Guidance for Industry

Handling and Retention of BA and BE Testing Samples

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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OGD

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I. INTRODUCTION

This guidance is intended to provide recommendations for study sponsors and/or drug manufacturers, contract research organizations (CROs), site management organizations (SMOs), clinical investigators, and independent third parties regarding the procedure for handling reserve samples from relevant bioavailability (BA) and bioequivalence (BE) studies, as required by 21 CFR 320.38 and 320.63. The guidance highlights (1) how the test article and reference standard for BA and BE studies should be distributed to the testing facilities, (2) how testing facilities should randomly select samples for testing and material to maintain as reserve samples, and (3) how the reserve samples should be retained. The guidance also clarifies and emphasizes points addressed in §§ 320.38 and 320.63.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Following the generic drug scandal in the 1980s, the FDA issued an interim rule in the *Federal Register* of November 8, 1990,[2] on the retention of BA and BE testing samples. The intent of the interim rule was to deter possible bias and fraud in BA and BE testing by study sponsors and/or drug manufacturers. Following public comments, a final rule was issued in the *Federal Register*

[1] This guidance has been prepared by the Division of Scientific Investigations in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.
[2] 55 FR 47034.

of April 28, 1993.[3] Implementing regulations are located in 21 CFR 312.57(d), 314.125(b)(17), 314.127(b), 314.150(b)(9), 320.31(d)(1), 320.38, and 320.63.

In the preamble to the final rule, the Agency stated that the study sponsor and/or drug manufacturer should not separate out the reserve samples of the test article and reference standard before sending the drug product to the testing facility.[4] This is to ensure that the reserve samples are in fact representative of the batches provided by the study sponsor and/or drug manufacturer for the testing. The study sponsor and/or drug manufacturer should send to the testing facility batches of the test article and reference standard so that the testing facility can ***randomly select*** samples for testing, and material to maintain as reserve samples. The drug product should also be maintained in the sponsor's and/or manufacturer's original container (see section III).

Also in the preamble to the final rule, the Agency noted that reserve sample retention is the responsibility of the organization that conducts the BA or BE study.[5] The intent is to eliminate the possibility of sample substitution by the study sponsor and/or drug manufacturer, or prevent the alteration of any reserve samples from a study conducted by a contractor before release of drug product samples to the FDA.

FDA's Division of Scientific Investigations (DSI) and field investigators from the Office of Regulatory Affairs (ORA) conduct inspections of clinical and analytical sites that perform BA and BE studies for study sponsors and/or drug manufacturers seeking approval of generic and new drug products. A frequent finding from these inspections is the absence of reserve samples at the testing facilities where the studies are conducted. In many cases, DSI finds that testing facilities return reserve samples to the study sponsors and/or drug manufacturers, against the direction of the regulations in 21 CFR 320.38 and 320.63. In other cases, study sponsors and/or drug manufacturers, SMOs, or contract packaging facilities designate the study test article and reference standard for each subject, and preclude the testing facilities from randomly selecting representative reserve samples from the supplies. DSI also finds that deviations from the regulations more often occur in BE studies with pharmacodynamic or clinical endpoints in which the studies are confused with clinical safety or efficacy studies. The pharmacodynamic or clinical endpoint BE studies are usually multisite, blinded studies conducted under contract (either directly with the study sponsor or drug manufacturer or through an SMO) by physicians or clinical investigators who use their own clinics or offices to conduct the studies. Moreover, some clinical investigators believe that they are not CROs and are not required to retain reserve samples. This guidance clarifies the responsibilities for retention of reserve samples.

