GUIDANCE FOR ORGANIZATIONS PERFORMING IN VIVO BIOEQUIVALENCE STUDIES

(August 2015)

REVISED DRAFT FOR COMMENT

Should you have any comments on the attached text, please send these to Dr S. Kopp, Dr S. Kopp, Group Lead, Medicines Quality Assurance, Technologies, Standards and Norms (kopps@who.int) with a copy to Ms Marie Gaspard (gaspardm@who.int) by 1 October 2015.

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BACKGROUND


The WHO Expert Committee on Specifications for Pharmaceutical Preparations agreed that in light of the new developments a draft for revision be prepared.

This guideline is being revised to take into consideration the revision for the multisource guideline, as well as the creation of a new guideline on good data management. It will also be revised to take into consideration the experience accumulated in the area of assessing and inspecting bioequivalence (BE) studies since 2006. The areas with recurrent inspection findings are being clarified and supplementary detail has been added in the area of bioanalysis. It also includes an increased level of insistence on subject safety and data integrity.

Based on the first working document:

http://www.who.int/medicines/areas/quality_safety/quality_assurance/BE-invivo-studies-guidance-QAS15-622_21052015.pdf?ua=1 this second version is suggested including the numerous comments and feedback received from the public consultation, the PQT as well as the consultation on data management, bioequivalence, GMP and medicines’ inspection held in 2015.

The PQT was started in 2001 to assure that medicinal products supplied for procurement meet WHO norms and standards with respect to quality, safety and efficacy (http://www.who.int/prequal/).

Specifically it is a requirement that the submitted product dossier with all its necessary contents is assessed and found acceptable, and that the manufacturing sites for the finished pharmaceutical product (FPP), as well as the active pharmaceutical ingredient (API), are both inspected and found to comply with WHO good manufacturing practices (GMP). Since products submitted to the PQT are usually multisource ("generic") products, therapeutic equivalence is generally demonstrated by performing a BE study, for example in a contract research organization (also known as a clinical research organization) (CRO). For prequalification of such a product it is vital that, in addition to the above-mentioned requirements, the CRO used by the sponsor for BE studies is compliant with respect to WHO good clinical practices (GCP) and considers relevant elements from WHO good laboratory practices (GLP) and good practices for quality control (QC) laboratories to ensure integrity and traceability of data. In addition, if local legal provisions exist, CROs should be licensed by the respective national medicines authority. Where required by national regulations, BE studies should be authorized by the national regulatory authority. Those involved in the conduct and analysis of BE studies with products to be submitted for prequalification therefore need to ensure that they comply with the mentioned WHO norms and standards to be prepared for any inspections by WHO.
INTRODUCTION

Multisource pharmaceutical products need to conform to the same standards of quality, efficacy and safety as required of the originator's (comparator) product. Specifically, the multisource product should be therapeutically equivalent and interchangeable with the comparator product. Testing the BE between a product and a suitable comparator...
pharmacologically equivalent or a pharmaceutical alternative) in a pharmacokinetic study with a limited number of subjects is one way of demonstrating therapeutic equivalence without having to perform a clinical trial involving many patients. In such a pharmacokinetic study any statement about the safety and efficacy of the test product will be a prediction based on measurement of systemic concentrations, assuming that essentially similar plasma concentrations of the drug will result in essentially similar concentrations at the site of action and therefore an essentially similar therapeutic outcome. The BE study thus provides indirect evidence of the efficacy and safety of a multisource drug product. Often this will be the only evidence that the product is safe and efficacious. It is therefore crucial that the BE study is performed in an appropriate manner. Several guidance documents stress the importance of onsite inspections to verify compliance with standards of GCP.\textsuperscript{1,4}

1. **SCOPE**

The objective of this document is to provide guidance to organizations that are involved in the conduct and analysis of in vivo BE studies. This guidance has been updated relative to the previous version of this document.

BE studies should be performed in compliance with the general regulatory requirements and good practices recommendations as specified in the WHO bioequivalence guideline\textsuperscript{1}, GCP\textsuperscript{2} and GLP\textsuperscript{3} guidelines. It is acknowledged that GLP formally only apply to non-clinical safety studies. However the WHO bioequivalence guidelines require that the validation of bioanalytical methods and the analysis of BE study samples be performed following the principles of GLP. This does not imply that the laboratory in charge of the bioanalytical part of the study should be monitored as part of a national GLP compliance programme.

The text below lists general recommendations for organizations (including CROs and laboratories) conducting BE studies and analysis of clinical trial samples. Recommendations for facilities and equipment are listed in the respective paragraphs. Recommended documents, standard operating procedures (SOP) and records are listed in Appendix 1, but this is not to be considered an exhaustive list − others may be necessary depending on each individual CRO’s functional and compliance needs.

This document provides information on:

− organization and management;
− study protocols;
− clinical phase of a study;
− bioanalytical phase of a study;
− pharmacokinetic and statistical analysis;
− study report;
− quality management system.

The present guideline targets organizations conducting BE studies and highlights certain important aspects of the activities of such organizations. This document does not replace the above-mentioned GCP or GLP guidelines, which are more complete. It is therefore not a stand-alone document.
2. GLOSSARY

The definitions given below apply to the terms used in this guidance. They may have different meanings in other contexts.

**adverse event.** Any untoward medical occurrence in a clinical trial subject administered a pharmaceutical product; it does not necessarily have a causal relationship with the treatment.

**audit of a trial.** A systematic examination, carried out independently of those directly involved in the trial, to determine whether the conduct of a trial complies with the agreed protocol and whether the data reported are consistent with the records on site, e.g. whether data reported or recorded in the case-report forms are consonant with those found in hospital files and other original records.

**bioequivalence.** Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and their bioavailabilities, in terms of rate ($C_{max}$ and $t_{max}$) and extent of absorption (area under the curve), after administration of the same molar dose under the same conditions, are similar to such a degree that their effects can be expected to be essentially the same.

**calibration curve samples (or calibration standards).** A matrix to which a known amount of analyte has been added or spiked. Calibration standards are used to construct calibration curves.

**case-report form.** A document that is used to record data on each trial subject during the course of the trial, as defined by the protocol. The data should be collected by procedures which guarantee preservation, retention and retrieval of information and allow easy access for verification, audit and inspection.

**comparator product (or reference product).** The comparator product is a pharmaceutical product with which the multisource product is intended to be interchangeable in clinical practice. The comparator product will normally be the innovator product for which efficacy, safety and quality have been established. If the innovator product is no longer marketed in the jurisdiction, the selection principle as described in Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products (WHO Technical Report Series, No. 992, Annex 8, 2015) should be used to identify a suitable alternative comparator product.

**contract.** A document, dated and signed by the investigator, institution and sponsor, that sets out any agreements on financial matters and delegation/distribution of responsibilities. The protocol may also serve as a contract when it contains such information and is signed. Contracts can also be signed with other parties such as vendors supplying services to the contract research organization.

**contract research organization.** A scientific organization (commercial, academic or other) to which a sponsor may transfer some of its tasks and obligations. Any such transfer should be defined in writing.

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In the context of this guidance document bioequivalence studies are often contracted by the sponsor to a contract research organization (CRO), which will perform some of the tasks of the sponsor, but which will also perform the trial. The investigator (clinical part of the study) and the study director (bioanalytical part of the study) are then employees of the CRO.

To facilitate reading, the term "CRO" is used throughout this document to designate any organization performing the trial, even though it is acknowledged that part or all of the study may be performed in-house by the sponsor itself or at a hospital.

**ethics committee**: An independent body (a review board or a committee, institutional, regional or national), constituted of medical professionals and non-medical members, whose responsibility is to verify that the safety, integrity and human rights of the subjects participating in a particular trial are protected and to consider the general ethics of the trial, thereby providing public reassurance. Ethics committees should be constituted and operated so that their tasks can be executed free from bias and from any influence of those who are conducting the trial.

