differences	Strict personnel requirements, which may not be feasible in international setting	Requires documented organizational chart, personnel policies, competency assessments, and job descriptions	No significant differences	Contains requirements for the use of sub-contractors
Equipment/instrumentation	No significant differences	No significant differences	Requires documented PM and calibration plans	Requires documented PM and calibration plans	No significant differences
Testing facilities operation	No significant differences	Requires that textbooks only supplement, not replace, SOPs	Requires annual review of procedural manuals. Documentation indicating staff has read and understands procedural documents	Requires a written document control log	No significant differences
Test and control articles	No significant differences	List specific labeling requirements for reagents/controls/solutions	List specific labeling requirements for reagents/controls/solutions	No significant differences	No significant differences
Verification of performance specifications	Not addressed	Verification of the following parameters for FDA approved system: accuracy, precision, reportable range, and reference intervals. Establishment of performance specifications for modified FDA cleared system or non-approved system: accuracy, precision, reportable range, reference intervals, sensitivity, and specificity	Verification of the following parameters for FDA approved system: accuracy, precision, reportable range, and reference intervals. Establishment of performance specifications for modified FDA cleared system or non-approved system: accuracy, precision, reportable range, reference intervals, sensitivity, and specificity	No significant differences	No significant differences
Records and reports	Retention for either: Two years post-submission of a research or marketing permit to the FDA, or 5 years post-submission of study results to the FDA in support of a research or marketing permit	Retention of records for 2 years	Retention of records for 2 years	No significant differences	No significant differences
Physical facilities	No significant differences	No significant differences	No significant differences	No significant differences	No significant differences
Specimen transport and management	Not addressed	No significant differences	No significant differences	No significant differences	Materials must be transferred to the sponsor, should the facility go out of business
Personnel safety	Not addressed	Compliance to state, local, and federal safety regulations	Annual review of all safety policies/procedures Requires compliance with OSHA Standards	No significant differences	No significant differences
Laboratory information systems	No significant differences	No significant differences	Validation only for transfusion medicine	Validation of all laboratory software used for collection, processing, recording, reporting, storage, or retrieval of examination data	Compliance with FDA 21 CFR part 11

This table illustrates some of the major differences in the reference materials. Most were utilized to generate the GCLP standards as they described key laboratory operational topics. Other differences, such as the personnel requirements mandated by CLIA, were not included due to non-applicability to globally operated facilities. The text inserted in the chart describes significant requirements or differences identified for the corresponding topics. The entry of "No significant differences" reflects no noteworthy differences between the compared regulation/guidance materials. An entry of "Not addressed" indicates that the topic is not described adequately by the material cited.

at multiple sites. A corollary of this infrastructure is that the data will be produced in an environment conducive to study reconstruction, enable prioritization between candidate product regimens and guide rational decision making for moving products forward into advanced clinical trials.

2. Standards for organization and personnel

Appropriately trained and well-organized laboratory staff are key to the successful operation of a research facility. Systems are required to drive organizational structure, training and ongoing competency assessment to ensure appropriate accountability and communication during study conduct.

2.1. Required activities and documentation

All personnel must receive direct and detailed training for the performance of all duties and tasks that they perform. Competency assessments must be conducted and recorded for all components of the employee's training and functional responsibilities upon completion of initial training. A clinical laboratory continuing education program that is adequate to meet the needs of all personnel must be documented, and evidence of ongoing adherence by all laboratory personnel must be readily available.

A testing laboratory must have the following documents stored in the laboratory or readily available for authorized personnel: organizational, departmental, and/or personnel policies that address such topics as orientation, training, continuing education requirements, performance evaluations, benefits, discipline, dress codes, holidays, security, communication, termination, and attendance [10–12]; job descriptions that define qualifications and delegation of duties for all laboratory positions [11–13]; personnel files that document each employee's qualifications, training, and competency assessments as they relate to job performance [14]; and the organizational chart(s) that represent the formal reporting and communication relationships that exist among personnel and management and between the main laboratory unit and satellite units [15].

2.2. Job-specific training, education, and assessments

The laboratory director must designate staff who has overall responsibility for the study and serves as the single point-of-contact for document control, staff training and familiarity with GCLP. All laboratory personnel must receive direct and detailed job-specific training and continuing education to perform all duties so that they understand and competently carry out the necessary functions [11,12]. Additionally, competency assessments must be conducted every 6 months during the first year of employment, and annually thereafter. Annual evaluations for the employee's overall performance of job responsibilities, duties, and tasks as outlined in the job description must be given to all laboratory personnel [16,17]. The laboratory must employ an adequate number of qualified personnel to perform all of the functions associated with the volume and complexity of tasks and testing performed within the laboratory [11,12,18,19].

All laboratory staff signatures, initials, or codes used as staff identifiers on any laboratory documentation must be linked to a printed name list. This laboratory's documented list should be a "controlled or traceable version" document that must be updated if changes occur in the laboratory. Signature logs should be archived so that those individuals who performed trial testing throughout the length of a trial are identifiable.

3. Standards for laboratory equipment

Proper maintenance of all laboratory equipment is necessary for assays to function within manufacturer's specifications. Internal preventative maintenance activities as well as vendor provided maintenance/repair for laboratory equipment is paramount in providing accurate and reliable results. The standards below provide direction on how to accomplish this.

