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*Themed Issue: Bioanalytical Method Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays*
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# Specificity and Selectivity Evaluations of Ligand Binding Assay of Protein Therapeutics Against Concomitant Drugs and Related Endogenous Proteins

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## ABSTRACT

Macromolecule drugs designed against specific target proteins/receptors have been applied in combination therapies, especially for complex and related diseases such as cancer for synergistic efficacy and alleviation of side effects. Protein therapeutics are typically measured using ligand binding assays (LBA). Evaluating the specificity and selectivity of LBA against their target proteins or in instances where concomitantly administered drugs are given was brought up during a conversation at the 3rd American Association of Pharmaceutical Scientists/US Food and Drug Administration Bioanalytical Workshop but was not discussed at the meeting sessions. The purpose of this article is to discuss the challenges related to this issue and present a few approaches and experiences to elicit further discussions.

Specificity and selectivity tests should be based on the anticipated levels of the individual therapeutics with reference to the dosing regimens defined in the clinical study protocol. When the concomitantly administered compound is available as a pure or well-defined material, various concentrations from zero to above the expected high levels are added to validation samples of the protein therapeutics to assess specificity. Recovery results from spiked samples of target patient populations on concomitant medications can also be compared with those from normal individuals for selectivity. If the drug has an endogenous counterpart, the baseline concentrations of each lot should be subtracted from the test samples in the selectivity assessment. This article illustrates a flexible approach to evaluating specificity and selectivity on samples from target patient populations receiving multiple medications.

**KEYWORDS:** Binding assay, protein therapeutics, concomitants, specificity, selectivity

## INTRODUCTION

The increased knowledge of biological pathways and permutation has led to the understanding of multiple causal factors of disease progression and various points of control to develop drugs based on mechanism. Drugs of different mechanisms of action have been used in combination therapies to provide synergistic effects, especially for complex and related diseases such as cancer and cardiovascular, hormonal, and immune disorders.[1-4] Because of synergistic, improved efficacy, doses can be lower than they would be for monotherapy of the individual drug, which alleviates side effects.[5,6] Many macromolecule drugs are designed against a specific target protein/receptor based upon a specific mechanism. Combination therapy using macromolecule drugs is increasingly popular. Large-molecule drugs can be used with small molecule and or another macromolecule drug in multitudes of dosing regimens. Information on dose-effect relationships from pharmacokinetic (PK) and pharmacodynamic modeling is important to aid decision making for patient treatment regimens. Ligand binding assays (LBA) are the major bioanalytical technique used to generate PK data for macromolecules. Ensuring the specificity and selectivity of LBA can be challenging given the presence of their target proteins and the use of concomitantly administered drugs. This issue was brought up in conversation during the 3rd American Association of Pharmaceutical Scientists/US Food and Drug Administration Bioanalytical Workshop but was not part of an open discussion at the meeting sessions. The purpose of this article is to discuss the challenges related to this issue and to present a few approaches and experiences in the form of case studies to elicit further discussions.

## CHALLENGES OF LBA FOR SPECIFICITY AND SELECTIVITY EVALUATIONS

Macromolecule protein therapeutics are of various types, including endogenous proteins or structurally similar analogs of endogenous proteins, monoclonal antibodies against a target protein or receptor, and peptides conjugated to a polymer or antibody. As opposed to small-molecule drugs, LBA used for macromolecules do not directly measure the molecule itself but indirectly measure a binding reaction with the reagents employed in the assay. This indirect measurement poses unique challenges when demonstrating specificity and selectivity, which require different considerations as compared with the

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typical bioanalytical chromatographic methods used to measure small-molecule drugs.

