# Handling and Retention of BA and BE Testing Samples

## Guidance for Industry

***DRAFT GUIDANCE****

**The draft portion of this guidance document is being distributed for comment purposes only.**

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

**March 2024**
**Generic Drugs**
**Revision 1**

*Section IV.B. of this document is issued as final guidance for immediate implementation. The remainder of the document is issued as draft guidance.

# Handling and Retention of BA and BE Testing Samples

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# Handling and Retention of BA and BE Testing Samples Guidance for Industry[1]

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

## I. INTRODUCTION

This guidance is intended to provide recommendations for **applicants**[2] of new drug applications (NDAs) and abbreviated new drug applications (ANDAs), including supplemental applications, and **contract research organizations (CROs)**, regarding the procedures for handling reserve samples from relevant bioavailability (BA) and bioequivalence (BE) studies, as required by §§ 320.38 and 320.63 (21 CFR 320.38 and 320.63),[3] and recommendations regarding responsibilities of each party involved in the study pertaining to reserve samples. In the context of §§ 320.38 and 320.63, the term applicant includes, as appropriate, **study sponsor** and/or drug manufacturer and the term CRO refers to any party contracted to help conduct BA or BE testing, including, as appropriate, **site management organizations (SMOs)**, **investigators**, and **testing sites**.[4]

The guidance highlights:

(1) How the test article (T) and **reference standard** (RS) for BA and BE studies should be distributed to the testing sites
(2) How testing sites should randomly select samples for testing and material to maintain as reserve samples
(3) How the reserve samples should be retained.

Additionally, this guidance:

[1] This guidance has been prepared by the Office of Generic Drugs, in cooperation with the Office of Scientific Investigations, the Office of Study Integrity and Surveillance, and the Office of Clinical Pharmacology in the Center for Drug Evaluation and Research, and the Office of Regulatory Affairs at the Food and Drug Administration.

[2] A Glossary of terms, as used in this guidance document, appears at the end of the document, and words found in the Glossary are bolded at first use.

[3] This includes retention of reserve samples for BA and BE studies conducted under an investigational new drug application (IND) as required by 21 CFR 312.57(d).

[4] This interpretation is consistent with the 1993 final rule, which states: "The final rule applies to domestic and foreign sponsors and applicants (hereinafter called a study sponsor) who perform in-house bioavailability or bioequivalence testing for new drug product approval under an NDA, ANDA, or supplemental application and to any domestic and foreign testing facility that performs such bioavailability or bioequivalence testing under contract (contract research organization) for a study sponsor." 58 FR 25918, 25918 (April 28, 1993).

(4) Addresses the requirement at 21 CFR 320.38(c) to retain reserve samples of sufficient quantity to permit FDA to perform five times all the release tests required in an application or supplemental application
(5) Describes the conditions under which the Agency does not generally intend to take enforcement action against an applicant or CRO for retaining less than the quantity of reserve samples of the test article and reference standard that were used in the BA or BE study as specified in 21 CFR 320.38(c).

The guidance also provides clarifying recommendations related to certain other relevant requirements in §§ 320.38 and 320.63.

This guidance is a revision of the final guidance *Handling and Retention of BA and BE Testing Samples* (May 2004) ("the 2004 Guidance"). This guidance is issued in part as final guidance and in part as draft guidance. Specifically, Section IV.B. of this guidance is issued as final guidance. It revises and supersedes the agency's compliance policy related to the quantity of BA and BE samples retained under § 320.38(c) described in the final guidance *Compliance Policy for the Quantity of Bioavailability and Bioequivalence Samples Retained Under 21 CFR 320.38(c)* (August 2020) ("the 2020 Compliance Policy"), which is hereby withdrawn. Section IV.B also describes the conditions under which the agency generally does not intend to take enforcement action against an applicant or CRO that retains less than the quantity of reserve samples (that is, samples of the T and RS that were used in an in vivo BA or in vivo or in vitro BE study) specified in the regulation. This revised compliance policy is for immediate implementation. It also supersedes statements related to quantity of reserve samples in section IX. Number of Reserve Samples for BA and BE Testing of the draft guidance *Nasal Aerosols and Nasal Sprays for Local Action* (April 2003).[5] This revised compliance policy is applicable to all reserve samples for BA and BE studies held to date, including reserve samples from previously completed BA or BE studies.

The rest of this guidance is issued as draft guidance for public comment purposes only. It discusses additional recommendations around the handling and retention of BA and BE testing samples. When finalized, it will represent the agency's current thinking on this topic.

In general, FDA's guidance documents 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

On November 8, 1990,[6] the FDA issued an interim rule in the *Federal Register* 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 a public comment

[5] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents
[6] 55 FR 47034.

period, a final rule was issued in the *Federal Register* of April 28, 1993.[7] 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.[8]

In the preamble to the 1993 final rule, the Agency stated that the study sponsor and/or drug manufacturer should not separate out the reserve samples of the T and RS before sending the drug product to the testing site.[9] This is to ensure that the reserve samples are in fact representative of the drug product provided by the study sponsor and/or drug manufacturer for the testing. The study sponsor and/or drug manufacturer should send **shipment**(s) of the T and RS to the testing site so that the testing site can *randomly select* samples to retain as reserve samples, and samples for testing. Generally, the drug product should also be maintained in the study sponsor's and/or drug manufacturer's **original container** (see section IV).

