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Research Article

Assessment of Incurred Sample Reanalysis for Macromolecules to Evaluate Bioanalytical Method Robustness: Effects from Imprecision

Theingi M. Thway,[1,4] Michael Eschenberg,[2] Dominador Calamba,[1] Chris Macaraeg,[1] Mark Ma,[1] and Binodh DeSilva[1,3]

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Abstract. Incurred sample reanalysis (ISR) is recommended by regulatory agencies to demonstrate reproducibility of validated methods and provide confidence that methods used in pharmacokinetic and toxicokinetic assessments give reproducible results. For macromolecules to pass ISR, regulatory recommendations require that two thirds of ISR samples be within 30% of the average of original and reanalyzed values. A modified Bland–Altman (mBA) analysis was used to evaluate whether total error (TE), the sum of precision and accuracy, was predictive of a method's passing ISR and to identify potential contributing parameters for ISR success. Simulated studies determined minimum precision requirements for methods to have successful ISR and evaluated the relationship between precision and the probability of a method's passing ISR acceptance criteria. The present analysis evaluated ISRs conducted for 37 studies involving ligand-binding assays (LBAs), with TEs ranging from 15% to 30%. An mBA approach was used to assess accuracy and precision of ISR, each with a threshold of 30%. All ISR studies met current regulatory criteria; using mBA, all studies met the accuracy threshold of 30% or less, but two studies (5%) failed to meet the 30% precision threshold. Simulation results showed that when an LBA has ≤15% imprecision, the ISR criteria for both the regulatory recommendation and mBA would be met in 99.9% of studies. Approximately 71% of samples are expected to be within 1.5 times the method imprecision. Therefore, precision appears to be a critical parameter in LBA reproducibility and may also be useful in identifying methods that have difficulty passing ISR.

KEY WORDS: incurred sample reanalysis; LBA; sample size selection; statistical analysis; total error.

INTRODUCTION

Pharmacokinetic (PK) and toxicokinetic (TK) assessments of therapeutic drugs are critical in all stages of drug development. Thus, the bioanalytical methods used in their assessment play a significant role in the evaluation of PK/TK parameters and require more stringent criteria to demonstrate the validity of their usefulness relative to those used for diagnostic or research purposes. Incurred sample reanalysis (ISR) is the most recent regulatory recommendation to ensure the reproducibility of bioanalytical methods used in the types of studies mentioned above (1,2). At the AAPS workshop held at Crystal City, VA, in 2008 (1), the FDA presented case studies in which there were discrepancies in results between the original and reanalyzed concentrations in multi-analyte assays (chromatographic assays) for small-molecule drugs. At the end of the workshop, the agency representatives strongly recommended the practice of ISR to reinforce confidence that bioanalytical methods produce valid and reproducible results. The agency's concern was that without such confidence, a valid conclusion could not be made on PK/TK assessments. It is critical that the regulated bioanalytical laboratories develop, validate, and implement robust bioanalytical methods that generate high-quality, reproducible results. ISR failure can delay clinical study timelines and further affect the drug development process.

The current recommendation is that ISRs should be conducted for all bioequivalence or comparability studies, for both drug/drug interaction studies as well as preclinical and clinical studies in which PK assessment is the primary endpoint (1,2). At least 5% to 10% of the samples must be reanalyzed to show method reproducibility. The initially recommended ISR acceptance criteria were that at least two thirds of reanalyzed samples must be within 30% of the originally reported concentrations for large-molecule therapeutics (within 20% for small-molecule therapeutics) (3). Later, the workshop committee recommended that at least two thirds of reanalyzed samples be within 30% of the averaged concentrations between original and repeats for macromolecules (within 20% of the averaged results for small-molecule therapeutics) (1). The current ISR recommendations focus on the agreement between two results since the accuracy of sample measurement is unknown. The