Bioanalytical methods for small-molecule drugs use liquid chromatography-tandem mass spectrometry (LC-MS/MS). Such analysis is preceded by a process where the analyte is extracted from the matrix components. The process usually involves protein precipitation with organic solvents, followed by either liquid-liquid or solid phase extraction. The extraction procedures can serve to concentrate the analyte while removing the extraneous matrix materials, which can increase the signal-to-noise ratio of the assay at low levels. High-performance chromatography of the extract with flexible choices of analytical columns and elution conditions further isolates the analyte before quantification in the mass spectrometer (MS). A triple-quadrupole MS in the multiple reaction mode is commonly used for small molecules. It filters out and unequivocally selects the accurate mass/charge ratios of the analyte molecular ion and its product ions for high specificity. In addition, the use of a stable heavy-isotope label of the analyte as an internal standard (IS) corrects for extraction recovery and ionization variability, and normalizes the matrix effect of the LC-MS/MS detection for high selectivity. On the other hand, with the exception of some small peptides, most LBA have no extraction since the extraction procedure for small molecules would denature most protein/peptide compounds. The processing step before the binding reaction is often a simple dilution with the assay buffer. LBA do not have a comparable means of correction for assay variability and matrix effect between the individual samples such as the use of an IS. Since an LBA measures the macromolecular therapeutic within the biologic matrix, the specificity and selectivity are dependent on the ligand binding reagents.

Unlike the small molecules, the catabolic species of macromolecule drugs are not well defined and/or purified for investigation of possible interferences caused by the biotransformed fragments. Therefore, the specificity test design for a concomitant protein drug would not be as straightforward as that of a small-molecule drug with known metabolites. The macromolecule drug can be an analog to an endogenous protein. Such proteins have a heterogeneous nature (ie, multiple isoforms or clipped forms may exist in the matrix), which complicates specificity assessments. The magnitude of interference impact on the measurement of the analyte is dependent on the abundance of the interfering material. Since the concentrations of the macromolecule drug are often much higher relative to the endogenous counterpart, in this case, interference may not be an issue.

Specificity and selectivity are dependent on the ligand reagents and the patient biology. If the macromolecule drug is a monoclonal antibody against a target protein, the presence of the soluble form(s) of the target protein may interfere with LBA; this would be especially true when the target protein is used as the ligand in the binding reaction. There are good examples where the concentrations of the endogenous protein or its soluble forms are increased because of a compensatory mechanism.[7-10] Investigation will be warranted in such scenarios. An understanding of the biology behind the therapeutic, including the compensatory mechanisms, can help define the investigation that should be used in examining the specificity of a particular method. An additional complicating factor may be the presence of an endogenous protein or receptor that binds the target protein, which may in turn interfere with LBA.

It is a challenge to the analyst to recognize the particular reactions between the reagents and the analyte and then to decide on the type of experiments to be employed to prove the specificity and selectivity of LBA during method validation. Our experience has shown that this should be a case-by-case decision dependent on the method and the concomitant drugs being administered. When possible and appropriate, a test plan describing the specificity and selectivity evaluation should be drafted as part of a validation plan before method validation experiments begin.

## Assessment of Interference

To date, there is no consensus on how specificity and selectivity should be evaluated and expressed for LBA of large molecules.[11-16]

The specificity of LBA is the ability of assay reagents (eg, antibodies) to distinguish between the analyte, which the reagents are intended to detect, and other structurally similar components. Lack of specificity caused by cross-reaction of the structurally similar compounds in the matrix often leads to false positives and/or overestimation of the analyte concentration. Selectivity is the ability of LBA to determine the analyte unequivocally in the presence of components that may be expected to be present in the sample. Lack of selectivity could result in inhibition or enhancement of the binding reactions, caused by agonistic or antagonistic factors in the matrix. In general, signal suppression from binding proteins occurs more often than does enhancement, resulting in a negative bias. The extent of interference is the product of the concentration of the interfering molecule and the cross-reactivity (or inhibition). However, the concentration-response relationship of LBA is nonlinear, and often the magnitude of cross-reactivity (or inhibition) is not monodispersed over the entire assay range. Specificity and selectivity are method-dependent for LBA. For example, if one of the ligand pairs is an antibody against the fragment cystalizable (FC) portion, interference may occur from a concomitant drug of the same immunoglobulin (Ig)G subclass. If the ligand is the target protein or its analog, the presence

of the endogenous target protein at high concentration in certain patients could interfere with the assay.