Also in the preamble to the 1993 final rule, the Agency noted that reserve sample retention is the responsibility of the organization that conducts the BA or BE study.[10] 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 Office of Study Integrity and Surveillance (OSIS) and field investigators from the Office of Regulatory Affairs conduct inspections of clinical and analytical sites that perform BA and BE studies for study sponsors and/or drug manufacturers seeking approval of drug products under NDAs and ANDAs. A frequent finding from these inspections is the absence of reserve samples at the testing sites where the studies are conducted. In many cases, OSIS finds that testing sites return reserve samples to the study sponsors and/or drug manufacturers, against the direction of the regulations in §§ 320.38 and 320.63. In other cases, study sponsors and/or drug manufacturers, SMOs, or contract packaging facilities designate the T and RS for each subject and preclude the testing sites from randomly selecting representative reserve samples from the supplies. OSIS also finds that deviations from the regulations often occur in comparative clinical pharmacodynamic or comparative clinical endpoint BE studies in which the studies are confused with clinical safety or efficacy studies. The comparative clinical pharmacodynamic or comparative 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 investigators who use their own clinics or offices to conduct the studies. As such, some investigators incorrectly believe their clinics or offices are not considered CROs required to retain reserve samples.

[7] 58 FR 25918.

[8] These citations reflect the current location of the implementing regulations. When originally codified in 1993, some might have appeared in different locations, e.g., the material currently at 21 CFR 312.57(d) appeared at 21 CFR 312.57(c) when originally codified in 1993.

[9] 58 FR 25918 at 25920.

[10] 58 FR 25918 at 25921.

This revised guidance[11] clarifies the recommendations related to the handling and retention of reserve samples:

- Section III clarifies the types of in vivo and in vitro studies for which an applicant or its CRO is required to retain reserve samples.
- Section IV.A. updates the sampling techniques recommended in the 2004 Guidance to reflect that generally reserve samples should be selected by the testing sites prior to conducting the BA or BE study and provides new recommendations on how to handle reserve samples for co-packaged products, blinded studies, and repackaged products.
- Section IV.B. describes the conditions under which FDA generally does not intend to enforce the requirement to retain a sufficient quantity to perform five times all the release tests required in the application or supplemental application. This section of the guidance is final and for immediate implementation.
- Section IV.C. clarifies FDA's recommendations for each testing site regarding the retention of reserve samples for multiple studies, multiple batches, and multiple shipments.
- Section IV.D. discusses FDA's recommendations regarding access to and storage of reserve samples, including the appropriate tracking and documentation of transfers of reserve samples.
- Section V clarifies the responsibilities of the stakeholders (i.e., study sponsor, drug manufacturer, testing site, investigator, CRO, SMO, etc.) for the handling and retention of reserve samples in various study settings.

## III. APPLICABILITY OF RESERVE SAMPLES REQUIREMENT

The study sponsor and/or drug manufacturer should clarify to the testing sites whether reserve samples are required to be retained under §§320.38 and 320.63. Where a study sponsor and/or drug manufacturer determines that reserve samples are required to be retained, FDA recommends, as a best practice, documenting a detailed plan for the handling and retention of reserve samples in the study protocol and describing the procedures followed and what was retained in the study report. It is recommended that the study protocol and study report include: the method for random selection of the reserve samples; the testing site staff responsible for selecting the reserve samples for retention; the total quantity of reserve samples; the number of shipments of study drug (T and RS) to each testing site; and the number of reserve samples from each shipment. This information will help support evaluation of the study's integrity.

### A. In vivo BA and BE studies

The applicant or, if testing is performed under contract, its CRO must retain appropriately identified reserve samples of the T and RS used in an in vivo BA or BE study in accordance with §§ 320.38 and 320.63. Generally, reserve samples must be retained for an in vivo BA or BE study that is required for approval. FDA recognizes that reserve samples are not required to be retained for all in vivo studies. For example, reserve samples are not required to be retained for

[11] As noted above, section IV.B. of this document is issued as final guidance for immediate implementation. The remainder the document is issued as draft guidance.

in vivo studies that assess irritation, sensitization, or stand-alone adhesion[12] of transdermal or topical delivery systems.

### B. 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.

The regulations for reserve samples apply to in vitro BE studies. Please note, however, that not all in vitro studies are BE studies, and in vitro studies that are not BE studies are not subject to the regulations for reserve samples. For example, applicants may conduct in vitro characterization studies that compare test and reference products but that are not in vitro BE studies. An in vitro BE study typically should have well-defined statistical equivalence criteria and endpoints. Generally, product-specific guidances (PSGs) explicitly describe in vitro tests as either in vitro BE studies or in vitro characterization studies. FDA recommends that reserve samples be retained for all studies that PSGs describe as in vitro BE studies. The in vitro BE studies recommended in PSGs for nasal aerosols and nasal sprays for local action are examples of this. Also, in vitro studies conducted to compare dissolution rates for different strengths of the same formulation are not subject to the reserve sample regulations because they are used as qualifying criteria for a biowaiver, not to establish BE.

## IV. HANDLING AND RETENTION OF RESERVE SAMPLES

### A. Sampling Techniques

We recommend that the party responsible for sending the T and RS to the testing site (usually study sponsor and/or drug manufacturer) send shipment(s) of the T and RS to the testing site packaged in such a way that the testing site can randomly select samples for BA or BE testing and samples to maintain as reserve samples in the original container(s). Reserve samples should not be selected by the study sponsor and/or drug manufacturer or other packaging site prior to reaching the testing site(s).[13] The reserve samples should be randomly selected from each

[12] Applicants may choose to evaluate transdermal delivery system (TDS) adhesion in studies performed to evaluate TDS adhesion only or in studies performed with a combined purpose (e.g., for the simultaneous evaluation of adhesion and bioequivalence with pharmacokinetic endpoints). As used in this guidance, stand-alone adhesion refers to a TDS study that only evaluates adhesion, without some other combined purpose. See draft guidances on *Assessment of Adhesion for Topical and Transdermal Systems Submitted in New Drug Applications* (July 2021) and *Assessing Adhesion with Transdermal and Topical Delivery Systems for ANDAs* (April 2023), which, when finalized, will represent FDA's current thinking on adhesion studies.