**final report**. A comprehensive description of the trial after its completion including a description of experimental methods (including statistical methods) and materials, a presentation and evaluation of the results, statistical analysis and a critical, ethical, statistical and clinical appraisal.

**good clinical practice**. A standard for clinical studies which encompasses the design, conduct, monitoring, termination, audit, analysis, reporting and documentation of the studies and which ensures that the studies are scientifically and ethically sound and that the clinical properties of the pharmaceutical product (diagnostic, therapeutic or prophylactic) under investigation are properly documented.

**good laboratory practice**. A quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

**informed consent**. A subject’s voluntary confirmation of willingness to participate in a particular trial and the documentation thereof. This consent should be sought only after all appropriate information has been given about the trial including an explanation of its status as research, its objectives, potential benefits, risks and inconveniences, alternative treatment that may be available, and of the subject’s rights and responsibilities in accordance with the current revision of the Declaration of Helsinki.

**inspection**. An officially-conducted examination (i.e. review of the conduct of the trial, including quality assurance, personnel involved, any delegation of authority and audit) by relevant authorities at the site of investigation and/or at the site of the sponsor in order to verify adherence to good clinical practices and good laboratory practices as set out in this document.

**internal standard**. Test compound(s) (e.g. a structurally similar analogue or stable isotope labelled compound) added to calibration standards, quality control samples and study samples at a known and constant concentration to correct for experimental variability during sample preparation and analysis.

**investigational labelling**. Labelling developed specifically for products involved in a clinical trial.
**investigational product (or study product).** Any pharmaceutical product (see definition) or placebo being tested or used as a reference in a clinical trial.

**investigator.** A person responsible for the trial and for the rights, health and welfare of the subjects in the trial. The investigator should have qualifications and competence in accordance with local laws and regulations as evidenced by an up-to-date curriculum vitae and other credentials. Decisions relating to, and the provision of, medical or dental care must always be the responsibility of a clinically competent person legally allowed to practise medicine or dentistry.

**lower limit of quantification.** The lower limit of quantification of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with pre-defined precision and accuracy.

**monitor.** A person appointed by, and responsible to, the sponsor or contract research organization for the monitoring and reporting of progress of the trial and for verification of data.

**pharmaceutical product.** Any substance or combination of substances which has a therapeutic, prophylactic or diagnostic use, or is intended to modify physiological functions, and is presented in a dosage form suitable for administration to humans.

**principal investigator.** The investigator serving as coordinator for certain kinds of clinical trials, e.g. multicentre trials. Note: "principal investigator" also has a specific, but different meaning in good laboratory practices, practically seldom used in bioequivalence studies. To avoid any misunderstanding, the words "principal investigator" will only be used in this guidance document with their good clinical practices meaning.

**protocol.** A document which states the background, rationale and objectives of the trial and describes its design, methodology and organization, including statistical considerations, and the conditions under which it is to be performed and managed. The protocol should be dated and signed by the investigator, the institution involved and the sponsor. It can also function as a contract.

**quality assurance relating to clinical trials.** Systems and quality control procedures that are established to ensure that the trial is performed and the data are generated in compliance with good clinical practices and good laboratory practices. These include procedures to be followed which apply to ethical and professional conduct, standard operating procedures, reporting, and professional qualifications or skills of personnel.

**quality control samples.** A spiked sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analysed in an individual batch.

**raw data.** All records or certified copies of original observations, clinical findings or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Such material includes laboratory notes, memoranda, calculations and documents, as well as all records of data from automated instruments or exact, verified copies, e.g. in the form of photocopies or microfiches. Raw data can also include photographic negatives, microfilm, magnetic media (e.g. computer diskettes) and optical media (CD-ROMs).
**serious adverse event.** An event that is associated with death, admission to hospital, prolongation of a hospital stay, persistent or significant disability or incapacity, or is otherwise life-threatening in connection with a clinical trial.

**sponsor.** An individual, a company, an institution or an organization which takes responsibility for the initiation, management and/or financing of a clinical trial. When an investigator initiates and takes full responsibility for a trial, the investigator then also assumes the role of the sponsor.

**standard operating procedures.** Standard, detailed, written instructions for the management of clinical trials. They provide a general framework enabling the efficient implementation and performance of all the functions and activities for a particular trial as described in this document.

**study director.** According to the Organisation for Economic Co-operation and Development principles of good laboratory practice: the individual responsible for the overall conduct of the nonclinical health and environmental safety study. In a bioequivalence study the individual responsible for the conduct of the bioanalytical part of the study.

**study product.** see investigational product

**test product.** Any pharmaceutical product (see definition) or placebo being tested against the reference in a clinical trial. In a bioequivalence study the multisource product being tested against the comparator product.

**trial subject.** An individual who participates in a clinical trial, either as a recipient of the pharmaceutical product under investigation or as a control. The individual may be:

- a healthy person who volunteers to participate in a trial;
- a person with a condition unrelated to the use of the investigational product;
- a person (usually a patient) whose condition is relevant to the use of the investigational product.

**upper limit of quantification.** The upper limit of quantification of an individual analytical procedure is the highest amount of analyte in a sample which can be quantitatively determined with predefined precision and accuracy.

**validation.** Action of proving and documenting, in accordance with the principles of good clinical practices and good laboratory practices, that any procedure, process, equipment (including the software or hardware used), material, activity or system actually and consistently leads to the expected results.

**verification of data.** The procedures carried out to ensure that the data contained in the final report match original observations. These procedures may apply to raw data, data in case-report forms (in hard copy or electronic form), computer printouts and statistical analysis and tables.
GENERAL SECTION

3. ORGANIZATION AND MANAGEMENT

Note: the acronym “CRO” is used throughout this document to refer not only to a contract research organization (CRO), but also to any organization involved in the conduct or analysis of in vivo BE studies.

3.1 Where national requirements exist as to the legal status of a CRO these have to be complied with. This also applies to the research unit which is a subsidiary of the manufacturer.

3.2 The CRO should have an organization chart reflecting key positions and the names of responsible persons. The organization chart should be dated, authorized and kept up to date.

3.3 There should be job descriptions for all personnel, including a description of their responsibilities. All job descriptions should be acknowledged and signed off by the staff member to whom it applies.

3.4 There should be a list of signatures of authorized personnel participating in each study.

3.5 For the bioanalytical part of the trial, the principles of GLP clearly establish the responsibilities of the test facility management. The CRO management should be aware that as the investigator is an employee of the CRO, some of the responsibilities usually assigned to the investigator would in a similar way reside with the CRO management. At a minimum, the CRO management should:

- ensure that the principles of GCP and GLP, as appropriate, are complied with in the CRO;
- ensure that a sufficient number of qualified personnel, appropriate facilities, equipment and materials are available for the timely and proper conduct of the study;
- ensure the maintenance of a record of the qualifications, training, experience and job description for each professional and technical individual;
- ensure that personnel clearly understand the functions they are to perform, and where necessary, provide training for these functions;
- ensure that appropriate and technically valid SOPs are established and followed, and approve all original and revised SOPs. Ensure the maintenance of a historical file of all SOPs;
- ensure that there is a quality assurance (QA) programme with designated personnel and assure that the QA responsibility is being performed in accordance with the principles of GLP and GCP, as appropriate;
- ensure that an individual is identified as responsible for the management of the archive(s), and ensure that the documents transferred to the archives are kept under adequate conditions for the appropriate duration;
- ensure that supplies meet requirements appropriate to their use in a study;
- establish procedures to ensure that computerized systems are suitable for their intended purpose, and are validated, operated and maintained in accordance with the principles of GCP and GLP, as appropriate.
4. COMPUTER SYSTEMS

Note: this section highlights only some of the requirements for computer systems that are specific to BE studies. Organizations involved in BE studies should ensure that the relevant principles of the following guidelines are appropriately followed:

- GAMP 5: A risk-based approach to compliant GxP computerized systems
- US FDA Code of Federal Regulations Part 11
- EU guidelines to Good Manufacturing Practice and Medicinal Products for Human and Veterinary Use Annex 11, Computerised systems
- WHO Good data management practices guidelines – [Note from the Secretariat: full reference will be added once finalized].