The conventional cross-reactivity estimation requires that a pure (or well-characterized) reference material of the potentially interfering substance be available. This is commonly done when LBA are employed to measure conventional low-molecular-weight drugs. Standard curves in a buffer solution of the analyte and the potentially interfering molecule are compared side by side. The percent cross-reactivity is calculated from the ratio of the midpoints of the 2 binding curves (50% of the effective dose [$ED_{50}$]). When binding curves are parallel, the expressed percent cross-reactivity would be equivalent regardless of which point, such as $ED_{20}$, $ED_{50}$, or $ED_{80}$, is chosen for the comparison. However, the binding reactions of the analyte and the interfering molecule are often dissimilar, with different slopes and asymptotes. Moreover, pure reference material of the combination therapy and its metabolites is not often available.

Knowledge of the expected concentrations of the analyte/interfering molecule may not be available to the analyst designing specificity and selectivity experiments. The concentrations of the analyte and the interfering molecule along the PK profile can vary. If the combination therapy is another macromolecule, incurred samples from monotherapy study of that compound would contain the biotransformed species, which could be used to test for interference. Although the exact concentrations of the interfering molecule are unknown, the dynamic ratio of analyte/interfering molecule can change over the time course of plasma concentrations. For example, at the $T_{max}$ the analyte might be high enough to overshadow the contribution from the interfering molecule, while this may not be true at the elimination phase or at a trough level. One option would be to use the incurred samples from monotherapy studies of the concomitant drug, including pooled samples from time points around the $T_{max}$, and others during the elimination phase or at trough levels. However, the availability of incurred samples could be an issue for this approach. This would be especially true if the therapeutics are from products of different companies or if there has been a substantial time lapse between the 2 development programs. Another option is to use validation samples (VS) in a checkerboard design with cross-mixtures of high- and low-concentration combinations of the analyte and the concomitant drug as test samples.

The compensatory feedback mechanism of the target protein and other related binding proteins can be affected by dosing regimens and could vary with time and patient population, producing differences in the extent of interference. In this instance, matrix samples from several individuals of the same target populations can be used for evaluation.

The following illustrations present a case-by-case approach on how to establish the specificity and selectivity of LBA for several protein drugs against concomitantly administered drugs and related endogenous proteins. The method used was enzyme-linked immunosorbent assays (ELISA).

## Case Illustrations

### *Specificity Test Assessing Recovery of Known Additions of Concomitantly Administered Drug to Validation Samples*

The following 2 examples illustrate a straightforward approach of adding known amounts of a concomitantly administered drug to VS at levels 1 time to 1000 times the upper limit of quantification (ULOQ) of the drug analyte. These levels are added to VS prepared at the lower limit of quantification (LLOQ) and the ULOQ. These VS are compared with control samples with none of the test compounds added. The capture and detector reagent of this ELISA method was both anti-idiotypic monoclonal antibodies.

The specificity of Compound A, a recombinant human protein developed for cancer adjuvant therapy, was tested using doxorubicin, ifosfamide, and pegfilgrastim as potentially interfering compounds. Human serum samples were spiked with these drugs at 0, 1.8, 18, 180, and 1800 ng/mL and mixed with equal amounts of VS spiked with the drugs at the following levels: zero, LLOQ (0.072 ng/mL), and ULOQ (1.8 ng/mL). As shown in Table 1, no false levels of Compound A were found in the blank samples spiked with the 3 test compounds over the range of 1.8 to 1800 ng/mL. The addition recoveries of the VS at both the LLOQ and the ULOQ in the presence of the spiked test compounds were similar to those of the zero spiked samples. All recoveries were accurate within 10% of the nominal VS concentrations.

Table 2 shows the specificity test of Compound B, a monoclonal antibody under development for cancer that will be measured in the presence of a marketed protein therapeutic. The test compound is another monoclonal antibody cancer drug that employs a different mechanism of action and is being codeveloped for combination therapy with Compound B. The range of the concentrations of the test compound covered up to more than 100 times the expected concentrations of ex vivo samples. The checkerboard design of the 5 levels in ng/mL of VS against 4 to 5 levels in μg/mL of the test compound is useful for assessing the effects of various concentration combinations that may occur in different clinical protocols of varying doses and dosing times. The results show that there was no effect on the addition recovery of the VS from all the tested ex vivo concentrations of the concomitantly administered drug. For this method, the capture agent of LBA is the target protein. The established specificity