III. SAMPLING TECHNIQUES

We recommend that the study sponsor and/or drug manufacturer send to the testing facility batches of the test article and reference standard packaged in such a way that the testing facility

[3] 58 FR 25918.
[4] 58 FR 25918 at 25920.
[5] 58 FR 25918 at 25921.

can randomly select samples for bioequivalence testing and samples to maintain as reserve samples. This will ensure that the reserve samples are in fact representative of the batches provided by the study sponsor and/or drug manufacturer and that they are retained in the study sponsor's original container. Because the study sponsor and/or drug manufacturer may provide a testing facility with a variety of container sizes and packaging, FDA is flexible in applying the representativeness requirement described in 21 CFR 320.38. For example, any of the following random sampling techniques might be used by the testing facility for the container size and packaging described[6] (bolded text is particularly relevant).

Single Container – If a single container of the test article and reference standard are provided to the testing facility, the testing facility should remove a sufficient quantity of the test article and reference standard from their respective containers to conduct the study; the remainder in each container should be retained as reserve samples in the original containers.

Multiple Containers – If multiple containers of the test article and reference standard are provided to the testing facility, the testing facility should ***randomly select*** enough containers of the test article and reference standard to conduct the study; the remaining containers of the test article and reference standard should be retained as the reserve sample in the original containers. Generally, multiple open bottles are discouraged. We encourage testing facilities to limit the number of open containers retained as study reserves.

Unit Dose – If the test article and reference standard are provided to the testing facility in unit dose packaging, the testing facility should ***randomly select*** a sufficient quantity of unit doses of the test article and reference standard to conduct the study; the remaining unit doses of the test article and of the reference standard should be retained as the reserve samples in the original unit dose packaging. ***Therefore, it would be inappropriate to provide the study medications in unit dose packaging and all the reserve samples in bulk containers.***

Blinded Study – If the study is to be blinded and the test article and reference standard are provided to the testing facility in unit dose packaging with each unit dose labeled with a randomization code, ***the study sponsor and/or drug manufacturer should provide the testing facility with a labeled set of the test article and reference standard sufficient to conduct the study and with additional, identically labeled sets sufficient to retain the "five times quantity" (see section V). The testing facility should randomly select a labeled set to conduct the study; the remaining labeled sets would be retained in their unit dose packaging as the reserve samples***. For a blinded study, we recommend that the study sponsor and/or drug manufacturer also provide to the testing facility a sealed code for use by FDA should it be necessary to break the code. The sealed code should be maintained at the testing facility.

IV. RETENTION FOR MULTIPLE STUDIES AND SHIPMENTS

If the same batches of the test articles and reference standards initially provided to the testing facility are used in performing more than one study, only one reserve sample of the test article and reference standard in sufficient quantity need to be retained. The reserve samples should be

[6] 58 FR 25918 at 25920.

identified as having come from the same batches as used in each study. However, if additional supplies of the test article and reference standard will be used by a testing facility to perform the same study or additional studies, the testing facility should retain a sufficient quantity of reserve samples from the subsequent shipment. If a CRO with multiple testing facilities conducts more than one BE study (e.g., fed and fasted studies) for the same drug product, and the study test article and reference standard are sent to the testing facilities in different shipments, we recommend that sufficient quantity of reserve samples be kept for each study at each testing facility. These approaches are to ensure that the reserve samples are in fact representative of the batch provided by the study sponsor and/or drug manufacturer to the testing facility.

V. QUANTITY OF RESERVE SAMPLES

The quantity of reserve samples should be sufficient to permit the Agency to perform five times all of the release tests required in the application or supplemental application. The rationale for requiring the *five times quantity* is provided in the final rule. The clinical investigator can obtain the amount that constitutes the five times quantity from the sponsor and/or drug manufacturer. For solid oral dosage forms (e.g., tablets, capsules), an upper limit of 300 units each for the test article and reference standard can be considered sufficient to meet the five times quantity. Because the Agency has limited experience with the retention and testing of non-solid oral dosage forms, the Agency is unable to recommend an upper limit for the retention of non-solid oral dosage forms at this time. In the case of a reference standard that is an extemporaneously compounded solution or suspension or a reconstitutable powder, we recommend that the pure active ingredient and the unconstituted powder be retained. For a multisite BA or BE study, we recommend that the total amount of reserve samples to be retained across ***all*** testing facilities satisfy the five times quantity requirement. Each site is asked to retain a reasonable amount of test article and reference standard to be determined by considering (1) the total number of testing facilities participating in the study, (2) the number of subjects expected to be enrolled at each testing facility, and (3) a minimum limit (e.g., 5 dose units) for each of the test articles and reference standards. If the reserve samples from more than one testing facility are transferred to an independent third party for storage, we recommend that the independent third party segregate the reserve samples from the various testing facilities so that any given reserve sample can be unambiguously associated with the testing facility from which it came.