General

4.1 Computer systems should be qualified and validated (hardware, software, networks, data storage systems and interfaces). Qualification is the planning, carrying out and recording of tests on equipment and systems which form part of the validated process, to demonstrate that it will perform as intended.

Hardware

4.2 There should be a sufficient number of computers to enable personnel to perform data entry and data handling, required calculations and compiling of reports.

4.3 Computers should have sufficient capacity and memory for the intended use.

Software

4.5 The software programs used to perform key steps detailed in this guideline should be suitable and validated for the intended use. Whether standard, off-the-shelf software is purchased or whether bespoke software is developed, developer, vendor and/or service provider qualification and/or validation certificates may be provided but it is the user’s responsibility to ensure that the software is validated for its intended use.

4.6 As software, computer systems and related equipment can be technically complex, the user should ensure that formal qualification and validation was carried out by the developer and that it was developed in a controlled manner in accordance with a system of quality assurance.

4.7 Performance qualification should take account of the specific user’s requirements, of regulatory/guideline requirements for BE studies, of the operating environment in which it will be used, and of how it will be used by an organization’s staff in the context of a study. Quality risk analysis should be applied when deciding which components need to be validated. All phases of
their life cycle should be considered. For example, when a CRO decommissions the software in use for high-performance liquid chromatography (HPLC) and mass spectrometric (MS) analysis (e.g. HPLC-MS/MS), it should ensure that the data collected by the system using this software remains fully readable. This could be done, for instance, by having the old software installed on a workstation for inspection/verification purposes only.

4.8 There should be SOPs in place for usage of each software program that is used to perform key steps of a BE study.

4.9 There should be a system in place for the implementation of regular updates to key software programs (e.g. such as those used for control and data processing of chromatographic and mass spectrometry systems) whenever required, following an appropriate risk assessment on the potential impact that it could have on current data and on qualification/validation status.

4.10 Software programs used, frequency of virus testing, storage of data and the making and archiving of back-ups should be specified in writing.

4.11 The programs used should be able to provide the required quality and management information, reliably and accurately. Necessary programs for data management include word processing, data entry, databases, graphics, pharmacokinetics and statistical programs. Self-designed software programs must be suitable and validated for their intended use.

4.12 Since data for BE studies is often transferred electronically between organizations involved in the studies, there should be a verification that the software used between each organization is compatible prior to commencing key study-related tasks.

4.13 These requirements apply to all systems used in clinical BE studies, e.g. subject database, electronic case report forms, electrocardiogram (ECG) recording software, HPLC-MS/MS software, software used for pharmacokinetic analysis, for statistical analysis, etc.

Networks

4.14 Networks, including the full client/server architecture and interfaces such as laboratory information management systems, when used, should be appropriately designed, qualified, managed and controlled.

4.15 Access to each component of the system by the different users at any given organization involved in the studies, should be appropriately defined, controlled and documented.

4.16 There should be a documented inventory of all computerized systems on the network, with a clear identification of those which are GxP regulated. Any changes to the network, including the temporary addition or removal of systems from the network, should be documented.

Data management

4.17 Data entry includes transfer of the data from case report forms (CRF) and analytical data to the computerized system for pharmacokinetic and statistical analysis and reporting.

4.18 Data entry procedures should be designed to prevent errors. The data entry process should be specified in the SOP.
4.19 Double entry of the data should be performed. Data validation methodology (proof-reading, double data entry, electronic logical control) should be specified in writing.

4.20 Changes made to data entered in the database should be made by authorized persons only. Changes should be specified and documented.

4.21 Electronic data should be backed up at regular intervals. The reliability and completeness of these back-ups should be verified – data should not be selected but comprehensively backed up.

4.22 All of the raw electronic data must be kept. This includes:

– all meta data associated to a computerized system and the equipment that is associated to it (which includes the audit trails for integration, for projects and for the entire instrument);
– validation data and meta data in the form of their source electronic files.

PDF copies are not sufficient on their own, unless it can be demonstrated that these are the raw data and that no alteration was possible after they were generated.

4.23 All electronic records obtained from HPLC and MS analysis (e.g. HPLC-MS/MS) are required to be retained, maintained and backed-up. It should be ensured that back-up data are exact and complete and that they are secure from alteration, inadvertent erasures or loss shall be maintained. The printed paper copy of the chromatogram would not be considered a “true, exact and complete copy” of the entire electronic raw data used to create that chromatogram. Printed chromatograms do not generally include, for example, the sample sequence, instrument method, processing method, integration settings or the full audit trail, of which all were used to create the chromatogram or are associated with its validity. Therefore there should be a higher emphasis on conservation of electronic data than paper data, as paper data is usually not considered the true source data, except in the case of paper logbooks where the original record was handwritten, for instance.

4.24 If data is transformed during processing steps (such as in the example of re-integration of chromatographic data), it should always be possible to compare the original data with the processed data.

5. QUALITY MANAGEMENT

5.1 The CRO should have appropriate QA and QC systems with written SOPs to ensure that trials are conducted and data are generated, documented and reported in compliance with the protocol, GCP, GLP and the applicable regulatory requirements.

5.2 QA personnel should be independent of the work they are quality assuring, including:

– conducting or monitoring of the trial;
– conducting bioanalysis;
– performing reporting and pharmacokinetic and statistical analyses.

As a consequence, QA personnel should not be directly involved in trial-related activities, and an in-process audit by QA personnel does not replace oversight by another person when required.

5.3 The QA unit should be responsible for:
verifying all activities undertaken during the study;
- ensuring that the quality management systems, are followed, reviewed and updated;
- determining that the protocol and SOPs are made available to study personnel and are being followed;
- checking all the study data for reliability and traceability;
- planning and performing self-inspections (internal audits) at regular and defined intervals in accordance with an SOP, and following up on any corrective action as required, to determine if all studies are conducted in accordance with GCP and GLP;
- ensuring that contract facilities adhere to GCP and, if applicable, to GLP. This would include auditing of such facilities, and following up on any corrective action as required;
- verifying that the trial report accurately and completely reflects the data of the study and the methods and procedures followed;
- promptly reporting audit findings in writing to management, to the investigator and to the study director, as applicable.

5.4 The CRO should allow the sponsor to monitor the studies and to perform audits of the clinical and analytical study and sites and should provide suitable office space for these activities.

5.5 Both retrospective and in-process (e.g. in bioanalysis, as the samples and standards are being prepared and tested) QA verifications should be performed.

5.6 The quality management system should include root cause analysis, tracking for trends and ensuring all aspects of data integrity.

6. ARCHIVE FACILITIES

Note: The CRO should have sufficient and appropriately secure storage space, which should be fire proof, humidity-controlled and pest-controlled, for archiving of trial-related documentation and product samples. Archives should be protected from flooding.

6.1 An SOP should be in place for archiving.

6.2 Access to archive storage areas should be controlled and restricted to authorized personnel.

6.3 Records should be maintained of document access and return.

6.4 The length of period for which study documentation including raw data is kept in the archive should be defined in the SOP and may vary depending on country requirements. This duration should be specified in the contract between the sponsor and the CRO, which should include provisions for financing of the archival.