[1] Pharmacokinetics and Drug Metabolism, Amgen Inc, One Amgen Center Drive, Thousand Oaks, California 91320, USA.
[2] Biostatistics-Medical Sciences, Amgen Inc, One Amgen Center Drive, Thousand Oaks, California 91320, USA.
[3] Bristol-Myers Squibb, 345 Park Avenue, New York, New York 10154, USA.
[4] To whom correspondence should be addressed. (e-mail: tthway@amgen.com)

method acceptance criteria for a validated ligand-binding assay (LBA), a commonly used method of choice for bioanalysis of macromolecules, use two requirements (Table I): accuracy and precision, collectively called total error (TE). The TE of a validated LBA includes both random (assessed by imprecision or coefficient of variation [percent CV]) and systematic (assessed by accuracy or percent bias) errors, which are determined during prestudy validation by using serum samples spiked with known concentrations of therapeutic molecules (also known as quality control [QC]) and by analyzing the same spiked samples at least 30 times. Although these samples may differ from the actual study samples or *in vivo*-derived samples (incurred samples), it is reasonable to believe that method precision determined with the spiked samples should be similar to that of incurred samples. However, once the ISR is conducted, only two concentration values are available for each sample pair. The statistical method used in prestudy validation cannot be applied in ISR to assess accuracy and precision with an n of 2. The term "accuracy of ISR" in this report refers to the closeness between two observed values (original and reanalyzed results or sample pair), and "precision of ISR" refers to the CV across all sample pairs (see Table I for terms and definitions used in this report). Nonetheless, accuracy and precision of ISR can be evaluated by using a modified Bland–Altman (mBA) analysis. The original Bland–Altman (BA) analysis is a statistical method that is useful in assessing the agreement between two data sets with paired observations and in evaluating both systematic and random differences (4). It has been suggested that the agreement between original and reanalyzed results for ISR can be evaluated by using the assay acceptance criteria and an mBA statistical approach (5).

Before implementation of ISR, LBAs did not have repeated or reanalyzed results to determine method reproducibility. With a substantial amount of ISR data now available, this database serves as a resource for analyzing the parameters contributing to bioanalytical robustness and for seeking out those parameters that may contribute to ISR success. A 4% failure rate for small-molecule ISR was recently reported (2); the failure rate for large-molecule ISR has not been previously described.

Since late 2007, 26 studies at Amgen Inc. (Thousand Oaks, CA), and 11 studies at three different contract research organizations (CROs) were conducted for ISR. These 37 studies spanned four biotherapeutic protein forms and were intended for various therapeutic indications. Six of these were comparability studies, and the rest were PK or TK studies. The TEs of the 30 bioanalytical methods used in these studies were 15% (9 methods), 20% (16 methods), 25% (4 methods), and 30% (1 method).

This report illustrates the overall success rates of macromolecule ISRs assessed by using the current regulatory agency-recommended criteria while retrospectively evaluating ISR with an mBA analysis. We then assessed potential contributing factors for LBA robustness that can benefit future method development and prestudy validation of macromolecules and may also provide insight for future investigational guidelines in the event of ISR failure. This report also focuses on the statistical relevance of method acceptance criteria to current ISR criteria for macromolecules.

METHODS AND MATERIALS

ISR Sample Selection and Acceptance Criteria

The total numbers of subjects and samples for each ISR analysis varied depending upon the effective standard procedure at the time. In early studies, ISR evaluation was conducted with samples from at least three subjects/animals, one from each dose level, with the complete PK profile represented for each subject/animal. In more recent studies, ISR analysis was conducted by selecting ISR samples from at least two time points for each subject/animal, one around C_{max} and one from the terminal phase, for at least 20 subjects/animals. The acceptance criteria followed the regulatory recommendation that at least two thirds of repeat results must be within 30% of the originally reported results (for early studies) or at least two thirds of the repeat results must be within 30% of averaged results (for recent studies).

General Procedure for ISR

Sample selection was documented before analysis. Freeze/thaw stability of samples was ensured within the validated cycles before ISR analysis. In general, the primary vials were used to perform ISR if they had undergone an acceptable number of freeze/thaw cycles. Otherwise, the backup sample vials were used for ISR. The same sample dilution factor was used for both the original and ISR analyses in most samples. ISR was typically conducted within 3 to 6 months of the time the original analysis was performed.