VI. RESPONSIBILITIES IN VARIOUS STUDY SETTINGS

Because of the variety of study settings potentially involved in conducting BA and BE studies, several examples are provided here. These examples are not the only possible study settings. However, in ***all*** instances, the chain of custody of the reserve samples used in the study should be preserved. The sponsor and/or manufacturer and any storage facility should document and maintain the transfer records for Agency verification.

A. Studies Conducted at CROs, Universities, Hospitals, or Physicians' Offices

CROs are the most common study sites. Many BA/BE studies of oral dosage forms are conducted at CROs to support approval of abbreviated new drug applications (ANDAs), new drug applications (NDAs), and NDA supplements. CROs typically conduct single-site, open-label, crossover design studies with healthy volunteers as participants.

Study sponsors and drug manufacturers sometimes conduct BA and BE studies through a CRO, university faculty, hospitals, or clinical investigators in private practice. The testing facilities are usually clinical study units in universities, hospitals, or clinics run by physicians.

The responsibilities of the study sponsor and/or drug manufacturer include:

- Packaging, distributing, and shipping the test article and reference standard to the testing facility
- Monitoring the study if it is conducted under an investigational new drug application (IND) (rarely needed for most ANDA studies)

The responsibilities of the testing facility are as follows:

- The clinical investigator or designee (such as the study coordinator or research pharmacist of the testing facility) should randomly select sufficient test article and reference standard to conduct the study from the supplies received from the sponsor and/or drug manufacturer, and retain the remaining study samples as study reserves.
- The testing facility or the pharmacy of the testing facility should retain the reserve samples.
- If the testing facility does not have adequate storage, or goes out of business, the reserve samples can be transferred to an independent third party with an adequate facility for storage under conditions consistent with product labeling.

Note: When studies are conducted at universities, hospitals, or physicians' offices, the clinical investigator or physician conducting the study should ***not*** send the reserve samples back to the study sponsor and/or drug manufacturer. The goal is to eliminate the possibility for sample substitution by the study sponsor and/or drug manufacturer, and to preclude the alteration of a reserve sample from a study conducted by another entity before the release of the reserve sample to the FDA.

B. Studies Involving SMOs

When BA or BE studies are conducted by an SMO, they are frequently multisite, open-label studies of oral dosage forms in patients, or multisite, open-label studies of nonoral dosage forms with pharmacodynamic or clinical endpoints. Often, the study sponsor and/or drug manufacturer contracts with an SMO to recruit clinical investigators and to monitor a study. The SMO is involved directly or indirectly (i.e., by subcontracting to another party) in packaging and shipping of study test articles and reference standards to the testing facilities. The testing facilities are usually the clinical study units of CROs, universities, hospitals, or clinics run by physicians.

The responsibility of the study sponsor or drug manufacturer is to ship the test article and reference standard to the SMO under contract, or to the packaging facility under subcontract to the SMO.