Laboratory staff must conduct preventive maintenance and service per manufacturer specifications by following documented daily, weekly, and/or monthly routine maintenance plans for all equipment utilized to ensure that all equipment performs consistently and reproducibly during the conduct of the trial [20]. Additionally, the laboratory must document all scheduled preventive maintenance (PM), unscheduled maintenance, service records, and calibrations for all equipment utilized. This documentation should be readily accessible to operators [20–22]. As a follow-up step, the laboratory director or designee must consistently review, sign, and date all documentation at least monthly to establish an audit trail [11,12]. The laboratory must establish tolerance limits for equipment temperatures and other monitored conditions (e.g., $\%CO_2$, liquid nitrogen levels) that are consistent with manufacturers' guidelines and procedural activities because certain reagents and equipment perform optimally under specific conditions [23]. The lab should also maintain daily (or "dates of use") records of temperatures and other monitored conditions (e.g. humidity). For observations that fall outside of designated tolerance ranges, the laboratory must maintain appropriate documentation of corrective action for these out-of-range temperatures and other conditions.

4. Standards for test facility operation

4.1. Standard operating procedures

Standard operating procedures (SOPs) are critical for maintaining consistent test performance. The laboratory must write SOPs for all laboratory activities to ensure the consistency, quality, and integrity of the generated data. Current SOPs must be readily available in the work areas and accessible to testing personnel [24].

The laboratory must write these SOPs in a manner and language that is appropriate to the laboratory personnel conducting the procedures. SOPs should also be written in a standard format, such as the format recommended by the Clinical and Laboratory Standards Institute (CLSI) [25]. All laboratory personnel must document and maintain verification that they have reviewed and

understood all relevant SOPs so that there is evidence that all personnel are knowledgeable of appropriate laboratory SOPs [26].

4.2. Document control plan

The laboratory must maintain a written current document control plan that addresses and ensures the following vital elements of SOPs: a master list of SOPs currently used in the laboratory [25]; an authorization process that is standard and consistent, limiting SOP approvals to laboratory management [24]; assurance that all SOPs are procedurally accurate and relevant, as well as review of each SOP at appropriate time intervals [27]; removal of retired or obsolete SOPs from circulation and identification of them as retired or obsolete; and an archival system that allows for maintenance of retired or obsolete SOPs for a period defined by the laboratory that meets or exceeds the requirements of applicable regulatory bodies, such as the U.S. FDA [24].

5. Quality control program

The laboratory director or designee should be actively involved in the design, implementation, and oversight of a site-specific, written Quality Control (QC) program which defines procedures for monitoring analytic performance and consistent identification, documentation, and resolution of QC issues [11,12]. This is so as to be able to detect immediate errors as well as changes that occur over time and hence assure the accuracy and reliability of test results, particularly if the data are used for patient management or product advancement decisions. In addition, the laboratory director and/or designee must determine the number and frequency of QC tests, as well as the appropriate QC materials to use [28]. The quality control program supports functions in the following areas: Test standards and controls, reagents, test specimens, review of quality control data, quality control logs, labeling of quality control materials and reagents, inventory control, parallel testing, and water quality testing.

5.1. Test standards and controls

Individual assay controls must be in place to ensure assay performance. Control activities must be well defined and managed through an ongoing quality control (QC) program to capture immediate performance issues, as well as assay problems that can occur over time.

5.2. Reagents

For quantitative tests, it is necessary to use control materials at known values that span the reportable range of the assay where clinical or patient management decisions are made. For example, in the ELISpot assay, the use of Gag peptides or HIV-1 that traverses the assay dynamic range or negative sera that show a range of responses to Cytomegalovirus-derived peptides. For qualitative tests, include positive and negative controls with each run. For staining procedures, gram stains require both Gram-positive and Gram-negative control organisms to be used once per week and with each change of a lot number of any component in the stain procedure. Other stains require daily or day-of-use QC, using a positive reacting organism and a negative. The laboratory must establish and document site-specific tolerance limits for acceptance of control results because manufacturers tend to set wide ranges to accommodate a spectrum of laboratory settings [28]. All QC samples must be tested in the same manner as study-participant specimens and by the personnel who routinely perform study-participant testing [28].

5.3. Test specimens

The laboratory must maintain and document acceptance criteria to test specimens and must follow site-specific instructions defined in the QC plan to routinely monitor analytic performance and to identify, document and resolve QC analytical problems. The laboratory must report results of specimen testing after ensuring data integrity, quality, and accuracy as described in the QC plan. The latter also specifies how the laboratory must proceed when changes of critical analytes occur; how QC logs must document control results from tested specimens; how all QC materials and reagents must be prepared, labeled and stored following the manufacturer's specifications; how an inventory control system must be established and followed to maintain continuous supply of reagents and materials; how parallel testing for new lots of reagents must be conducted to bridge with existing reagents; and how to test water quality to ensure that it meets defined tolerance limits as set forth by the testing requirements.

5.4. Review of quality control data

QC must be performed and acceptable results obtained (as defined in the written QC program) before test results are reported [28] to ensure quality and accuracy of all aspects of the work performed and reported. QC must also be run and reviewed after a change of analytically critical reagents, major preventive maintenance/service, or change of a critical instrument component [28]. The laboratory personnel performing the testing must use the laboratory's QC program as a guide for selecting the appropriate corrective action to take for QC data that falls outside of established tolerance limits. Records should include detailed information of actions taken leading to resolution and include staff initials and dates. The laboratory must ensure a corrective action log is present to facilitate documentation and resolution of QC failures [29]. In the event the QC data is determined to be unacceptable, the laboratory must re-evaluate all study-participant test results since the last acceptable test run to determine if a significant clinical difference has occurred, in which case, the instrument QC should be re-established and the affected testing repeated [29].