Statistical Methods/Approaches

Modified Bland–Altman Tool

An mBA analysis was proposed by Rocci *et al.* (5). This method evaluates the closeness between the original and reanalysis values (sample pair) by computing ratio limits and evaluating whether these limits fall completely within a specified threshold. We used a threshold of 30% (between 0.70 and 1.43) to be consistent with the current regulatory recommendation of a 30% threshold for macromolecules. The precision of the reanalysis is evaluated by computing limits of agreement (LOA) and determining whether the LOA and 95% confidence intervals fall completely within the threshold used for the ratio limits. The analysis is performed by using an mBA plotter, which is a validated utility that was created with Microsoft Excel at Amgen Inc. (6).

Simulation of ISR Studies

A total of 980,000 ISR studies were simulated, with each study having a specified CV and sample size. Each simulated study creates paired values: original and reanalyzed. Each pair is randomly assigned a target value between 45 and 195. This target value is considered the "true" value for a sample. The original and reanalyzed values are then derived from the target value on the basis of the random error of the method (*i.e.*, percent CV). It is assumed that the original and reanalyzed values are normally distributed with a mean value

Table I. Terms and Naming of Criteria Used in This Report

Criteria	Method acceptance criteria guidance	Current regulatory recommendation	Modified Bland–Altman[a]
Stages to perform	Prestudy validation	In-study validation by ISR	In-study validation by ISR
Sample type used	Spiked with known nominal concentrations	*In vivo*-derived and true concentration not known	*In vivo*-derived and true concentration not known
Accuracy	Based on nominal values and to have ≤20%	Based on the difference of 2 observed values to be within 30% of their average	Based on the 2 observed values to be within 30%
Precision	Based from nominal values. At least 2/3 of the results be ≤20%	At least 2/3 of the paired results to be ≤30%	All of the paired results to be ≤30%

[a] For simplicity purpose, threshold or criteria are set as 30% for this research report

equal to the target value and a standard deviation that is based on the method CV. Each simulated study has a sample size between 20 and 150 paired samples (in increments of 10 samples) and percent CV between 10% and 40% (in increments of 5 samples). This equates to 10,000 studies for each CV and sample size combination. As an example, for a CV of 10% and a study sample size of 20 sample pairs, there are 10,000 studies simulated.

For each study, an evaluation is performed on whether the ISR passes on the basis of the current regulatory recommendation (two thirds of reanalyzed values agree within 30% of their average for evaluation of accuracy) and on the basis of the mBA criteria for mean ratio (evaluation of accuracy) and LOA (evaluation of precision) between 0.70 and 1.43. Concordance between the two criteria is then summarized by method CV and sample size. An estimate of the ISR pass rate for each method CV and sample size is made on the basis of the simulation results. Data from a simulation study with percent CV ranging from 10% to 20% are illustrated.

Simulation of ISR Samples

Five million paired samples were simulated on the basis of the target values and the method CVs: one million pairs with a 10% CV, one million with a 15% CV, one million with a 20% CV, one million with a 25% CV, and one million with a 30% CV. Each pair (original and reanalyzed values) was created with a randomly selected target value between 40 and 240 (same target value for both original and reanalyzed values). It is assumed that the original and reanalyzed values are normally distributed with a mean value equal to the target value and a standard deviation that is based on the method CV. Each pair was then evaluated to determine the percent difference from the average of the pair by using the following formula: (Reanalyzed−Original)/0.5×(Original+Reanalyzed). For each CV level, the percent difference between the average of the original and reanalyzed value was compared for each specified criterion limit (10%, 15%, 20%, 25%, 30%, 40%, 50%, and 60%) to determine whether the percent difference for the pair was less than the criterion limit. The percentage of pairs that were less than or equal to the criterion limit was then calculated (*e.g.*, for a 15% method CV, the percentage of paired samples that has a difference of ≤15% between their averaged values).