[13] Where a study uses a central pharmacy to manage multiple testing sites, reserve samples should not be selected by the central pharmacy, but by the actual testing sites.

shipment at each testing site before administering or dispensing samples from that shipment for use in the BA or BE study. FDA inspections have revealed that selecting reserve samples from leftover testing samples (samples not administered to subjects or used in the study) has left some study sites with insufficient quantities to retain as reserve samples, and improper storage of reserve samples not in the original containers or in multiple open containers. As such, leftover samples should not be retained as reserve samples, unless the study involves use of a single container of bulk packaged single-dose unit product as described below. This will help ensure that the reserve samples are in fact representative of the drug product provided by the study sponsor and/or drug manufacturer and used in the BA or BE study, that they are retained in sufficient quantity and in accordance with storage requirements, and that they are retained in the study sponsor's and/or drug manufacturer's original container. Because the study sponsor and/or drug manufacturer may provide a testing site with a variety of container sizes and packaging, FDA considers the representativeness requirement described in § 320.38(a) to inherently include flexibility in the technique used to randomly select the reserve samples depending on the size and type of packaging used for the drug product. For example, any of the following random sampling techniques might be used by the testing site for the container size and packaging described (italicized text is particularly relevant).

Single Container of Bulk Packaged Single-Dose Unit Product – If a single container of bulk packaged **single-dose unit** product for each of the T and RS are provided to the testing site (e.g., one bottle of 500 tablets), the testing site should remove a sufficient quantity of the T and RS from their respective containers to conduct the study; the remainder in each container should be retained as reserve samples in the original containers in accordance with the recommendations for handling and retaining reserve samples (quantity, access, storage, etc.) discussed below. However, if removal of the quantity necessary to conduct the study leaves the container with a remainder that is less than the recommended minimum quantity as described in section IV.B., more than one container should be shipped to the testing site and the techniques for multiple containers described below should be applied.

Multiple Containers – If multiple containers of the T and RS are provided to the testing site, the testing site should *randomly select* a sufficient number of containers of the T and RS to retain as reserve samples in the original containers; the remaining containers of the T and RS should be used to conduct the study.

Unit Dose – If the T and RS are provided to the testing site in **unit** dose packaging, the testing site should *randomly select* a sufficient quantity of unit doses of the T and RS to retain as reserve samples in the original unit dose packaging; the remaining unit doses of the T and RS should be used to conduct the study. *Providing the study medications (T and RS) in unit dose packaging and all the reserve samples (T and RS) in bulk containers is not recommended because it inappropriately precludes the testing site from randomly selecting reserve samples.*

Co-Packaged Products – If the BA or BE study involves testing co-packaged products (e.g., injector and vial), the testing site should *randomly select* a sufficient quantity of the T and RS co-packaged products (e.g., both drug and device) to retain as reserve samples in the original containers; the remaining samples of co-packaged products (e.g., drug and device) should be used to conduct the study.

Blinded Study – If the study is to be blinded and the T and RS (and placebo, (P), as applicable) are provided to the testing site in unit dose packaging with each unit dose labeled with a randomization code, the study sponsor and/or drug manufacturer should provide the testing site with a labeled set of the T and RS (and P, as applicable) sufficient to conduct the study and with additional, identically labeled sets sufficient for the testing site to retain the recommended minimum quantities for reserve samples identified in section IV.B. below.

FDA is aware that many study sponsors and/or drug manufacturers (or their SMOs) now use interactive response technology (IRT) for randomization. During inspections, we have seen instances where the study sponsor and/or drug manufacturer or SMO uses IRT to determine which containers to select as reserve samples or otherwise instructs the testing site to select specific pre-numbered containers as reserve samples. As noted above, reserve samples should not be selected by the study sponsor and/or drug manufacturer or other packaging site prior to reaching the testing sites. SMOs should not select reserve samples or tell testing sites which pre-numbered containers to retain as reserve samples (even when the selection is based on the use of IRT), because SMOs involved in packaging the T and RS (and P, as applicable) would know the identity of the individually numbered containers, and therefore such pre-selection could compromise the integrity of the study. This disrupts random selection and sometimes breaks the blinding of samples absent a clinical safety issue necessitating the unblinding. Study sponsors and/or drug manufacturers, SMOs, or other individuals should not instruct testing sites to select a specific pre-numbered container(s) as reserves; rather, testing sites should *randomly select* a sufficient quantity of the pre-numbered containers to retain as reserve samples.

Additionally, for blinded studies, the T and RS (and P, as applicable) are often shipped to the testing site in blocks (also referred to as blinded kits or labeled sets), so it is important that the testing site selects and retains intact blocks of product (which consist of T, RS, and P, as applicable) or that the process for selecting reserve samples permits the site to retain sufficient representative samples from blocks of T, RS, and P without un-blinding the samples. FDA recommends that the testing site *randomly select* a sufficient quantity of blocks from each shipment of product used in the study to retain as reserve samples in their original packaging and then use the remaining blocks to conduct the study.

We also recommend that the study sponsor and/or drug manufacturer provide to the testing site a sealed code (e.g., IRT blinded code or blinded label) with the blinded drug product at the beginning of the study for use by FDA should it be necessary to break the code. The sealed code should be maintained at the testing site. Alternatively, the sealed code may be archived with the study documents offsite once the study has been completed but should be readily accessible for use upon request by FDA.