5.5. Quality control logs

QC logs must document control results assayed with each test to determine the acceptability of the QC run and to aid in

detection of shifts and trends in control data [28,30]. QC records must be readily available to the staff performing the test. Results of controls must be recorded or plotted in real time (e.g., Levy Jennings [LJ] charts or control charts) to readily detect a malfunction in the instrument or in the analytic system. Laboratory personnel who perform QC runs, record results, and plot data on graphs must record their initials, date, and time as testing is performed. QC records should contain detailed information to reconstruct establishment of ranges for each QC material used for monitoring analytic performance. Information should include, but is not limited to: package insert (containing material name, manufacturer, concentration, lot numbers, etc.), opened dates, expiration dates, dates of testing, testing personnel, raw data, evaluation, approval, and other appropriate information. Laboratory supervisory personnel must regularly review, sign, and date QC records and corrective action logs at least monthly [11,12]. QC record retention time periods established by the laboratory must meet or exceed the requirements set forth by the product sponsor and/or any applicable regulatory bodies such as the FDA [31].

5.6. Labeling of quality control materials and reagents

All QC materials and reagents currently in use must be prepared and stored as required by the manufacturer. If ambient temperature is indicated for storage or use, there must be documentation that the defined ambient temperature is maintained and that corrective action is taken when tolerance limits are exceeded [23].

All QC materials and reagents must be properly labeled for content and include storage requirements, date opened, prepared, or reconstituted by the laboratory, the initials of personnel who prepared/reconstituted the QC material and reagents, and the expiration date [23]. An expiration date must be assigned to QC materials and reagents that do not have a manufacturer-provided expiration date or an expiration date that changes upon reconstitution or use. The manufacturer should be consulted should this situation arise (exception: microbiological organisms—storage and sub-culturing techniques will determine time of use) [32]. Deteriorated or outdated (expired) QC materials and reagents must not be used because this may jeopardize the quality of collected data [23].

5.7. Inventory control

The laboratory must have an established documented inventory system to maintain an appropriate amount of "working" supplies and reagents and to prevent delays in testing of specimens due to lack of required reagents [20,33]. There must be evidence of a system which highlights the need to place supply orders, tracks orders (once placed), and defines alternate plans for delayed deliveries of supplies and recovery procedures for "out-of-stock" conditions (a system that details steps to ensure minimal lapse in ability to perform testing).

5.8. Parallel testing

For each new lot, batch or kit of reagents, the laboratory must document that samples, manufacturer-provided reference materials or proficiency testing materials are tested in parallel with both the current lot and the new lot to assess test comparability before or concurrently with being placed into service [34]. For quantitative tests, parallel testing should be performed by assaying the same samples or reference materials with both the old and new lot numbers to assess comparability. Quality control materials should also be tested when comparing old and new lots. For qualitative tests, parallel testing must include re-testing at least one known positive (or abnormal) and one known negative (or normal) sample.

5.9. Water quality testing

If specific water types are required per manufacturer for certain testing procedures, the laboratory must ensure that records of water quality testing are complete and/or indicate that the required standards for water quality (e.g., pH and resistivity) are consistently met [35]. The laboratory must document evidence of corrective action taken when water testing does not meet defined tolerance limits [23].

6. Standards for verification of performance specifications

Validation of manufacturer provided performance specifications, or the development of such specifications can be challenging. The assay development and approval status defines what parameters are required in a formal validation study. The standards below offer guidance on how to validate an assay.

6.1. Required activities and documentation

The laboratory must verify and document optimal performance of non-waived CLIA tests used to acquire study-participant results following pre-defined specifications that are equivalent to the ones provided by the manufacturer. The definition of the normal range must include specifications for the analytical measurement range (AMR) and the clinically reportable range (CRR) of each test used. The laboratory must also include a correction factor for each test to account for systematic errors that occur between tests. The inclusion of correction factors ensures data comparability when multiple tests are conducted to measure the same analyte in support of study-participant results.

6.2. Verification of performance standards

Before reporting study-participant results, each laboratory that introduces a non-waived (a CLIA designation) test such as an ELISA test, must demonstrate performance specifications comparable to those established by the manufacturer (as found in manufacturer's publications such as user manuals or package inserts) to ensure the assay is performing optimally within the

proposed testing environment [36]. Documentation of experiment results and approval should be readily accessible [36]. Methods that are defined as waived by CLIA do not require method validation, unless otherwise instructed by the sponsor. Laboratories are not required to verify or establish performance specifications for any analytical test system used by the laboratory before 24 April 2003 [36]. Verification and documentation of normal responses for each test system including the AMR and CRR and normal range(s) must be established to determine the usable and reliable range of results produced by that system [37]. For FDA-cleared/approved tests, analytical sensitivity documentation may consist of data from manufacturers or the published literature. If non-FDA approved methods are utilized, such as to monitor immunogenicity to a candidate vaccine, the laboratory must define, test and document the parameters described in the ICH Guidelines, Validation of Analytical Procedures: Text and Methodology, Q2(R1) document that includes the original Q2A and Q2B documents [38], or the Bioanalytical Method Validation Guidelines provided for the Industry by the FDA [39] to validate a bioanalytical assay. Examples of bioanalytical assays that have been validated for use in human clinical trials, using the ICH Guidelines are the ELISpot [6,40] and ICS assays [7]. These include accuracy, precision, analytical sensitivity, analytical specificity, reportable range, reference intervals, and any other parameter required for test performance.

If the test system to be validated is an unmodified, FDA-approved method, the manufacturer's reference range may be verified for the appropriate testing population. If the test is modified, or not FDA-approved, the reference range must be established [36]. The reference range must be established or verified for each analyte and specimen source/type (e.g., blood, urine and cerebrospinal fluid) when appropriate [41]. The laboratory may use the manufacturer's reference range when appropriate specimens are difficult to obtain (e.g., 24-h urine specimens, 72-h stool specimens, urine toxicology specimens) provided the range is appropriate for the laboratory's study participant population. In cases where the appropriate specimens are difficult to obtain and the manufacturer has not provided reference ranges appropriate for the laboratory's study participant population, the laboratory may use published reference range(s).