The responsibilities of the SMO include:

- Packaging, distributing, and shipping the test article and reference standard to all testing facilities (or subcontracting a packaging facility to perform this function)
- Monitoring the study at different sites if it is conducted under an IND (rarely needed for most ANDA studies)

The SMO should ***not*** randomly select and retain reserve study samples. As explained in the preamble to the final rule, the Agency intended that sufficient test article and reference standard to conduct the study should be randomly selected at each testing facility, and that each testing facility should retain the remaining study samples as reserves. [7]

The responsibilities of the testing facilities are as follows:

- The clinical investigator or designee (such as the study coordinator or the research pharmacist of each testing facility) should randomly select sufficient test article and reference standard to conduct the study from the supplies received from the SMO under contract, or from the packaging facility under subcontract with the SMO, and retain the remaining study samples as study reserves.
- Each testing facility or the pharmacy of each testing facility should retain the reserve samples.
- Following the completion of the study, if one or more of the testing facilities do not have adequate storage, reserve samples can be transferred to an independent third party with an adequate facility for storage under conditions consistent with product labeling. The reserve samples should not be transferred back to an SMO or any other organization that deals with packaging the test articles and reference standard for storage. This is to eliminate the possibility of commingling reserve samples from packaging activities (21 CFR 211.84 and 211.170) and bioequivalence studies (21 CFR 320.38 and 320.63). As stated in subsection VI.A above, the reserve samples should ***not*** be shipped back to the sponsor or manufacturer.

C. Blinded Studies With Pharmacodynamic or Clinical Endpoints Involving an SMO

Blinded BE studies are often conducted at multiple sites and involve nonoral dosage forms with pharmacodynamic or clinical endpoints. Often, the study sponsor and/or drug manufacturer contracts with an SMO to recruit clinical investigators and monitor the study. The SMO is involved directly or indirectly (i.e., by subcontracting to another party) in packaging and shipping study test articles and reference standards to the testing facilities. The testing facilities are usually the clinical study units of CROs, universities, hospitals, or clinics run by physicians.

[7] 58 FR 25918 at 25920.

In multisite, blinded BE studies, the sponsor and/or drug manufacturer needs to consider whether the study design will allow for selection and retention of reserve samples in accordance with 21 CFR 320.38 and 320.63 and the final rule. If the study design is too complex to meet the regulatory requirements for reserve samples, the study design may need to be reconsidered.

The responsibility of the study sponsor and/or drug manufacturer is to ship the test article and reference standard to the SMO under contract, or to the packaging facility under subcontract to the SMO.

The responsibilities of the SMO include:

- Packaging, distributing, and shipping test article and reference standard to all testing facilities (or subcontracting a packaging facility to perform this function). We recommend that the SMO provide the testing facilities with enough code-labeled sets to conduct the study and to retain the five times quantity. Based on inspection experience, DSI does not recommend that test article and reference standard be prenumbered for subjects, because assigning unit doses to a designated subject number precludes the random selection of drug used for dosing and drug used for reserve samples (see example below for illustration).
- Monitoring the study at different sites if it is conducted under an IND (rarely needed for most ANDA studies)

Note: The SMO should not select reserve samples. In addition, the reserve samples should not be transferred by the testing facility back to an SMO or any other organization that deals with packaging the test articles and reference standard for storage.

The responsibilities of the testing facilities are as follows:

- The clinical investigator or designee (such as the study coordinator or the research pharmacist) of each testing facility should randomly select sufficient test article and reference standard to conduct the study from the supplies received from the SMO under contract, or from the packaging facility under subcontract with the SMO, and retain the remaining study samples as study reserves. The clinical investigator should be aware of the sampling techniques used for blinded studies as described in section III.
- Each testing facility or the pharmacy of each testing facility should retain the reserve samples. Please note that if a placebo is used in blinded BE studies, reserve samples for the placebo should be retained along with the test article and reference standard reserves. The sealed treatment code of the study should be kept at the testing facility. This is applicable even if the reserve samples are forwarded to an independent third party (see paragraph below).

3. If one or more of the testing facilities do not have adequate storage, or go out of business, the reserve samples can be forwarded to an independent third party with an adequate facility for storage under conditions consistent with product labeling.