Repackaged Products – Study sponsors and/or drug manufacturers that repackage T or RS drug products prior to shipping them to the testing sites (for example, in order to effectively blind a study when the RS is a commercially marketed drug product obtained in its original manufacturer's packaging) should ensure that the repackaged product retains the appropriate **batch** or **lot** number and expiration date associated with the product to allow for positive identification that the reserve samples are representative of what was used in the study.

Additionally, as a reminder, as with other drug products, the containers of the repackaged product must meet all container closure requirements in accordance with 21 CFR 211 and all requirements related to the product's labeling. Each testing site (not the repackager, study sponsor and/or drug manufacturer) conducting a BA or BE study should randomly select and retain reserve samples of the drug product used for the study from the study sponsor's and/or drug manufacturer's original container (which may have been previously repackaged from another manufacturer's container). Regardless of whether the T and RS are repackaged, each reserve sample must be stored under conditions consistent with product labeling, and must be adequately labeled so that the reserve sample can be positively identified as having come from the same sample as used in the specific BA or BE study. Adequate repackaging records (original manufacturer's container to repackaged container) should be maintained by the repackager, study sponsor and/or drug manufacturer and made available to the Agency upon request to help make a positive identification that the reserve sample was obtained from the same sample as used in the specific BA or BE study.

### B. Quantity of Reserve Samples[14]

The regulations at §§ 320.38 and 320.63 require the applicant or, if testing is performed under contract, its CRO to retain reserve samples of the T and RS used in conducting certain in vivo BA studies or an in vivo or in vitro BE study in a sufficient quantity to permit FDA to perform five times all the release tests required in the application or supplemental application. Because of technological advances in FDA's ability to test these products using methods that are less destructive and more sensitive, FDA can now detect the identity and composition of the test article and reference standard with smaller volumes of samples. As such, FDA finds it may be appropriate for applicants (or their CROs) to retain a lesser quantity of samples than what is specified in § 320.38(c), as long as it is still sufficient for FDA to conduct the necessary "chemical and physical examination of the samples to assure the identity and composition of the test article and reference standard" as intended by the regulation.[15] Under current physicochemical testing methods, the Agency generally needs the quantities described below to conduct the necessary testing of the samples. Accordingly, at this time and based on our current understanding of the risks involved, FDA generally does not intend to enforce the requirement to retain a sufficient quantity to perform five times all the release tests required in the application or supplemental application, so long as the identified lower quantities below are retained:

[14] As explained in the introduction, this section of the guidance is final and for immediate implementation. The conditions described in this section, under which FDA does not generally intend to take enforcement action against an applicant or CRO for retaining less than the quantity of reserve samples of the test article and reference standard that were used in the BA or BE study specified in § 320.38(c), supersede FDA's previous statements on the topic in the 2004 Guidance, the 2020 Compliance Policy (hereby withdrawn), and in section IX. Number of Reserve Samples for BA and BE Testing of the draft guidance *Nasal Aerosols and Nasal Sprays for Local Action* (April 2003). FDA is implementing this section of the guidance without prior public comment because the Agency has determined that prior public participation is not feasible or appropriate (see section 701(h)(1)(C)(i) of the FD&C Act and 21 CFR 10.115(g)(2) and (g)(3)). FDA made this determination because the approach in the guidance presents a less burdensome policy that is consistent with public health.

[15] 58 FR 25918, at 25923.

For in vivo studies: A minimum quantity of 30 single-dose (SD) or 3 **multi-dose (MD) units** each of the T and RS (and P if applicable) in the original container in total across all testing sites with at least 1 unit in the original container per treatment (or blinded kit, as applicable) retained from each shipment[16] used in the BA or BE study.[17]

For in vitro studies: A minimum quantity of 30 SD or 3 MD units in the original container *per batch* each of the T and RS in total for all in vitro studies conducted at the testing site with at least 1 unit in the original container each of the T and RS retained from each shipment used in the BE studies.

This compliance policy is applicable to all reserve samples for BA and BE studies held to date, including reserve samples from previously completed BA or BE studies.

The quantities above reflect differences between in vitro and in vivo studies. For example, because, unlike in vitro studies, in vivo studies often involve multiple testing sites, the lower quantities are reflective of the recommended minimum total quantity to be retained for the study across all testing sites for in vivo studies. Additionally, because FDA often recommends testing three different batches of drug product in in vitro BE studies, we recommend that the testing site randomly select reserve samples from each batch used in the BE study to help ensure random selection, prevent sample manipulation by sponsors, and avoid the potential for biased testing or sampling. Overall, the lower quantities described above help ensure the Agency will be able to collect a sufficient quantity of reserve samples for testing the identity and composition of the drug products used in each BA or BE study when necessary.

Testing sites should not open containers to retrieve the recommended minimum quantity for reserve samples. Rather, reserve samples should be retained in the original container as defined in the glossary. Depending on the study sponsor's and/or drug manufacturer's study design (i.e., number of testing sites, number of shipments of drug product to each testing site, etc.) and packaging of the drug product, the reserve quantity necessary to be retained may exceed the recommended quantity of 30 SD units or 3 MD units listed above. For example, if an in vivo BE study for a MD unit product has ten testing sites and each testing site receives five shipments of drug products, then it is recommended that at least one MD unit per treatment (or blinded kit, as applicable) be retained from each shipment, resulting in a total of at least 50 units per treatment (or blinded kits, as applicable) to be retained for the entire study. As another example, if an in vivo BE study for a SD unit product has five testing sites and each testing site receives two shipments of drug products that are packaged into ten-count bottles, then it is recommended that at least one bottle of SD unit per treatment (or blinded kit, as applicable) be

[16] If no subject of a BA or BE study received study drug from a shipment received at a testing site, then the site does not need to retain reserve samples from that shipment.