An appropriate number of specimens must be evaluated to verify the manufacturer's claims for normal values or, as applicable, the published reference ranges. Typically, 20 specimens are required to verify the manufacturer's or published ranges. These specimens should be appropriately collected from patients that have been predetermined as "normal" by established inclusion/exclusion criteria (e.g., HIV-negative, HBsAg-negative). The specimens should be representative of the population (age, gender, genetics, geographic area, etc.) [42].

An appropriate number of specimens must be evaluated to establish reference ranges. Typically, the minimum number of specimens required to establish reference ranges is 120 specimens per demographic group (e.g., if the laboratory wishes to establish gender-specific reference ranges, then the minimum number of specimens would be 240: 120 normal male and 120 normal female) [42]. Reference intervals must be evaluated at the following times: upon introduction of a new analyte to the test offerings by a laboratory, with a change of analytic methodology, or with a change in study-participant population [42].

6.3. Correction factors

Correction factors represent adjustments made to compensate for constant and proportional errors when more than one assay format is being used to report study participant data. To ensure interchangeability of the data from any assay used, a correction factor must be incorporated into the relevant test procedure and reflected in the appropriate SOPs if the laboratory has determined the need for correction factors based on the validation exercises.

7. Standards for records and reports

The laboratory must define and maintain a system to provide and retain all clinical trial data records and reports for a period of time to troubleshoot potential problems, or if it is necessary to reconstruct the study for auditing purposes. These records may include specimen tracking forms, laboratory requisitions, chain-of-custody documents, laboratory reports, equipment service and maintenance records, and instrument printouts [31].

7.1. Required activities and documentation

Adequate manual or electronic systems must be in place to ensure assay results and other study participant specific data (e.g., participant identifier) are accurately and reliably sent from the point of data entry (whether entered via an analyzer interface or manually) to the final report destination in an accurate and timely manner, or according to specifications detailed within protocols and/or the study/analytical plan [43]. Assay results must be released only to authorized persons and, if applicable, the individual responsible for requesting the test(s) [43].

The laboratory director must define alert or critical values in consultation with study-related clinicians [43]. Complete procedures must be in place for immediate notification of key study personnel/responsible clinic staff when assay results fall within established alert or critical ranges [43]. The laboratory must, upon request, make available a list of test assays employed by the laboratory and, as applicable, the performance specifications established or verified [43]. When the laboratory cannot report study-participant test results within the time frames established by the laboratory, the laboratory must notify the appropriate individual(s) of the delays [43].

The laboratory referring study participant specimens for testing to another laboratory must not revise results or information directly related to the interpretation of results provided by the testing laboratory and must retain the testing laboratory's report for the period of time defined by the laboratory [43].

7.2. Report format

Reports generated by the Laboratory Information System (LIS), and those created by other means, must be concise, readable, standardized in format, and chronological. The laboratory's

test report must indicate the following items [43]: either the study participant's name and/or a unique identifier; the name and address of the laboratory location where the assay was performed; the date and time of specimen receipt into the laboratory; the assay report date; the name of the test performed; specimen source (e.g., blood, cerebrospinal fluid and urine); the assay result and, if applicable, the units of measurement or interpretation or both; reference ranges along with age and gender of study-participants, if these affect the reference range; any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability; and the records and dates of all assays performed.

7.3. Result modification log and errors in test results

The laboratory must promptly notify the appropriate clinician and/or clinic staff member if an erroneous result is reported and then corrected as decisions about the clinical trial product and patient/study participant management depend on these data. It is important to replicate all of the previous information (test results, interpretations, reference intervals) for comparison with the revised information and to clearly indicate that the result has been corrected [43]. Additionally, the laboratory must have a system that identifies the analyst performing and completing the test result modification, along with the date and time. A log or other appropriate record must be kept for result modifications. The laboratory director or designee must review, sign, and date the Result Modifications/Corrective Action Logs at least monthly [44]. The laboratory must maintain copies of the original report as well as the corrected report [43]. Proper error correction techniques (e.g., single line through error, signature, and date, or electronic equivalent) must be utilized at all times by the laboratory.

7.4. Archiving reports or records

All clinical trial data records and reports must be safely and securely (e.g., fire-proof storage with limited access) retained by the laboratory for a period of time that has been defined by the laboratory to be able to fully reconstruct the study, if necessary. Retention time periods established by the laboratory must meet or exceed the requirements set forth by the product sponsor and/or any applicable regulatory bodies such as the FDA [31]. The laboratory may archive test reports or records either on- or off-site. Stored study-participant result data and archival information must be easily and readily retrievable within a time frame consistent with study/trial needs (e.g., within 24 h).

8. Standards for physical facilities

The environment in which laboratory testing is performed must be conducive to efficient operations that do not compromise the safety of the staff or the quality of the pre-analytical, analytical and post-analytical processes.

8.1. Required activities and documentation

The laboratory design must account for equipment placement, proper ventilation, and have a designated area for reagent storage as well as archiving of data in a secure fire-proof (preferred), fire-resistant, or fire-protected environment with access to only authorized personnel.

8.2. General space

Laboratory work areas must have sufficient space so that there is no hindrance to the work or employee safety [45,46]. Laboratory room (ambient) temperature and humidity must be controlled so that equipment and testing is maintained within the tolerance limits set forth by the manufacturer [23]. Ambient temperature logs should be utilized to document the acceptable ambient temperature range, record daily actual temperatures, and allow for documentation of corrective action taken should the acceptable temperature ranges be exceeded [47]. All floors, walls, ceilings, and bench tops of the laboratory must be clean and well maintained [48].