**Table 1.** Effect of Concomitantly Administered Test Compounds on the Accuracy of Addition Recovery of Compound A Validation Samples*

| | | Validation Sample Compound A Conc† | | | | |
|---|---|---|---|---|---|---|
| | | Blank | 0.072 ng/mL | | 1.8 ng/mL | |
| Test Compound | Test Compound Conc (ng/mL) | Observed Conc (ng/mL) | Observed Conc (ng/mL) | % Diff From Nominal | Observed Conc (ng/mL) | % Diff From Nominal |
| doxorubicin | 0 | BQL | 0.065 | –9.7 | 1.79 | –0.7 |
| | 1.8 | BQL | 0.074 | 2.8 | 1.85 | 3.0 |
| | 18 | ND | 0.068 | –5.6 | 1.88 | 4.4 |
| | 180 | BQL | 0.068 | –5.6 | 1.81 | 0.6 |
| | 1800 | BQL | 0.065 | –9.7 | 1.91 | 6.2 |
| ifosfamide | 0 | BQL | 0.065 | –9.7 | 1.79 | –0.7 |
| | 1.8 | BQL | 0.073 | 1.4 | 1.74 | –3.2 |
| | 18 | ND | 0.066 | –8.3 | 1.78 | –1.3 |
| | 180 | BQL | 0.071 | –1.4 | 1.71 | –5.0 |
| | 1800 | BQL | 0.065 | –9.7 | 1.81 | 0.3 |
| pegfilgrastim | 0 | BQL | 0.065 | –9.7 | 1.79 | –0.7 |
| | 1.8 | BQL | 0.076 | 5.6 | 1.72 | –4.3 |
| | 18 | ND | 0.071 | –1.4 | 1.70 | –5.3 |
| | 180 | BQL | 0.073 | 1.4 | 1.70 | –5.6 |
| | 1800 | BQL | 0.068 | –5.6 | 1.76 | –2.2 |

*Compound A validation samples at zero (blank), lower limit of quantification (0.072 ng/mL), and upper limit of quantification (1.8 ng/mL) with and without the addition of the test compounds (doxorubicin, ifosfamide, and pegfilgrastim) in human serum were analyzed with a validated enzyme-linked immunosorbent assay. Assay acceptance criterion of the VS in this method were within 20% of the nominal value. Conc indicates concentration; diff, difference; BQL, below quantifiable limit; ND, not done.
†Each data point from the mean of 3 determinations.

against the test compound also confirms the separate mechanism of action of the combination therapy.

### *Specificity Test for a Monoclonal Antibody Against a Similar Class of Immunoglobulin and Selectivity Test Against an Endogenous Protein*

This example illustrates the approach to test both the specificity of the ELISA method against a structurally similar immunoglobulin and the selectivity against a soluble form of the target receptor protein for a monoclonal antibody. Anti-idiotypic monoclonal antibodies were used for the capture and detector reagent of this method.

Compound C is a fully human monoclonal antibody of the $IgG_1$ subclass. It blocks a target protein binding to receptor X. The ectodomain of receptor X can be found in the circulation as a soluble protein X (PX). The 2 potential cross-reactive proteins, PX (in a recombinant form, rPX) and another in-house compound of the same $IgG_1$ subclass, were tested at 0.1, 1, 10, 100, 1000, 10 000, and 100 000 ng/mL. Compound C VS at 0, 15.5 (LLOQ), and 901 ng/mL (ULOQ) were spiked with the test compounds at these 7 concentrations. The mean concentrations of all blank matrices (0 ng/mL Compound C) prepared with either rPX or $IgG_1$ were found to be below the LLOQ, indicating the lack of cross-reactivity from the test compounds in the assay. As shown in Figure 1, left panel, $IgG_1$ at all test concentrations did not cause unacceptable bias at both LLOQ and ULOQ concentrations. The right panel of Figure 1 shows that rPX