[17] Requests for a reduction in the quantity of reserve samples beyond the recommended minimum quantity described in this guidance for BA or BE studies involving multiple shipments and testing sites (e.g., comparative clinical endpoint studies) should include a detailed description, along with supportive documentation, of any *unusual* circumstances that may prevent a particular study from retaining samples from each shipment and will be addressed on a case-by-case basis.

retained from each shipment, resulting in a total of at least 100 units per treatment (or blinded kits, as applicable) to be retained for the entire study.

FDA strongly advises all study sponsors and/or drug manufacturers to retain or direct the retention of no fewer than the recommended minimum quantities as described above. If a testing site fails to retain any samples in a BA or BE study, the study data generated from that particular testing site may not be included in the Agency's assessment for BA or BE, which may result in an inadequate sample size for the required BA or BE study and trigger the need to redo the study.

### C. Retention for Multiple Shipments, Batches, and Studies

To ensure that reserve samples are representative of what was used in the BA or BE study as required by §§ 320.38(a) and 320.63, generally each testing site should randomly select a sufficient quantity as described above in section IV.B. *from each shipment* for each BA or BE study. We recognize that certain BE studies, such as comparative pharmacodynamic or comparative clinical endpoint BE studies, usually require multiple testing sites and multiple shipments of drug product to testing sites to complete the study. FDA recommends that applicants consider planning or modifying shipping patterns and/or the study design to minimize the number of shipments of drug products to testing sites to the extent possible in order to avoid having to retain excessive quantities of reserve samples. In the rare instance where there may be *unusual* circumstances that prevent a study from retaining the recommended minimum quantity of reserve samples as described in section IV.B. above, FDA recommends study sponsors and/or drug manufacturers submit their reserve sample plan proposal and justification for the proposal to the Agency for feedback prior to conducting the study.[18]

For in vivo studies, testing sites do not need to retain reserve samples from every lot or batch, within a single shipment or across multiple shipments, of T and RS used in a BA or BE study. However, FDA recommends as a best practice that study sponsors and/or drug manufacturers should send samples to testing sites in such a manner that the testing sites are able to randomly select reserve samples from the same batch. For example, if there are three different batches of drug product to be used in an in vivo BE study, the study sponsor and/or drug manufacturer could ensure that each shipment to the testing site(s) consists of only one batch of product. This would help ensure that the randomly selected reserve samples by the testing sites are representative of the drug products used in the study.

The quantity described in section IV.B. for in vivo studies should be kept per study, except in the limited circumstances where: more than one study with the same T and RS products is conducted at the same testing site (e.g., an in vivo BE or BA study under fasting conditions and an in vivo BE or BA study under fed conditions); the T and RS for the studies are provided to the testing site in the same shipment; and the T and RS used in each study are from the same batch or batches. In that case, the testing site could retain a single set of reserve samples of the T and RS in sufficient quantity across all the studies. In other words, if the same T and RS products provided to the testing site in the initial shipment are used in performing more than one study,

[18] See footnote 17.

only one set of reserve samples of the T and RS in sufficient quantity needs to be retained. The reserve samples should be identified as having come from the same batch or batches as used in each study. However, if one or more of those studies for which the testing site retained reserve samples from that initial shipment subsequently need an additional shipment(s) of the T and RS to complete the study(ies), the testing site should retain a sufficient quantity of reserve samples from each subsequent shipment to ensure that the reserve samples are representative of what was used in the BA or BE study(ies).

If a CRO with multiple testing sites conducts more than one BA or BE study (e.g., fed and fasted BE studies) for the same drug product, and the T and RS are shipped to the testing sites in multiple shipments, we recommend that a sufficient quantity of reserve samples be kept separately for each study at each testing site, as described in section IV.B.

These approaches are to ensure that the reserve samples are in fact representative of the drug product provided to the testing site and used in the study.

### D. Storage of and Access to Reserve Samples

In accordance with §§ 320.38(e) and 320.63, reserve samples must be stored for a period of at least 5 years following the date on which the application or supplemental application is approved, or the date of completion of the BA or BE study if such application or supplemental application is not approved. Additionally, reserve samples must be stored under conditions consistent with product labeling and in an area segregated from the area where testing is conducted and with access limited to authorized personnel. When there are multiple shipments, reserve samples selected from each shipment should not be commingled, but rather should be segregated and labeled to identify the shipment from which the samples were pulled. After the reserve samples have been randomly selected by the testing site, they may be sent to a separate facility for storage owned by an independent third party in accordance with § 320.38(h) and (i), with appropriate tracking and documentation. An **independent third party** means, at a minimum, having independent management (control) from the applicant/study sponsor and/or drug manufacturer to ensure that substitution of samples does not take place. Testing sites should ensure that any reserve samples transferred to a separate storage facility are not commingled with reserve samples from other testing sites so that any given reserve sample can be unambiguously associated with the testing site from which it came.