8.3. Molecular amplification work areas

Molecular amplification procedures within the laboratory that are not contained in closed systems must have a unidirectional workflow. This must include separate areas for specimen preparation, amplification, detection, and as applicable, reagent preparation to avoid contamination and mix-ups between test and control articles.

9. Standards for specimen transport and management

The accuracy of all laboratory test results depends on the identity and integrity of the specimen submitted. The establishment of a sound specimen chain of custody from collection through to reporting of test results is paramount in ensuring quality data.

9.1. Required activities and documentation

The laboratory must have documented procedures for collection, transportation, and receipt of specimens because the accuracy of all laboratory tests is dependent on specimen quality [23]. A laboratory can only ensure specimen integrity when following appropriate specimen management and transportation procedures. A properly completed request form must accompany each study-participant sample to the laboratory. The request form must document unique study-participant identifiers, specimen collection date and time, study participant demographics, specimen type, and the collector's (phlebotomist's) identity [49]. The specimen inspection process must involve verification of the specimen container label information with the request form or log sheet [50]. Any discrepant or missing information must be verified promptly, before specimens are processed or stored by laboratory personnel. The laboratory must have documented specimen acceptance/rejection criteria for evaluation of sample adequacy and integrity [24,51]. The

laboratory must maintain an audit trail for every specimen from collection to disposal or storage. Audit trails must verify the date and time an activity was performed and the personnel responsible for that activity. All audit trails must be documented [51]. A shipping procedure must be documented that addresses preparing shipments by following all federal and local transportation of dangerous goods regulations (e.g., International Air Transport Association (IATA)) by laboratory personnel who are certified in hazardous materials/dangerous goods transportation safety regulations [52]. Twenty-four-hour monitoring of storage conditions (using personnel and/or electronic monitoring with alert systems) and SOPs for response to alerts must be in place to ensure the integrity of samples is maintained.

10. Standards for personnel safety

The safety of all laboratory staff is reliant upon the avoidance of avoid laboratory accidents that may pose a high risk of acquisition of infectious agents through handling of blood, as an example. Although exposure cannot always be avoided, every precaution must be taken to provide a safe work environment.

10.1. Required activities and documentation

Safety policies defined according to regulatory organizations such as the Occupational Safety and Health Administration (OSHA) [53] or the International Organization for Standardization (ISO) [3] must be present in the laboratory. The following safety policies must be in place to ensure the safety of laboratory staff and any authorized individuals [45,54]: Standard Precautions/Universal Precautions Policy, Chemical Hygiene/Hazard Communication Plan, Waste Management Policy, Safety Equipment, and general safety policies (these policies address less specific topics as they relate to laboratory safety, such as fire and back safety).

10.2. Safety equipment and material safety data sheets

Fire extinguishers, emergency shower, eye wash, and sharps containers must be present in each laboratory, in compliance with general safety/local laws. Periodic inspection and/or function checks of applicable safety equipment must be documented [54]. The employer must assess the workplace to determine if hazards are likely to be present which necessitate the use of Personal Protective Equipment (PPE) and provide access to PPE to all laboratory staff at risk [55]. All laboratory employees must use PPE if there is a potential for exposure to blood or other potentially infectious material through any route (e.g., skin, eyes, other mucous membranes) [55].

The laboratory must have Material Safety Data Sheets (MSDS) or equivalent in the workplace for each hazardous chemical that they use [56].

10.3. Safety training

All laboratory staff must receive safety training. At a minimum, the safety training must include [52,54–57]: Blood-borne pathogens, PPE, Chemical Hygiene/Hazard Communications, use of safety equipment in the laboratory, use of cryogenic chemicals (e.g., dry ice and liquid nitrogen), transportation of potentially infectious material, waste management/biohazard containment, and general safety/local laws related to safety. Safety training must be documented and maintained. Safety training must be completed before any employee begins working in the laboratory and on a regular basis thereafter. Ongoing safety training must take place each calendar year. Documentation of this training must be signed and dated by the employee.

10.4. Safety incident reporting

Safety-related incidents must be documented, submitted, reviewed, and signed by the Laboratory Manager or designee on a regular basis, not to exceed 1 month from time of submission. Safety reports must be incorporated into the Quality Management (QM) program allowing the laboratory to note trends and correct problems to prevent recurrence [58].

11. Standards for laboratory information systems (LIS)

An LIS is a powerful tool to manage complex processes, ensure regulatory compliance and promote collaborations between multiple laboratories. Usually an LIS is capable of consolidating disparate scientific processes into a single, compliant platform with comprehensive reporting, surveillance and networking capabilities. The result is vastly enhanced data management and data sharing-within the laboratory and across laboratories.

11.1. Required activities and documentation

The purpose of an LIS, the way it functions, and its interaction with other devices or programs must be documented with validation data and results including data entry, data transmission, calculations, storage and retrieval [59]. Since patient management decisions and product advancement decisions are based on laboratory data, appropriate steps must exist to ensure data quality and integrity through documentation. Both abnormal and normal data must be used to test the system. Any changes or modifications to the system must be documented, and the laboratory director or designee must approve all changes before they are released for use. Computer time-stamped audit trails must be used by the LIS [59]. The laboratory's LIS policies must ensure that LIS access is limited to authorized individuals [59,60]. The laboratory must maintain a written SOP for the operation of the LIS and should be appropriate and specific to the day-to-day activities of the laboratory staff as well as the daily operations of the Information Technology staff [59]. Documentation must be maintained indicating that all users of the computer system receive adequate training both initially and after system modification [59]. Documented procedures and a disaster-preparedness plan must exist for the preservation of data and equipment in case of an unexpected destructive event (e.g., fire and flood) or software failure and/or hardware failure, allowing for the timely restoration of service [59,61].