RESULTS

Accuracy of ISRs Conducted According to Current Regulatory Guidelines

To determine the reproducibility of each bioanalytical method, ISR analysis was conducted with predefined acceptance criteria on the basis of the current regulatory recommendation (at the time of analysis) specified in the standard procedure. Figure 1 shows summaries with the number of ISR samples (in parentheses) and the percentage of sample pairs (original and reanalyzed results) that fell within the 30% difference in each study. The different panels include studies in which bioanalytical methods with a TE of 15% (Fig. 1a, studies A1–A11), 20% (Fig. 1b, studies B1–B21), or 25% to 30% (Fig. 1c, studies C1–C4 and D1) were applied. Each ISR analysis result met the current regulatory recommendations: at least two thirds (67%) of reanalyzed samples were within 30% of the original or the averaged results. In all studies, more than 79% of ISR results were within 30% of the averaged values, regardless of method TE (Fig. 1a–c). In 11 studies, among three categories (ISR studies using either 15%, 20%, or 25/30% TE methods), all ISR results were within 30% of the original or the averaged values. Regardless of the bioanalytical method TE or total ISR sample number, all ISR studies met the regulatory recommendations. Thus, the reported bioanalytical results were considered to be reproducible.

ISR Success Rate in LBA and the Concordance between Regulatory and mBA Criteria

The current regulatory ISR criteria are based strictly on an evaluation of the closeness of two concentrations within a limit: two thirds of results need to be within 30% of the averaged values. The mBA approach was used to assess both accuracy (ratio limits) and precision (LOA), as well as to retrospectively evaluate ISR accuracy and precision. A threshold of 30% was applied to each parameter. The concordance of successful ISR rates between the current ISR criteria and the mBA approach was then determined. Of 37 ISR studies conducted, all met the current ISR criteria, and 35 of the 37 studies (95%) met the mBA