Some in vitro BE studies are conducted at the same place where the test articles are manufactured (in-house in vitro BE study). More rarely, some study sponsors and/or drug manufacturers conduct in-house in vivo BA and BE studies. In these cases, the study sponsor and/or drug manufacturer may store the reserve samples in the same facility, as long as the storage area is segregated from the area where the test articles are manufactured and testing is conducted, and access to the storage area is limited to authorized personnel in accordance with § 320.38(e). The study sponsor and/or drug manufacturer should have proper tracking and accountability, including access restrictions. Generally, manufacturing sites that store reserve samples should not commingle samples from manufacturing and packaging activities required under part 211 (21 CFR part 211) and reserve samples required under part 320 (21 CFR part 320). However, if the part 211 samples are from the same batch as the batch used in an in-house

in vitro BE study (or, in the more rare case, an in-house in vivo BA or BE study), the study sponsor and/or drug manufacturer can use the same samples to satisfy the samples requirements for both parts 211 and 320. The study sponsor and/or drug manufacturer must ensure the samples retained for both parts 211 and 320 are retained for the required duration in § 211.172 and § 320.38(e), whichever is longer, and are of sufficient quantity to meet the requirement at § 211.170(a) and the recommendations in section IV.B. of this guidance, whichever is greater.

Access to reserve samples should be limited to personnel authorized to manage and store the reserve samples. FDA also recommends that each testing site establish and maintain appropriate tracking of who accessed the reserve sample storage area, including when and why, for drug accountability. Any facility (e.g., testing site or independent third-party storage facility) storing reserve samples should document and maintain the transfer records for Agency verification. To ensure appropriate tracking and documentation of transfers of reserve samples, FDA recommends that the transfer records establish a chain of custody that is sufficient to allow FDA to trace the handling of those samples from what was used in the study back to the study sponsor and/or drug manufacturer of the product. Transfer records should include, among other supportive information:

- Dosing records (for in vitro studies, records of what was used in the analysis)
- Shipping records
- Temperature controls during transportation
- Sample records (quantity, unique sample numbers, batch number, expiration date)
- The dates of all activities (shipment and/or receipt, administration or dispensing of drugs)
- Quantity of reserve samples sent to third party for storage
- The name and address of the shipper and recipient of each shipment

Ultimately, the study sponsor and/or drug manufacturer should ensure the integrity of the shipments to the testing sites and proper storage of reserve samples.

## V. EXAMPLES OF TYPICAL ROLES IN VARIOUS STUDY SETTINGS

Because of the variety of study settings potentially involved in conducting BA and BE studies, several examples of study settings and associated typical roles for different entities 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. Where the study sponsor and/or drug manufacturer repackages samples prior to shipping them to the testing sites, the study sponsor and/or drug manufacturer should maintain adequate repackaging records (original manufacturer's container to repackaged container). Such records should be made available to the Agency to help make a positive identification that the reserve sample was obtained from the same sample as used in the specific BA or BE study. If a BA or BE study includes a P in addition to T and RS (e.g., comparative clinical endpoint study), the discussion in this section applicable to T and RS also applies to P where samples are blinded or where P is co-packaged with T and RS. Testing site(s) should not unblind samples or open co-packaged samples to avoid retaining P as reserve samples.

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

Study sponsors and/or drug manufacturers sometimes conduct BA and BE studies through CROs such as university faculty, hospitals, or investigators in private practice. For example, a study sponsor and/or drug manufacturer may contract with clinical study units in universities, hospitals, or clinics run by physicians. A study sponsor and/or drug manufacturer may also contract directly with a physician (investigator), who independently conducts a study at universities, hospitals, or clinics.

Many BA/BE studies of oral dosage forms are conducted at such CROs to support approval of ANDAs and NDAs (including ANDA and NDA supplements). Such studies are often conducted as single-site, open-label, crossover design studies with healthy volunteers as participants. These CROs conducting such BA/BE studies are considered the testing sites and their typical roles, relative to the role of the study sponsor and/or drug manufacturer, are described below.

The typical role of the study sponsor and/or drug manufacturer includes:

- Packaging, distributing, and shipping the T and RS to the testing site
- Monitoring the study if it is conducted under an investigational new drug application (IND) (rarely needed for most studies to support approval of an ANDA)

The typical role of the testing site includes:

- The investigator or designee (such as the study coordinator or research pharmacist of the testing site) should randomly select, as discussed in section IV.B., a sufficient quantity of T and RS from the supplies received from the study sponsor and/or drug manufacturer to retain as reserve samples in the original containers and use the remaining study samples to conduct the study (unless the testing site receives only a single container of bulk packaged single-dose unit product to perform the study, in which case a sufficient quantity should be removed from the container to conduct the study and the remainder in the container should be retained as reserve samples).
    - Each testing site should randomly select and retain its own reserve samples, even where multiple testing sites may be managed by the same CRO, to maintain representativeness of the samples used in the BA or BE study.
- The testing site should retain the reserve samples from each shipment.
- If the testing site does not provide storage for the reserve samples, 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 investigator or physician conducting the study should *not* send the reserve samples back to the study sponsor and/or drug manufacturer or any other organization that deals with manufacturing, distributing, or packaging the T and RS. 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

In vivo BA and BE studies managed by an SMO are frequently multisite, open-label studies of oral dosage forms in patients, but may also be multisite, blinded or open-label comparative pharmacodynamic or comparative clinical endpoint BE studies of nonoral dosage forms. Often, the study sponsor and/or drug manufacturer contracts with an SMO to recruit investigators and to monitor a study. The SMO is involved directly or indirectly (i.e., by subcontracting to another party) in packaging and shipping the T and RS to the testing sites. The testing sites are usually the clinical study units of universities, hospitals, other healthcare facilities, or other CROs. Some of these clinical study units may utilize a pharmacy on site to receive the drug products from the SMO or subcontracted packaging facility, dispense the drug products to the investigator for use in the study, and store the reserve samples.

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

The typical role of the study sponsor and/or drug manufacturer is to ship the T and RS to the SMO under contract, or to the packaging facility under subcontract to the SMO. Although the SMO is either directly or indirectly involved in packaging and shipping the T and RS to the testing sites, the study sponsor and/or drug manufacturer should remain responsible for maintaining the integrity of the drug products (T, RS, and, where applicable, P) during shipment to the testing sites.