12. Standards for Quality Management

An overarching Quality Management (QM) Program is essential to ensure safety of study participants and maintenance of quality laboratory operations. The QM Program is a systematic approach to plan the achievement of quality objectives, comply with approved procedures, and assign specific functional responsibilities to laboratory staff. The QM Program should also include an External Quality Assurance (EQA) program, which is set up to externally evaluate the laboratory's analytical performance by comparing performance, using coded reagent panels with peer laboratories.

12.1. Required activities and documentation

The laboratory must have a documented QM Program designed to monitor, assess and correct problems identified in pre-analytic, analytic, and post-analytic systems as well as overall laboratory scope [30,44,62,63]. A key component of the QM Program is the Quality Assurance Unit (QAU). The QAU must monitor for GCLP compliance, oversee the development of the QM Program, resolve quality-related problems as described above, submit status reports to management, and prepare and respond to external audits [30,44,62,63]. The laboratory must provide evidence of implementation of the QM Program (i.e., minutes of committee meetings, results of ongoing measurement, and documentation-related complaint investigations) [64]. The laboratory must be able to provide evidence of appraisal of its QM Program (i.e., revisions to laboratory policies and procedures and to the QM Program) [65]. The QM Program documentation must demonstrate regular (at least annual) review by the laboratory director or designee(s) [11,12]. The laboratory should enroll in EQA programs that cover all study protocol analytes [66].

12.2. Quality management program

The laboratory must have a documented QM Program which must incorporate the following elements: developed and maintained by the QAU staff; integrated with the institutional QM Program; describe the operational plan with QM Program's goals and objectives; accessible to all staff; designed to monitor, evaluate and correct problems in areas of quality; address monitoring to include complaints and incidents; include all aspects of the laboratory's scope of care; address problems that would interfere with study-participant care or safety while addressing risk assessment; describe procedures for collection and communication of quality and safety information; include control activities (e.g., QC and EQA); include key indicators of quality of laboratory operations that target improvement (e.g., test turnaround time, specimen acceptability, test order accuracy, and safety events) and; demonstrate regular review by the laboratory director or designee. The laboratory's QM Program must include results of ongoing measurement activities of key indicators of quality of laboratory operations compared with internal or external benchmarks and trended over time. The laboratory must be able to use the QM Program for guidance when conducting annual appraisals of effectiveness and must provide evidence of its implementation.

12.3. Internal audits, testing turnaround times, and laboratory communication plan

The laboratory's monitoring of the QM Program must include an internal auditing program. Internal audits involve an individual or a group of laboratory personnel performing a self-assessment comprised of a comparison of the actual practices within the laboratory against the laboratory's policies and procedures (e.g., personnel files, training documentation, QC performance, review of SOPs). These audits may also compare the laboratory's practices against a standard set of guidelines or standards. All findings (compliance, noncompliance, or deficiencies) that result from the internal audit should be documented in an organized format to allow for appropriate corrective actions and follow-up through resolutions.

The laboratory must have a list of assay turnaround times readily available to all laboratory staff as well laboratory customers. The laboratory must also have a non-retaliatory policy for employees to communicate concerns regarding testing quality or laboratory safety to laboratory management.

12.4. External quality assurance

EQA programs serve three purposes: (1) to provide an internal measurement tool for ensuring that the information a laboratory generates and provides is accurate, timely, clinically appropriate and useful; (2) to provide the sponsoring and regulatory agencies with confidence that individual laboratories are generating data with a rigor that will support product licensure; (3) to ensure that clinical trial volunteer specimens will be analyzed in a system that provides accurate and reliable information to ensure trial volunteer safety. This external evaluation of the laboratory's analytical performance is vital to ensure a complete quality assessment of laboratory operations. Therefore, it is critical that laboratories enroll in EQA programs that cover all study protocol analytes [11,12]. The laboratory director or designee must review all external QA data and evidence of supervisory review of EQA program results must be available (e.g., signature and date of reviewed results and documentation of corrective or preventive actions taken upon unacceptable results) [11,12]. EQA specimens must be analyzed, quality assured and reported just as study-participant specimens are tested in the laboratory. As an example, most of the HIV-1 protocol-mandated safety assays are covered by EQA programs administered through the CAP and other organizations. Until recently no EQA programs existed for immunogenicity endpoint assays. Through efforts pioneered by the Division of AIDS, EQA programs for ELISpot and intracellular cytokine flow cytometry have been established for laboratories involved in the testing of HIV-1 vaccines [67,68]. The results of ELISpot and ICS EQA programs have been published, are continually being refined and are becoming open to more participants via commercialization [67,68]. Eventually these EQA programs will mature and participating laboratories

will be assessed bi-annually and provided feedback on their performance.

13. Conclusions

The GCLP standards were developed to bring together multiple guidance and regulatory information, as they apply to clinical research and to fill a void of a single GCLP reference for global clinical research laboratories with regard to laboratories that support clinical trials such as those that perform protocol-mandated safety assays, process blood, and perform immune monitoring assays for candidates on a product licensure pathway. To maintain a GCLP environment for a clinical trial it is critical that all of the key GCLP elements are in place and operational. These elements include organization and personnel, testing facilities, appropriately validated assays, relevant positive and negative controls for the assays, a system for recording, reporting and archiving data, a safety program tailored to personnel working in the laboratory, an information management system that encompasses specimen receipt/acceptance, storage, retrieval and shipping and an overall quality management plan. The most appropriate way to ensure compliance with GCLP guidance is to audit laboratories. Because key decisions regarding the advancement of products are based on laboratory-generated data obtained from specimens collected during the trials, GCLP compliance is critical. Such compliance will assist laboratories in ensuring, accurate, precise, reproducible data are produced that guarantee sponsor confidence, and stand under regulatory agency review.