Below is a suggested packaging and random selection plan for a blinded, multisite study of a dermatological cream product involving a SMO:

The study enrolls 300 subjects with approximately 60 subjects at five testing facilities. The five times quantity for the test article and reference standard is 50 tubes for each product. In preparation for conducting the study, the SMO prepares 200 boxes that contain one code-labeled tube of test article and one code-labeled tube of reference standard in each box. The SMO randomly distributes 40 boxes to each clinical testing facility. The clinical facility randomly selects 30 of the boxes to dose 60 subjects. The remaining 10 boxes serve as the reserve samples. In this example, staff (e.g., a pharmacist) not involved with the study may be recommended to ensure the study remains blinded. This packaging system ensures that an equal number of test article and reference standard are administered to the subjects at each site, and that an equal number of test article and reference standard will be maintained as reserve samples. Since 10 boxes are kept at each of 5 testing facilities, 50 tubes each of test article and reference standard are retained and the five times quantity reserve sample requirement is met. In addition, the requirement of random selection by each testing facility is also met.

D. In-House Studies Conducted by a Study Sponsor and/or Drug Manufacturer

Only about 7 percent of all sites inspected by DSI from 1997 to 2002 conducted in-house BA and BE studies. If a study sponsor and/or drug manufacturer conducts such a study, manufacturing reserve samples (21 CFR 211.170) and BE study reserve samples (21 CFR 320.38 and 320.63) should be separated. The in-house clinical research unit should operate as an independent unit for the purposes of sample retention. All matters (e.g., manufacturing, purchasing, packaging, transfer records) concerning the test article and reference standard should be clearly documented and available to FDA investigators during an inspection. Standard procedures concerning security and accountability of the test article and reference standard for each study should be established to eliminate the possibility of sample substitution. Sponsors conducting in-house studies can engage an independent third party to store reserve samples. If an independent third party is not used, there should be (1) a totally segregated and fully compliant in-house storage area; (2) procedures and policies in place to show that adequate test article and reference standard are retained; (3) controlled access to the reserve samples; (4) a rigorous and unbroken chain of custody for the reserve samples.

The study sponsor and/or drug manufacturer (clinical research department) should be responsible for packaging and transferring the test article and reference standard to the in-house clinical study unit.

The testing facility (in-house clinical study unit) should be responsible for:

- Documentation of all matters concerning the transfer and receipt of the test article and reference standard
- Random selection of sufficient test article and reference standard to conduct the study, and retention of the remaining study samples as reserves. The selection is generally

made by the clinical investigator, study coordinator, or research pharmacist (if available) in the clinical study unit. We recommend that a staff member (e.g., a study nurse) witness the random selection process and dosing.

- Retention of reserve samples in a secure area. To ensure the authenticity of the reserve samples, access to this area should be limited. We encourage maintenance of an entry log to the storage area.
- Preparation for adequate storage of reserve samples. If the in-house testing facilities do not have adequate storage, or go out of business, the reserve samples can be forwarded to an independent third party with an adequate facility for secure storage under conditions consistent with product labeling.

E. In Vitro BE Studies

21 CFR 320.63 states:

> The applicant of an abbreviated application or a supplemental application submitted under section 505 of the Federal Food, Drug, and Cosmetic Act, or, if bioequivalence testing was performed under contract, the contract research organization shall retain reserve samples of any test article and reference standard used in conducting an in vivo or in vitro bioequivalence study required for approval of the abbreviated application or supplemental application.

Thus, the regulations for reserve samples apply to in vitro BE studies. The in vitro BE studies required for approval of nasal aerosols and nasal sprays for local action are an example of this. Note that in vitro studies conducted to compare dissolution rates for different strengths of the same formulation are not subject to the reserve sample regulations. For an in vitro BE study, the roles of the study sponsor and/or drug manufacturer and the testing facility are similar to those described for in vivo BE studies conducted by CROs and in the examples of in vivo BE studies conducted in-house by a study sponsor and/or drug manufacturer.