6.5 All data, including both paper and electronic, should be easy to retrieve and traceable.

7. PREMISES

7.1 The facilities should be maintained clean and should have adequate conditions of lighting, ventilation and, if required, environmental control. Floor, walls and working benches surfaces should facilitate the cleaning and decontamination.
7.2 Clinical trials must be carried out under conditions which ensure adequate safety for the subjects. The site selected should be appropriate to the potential risk involved.

7.3 The CRO should have sufficient space to accommodate the personnel and activities required to perform the studies. The site must have adequate facilities, including laboratories, and equipment. The facilities used for the clinical phase of the study, including areas listed in paragraph 9.6 should be well organized in order to carry out the activities in logical order.

7.4 The entry to the facility should be restricted and controlled. There should be alarm systems to detect the exit of subjects from clinical facilities or the doors should be locked (however, doors should be locked only if emergency evacuation can still be ensured). Any entry and exit to the facility should be recorded.

7.5 Sites involved in clinical activities should include a pharmacy where investigational products should be stored under appropriate conditions with entry and exit restricted by access control. Appropriate entry/exit records of each visit to the pharmacy should be maintained.

7.6 Utilities such as water, air, gas and electricity should be adequate, stable and uninterrupted.

7.7 There should be access to telephone, email and facsimile facilities to ensure proper communication. The CRO should have the necessary office equipment (printer, copy machine) to perform the required activities.

7.8 Laboratory premises should be designed to suit the operations to be carried out in them. Sufficient space should be given to avoid mix-ups, contamination and cross-contamination. There should be adequate suitable storage space for samples, standards, solvents, reagents and records.

7.9 Laboratory premises should be designed to provide adequate protection to all employees and visitors, including inspectors or auditors, by ensuring their safety while handling or working in the presence of chemicals and biological samples. Improper working conditions can negatively impact on the quality of the work performed and of the data generated.

The following general rules for safe working in accordance with national regulations and SOPs normally include the following requirements:

(a) safety data sheets should be available to staff before testing is carried out; staff working in the laboratory should be familiar with and knowledgeable of the material safety data sheets for the chemicals and solvents that they are handling;
(b) smoking, eating and drinking in the laboratory should be prohibited;
(c) staff should be familiar with the use of fire-fighting equipment, including fire extinguishers, fire blankets and gas masks;
(d) staff should wear laboratory coats or other protective clothing, including eye protection;
(e) special care should be taken, as appropriate, in handling, for example, highly potent, infectious or volatile substances;
(f) highly toxic and/or genotoxic samples should be handled in a specially designed facility to avoid the risk of contamination;
(g) all containers of chemicals should be fully labelled and include prominent warnings (e.g. “poison”, “flammable”, “radioactive”) whenever appropriate;
(h) adequate insulation and spark-proofing should be provided for electrical wiring and equipment, including refrigerators;
(i) rules on safe handling of cylinders of compressed gases should be observed and staff should
be familiar with the relevant colour identification codes;
(j) staff should be aware of the need to avoid working alone in the laboratory;
(k) first-aid materials should be provided and staff instructed in first-aid techniques, emergency
care and the use of antidotes;
(l) containers containing volatile organic solvents, such as mobile phases or liquid/liquid
extraction solvents, should be closed with an appropriate seal;
(m) volatile organic chemicals should be handled under certified fume-hoods or air extractors
and safety and eye showers should be available in the laboratory.

7.10 Premises should have suitable systems in place to dispose of waste, to treat fumes and to
protect the environment in conformance to local or national regulation.

8. PERSONNEL

8.1 There should be a sufficient number of qualified and appropriately trained medical,
paramedical, technical and clerical staff to support the trial and to be able to respond effectively to
all reasonably foreseeable emergencies. The number of members of staff required depends on the
number and complexity of the trials performed by the CRO. At all stages during the trial, including
at night, there should be a sufficient number of appropriately qualified and trained personnel to
ensure that the rights, safety and well-being of the subjects are maintained, and to take care of the
subjects in emergency situations.

8.2 The delegation of significant trial-related duties should be documented in writing.

8.3 Contract workers may be employed to perform certain activities. All contract workers
having access to the clinical or bioanalytical areas or performing trial-related activities should be
provided with adequate information, training and job descriptions. Their contracts should be signed
before the performance of work.

8.4 Current curriculum vitae and training records should be kept for full-time and contract
workers.

8.5 The personnel responsible for the planning and conduct of the study should have
appropriate qualifications and sufficient knowledge and experience in the relevant field. They
should receive the study-specific information and training required for the performance of their
work.

8.6 Records for training and assessment of knowledge of GCP, GLP and any other relevant area
or technique should be maintained.

8.7 There should be adequate measures in place to protect personnel from accidental
contamination (e.g. from accidental needle pricks) while obtaining blood samples from subjects or
while handling the samples that are derived from blood products (e.g. plasma and its extracts) or
while handling or disposing of infectious waste.
9. CLINICAL PHASE

Note: As in vivo BE trials are considered as clinical trials, specifically a Phase I study, the general requirements and recommendations of GCP apply to all BE trials. Clinical trials must be carried out under conditions which ensure adequate safety of the subjects. The clinical phase of the study can be performed in the premises of a CRO or by contracting suitable premises in a hospital.

9.1 A CRO should have rooms meeting the requirements listed in the sections below.

9.2 There should be sufficient space to accommodate the study subjects.

9.3 Where appropriate, beds should be available for the subjects. The necessity of beds and overnight stay depends on the type of trial and investigational drug and should be specified in the trial protocol. Overnight stays are usually required during the night prior to dosing to ensure adequately controlled conditions and that there was no outside food/medication intake within the number of hours that is specified in the trial protocol.

9.4 Systems should be in place in the housing facilities and toilets so that subjects can alert CRO staff in case of need.

9.5 Facilities for changing and storing clothes and for washing and toilet purposes should be clean, well ordered, easily accessible and appropriate for the number of users. Closed toilets should be alarmed and doors should be designed to ensure that they can be opened from the outside should there be a medical emergency.

9.6 The study site should have the following facilities which should be separate areas where appropriate:

- rooms (areas) for subjects registration and screening;
- room (area) for individual subjects to obtain informed consent without compromising privacy;
- room for subjects housing;
- room (area) for subjects (recreation area);
- restricted-access room for pharmaceutical operations (e.g. storage, repacking, dispensing, documentation) (see also section 14);
- rooms (areas) for administration of the drug(s) under investigation and sample collection;
- room (area) for sample processing (e.g. plasma separation) and storage (freezer);
- controlled access storage areas for study materials, medication and documentation including CRFs;
- rooms (areas) in which to prepare standardized meals and a dining hall;
- availability of emergency or first-aid equipment and appropriate rescue medication for use in emergencies;
- adequate facilities for the proper care of subjects who require emergency or other medical care;
- archiving facilities.
9.7 Provisions should be made for the urgent transportation of subjects to a hospital or clinic equipped for the emergency care of subjects, if required by their condition.

9.8 Access to key documents, such as the randomization list, should be restricted to only certain specific members of personnel such as the pharmacist in charge of the study. Such documents should be password-secured (if electronic) or kept under lock and key (if distributed as a hard-copy) and their distribution should be documented.

9.9 Equipment used to obtain clinical measurements should be appropriately calibrated at predefined intervals.

9.10 The adequate function and performance of emergency-use equipment (e.g. defibrillators) should be verified at appropriate intervals.

10. CLINICAL LABORATORY

10.1 A suitable qualified clinical laboratory should be used for analysing samples. An accredited laboratory should be used whenever possible.

10.2 Haematological tests, urine analysis and other tests should be performed during the clinical trial as specified in the study protocol.