References

[1] 21 CFR, vol. 1, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies Reference, U.S. Government Printing Office via GPO Access, April 2005. http://www.access.gpo.gov/nara/cfr/waisidx_05/21cfr58_05.html.

[2] 42 CFR, vol. 3, Part 493, Laboratory Requirements, U.S. Government Printing Office via GPO Access, October 2005. http://www.access.gpo.gov/nara/cfr/waisidx_05/42cfr493_05.html.

[3] ISO 15189: Medical laboratories, particular requirements for quality and competence, International Organization for Standardization, 2003, http://www.iso.org/iso/en/CatalogueDetailPage.CatalogueDetail?CSNUMBER=26301.

[4] T. Stiles, V. Grant, N. Mawby, Good Clinical Laboratory Practice (GCLP): a quality system for laboratories that undertake the Analyses of Samples from Clinical Trials, BARQA, 2003, http://www.barqa.com/.

[5] M. Bull, D. Lee, J. Stucky, Y.L. Chiu, A. Rubin, H. Horton, M.J. McElrath, J. Immunol. Methods 322 (2007) 57–69.

[6] N.D. Russell, M.G. Hudgens, R. Ha, C. Havenar-Daughton, M.J. McElrath, J. Infect. Dis. 187 (2003) 226–242.

[7] H. Horton, E.P. Thomas, J.A. Stucky, I. Frank, Z. Moodie, Y. Huang, Y.L. Chiu, M.J. McElrath, S.C. De Rosa, J. Immunol. Methods 323 (2007) 39–54.

[8] K. Travers, P.J. Kanki, in: A. Aldovini, B.D. Walker (Eds.), Techniques in HIV Research, Stockton Press, New York, 1990, pp. 6–10.

[9] DAIDS Guidelines for Good Clinical Laboratory Practice Standards-Training, 2007, http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/labs/GCLP.pdf.

[10] College of American Pathologists, Laboratory General Checklist, GEN.54100 (College of American Pathologists, October 2006), http://www.cap.org/apps/docs/laboratory_accreditation/checklists/laboratory_general_october2006.pdf.

[11] 42 CFR, Part 493, Subpart M (493.1407), Standard; Laboratory director responsibilities, October 2005.

[12] 42 CFR, Part 493, Subpart M (493.1445), Standard; Laboratory director responsibilities, October 2005.

[13] College of American Pathologists, Laboratory General Checklist, GEN.54400 (College of American Pathologists, October 2006).

[14] College of American Pathologists, Laboratory General Checklist, GEN.54300 (College of American Pathologists, October 2006).

[15] College of American Pathologists, Laboratory General Checklist, GEN.54000 (College of American Pathologists, October 2006).

[16] 42 CFR, Part 493, Subpart M (493.1413), Standard; Technical consultant responsibilities, October 2005.

[17] 42 CFR, Part 493, Subpart M (493.1451), Standard: Technical supervisor responsibilities, October 2005.

[18] 42 CFR, Part 493, Subpart M (493.1423), Standard; testing personnel qualifications, October 2005.

[19] 42 CFR, Part 493, Subpart M (493.1487), Condition: laboratories performing high complexity testing; testing personnel, October 2005.

[20] 42 CFR, Part 493, Subpart K (493.1254), Standard: maintenance and function checks, October 2005.

[21] College of American Pathologists, Chemistry and Toxicology Checklist, CHM.23100 (College of American Pathologists, October 2006). http://www.cap.org/apps/docs/laboratory_accreditation/checklists/chemistry_and_toxicology_october2006.pdf.

[22] College of American Pathologists, Chemistry and Toxicology Checklist, CHM.23300 (College of American Pathologists, October 2006).

[23] 42 CFR, Part 493, Subpart K (493.1252), Standard: test systems, equipment, instruments, reagents, materials, and supplies, October 2005.

[24] 42 CFR, Part 493, Subpart K (493.1251), Standard: Procedure Manual, October 2005.

[25] Clinical and Laboratory Standards Institute, Laboratory Documents: Development and Control; Approved Guideline-Fifth ed. Document GP2-A5 (Clinical and Laboratory Standards Institute, 2006).

[26] College of American Pathologists, Chemistry and Toxicology Checklist, CHM.11300 (College of American Pathologists, October 2006).

[27] College of American Pathologists, Chemistry and Toxicology Checklist, CHM.11100 (College of American Pathologists, October 2006).

[28] 42 CFR, Part 493, Subpart K (493.1256), Standard: control procedures, October 2005.

[29] 42 CFR, Part 493, Subpart K (493.1282), Standard: corrective actions, October 2005.

[30] 42 CFR, Part 493, Subpart K (493.1289), Standard: analytic systems quality assessment, October 2005.

[31] 42 CFR, Part 493, Subpart J (493.1105), Standard: retention requirements, October 2005.

[32] College of American Pathologists, Chemistry and Toxicology Checklist, CHM.12600 (College of American Pathologists, October 2006).

[33] College of American Pathologists, Laboratory General Checklist, GEN.61900 (College of American Pathologists, October 2006).

[34] College of American Pathologists, Chemistry and Toxicology Checklist, CHM.12900 (College of American Pathologists, October 2006).