**Table 2.** Specificity of Enzyme-Linked Immunosorbent Assay of Compound B, a Monoclonal Antibody, Against Another Concomitantly Administered Monoclonal Antibody*

| | Concentration of Compound B Validation Samples (ng/mL) | | | |
|---|---|---|---|---|
| Concentration of Test Compound (μg/mL) | 20 | 50 | 250 | 1250 |
| | % Recovery | | | |
| 0 | 92.9 | 114.8 | 94.7 | 98.4 |
| 25 | 115.1 | 107.6 | 97.4 | 108.2 |
| 100 | 92.0 | 121.2 | 99.6 | 96.9 |
| 200 | 99.2 | 117.6 | 100.7 | 94.7 |

*The prestudy validation specificity test was performed with 4 concentrations of Compound B across the assay range, with and without the addition of the test compound at 3 levels. The highest level is at least 100 times the expected high concentration from ex vivo samples.

at higher than 1000 ng/mL caused unacceptable negative bias, which increased with concentration. It is expected that rPX at high concentrations could bind significant amounts of Compound C to cause the negative bias. However, the low physiological PX concentrations would not be sufficient to cause the interference. Since the concentrations of PX in serum can be tracked in the pharmacodynamic portion of the clinical study, any nonphysiologically high concentrations at greater than 1000 ng/mL can be recognized and further investigated for their impact on the PK data.

### *Selectivity Test for an Endogenous Protein Drug Against Drugs Expected To Be Given in Cancer Patients on Combination Therapies*

This example illustrates a selectivity test for epoetin alpha (a protein drug with an endogenous counterpart) using serum samples from cancer patients on combination therapies. The Quantikine IVD Erythropoietin ELISA Kit (R&D Systems, Minneapolis, MN) was modified by substituting the capture antibody with an in-house erythropoietin-specific antibody for this method. Samples from 10 individual patients with breast, prostate, colon, and head and neck cancer on various medications were tested in comparison with a noncancerous population of smokers and nonsmokers as controls. Tests were performed with and without the addition of epoetin alpha at 6 and 60 mU/mL, the low- and high-QC concentrations of the assay. Spiked recovery of each lot was calculated after subtraction of its own basal level and expressed as percent difference from the nominal spiked concentration. At shown in Table 3, the basal levels varied a lot from individual to individual, especially among the smokers (4.14-60.3 mU/mL). However, the baseline-subtracted concentrations within and among all 3 populations were similar, with coefficient of variance (%CV) ranges of 3.4% to 7.7% and 13.3% to 14.5% for 60 and 6 mU/mL spiked samples, respectively. The mean values of the corrected concentrations from cancer patients on various combination therapies were similar to those of the noncancerous population. The results show that no difference was observed on the assay in test sera from patients on combination therapies such as the cytotoxic drugs and trastuzumab, as listed in Table 3.

There was an overall negative bias for the percent recoveries of epoetin alpha from all serum samples, with approximately –20% for cancer patients and –30% for noncancerous patients. This could be a combination of preparative systematic bias and matrix effect, since the standards in this method were prepared in a protein buffer solution. For the assessment of specificity and selectivity, rather than evaluate from an absolute recovery based on the nominal concentration, it is more appropriate to compare the experimental results against the control set. For evaluation of multiple lots, the mean of the lots can be used instead of the nominal concentration to eliminate the systematic bias from spiking. Using the mean of the nonsmokers as a control, the cancer patient mean results in Table 3 were 16.8% and 7.3% different for 60 and 6 mU/mL spiked samples, respectively.

## CONCLUSIONS

The evaluation of assay specificity and selectivity should be clinically relevant. Because there are many variables in clinical trial protocol design and the types of concomitantly administered drugs, an understanding of the possible problem and rational approaches should be used to investigate and establish assay specificity and selectivity. Clinical trial protocols that evaluate combination therapies of macromolecule drug(s) vary with the type of mechanism of action, which