10.3 Sample labelling, receipt storage and chain of custody should ensure full traceability and sample integrity.

10.4 The CRO should be supplied with information about analytical methods used in the laboratory, a dated list of laboratory normal ranges and accreditation certificate of the laboratory, if available. These should be available for inspection by regulatory authorities, if required.

10.5 A current and signed curriculum vitae of the responsible analyst should be available in the laboratory information file.

10.6 Individual reports should be established by the laboratory for each subject and should be included in the CRFs. Source or raw data for all tests performed should be archived by the laboratory in electronic or paper formats, depending on their source and storage capacity. Electronic formats are preferred.

10.7 Data integrity requirements apply to all tests related to the study (full reference to be confirmed once the WHO data integrity guidelines are finalized.) For instance, raw data should be adequately protected from modification or deletion.

11. ETHICS

11.1 Independent ethics committee

Trials must be approved by an independent ethics committee (IEC) (or equivalent) before a study is conducted, according to WHO Operational guidelines for Ethics Committees that review biomedical research and to the enforced legislation. This committee must be independent from the sponsor, the investigator and of the CRO. The discussions, recommendations and decisions of the
IEC meetings should be documented in detailed minutes of the meeting. The IEC should be given sufficient time for reviewing protocols, informed consent forms (ICFs) and related documentation.

11.2 Informed consent

- Information for study participants should be given in a language and on a level of complexity appropriate and understandable to the subject, both orally and in writing.

- Informed consent must always be given by the subject and documented in writing before the start of any trial-related activities, in accordance with GCP. If informed consent is also recorded by video this recording should be retained following local legal requirements.

- The information must make clear that participation is voluntary and that the subject has the right to withdraw from the study on his or her own initiative at any time, without having to give a reason (compensation should be paid *prorata temporis*). If subjects who withdraw from the study offer their reasons for doing so, those reasons should be included in the study records.

- The subject must have access to information about insurance and other procedures for compensation or treatment should he or she be injured or disabled by participating in the trial or during screening.

- The volunteers/subjects should be given opportunity to discuss their concerns with a physician regarding potential side effects or reactions from the use of the investigational products before participation in the trial. They should also be given the opportunity and sufficient time to discuss their concerns with their participation in the trial with individuals outside of the CRO, such as friends and family members, if they wish.

- If the ICF is available in several languages (e.g. in English and in the local language, or in several vernacular languages), it should be ensured that all versions of the form contain the same information.

12. MONITORING

*Note: monitoring is an essential activity to ensure the quality of the clinical trial.*

12.1 The monitor should be qualified (see section 8, Personnel). The main responsibility of the monitor for a BE study is to ensure that the study is conducted in accordance with the protocol, GCP, GLP and applicable ethical and regulatory requirements. This includes verification of the use of correct procedures for completion of CRFs and verification of the accuracy of data obtained.

12.2 The sponsor can delegate the monitoring function to the CRO. In such cases the CRO should be able to arrange for the monitoring of the trial according to regulatory requirements. In such a case attention should be paid to the independence of the monitoring function in order to avoid conflicts of interest and pressure on the monitors. The monitoring reports should always be provided to the sponsor.

12.3 A risk-based approach to monitoring can be considered. However, a pre- and post-study visit as well as a monitoring visit during the conduct of the trial are usually performed. The monitor should prepare a written report after each site visit and communicate any issues to the CRO and to
the sponsor as promptly as possible, even during conduct of the study if possible, to enable prompt corrective action. Such communications and corrective actions should be documented.

12.4 When the monitoring is delegated to the CRO, SOPs should be available to describe:

- the designation of monitors, who should be independent from the personnel performing the trial;
- monitoring visit procedures;
- the extent of source data verification, including with regards to accountability of the investigational products and adherence to the protocol.

The extent of the monitoring, including the number of visits to be performed, should be agreed upon with the sponsor.

12.5 Separate SOPs (with checklists for the monitor) for the initiation visit, routine monitoring visits and a closing visit are recommended.

12.6 Appropriate entry/exit record of each monitoring visit should be maintained.

13. INVESTIGATORS

13.1 The principal investigator (PI) should have the overall responsibility for the clinical conduct of the study, including clinical aspects of study design, administration of the products under investigation, contacts with local authorities and the ethics committee and for signing the protocol and the final study report.

13.2 The investigator(s) should have appropriate qualifications, be suitably trained and have experience in the conduct of BE studies (the legal status of persons authorized to act as investigators differs between countries) and at least one investigator must be legally allowed to practise medicine.

13.3 The medically-qualified investigator should be responsible for the integrity, health and welfare of the subjects during the trial and the accurate documentation of all trial-related clinical data.

13.4 The CRO is responsible for selecting investigator(s). In cases where the investigators are not permanent employees of the CRO external investigators should be contracted and adequately trained.

14. RECEIVING, STORAGE AND HANDLING OF INVESTIGATIONAL DRUG PRODUCTS

14.1 CROs should document all the information concerning the receipt, storage, handling and accountability of investigational and comparator products at any stage of the trial. CROs must keep records of information about the shipment, delivery, receipt, storage (including storage conditions), dispensing, administration, reconciliation, return and/or destruction of any remaining pharmaceutical products. Detail of the pharmaceutical product used should include dosage form and strength, lot number, expiry date and other coding that identifies the specific characteristics of the product tested.
14.2 A suitable location within the CRO or a local pharmacy or hospital pharmacy should assume responsibility for storage, delivery, return and record keeping of the investigational products.

14.3 Pharmaceutical products should be stored under appropriate storage conditions as specified in the official drug information provided by the sponsor.

14.4 All study medication should be kept in a securely locked area accessible only to authorized persons.

14.5 Randomization should be performed in accordance with an SOP and records should be maintained, including the randomization list and seed, if applicable. Under normal operations the randomization list should be accessible only to the person who generates it, a dispensing pharmacist and the statistician and should not be circulated or made available to other staff members via any medium. A system should be in place which allows the PI or delegated staff to access the randomization list in case of emergencies.

14.6 Labelling should be performed in accordance with the following requirements:
- the printing step should be done in a manner that reduces potential risks of mislabelling and should be done in accordance with a SOP;
- each label should include the following information:
  - name of the sponsor,
  - a statement of “for clinical trial use only”,
  - trial reference number or study number,
  - batch number,
  - subject identification number (to which the product is destined to be given to),
  - period,
  - active ingredient and dosage,
  - the storage conditions,
  - expiry date (month/year) or retest date,
  - identification of the product (test or reference).
- compliance of all labels with the randomization list should be verified once printed, prior to labelling of the containers;
- labelling should be done on the container, not on the lid, to ensure that the information is not lost once the lid is removed;
- the system used for labelling and documenting the administration of the product should make it possible to verify that each subject indeed received the product dispensed for him, for instance, by using labels with a tear-off portion. In such a case, labels should be designed in such a way that two identical labels are pasted to the container and that the second label can be easily cut or detached and pasted onto the CRFs at the time of dosing (e.g. two labels printed side by side, with only one that is actually pasted onto the container and the other one which remains attached but unpasted – it should be torn off or cut with scissors at the time of dosing. Using two independent labels – one stuck on the container, one kept loose – should be avoided due to the risk of mix-ups);
- the empty containers should be labelled separately for the test and the reference investigational products and should remain adequately segregated and placed in a secure area under lock and key, to ensure absence of risk of any potential mix ups, until the dispensing stage;
label reconciliation should be performed;  
appropriate, detailed records should be maintained for each of the above steps.  