VII. EXCEPTION FOR INHALANT PRODUCTS

As stated in 21 CFR 320.38(c), each reserve sample shall consist of a sufficient quantity of samples to permit FDA to perform five times all of the release tests required in the application or supplemental application. Dose content uniformity or spray content uniformity release tests alone usually take 30 units (canisters or bottles) per batch. Performance of other release tests can suggest a need for additional units. The number of reserve sample units to be retained for three batches of test article and reference standard could exceed 1000 units (up to 250 units for each batch of the test article and reference standard) based on the five times quantity requirement. The Agency has determined that in lieu of the "five times quantity" requirement, the quantity of inhalant (nasal aerosol or nasal spray) test article and reference standard retained for testing and analyses should be at least 50 units for each batch (see the preamble to the final rule).[8]

[8] 58 FR 25918 at 25924.

For NDAs, at least 50 units of each of the three batches of nasal aerosol or nasal spray needed for BA studies should be retained. However, where the reference standard is another nasal aerosol or nasal spray, at least 50 units of that batch should also be retained. For ANDAs, at least 50 units of each of three batches should be retained for each of the test articles and reference standards used for in vivo or in vitro BE studies. If multiple testing facilities are used in a BA or BE study, the total amount of reserves for each product across ***all*** testing facilities would be at least 50 units, and each testing facility should retain a reasonable amount of test articles and reference standards (see section V for more details). For NDAs and ANDAs, if the in vivo or in vitro studies include placebo aerosols or sprays, at least 50 units of each placebo batch should also be retained. These recommendations apply only to nasal aerosol and nasal sprays for local action that are to be marketed as multiple dose products, typically labeled to deliver 30 or more actuations per canister or bottle.

GLOSSARY

Clinical Investigator – An individual who actually conducts a clinical investigation (i.e., under whose immediate direction the drug is administered or dispensed to a subject) (21 CFR 312.3(b)). In this guidance, when a clinical investigation involves BA or BE studies, the clinical investigator has the responsibility of retaining the reserve samples at the testing facility or through an independent third party.

Contract Research Organization (CRO) – An independent contractor of the sponsor or manufacturer that assumes one or more of the obligations of a sponsor (e.g., design of a protocol, selection or monitoring of investigations, evaluation of reports, and preparation of materials to be submitted to the FDA) (21 CFR 312.3(b)). This guidance addresses BA and BE studies submitted to support approvals of new and generic drugs. These studies are usually conducted by CROs under contract to study sponsors and/or drug manufacturers. Many CROs have their own testing facilities, with physicians (to serve as clinical investigators) and clinical support staff (e.g., nurses, medical technologists) to conduct the BA and BE studies.

Independent Third Party – In this guidance, *independent third party* indicates a person that has no affiliation with the study sponsor and/or drug manufacturer.

Reference Standard – In this guidance, *reference standard* refers to the reference product used in a BE study. It is usually the innovator's product or a marketed product of the drug under investigation. For BA studies, the reference standard can be an oral solution of the drug under investigation.

Reserve Samples – In this guidance, reserve samples and retention samples are used interchangeably.

Site Management Organization (SMO) – In this guidance, *site management organization* refers to an organization that manages clinical study sites on behalf of the sponsor and/or drug manufacturer.

Sponsor-Investigator – An individual who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. The term does not include any person other than an individual (21 CFR 312.3(b)).

Study Sponsor – A person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization. The sponsor does not actually conduct the investigation unless the sponsor is a sponsor-investigator (21 CFR 312.3 (b)).

In this guidance, the term *study sponsor and/or drug manufacturer* is used in recognition of the fact that most study sponsors are pharmaceutical companies that manufacture the drugs under investigation.

Testing Facility – The entity performing the BA or BE (in vivo or in vitro) study. The testing facility can be a CRO, university, hospital, clinic of a clinical investigator, or in-house clinical study unit of a study sponsor and/or drug manufacturer, where dosing and sampling (i.e., blood, urine, or clinical endpoints) are performed. In issuing the final rule, the Agency intended that reserve samples should be kept at the testing facility.