The typical role of the SMO includes:

- Packaging, distributing, and shipping the T and RS to all testing sites (or subcontracting a packaging facility to perform these functions). For blinded studies, we recommend that the SMO provide the testing sites with enough code-labeled sets to conduct the study and retain a sufficient quantity of reserve samples. Based on FDA's inspection experience, the Agency does not recommend prenumbering the T and RS 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 studies to support approval of an ANDA)

The SMO should *not* randomly select and retain reserve samples. In addition, the SMO, and any other organization that deals with manufacturing, distributing, or packaging the T and RS, should not have the reserve samples transferred back to them for storage. As explained in the preamble to the 1993 final rule, the Agency intended for a testing site to be supplied with sufficient T and RS for the testing site to randomly select T and RS for retention as reserve samples and to conduct the study.[19]

The typical role of the testing sites includes:

- The investigator or designee (such as the study coordinator or the research pharmacist of each testing site) should randomly select, as discussed in section IV.B., sufficient T and RS to retain as reserve samples (in the original containers) from the supplies received from the SMO under contract, or from the packaging facility under subcontract with the SMO, and use the remaining study samples to conduct the study (unless the testing site receives only a single container of bulk packaged single-dose unit product to perform the study, in which case a sufficient quantity should be removed from the container to conduct the study and the remainder in the container should be retained as reserve samples). For blinded studies, the investigator should be aware of the sampling techniques used for blinded studies as described in section IV.A.
- Each testing site or the pharmacy of each testing site should retain the reserve samples, or arrange for storage by an independent third party. The reserve samples should *not* be transferred back to an SMO, study sponsor and/or drug manufacturer, or any other organization that deals with manufacturing, distributing, or packaging the T and RS, for storage. This is to eliminate the potential for fraud and avoid commingling samples from manufacturing and packaging activities (§§ 211.84 and 211.170 (21 CFR 211.84 and 211.170)) with reserve samples from BA or BE studies (§§ 320.38 and 320.63).
- The sealed treatment code of the study should be kept at the testing site. This is applicable even if the reserve samples are forwarded to an independent third party.

Below is a suggested packaging and random selection plan for an open-label, multisite study of a tablet product involving an SMO:

> The study enrolls 300 subjects with approximately 60 subjects each at five testing sites. In preparation for conducting the study, the SMO prepares 310 14-count bottles of T and repackages 100-count bottles of RS into 310 14-count bottles of RS and plans to make two shipments to each clinical testing site over the course of the study. The SMO ships 31 bottles each of T and RS to each clinical testing site in each shipment. Each testing site randomly selects one bottle each of T and RS per shipment to retain as reserve samples and uses the remaining 30 bottles each of T and RS to dose 60 subjects. Since one bottle each of T and RS are kept per shipment at each of five testing sites (with two shipments to each testing site), 10 14-count bottles (140 tablets) each of T and RS are retained to satisfy the recommended minimum quantity (i.e., 30 SD units across all testing sites with at least 1 unit per treatment from each shipment) described above in

[19] 58 FR 25918 at 25920.

section IV.B. In addition, the sampling process used here permits representative reserve samples and random selection by each testing site.

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

> The study enrolls 300 subjects with approximately 60 subjects at each of five testing sites. In preparation for conducting the study, the SMO blinds the samples by preparing 105 blocks of drug products that contain one code-labeled tube of T, one code-labeled tube of RS, and one code-labeled tube of P in each block. The SMO plans to ship all products for the study in a single shipment to each testing site. The SMO ships 21 blocks of drug products to each clinical testing site. Each testing site randomly selects one block to retain as reserve samples and uses the remaining 20 blocks to dose 60 subjects. In this example, staff (e.g., a research pharmacist) not involved with the study should ensure the study remains blinded. This packaging system ensures that an equal number of T, RS, and P are administered to the subjects at each site, and that an equal number of T, RS, and P will be maintained as reserve samples. Since one block is kept at each of five testing sites, five tubes each of T, RS, and P are retained in total to satisfy the recommended minimum quantity described above in section IV.B. (i.e., three MD units in total across all testing sites with at least 1 unit from each shipment). In addition, the sampling process used here permits representative reserve samples and random selection by each testing site.

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

If a study sponsor and/or drug manufacturer conducts an in-house BA or BE study, samples from manufacturing and packaging activities (required under § 211.170) and BA or BE study reserve samples (required under §§ 320.38 and 320.63) should be stored separately, except in the limited circumstances described in section IV.D. above. The in-house clinical research unit, for purposes of this guidance, is considered to be the testing site and should operate as an independent unit for the purposes of sample retention. All matters (e.g., manufacturing, purchasing, packaging, transfer records) concerning the T and RS should be clearly documented and available to FDA investigators during an inspection. Standard procedures concerning security and accountability of the T and RS for each study should be established to eliminate the possibility of sample substitution. Study sponsors and/or drug manufacturers conducting in-house studies can engage an independent third party to store reserve samples in accordance with the recommendations described in section IV. above. If a study sponsor and/or drug manufacturer conducting in-house studies chooses not to utilize an independent third party to store reserve samples, they should ensure that reserve samples are retained in accordance with section IV. above and there should be (1) a totally segregated and fully compliant in-house storage area; (2) procedures and policies in place to show that adequate T and RS are retained; (3) controlled access to the reserve samples limited to personnel authorized to manage and store the reserve samples; (4) appropriate tracking of who accessed the reserve sample storage area, including when and why, for drug accountability; and (5) a rigorous and unbroken chain of custody for the reserve samples.