14.7 Dispensing/packaging should be performed in accordance with the following requirements:  
the surface area on to which the product will be handled should be thoroughly cleaned prior  
to bringing bottles of the product in the area. Any product container (full or empty), lone  
dosage formulations, labelling materials contaminants/dirt/debris should be removed from  
the area;  
a second person should verify that the surface area (otherwise referred to as “line”) is indeed  
clear and clean prior to bringing and opening containers of the product;  
test and reference products should be handled using an appropriate instrument, such as a  
spatula or spoon, as opposed to gloved hands;  
tablets should be distributed in each container in accordance with the randomization list  
either for the comparator or for the test product. Both products should never be handled at  
the same time. This also applies to the labelled containers;  
records should be made of this step in a manner that is similar to manufacturing batch  
records, as described in WHO GMP guidelines, i.e. each and every step should be recorded  
sequentially in detail;  
the surface area used to handle the product and its surroundings should be cleared and  
cleaned immediately after and/or prior to initiating the dispensing of the next product (it is  
important to note that this also applies to different products used in the same study);  
drug accountability and dispensing records should be maintained at all times. Each activity  
should be documented at the time it is performed. This includes  
records of doses dispensed and returned or destroyed,  
records of cleaning and clearance of the area prior to dispensing,  
record of verification of adequate cleaning and clearance of the area,  
record of verification by a second person of each step;  
any factors that could affect the integrity of the data relating to investigational medicinal  
products and comparators should be recorded, monitored and controlled.  

[Please refer for further guidance on labelling and dispensing to the WHO good manufacturing  
practices: supplementary guidelines for the manufacture of investigational pharmaceutical  

14.8 Dosing should be performed in accordance with the following requirements:  
dosing should be performed in accordance with a SOP;  
it should be performed under the supervision of the investigator or of qualified staff to  
whom this task has been explicitly delegated in writing;  
whenever possible, just prior to dosing, a check should be performed of vial contents  
matching the information on the label;  
the exact time of dosing should be documented;  
in order to ensure that the subject has swallowed the product, a mouth check should be  
performed by looking under the tongue, under the lips, in the corners of the mouth and  
between gums and cheeks, using a tongue depressor or a spatula and a flashlight, in the case  
of solid oral dosage forms. For other types of dosage forms verification of adequate  
administration should be performed by other suitable means. It should be documented;  
if more than one dosage unit is administered this should be clearly documented;
dosing can be documented directly in the case report forms. If retranscribed in the case of
report forms from other documents the original documents should be retained;
– drug reconciliation, after dosing, should be verified by a second responsible person.

14.9 The investigator should follow the protocol requirements, randomization scheme and where
required, blinding. The investigator should ensure that the investigational product use is
documented in such a way as to ensure appropriate dosage.

14.10 Samples of the product in the original container should be retained for possible
confirmatory testing in the future for a period of at least one year after the expiry date of the newest
product (test or reference) or in compliance with the applicable national requirements or
international recommendations, as appropriate. Sample retention should be defined and described in
an SOP and be specified in the contract between the sponsor and the CRO. Dispensed products that
were not administered should also be retained.

15. CASE REPORT FORMS

15.1 CRFs should be used to record data on each subject during the course of the trial.

15.2 The CRO should have a procedure for designing CRFs if the sponsor requests the CRO to
design them. It is recommended to use a standardized format or template which should be amended
for each study protocol in accordance with the requirements for the particular study. The CRF
should be reviewed against other trial documentation such as the protocol and trial database to
ensure that appropriate information and data is captured and that the CRF is consistent with other
trial documentation.

15.3 The required data to be collected on each volunteer should be specified in the trial protocol.
Any data to be recorded directly on the CRF (i.e. no prior written or electronic record of data), and
to be considered to be source data, should be identified in the protocol.

15.4 CRFs should reflect the actual results obtained during the study and allow easy access to
verification, audit and inspection of the data.

15.5 Appropriate procedures should be established and followed to document the investigator's
certification of the accuracy of CRFs. Any errors or omissions should be clarified with the
investigator, corrected, dated and signed and explained on the CRF.

15.6 Copies of the clinical laboratory reports and all ECGs should be included with the CRFs for
each subject and should be submitted along with the dossier, if applicable, according to
requirements of the regulatory authority to whom the dossier is submitted.

16. VOLUNTEERS, RECRUITMENT METHODS

Note: The selection of subjects should be performed sufficiently in advance to ensure that a
sufficient number of subjects will be available. The last-minute selection of additional
subjects may result in non-compliance with the inclusion and exclusion criteria,
possibly compromising the safety of the subjects and the integrity of the trial data. The
use of a generic screening process to select a pool of subjects which can be enrolled in
any bioequivalence study conducted at the CRO (unless the protocol foresees specific
inclusion or exclusion criteria) can help to achieve this goal.
16.1 Procedures for the recruitment of volunteers should be available and should include a description of the potential methods that can be used by the CRO for recruitment of volunteers. A database should be maintained for volunteers to avoid cross-participation and to specify a minimum time interval between a volunteer’s participation in two studies. Access to the database should be password controlled in order to secure confidential volunteer/subject information.

16.2 Volunteer and subject identification should be ensured by reliable means. If a biometric system is used for the identification of volunteers this system should be validated on a periodic basis as well as after any change made to the validated system that could impact its function.

16.3 Informed consent of potential subjects should be obtained for any screening procedures required to determine eligibility for the study, in addition to informed consent for participation in the research portion of the study.

16.4 Subject selection criteria (inclusion and exclusion criteria) and recruitment procedures should be described in the clinical trial protocol.

16.5 Subject screening results and trial participation should be recorded in a validated database maintained by the CRO. If a regional or national volunteer database exists then trial participation should be checked and participation data uploaded to this central repository to prevent over-volunteering. Access to the database should be password controlled in order to secure confidential subject information.

16.6 Ideally the CRO’s database should record and allow the users to query:

- contact details;
- gender;
- status: eligible, disqualified, not eligible, quarantined, etc., and the reason for this status if applicable;
- date and place of last study participation, if applicable/if known;
- date of last screening;
- a unique code assigned to the subject which will never change;
- outcome of last trial: Completed, randomised but not dosed, withdrawn for personal reason, withdrawn for medical reason, etc.

These data should be backed up daily and be available for review at any time.

16.7 Medical records should be generated for each subject and should include information obtained during each screening visit and each study participation, which could be relevant for the inclusion and follow-up of the subject into subsequent trials. Access to previous medical records for individual subjects should be available and a consistency check conducted where trial-specific medical records are generated. This is important to ensure safety issues can be assessed before enrolment in a study.

17. FOOD AND FLUIDS

17.1 As meals can significantly affect absorption of drugs fasting and meals should be standardized and adequately controlled and scheduled during the study days. The CRO should be able to arrange for standardized meals, snacks and drinks to study subjects as described in the clinical trial protocol.
17.2 Records should be maintained for timing, duration and amount of food and fluids consumed. Subjects should be asked for food and drink consumption prior to ambulatory samples being obtained, if the protocol contains specific requirements.

17.3 Standardized meals should be designed by a dietician with appropriate qualification, training and experience. If such services are contracted out a formal contract with terms of reference should be available.

18. SAFETY, ADVERSE EVENTS, ADVERSE EVENT REPORTING

18.1 Appropriate study planning includes adequate evaluation of risk to the subjects. The study should be planned, organized, performed and monitored so that the safety profile will be acceptable, including to the volunteers.

18.2 First-aid emergency equipment and appropriate rescue medication should be available at the study site and adequate facilities of the proper care of subjects who require emergency or other medical care. Any treatment given to a subject should be documented and included in the CRF and supporting documentation as necessary.

18.3 A medical doctor should be responsible for medical decisions in case of adverse events and for notifying the relevant health authorities, the sponsor and, when applicable, the Ethics Committee, without delay in the case of serious adverse events. Appropriate timelines should be respected as governed by national regulations.