[35] Clinical and Laboratory Standards Institute, Preparation and Testing of Reagent Water in the Clinical Laboratory-Fourth Edition. Document C3-A4 (Clinical and Laboratory Standards Institute, 2006).

[36] 42 CFR, Part 493, Subpart K (493.1253), Standard: establishment and verification of performance specifications, October 2005.

[37] College of American Pathologists, Laboratory General Checklist, GEN.42085 (College of American Pathologists, October 2006).

[38] ICH Guidelines. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. Q2(R1): Validation of Analytical Procedures: Text and Methodology. 2005. http://www.ich.org/cache/compo/276-254-1.html.

[39] U.S. Food and Drug Administration, Guidance for industry: bioanalytical methods validation, 2001, http://www.fda.gov/CDER/GUIDANCE/4252fnl.htm.

[40] S. Dubey, J. Clair, T.M. Fu, L. Guan, R. Long, R. Mogg, K. Anderson, K.B. Collins, C. Gaunt, V.R. Fernandez, L. Zhu, L. Kierstead, S. Thaler, S.B.

Gupta, W. Straus, D. Mehrotra, T.W. Tobery, D.R. Casimiro, J.W. Shiver, J. Acquir. Immune Defic. Syndr. 45 (2007) 20–27.
[41] College of American Pathologists, Laboratory General Checklist, GEN.42162 (College of American Pathologists, October 2006).
[42] J.O. Westgard, Basic Method Validation, 2nd ed., AACC Press, Washington, DC, 2003.
[43] 42 CFR, Part 493, Subpart K (493.1291), Standard: test report, October 2005.
[44] 42 CFR, Part 493, Subpart K (493.1299), Standard: postanalytic systems quality assessment, October 2005.
[45] 42 CFR, Part 493, Subpart J (493.1101), Standard: facilities, October 2005.
[46] College of American Pathologists, Laboratory General Checklist, GEN.60000 (College of American Pathologists, October 2006).
[47] College of American Pathologists, Chemistry and Toxicology Checklist, CHM.27700 (College of American Pathologists, October 2006).
[48] College of American Pathologists, Laboratory General Checklist, GEN.61600 (College of American Pathologists, October 2006).
[49] 42 CFR, Part 493, Subpart K (493.1241), Standard: test request, October 2005.
[50] 42 CFR, Part 493, Subpart K (493.1232), Standard: specimen identification and integrity, October 2005.
[51] 42 CFR, Part 493, Subpart K (493.1242), Standard: specimen submission, handling, and referral, October 2005.
[52] 49 CFR, Part 173, Subpart E (173.199), Category B infectious substances, October 2006, http://www.access.gpo.gov/nara/cfr/waisidx_06/49cfr173_06.html.
[53] U.S. Department of Labor, Occupational Safety & Health Administration, Compliance and Guides, http://www.osha.gov/html/comp-guides.html.
[54] 29 CFR, Part 1910, Subpart Z (1910.1030), Bloodborne pathogens, July 2005, http://www.access.gpo.gov/nara/cfr/waisidx_05/29cfr1910a_05.html.
[55] 29 CFR, Part 1910, Subpart I (1910.132), General requirements, July 2005, http://www.access.gpo.gov/nara/cfr/waisidx_05/29cfr1910_05.html.
[56] 29 CFR, Part 1910, Subpart Z (1910.1200), Hazard communication, July 2005.
[57] 29 CFR, Part 1910, Subpart L (1910.157), Portable fire extinguishers, July 2005.
[58] College of American Pathologists, Laboratory General Checklist, GEN.70150 (College of American Pathologists, October 2006).
[59] 21 CFR, Part 11, Subpart B (11.10), Controls for closed systems, April 2005, http://www.access.gpo.gov/nara/cfr/waisidx_05/21cfr11_05.html.
[60] 21 CFR, Part 11, Subpart C (11.300), Controls for identification codes/passwords, April 2005.
[61] College of American Pathologists, Laboratory General Checklist, GEN.43946 (College of American Pathologists, October 2006).
[62] 42 CFR, Part 493, Subpart K (493.1239), Standard: general laboratory systems quality assessment, October 2005.
[63] 42 CFR, Part 493, Subpart K (493.1249), Standard: preanalytic systems quality assessment, October 2005.
[64] College of American Pathologists, Laboratory General Checklist, GEN.16902 (College of American Pathologists, October 2006).
[65] College of American Pathologists, Laboratory General Checklist, GEN.20369 (College of American Pathologists, October 2006).
[66] 42 CFR, Part 493, Subpart H (493.801), Condition: enrollment and testing of samples, October 2005.
[67] J.H. Cox, G. Ferrari, S.A. Kalams, W. Lopaczynski, N. Oden, M.P. D'souza, AIDS Res. Hum. Retroviruses 21 (2005) 68–81.
[68] H.T. Maecker, A. Rinfret, P. D'Souza, J. Darden, E. Roig, C. Landry, P. Hayes, J. Birungi, O. Anzala, M. Garcia, A. Harari, I. Frank, R. Baydo, M. Baker, J. Holbrook, J. Ottinger, L. Lamoreaux, C.L. Epling, E. Sinclair, M.A. Suni, K. Punt, S. Calarota, S. El-Bahi, G. Alter, H. Maila, E. Kuta, J. Cox, C. Gray, M. Altfeld, N. Nougarede, J. Boyer, L. Tussey, T. Tobery, B. Bredt, M. Roederer, R. Koup, V.C. Maino, K. Weinhold, G. Pantaleo, J. Gilmour, H. Horton, R.P. Sekaly, BMC Immunol. 6 (2005) 1–18.