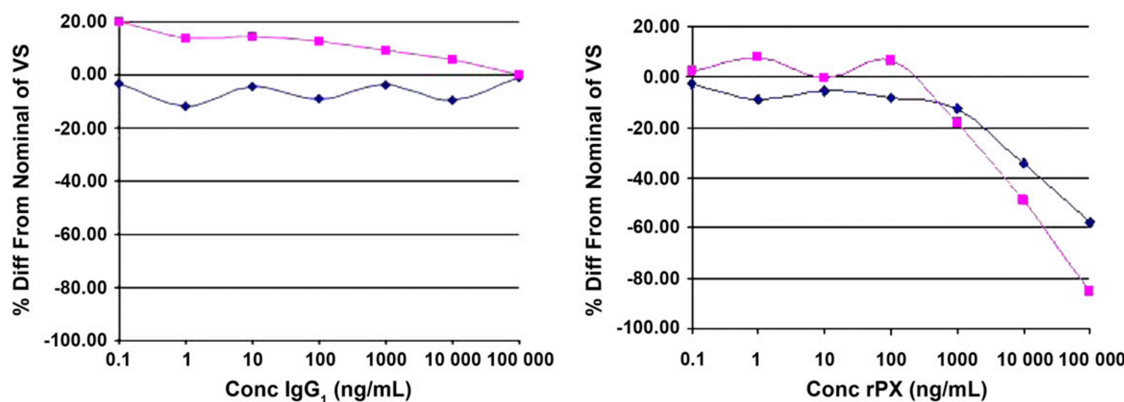


**Figure 1.** Specificity test of Compound C against another IgG$_1$ compound and the target protein receptor in a recombinant form, rPX. rPX and IgG$_1$ at 0.1, 1, 10, 100, 1000, 10 000, and 100 000 ng/mL were tested against Compound C validation samples at 0, 15.5 (LLOQ), and 901 ng/mL (ULOQ). The rPX used in the experiment was derived from a mouse myeloma cell line expressing a DNA sequence encoding the extracellular domain of the receptor. Symbols: diamonds, LLOQ; squares, ULOQ. Left panel: IgG$_1$; right panel: rPX. Diff indicates difference; VS, validation samples; rPX, recombinant protein X; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

**Table 3.** Selectivity Test for Epoetin Alpha*

| | | | Spiked rEPO 60 mU/mL | | | Spiked rEPO 6 mU/mL | | |
|---|---|---|---|---|---|---|---|---|
| | **Treatment** | **Basal Conc** | **Obs Conc** | **Corr Conc** | **Spike Recovery** | **Obs Conc** | **Corr Conc** | **Spike Recovery** |
| **Patient Type** | **Medications** | **(mU/mL)** | **(mU/mL)** | **(mU/mL)** | **% Diff From Nominal** | **(mU/mL)** | **(mU/mL)** | **% Diff From Nominal** |
| Breast cancer | doxorubicin, docetaxel, capecitabine | 12.2 | 59.1 | 46.8 | –21.9 | 17.1 | 4.88 | –18.7 |
| Colon cancer | 5-FU | 13.6 | 60.2 | 46.7 | –22.2 | 18.0 | 4.42 | –26.4 |
| Prostate cancer | zoledronic acid, leuprorelin, docetaxel | 16.0 | 64.4 | 48.4 | –19.3 | 21.0 | 4.96 | –17.4 |
| Breast cancer | trastuzumab, docetaxel, carboplatin | 16.3 | 65.9 | 49.6 | –17.3 | 21.6 | 5.37 | –10.5 |
| Prostate cancer | docetaxel | 18.5 | 67.1 | 48.6 | –19.0 | 24.6 | 6.09 | 1.5 |
| Colon cancer | carboplatin | 20.7 | 67.7 | 47.0 | –21.6 | 24.5 | 3.77 | –37.1 |
| Breast cancer | zoledronic acid, docetaxel, gemcitabine | 24.4 | 71.9 | 47.6 | –20.7 | 28.8 | 4.49 | –25.2 |
| Breast cancer | zoledronic acid, gemcitabine, abraxane | 27.6 | 75.8 | 48.2 | –19.7 | 32.0 | 4.40 | –26.7 |
| Breast cancer | capecitabine, zoledronic acid | 29.2 | 73.6 | 44.4 | –25.9 | 33.5 | 4.33 | –27.9 |
| Head and neck cancer | darbepoetin alpha, paclitaxel | 41.0 | 86.1 | 45.1 | –24.8 | 45.0 | 4.05 | –32.5 |
| Mean (range) of 10 patient lots | | 21.9 (12.2-41.0) | | 47.2 (3.4)† | –21.3 | | 4.68 (14.4)† | –22.1 |
| Mean (range) of 14 lots from noncancerous smokers | | 15.2 (4.14-60.3) | | 39.6 (7.7)† | –34.0 | | 4.21 (14.5)† | –29.8 |
| Mean (range) of 25 lots from noncancerous nonsmokers | | 9.18 (1.38-22.7) | | 40.4 (5.6)† | –32.7 | | 4.36 (13.3)† | –27.3 |