18.4 The CRO should have appropriate adverse event registration and reporting forms, which should be provided to the investigator; these forms can be part of the CRF. If required the respective sponsor's forms may be used.

BIOANALYTICAL SECTION

Note: The analysis of drug concentrations may be performed in the same CRO which conducted the clinical study, or may be contracted to another laboratory or CRO.

19. METHOD DEVELOPMENT

19.1 The bioanalytical laboratory should provide detail on how a bioanalytical method was developed. The laboratory should keep a copy of any publication used to develop the bioanalytical method. The modifications and adaptations to the published method made by the laboratory should be documented.

19.2 Selection of the internal standard should be justifiable by sound scientific principles. In general, chemical and physical properties of the internal standard should be as close to those of the analyte as possible. Both stable isotope-labelled and non-isotope labelled internal standards are acceptable, though the use of stable isotope-labelled internal standard is recommended when MS methods are used. The selection of a stable isotope labelled internal standard should take into consideration factors such as the isotope labelling positions in order to limit the risk of exchange reactions.
19.3 Method development should ensure that methods are created in a manner which will minimize any potential human error.

20. **METHOD VALIDATION**

The most up-to-date guidelines from stringent regulatory authorities (SRAs) on the topic of bioanalytical method validation should be followed.

20.1 Validation requirements for the analytical method should be described in the protocol. There should be separate SOPs for analytical method validation.

20.2 Data to support the stability of the samples under the stated conditions and period of storage should be available preferably before the start of the study.

20.3 Method validation should be performed with at least one run that is comparable in length to those that are expected to be used for analysis of samples.

21. **SAMPLE COLLECTION, STORAGE AND HANDLING OF BIOLOGICAL MATERIAL**

21.1 The specification of the samples (serum, plasma or urine), sampling method, volume and number of samples should be stated in the clinical trial protocol and the information provided to the volunteer.

21.2 There should be documented procedures for the collection, preparation, transport or shipping and storage of samples.

21.3 Any specific lighting conditions foreseen by the protocol or other documents should be complied with. This should be documented.

21.4 Actual sampling times and deviations from the prespecified sampling times should be recorded. Deviations should be reported in the study report and should be taken into consideration when calculating the pharmacokinetic parameters.

21.5 Labelling of collected samples should be clear to ensure correct identification and traceability of each sample.

21.6 The storage conditions of samples depend on the investigational drug. However, all storage conditions (e.g. freezer temperature) should be specified in the study protocol, controlled, monitored and recorded throughout the storage period and transportation. Procedures should be in place to ensure sample integrity in case of system failures.

21.7 Records for the storage and retrieval of samples should be maintained.

21.8 It is recommended to keep duplicate or back-up samples, and store and ship them separately.

21.9 The duration of storage of bioanalytical samples should be specified in the contract between the sponsor and the CRO.
21.10 Local requirements for the handling and destruction of remaining biological materials should be followed.

22. ANALYSIS OF STUDY SAMPLES

The most up-to-date guidelines from SRAs on the topic of bioanalytical method validation should be applied. Additionally:

22.1 The results of the method validation should be available before the initiation of study sample analysis, with the possible exception of the evaluation of the long-term stability of the analyte in matrix. However these results should be available before the study report is issued and should be submitted with the validation report in the application.

22.2 Each analytical run should include calibration curve (CC) standards, QC samples and subject samples processed simultaneously. The exact sequence of processing should be documented. All samples collected from a given subject during all trial periods should be analysed in the same run unless scientifically justified (e.g. due to the limited stability of samples, requiring the analysis of period one samples before period two is conducted).

22.3 Equipment with an adequate capacity should be used to be able to process all samples in a run simultaneously, rather than splitting the samples into several extraction batches. However, if using several extraction batches within a single analytical run cannot be avoided, each batch should include QC samples. The acceptance criteria for the analytical run should be defined in a SOP first for the full run, then if the run is acceptable, for each individual extraction batch.

22.4 Every effort should be made during method development to avoid carry-over effects. If carry-over cannot be avoided, procedures should be implemented to limit its influence, for instance, by inserting wash samples into runs after samples with a high level of concentration.

22.5 With regards to the use of blank plasma in the preparation of CCs and QCs:

- the number of freeze-thaw cycles and the storage duration that a given blank plasma sample can be submitted to, should be limited as much as possible to ensure absence of degradation and/or change of its properties. Freezing blank plasma in small volumes should be considered to help limit the number of freeze-thaw cycles for any given blank plasma sample;
- the anticoagulant that was used for the blank plasma should be documented. It should match the anticoagulant that was used in study samples, in nature and in proportion.

22.6 With regards to incurred sample reanalysis:

- incurred sample reanalysis should be performed in line with the European Medicines Agency (EMA) Guideline on Bioanalytical Method Validation (2011);
- large differences between results may indicate analytical issues and should be investigated.

23. DATA PROCESSING AND DOCUMENTATION

23.1 Integration settings should be science-based and fully justifiable. Smoothing should be kept low enough not to mask possible interferences and changes in peak geometry.
23.2 The different iterations used to obtain a CC should be saved – if a given CC fails, it is not acceptable to exclude CCs which meet acceptance criteria or similarly, to include CC standards which do not meet criteria, just to make the calibration or the QC standards pass. The source data should contain the original, first evaluation of runs (containing all calibration samples). If several calibration samples are excluded sequentially the CC obtained at each step should be retained to document that the criteria to exclude the next sample were met. If electronic raw data are used it is acceptable to only save the final calibration if it is possible to revert to the initial calibration during an inspection. The process and criteria for acceptance and exclusion of CC standards should be described in an SOP.

23.3 If the first or last calibration sample is rejected the calibration range should be truncated, i.e. the second calibration sample becomes the lower limit of quantification (LLOQ) in that run (or the one before last calibration sample becomes the upper limit of quantification (ULOQ). Samples with a concentration below the revised LLOQ (or above the revised ULOQ) should be reanalysed. Alternatively, the whole run may be repeated but this is not the preferred option.

23.4 Internal standard variation should be trended and used as part of the verifications of result validity. Significant changes in internal standard response could signal an analytical problem which could require an investigation and/or sample reanalysis. Significant differences between the internal standard results of CC standards or QC standards vs samples could also signal problems affecting the reliability of the results.

23.5 Full audit trails should be activated at all times and on all analytical instruments in a given facility, both prior, during and after the method validation and the study of interest.

23.6 All original analytical raw data (e.g. calculations, chromatograms and their associated audit trails, etc.) should be documented in a manner that will ensure traceability with respect to the sample number, equipment used, date and time of analysis and the name(s) of the technician(s). If several audit trail files are generated all should be retained (e.g. results table audit trail, project audit trail, instrument audit trail).

23.7 Each data point should be traceable to a specific sample, including sample number, time of collection of the sample, time of centrifugation, if applicable, time when the sample was placed in the freezer, time of sample analysis, etc., to be able to determine whether any aberrant results might have been due to sample mishandling.

24. GOOD LABORATORY PRACTICES

24.1 Although most GLP guidelines apply formally only to non-clinical safety studies, general principles of GLP should also be followed during the bioanalytical part of bioequivalence studies.

24.2 Analysis should be performed in a laboratory with established QA systems.

24.3 Key sample storage systems or other areas requiring environmental controls should be adequately qualified, calibrated and maintained. There should be an alarm system or an adequate monitoring system to control the temperature of the critical stage areas and key sample storage systems, such as freezers. If there is an automatic alarm system it has to be tested regularly for its functionality. The daily monitoring and all the alarm checks should be documented. There should be a system in place to ensure that timely and appropriate action is taken following an alarm.
24.4 For purposes of qualification and requalification the temperature mapping of the freezers and refrigerators should be run for between 24 and 72 hours, or more if justified. Remapping should be done after any significant modifications to the storage units.