The typical role of the study sponsor and/or drug manufacturer (clinical research department) includes packaging and transferring the T and RS to the testing site (in-house clinical study unit).

The typical role of the testing site (in-house clinical study unit) includes:

- Documenting all matters concerning the transfer and receipt of the T and RS
- Randomly selecting sufficient T and RS to retain as reserve samples and using the remainder to conduct the study (unless the testing site receives only a single container of bulk packaged single-dose unit product to perform the study , in which case a sufficient quantity should be removed from the container to conduct the study and the remainder in the container should be retained as reserve samples). The selection is generally made by the 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.
- Retaining 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.
- Preparing adequate storage of reserve samples. If the in-house testing sites 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.

### D. In Vitro BE Studies

For an in vitro BE study, the typical roles of the study sponsor and/or drug manufacturer and the testing site are similar to those described above for in vivo BA and BE studies conducted by CROs and in the examples of in vivo BA and BE studies conducted in-house by a study sponsor and/or drug manufacturer. As discussed above in section IV.B., the testing sites should randomly select and retain a recommended minimum quantity of 30 SD or three MD units in the original containers *per batch* each of the T and RS in total for all in vitro studies conducted at the testing site with at least one unit each of the T and RS retained from each shipment used in the BE studies. In a typical in vitro BE study that involves testing three separate batches of the T and RS, there should be 30 SD or three MD units in the original containers retained for each of the three T and RS batches as reserve samples, with at least one unit each of the T and RS retained from each shipment. FDA may need to differentiate between the RS and the three different batches of T in the course of investigating or assessing an in vitro BE study. We also recommend retaining reserve samples per batch for in vitro studies to help ensure random selection to the extent possible where the batches are openly identified for purposes of the in vitro studies, prevent sample manipulation by sponsors, and avoid the potential for biased testing or sampling. For purposes of this guidance, the typical roles of an investigator as discussed in the above sections apply to the principal investigator of an in vitro BE study to the extent applicable to an in vitro study.

## GLOSSARY

For purposes of this guidance,[20] key terms are defined as follows:

**Applicant** – any person who submits an NDA (including a 505(b)(2) application) or ANDA or an amendment or supplement to an NDA or ANDA under Part 314 to obtain FDA approval of a new drug and any person who owns an approved NDA (including a 505(b)(2) application) or ANDA (see 21 CFR 314.3).

In the context of §§ 320.38 and 320.63, the term *applicant* includes, as appropriate, *study sponsor and/or drug manufacturer*.

**Batch -** a specific quantity of a drug or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture.

**Container -** the immediate unit, bottle, vial, ampule, tube, or other receptacle containing the drug product (test article or reference standard). As used in this guidance, container does not refer to the shipping container within which the samples were shipped to the testing site.

**Contract Research Organization (CRO)** –an independent contractor of the study sponsor or drug manufacturer that assumes one or more of the obligations of a study 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). This guidance addresses BA and BE studies submitted to support approvals of drug products under NDAs and ANDAs. These studies are usually conducted by CROs under contract to study sponsors and/or drug manufacturers. Many CROs have their own testing sites, with physicians (to serve as investigators) and clinical support staff (e.g., nurses, medical technologists) to conduct the BA and BE studies.

In the context of §§ 320.38 and 320.63, the term *CRO* refers to any party contracted to help conduct BA or BE testing, including, as appropriate, *site management organizations* (SMOs), *investigators*, and *testing sites*.

**Independent Third Party** –an entity or site that is not overseen or directed by the applicant/ study sponsor and/or drug manufacturer.

**Investigator** –an individual who actually conducts a BA or BE investigation (for example, a physician under whose immediate direction the drug is administered or dispensed to a subject). When conducting a BA or BE study, the investigator should select the reserve samples from each shipment and ensure the reserve samples are appropriately retained at the testing site or through an independent third party.

[20] The definitions provided here are intended solely for purposes of this guidance and reflect FDA's interpretation of these terms as used in 21 CFR 320.38 and 21 CFR 320.63. The same terms may have different meanings in other contexts.

**Lot –** A batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by continuous process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits.

**Multi-Dose (MD) Unit –**a unit that has sufficient amount of the drug product (test article or reference standard) to deliver more than a single dose. For example, a single tube of ointment that contains multiple doses, or an inhaler device that delivers multiple doses of the drug from a single canister.

**Original Container –**the study sponsor's or drug manufacturer's container received by the testing site.

**Reference Standard** *–reference standard (RS)* is intended to be consistent with its usage and/or meaning in 21 CFR 320.38 and 21 CFR 320.63. For in vivo BE studies, reference standard has the meaning in 21 CFR 314.3(b).

**Shipment –**all the drug product (test article and reference standard) that is shipped together to a testing site at one time.

**Single-Dose (SD) Unit** –a unit that only contains the amount of the drug product (test article or reference standard) to deliver a single dose of the drug product. For example, tablets or capsules (packaged in bottles or unit dose blisters), or an inhaler device that requires the patient to insert an individual capsule into the device for each dose would be considered a single-dose unit.

**Site Management Organization (SMO)** –an organization that manages clinical testing sites on behalf of the study sponsor and/or drug manufacturer.

**Study Sponsor** – A person who takes responsibility for and initiates a BA or BE (in vivo or in vitro) study. The study sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization.

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 Site(s)**– the site(s) where the BA or BE (in vivo or in vitro) study is conducted. The testing site can be at a university, hospital, clinic of an investigator, or other CRO, 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 1993 final rule, the Agency intended that reserve samples should generally be kept at the testing site.

**Unit** - the individual drug product to be dispensed or administered to study subjects. For example, 30 units of an oral tablet means 30 oral tablets.