*The noncancerous donors included 14 smokers and 25 nonsmokers of apparently healthy status. Ten serum lots from cancer patients who were on various medications were tested. Each value of the noncancerous lot was from 1 determination (2 wells), while that of the cancer patients was the mean of 2 determinations (2 × 2 wells). Corr conc = obs conc – basal conc of each serum lot. Spike recovery % diff from nominal = (Corr conc/nominal conc – 1) × 100. rEPO indicates recombinant erythropoietin; conc, concentration; obs, observed; corr, corrected; diff, difference; 5-FU, 5-fluorouracil.
†Parenthetical values are percent coefficient of variance.

determines the target proteins and can lead to varying matrix effects in LBA. Therefore, traditional cross-reactivity testing of the concomitantly administered drug in LBA would be insufficient. However, it can be used as an initial specificity assessment during method development. The specificity tests can be performed with the available reference material from the known compounds. If the interfering species are unknown and/or reference material of the test compounds are unavailable, selectivity tests can be performed with samples obtained from the target population and incurred samples from monotherapy of the concomitantly administered drug, if available. The overall approach for specificity and selectivity tests is summarized in a flow diagram in Figure 2.

No single approach will fit all the scenarios of combination therapies and protocol design varieties. We presented only a few examples above, where specificity and selectivity were tested in VS of the analyte in biological matrix using a checkerboard design. Multiple concentrations of the VS are tested against various levels, from the trough to concentrations above the expected high levels of the test compounds. If a pure reference material of the test compound is not available to conduct the test, and/or the expected concentrations of the concomitant drugs are unknown for the particular protocol, samples from patients dosed with the concomitantly administered drugs can be used for tests such as the experiments shown in Table 3. Protein therapeutics with endogenous

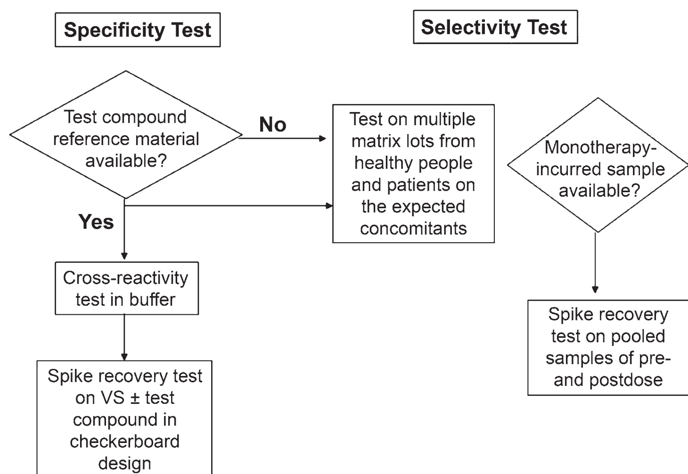


**Figure 2.** Flow diagram of specificity and selectivity tests for protein drug ligand binding assays against concomitantly administered drugs and related endogenous proteins. VS indicates validation samples. Test compound can be a concomitant or related endogenous protein.

counterparts require special attention in that the basal values should be determined for the additional recovery test and a sufficient number of individual lots in the target population should be tested. When appropriate, a test plan of specificity and selectivity evaluation should include an a priori validation plan before the experiments are performed, taking into consideration the upcoming protocol. Along the course of drug development, specificity and selectivity tests should be updated by considering any new concomitantly administered drugs that are to be employed in future protocols.

Further discussions and input from the industry will be valuable for providing the best practices for the design of specificity and selectivity tests.

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