24.5 Appropriate repairs and/or sample transfer to other equivalent storage units should be considered whenever an analysis of temperature monitoring records show unexplained variability outside normal operating limits.

24.6 Balances, other measuring devices and equipment/instruments used during the conduct of a trial should be periodically calibrated and verified before use. They should be fit for their intended purpose.

24.7 There should be SOPs for the operation, use, calibration, checks and preventive maintenance of equipment. Records should be maintained. Equipment used during the course of the trial should be identified to be able to verify that they have been appropriately qualified and calibrated.

24.8 Chemicals, reference substances, reagents, solvents and solutions should be labelled to indicate identity, purity concentration (if appropriate), expiry date and specific storage instructions. Information concerning source, preparation date and stability should be available.

PHARMACOKINETIC, STATISTICAL CALCULATIONS AND REPORTING SECTION

25. PHARMACOKINETIC AND STATISTICAL CALCULATIONS

25.1 The statistical model underlying any primary BE analysis should be stated in the protocol and/or a statistical analysis plan. It should be made clear which factors are fixed and which are random. It should be stated if the model is a mixed effects model, a normal linear model, etc. If the methods of statistical analysis are amended following protocol approval then this should be documented in a protocol amendment and should also be reported in the clinical study report including the reason for change.

25.2 Calculations should be made by qualified persons (see Section 8: Personnel).

25.3 The means of performing pharmacokinetic and statistical calculations (both software and scripts) should be specified in the study protocol and/or a pharmacokinetic analysis plan and a statistical analysis plan. Data analysis should conform to these requirements. This should include the manner in which $AUC_{\text{inf}}$ is derived (i.e. how the points used for extrapolation are selected).

25.4 Calculations should be made using validated software and scripts. Software and scripts should be validated or qualified using an SOP, ideally with datasets of varying complexity and with the alpha level(s) actually in use. Self-designed software should be demonstrated as suitable for intended use. For guidance on the use of computerized systems (5) (see Section 4: Computer Systems).

25.5 Data values input should be double-checked by a second qualified person as per an SOP.
25.6 A database of trial records should be maintained and it should preferably be locked as soon as possible after completion of the study. Once it is locked the study can be unblinded and statistical analysis performed. The dates of locking and statistical analysis should be documented, mentioned in the study report and the process should be defined in a suitable procedure.

26. STUDY REPORT

26.1 The clinical study report should reflect the complete study procedures and results in an accurate manner.

26.2 The clinical study report should be well written and presented. All deviations from the protocol in the performance of the study should be reported.

26.3 There should be no discrepancies between the results stated in the report and the actual original (raw) data.

26.4 The report should comply with regulatory requirements as applicable and be presented in a standard format.

26.5 The study report should include a report on the bioanalytical part of the trial, including a description of the bioanalytical method used and the validation report of this method.

26.6 The procedure for approval of the clinical study report by the investigator and sponsor and for approval of the bioanalytical report by the study director should be specified.

26.7 The report should be approved (signed and dated) by the responsible persons.

26.8 All monitoring and audit reports should be available before release of the final study report.

REFERENCES


6. WHO Operational guidelines for Ethics Committees that review biomedical research (7).

7. WHO Good data management practices guidelines (full reference to be confirmed once finalized.)


10. EU guidelines to Good Manufacturing Practice and Medicinal Products for Human and Veterinary Use Annex 11, Computerised systems.
APPENDIX 1 (REVISED)
EXAMPLES OF THE LIST OF STANDARD OPERATING PROCEDURES AT A CONTRACT RESEARCH ORGANIZATION

The following are examples of the list of standard operating procedures (SOPs) that should be used at contract research organizations (CROs). This list is not exhaustive as other procedures may be necessary depending on the functional and compliance requirements at each facility.

All of the documents at the CRO related to a bioequivalence (BE)/clinical trial should be controlled (version date, approved, etc.) documents. This control is easier if the documents are in the SOP format or are appended to SOPs.

SOPs should be in place at least for all the critical and major operations in the BE/clinical trial.

Number and name of SOP

1. Conduct of BE study.
2. Archiving and retrieval of documents related to a BE study.
3. Quality assurance of a BE study; audits of clinical and bioanalytical part of the study and the study report.
4. Study files.
5. Preparation and review of the protocol for the study.
6. Amendment to the protocol for the study.
8. Sponsor/CRO quality assurance agreement in conducting the BE study.
9. Study approval process by ethical committee.
10. Bioavailability (BA)/BE report.
11. Study report.
12. Written informed consent.
13. Obtaining written informed consent for screening from study volunteers.
14. Allotment of identification numbers to volunteers at various stages in BE study.
15. Investigator’s brochure.
17. Preparation of CRF, review and completion.
18. Data collection and CRF completion.
19. Adverse/serious adverse event monitoring, recording and reporting.
20. Organization chart of the study.
21. Training of the personnel.
22. Responsibilities of the members of the research team.
23. Monitoring of the study by the sponsor.
24. Conduct of pre-study meeting.
25. Study start-up.
26. Subject management.
27. SOP on mobilization of individuals for registration into volunteer bank.
28. Eligibility criteria for registration and registration of individuals into volunteer bank.
29. Handling of subject withdrawal.
30. Allotment of identification numbers to volunteers at various stages in the biostudy.
31. Screening of enrolled volunteers for the study.
32. Collection of urine samples of subjects for detection of drugs of abuse and transportation of samples to pathology laboratory.
33. Custodian duties.
34. Payments to research subjects for BA/BE studies.
35. Procedures for entry into and exit from clinical unit.
36. Handling of subject check-in and check-out.
37. Housekeeping at clinical unit.
38. Planning, preparation, evaluation and service of standardized meals for bio-studies.
39. Distribution of meals to study subjects.
40. Operation and maintenance of nurse calling system.
41. Administration of oral solid dosage form of the drug to human subjects during BA/BE study.
42. Cannulation of study subjects.
43. Collection of blood samples from study subjects.
44. Identification of biological samples.
45. Recording of vital signs of subjects.
46. Operation and verification of fire alarm system.
47. Oxygen administration to subject from medical oxygen cylinder.
49. Availability of ambulance during BA/BE study.
50. Centrifugation and separation of blood samples.
51. Storage of plasma/serum samples.
52. Segregation of bio-samples.
53. Transfer of plasma/serum samples to bioanalytical laboratory.
54. Procedures for washing glassware.
55. Recording temperature and relative humidity of rooms.
56. Instruction on operation and maintenance procedures for all the equipment in the clinical unit.
57. Numbering the equipment and log books for use in the clinical unit.
58. Control of access to pharmacy.
59. Pharmacy area requirements.
60. Authorization related to drug storage, dispensing and retrieval from storage for BE study.
61. Study drug receipt, return and accountability documentation.
62. Study drug receipt and return procedures.
63. Storage of drugs in the pharmacy.
64. Line clearance before and after dispensing.
65. Documentation of line clearance and dispensing; packaging records and release of dispensed drugs.
66. Retention of samples of study drugs.
67. Disposal of archived study drugs.
68. Disposal of biological materials.
69. Procedures for bioanalytical laboratory (SOPs for the different equipment, analytical methods, reagent preparation).
70. Out-of-specification in the laboratory.
71. Acceptance criteria for analytical runs: acceptance of calibration curves, acceptance of the runs based on quality control samples results.
72. Chromatographic acceptance criteria, chromatogram integration.
73. Sample re-assay.
74. Pharmacokinetic data from bioanalytical data.
75. Statistics in a BE study.

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