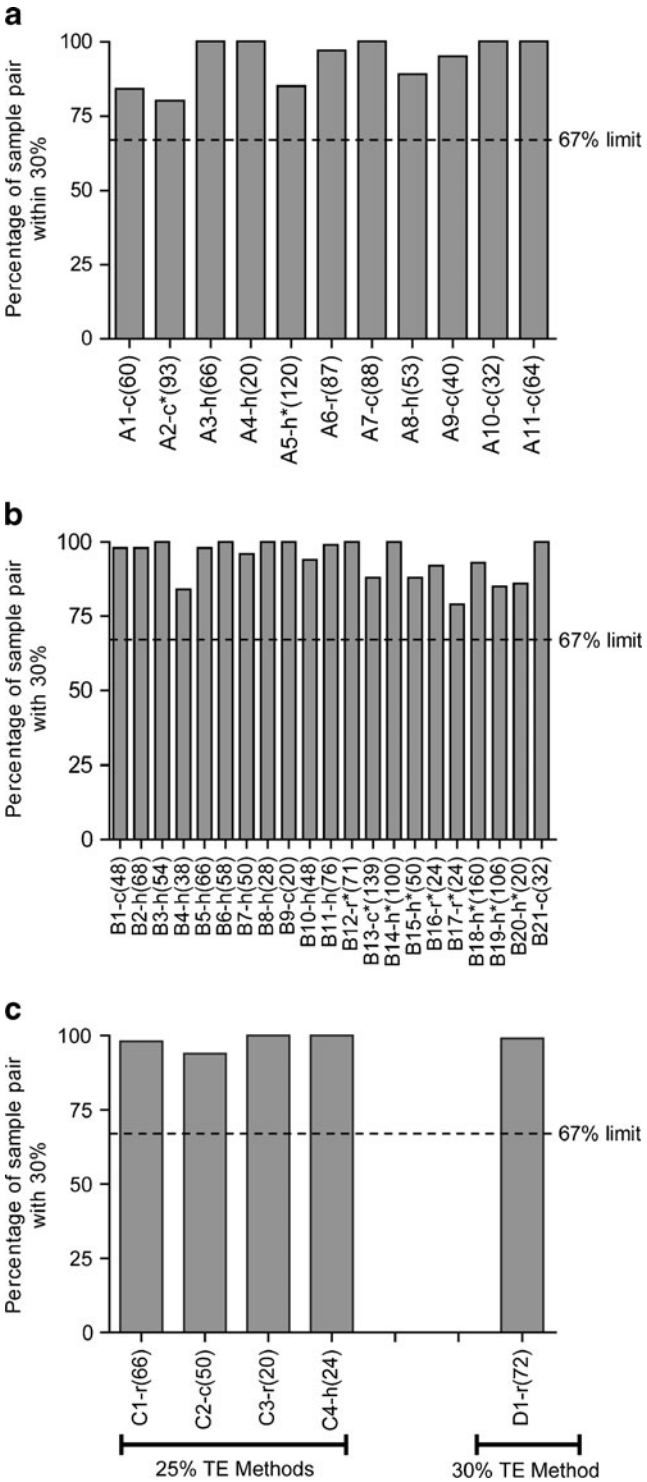

Fig. 1. ISRs categorized by method TEs of either 15% (Fig. 3a), 20% (Fig. 3b), or 25% and 30% (Fig. 3c). Each panel shows the percentage of ISR samples that fell within a 30% difference (*filled bar*), with the identifying study numbers and number of ISR samples noted in *parentheses*. Study IDs with *A*, *B*, *C*, and *D* used method TEs of 15%, 20%, 25%, and 30%, respectively. *Dashed lines* represent the minimum percentage (67%) of ISRs within a 30% difference required to meet the acceptance criteria. *Alphabetical initials* next to the study ID indicate the species used in each study (*c* cynomolgus monkey, *h* human, *r* rat). *Asterisk* indicates the studies conducted at CROs. *B2–B5* studies used the same analytical methods

criteria. The results demonstrated a 5% discordance between the passing rates observed with the different analysis approaches.

Accuracy and Precision Assessments of ISR Conducted with mBA Analysis

To identify potential contributing factors critical for macromolecule bioanalytical method robustness, the agreement of accuracy and precision between original and reanalyzed results from ISR experiments was examined for each ISR. Figures 2, 3, and 4 illustrate the accuracy (panels a) and precision (panels b) determined by the mBA analysis for each ISR study. Each figure shows data from studies in which bioanalytical methods with 15%, 20%, or 25%/30% TE were applied.

For the 15% TE methods (Fig. 2), the accuracy of all ISRs was within the 30% threshold (Fig. 2a). In terms of precision, all studies were within the 30% threshold, except for study A2-c*, which had the widest imprecision range and exceeded the 30% limit (Fig. 2b). Overall, the ratio limit was much tighter than the imprecision.

For the 20% TE methods (Fig. 3), the accuracy of all ISRs was within the 30% threshold (Fig. 3a). In terms of precision, all studies were within the 30% threshold, except for study B4-h, which had the widest LOA range and exceeded the 30% limit (Fig. 3b). Once again, we observed that the ratio limit was much tighter than the imprecision.

Since fewer methods with 25% or 30% TE were available, all ISR studies in which bioanalytical methods had 25% or 30% TE were combined for evaluation (Fig. 4). For the limited number of studies, both accuracy and precision were found to be within the 30% threshold, even though the bioanalytical method TE was ≥25% (Fig. 4a, b).

Each ISR study met the current regulatory recommendation regardless of the method acceptance criteria. However, by using the 30% threshold and applying the criteria to the evaluation of both accuracy and precision with an mBA analysis, 95% of studies passed ISR evaluation and 5% failed due to larger imprecision.

Minimum Precision Requirement During Prestudy Validation for Successful ISR with In-Study Validation

On the basis of evaluations indicating that the imprecision of an LBA method could affect method reproducibility, it is important to understand the acceptable limits of method imprecision during prestudy validation before finalizing the method acceptance criteria for the study. Acceptable limits for a successful ISR study were determined by using a simulation model. Results from the simulation studies are shown in Fig. 5. If an LBA method has ≤15% CV, ISR criteria both for regulatory recommendation and for the mBA approach would be met in >97% of the studies (Fig. 5a, b) when the sample size is ≥20. If the imprecision of a bioanalytical method reaches 20%, ISR sample sizes must be increased to unreasonable levels (>160) to have a ≥97% chance of passing the ISR evaluation using either the regulatory agency-recommended criterion or mBA analysis with a 30% threshold for a given study. A smaller sample size (Fig. 5b) than that of the currently recommended criteria is

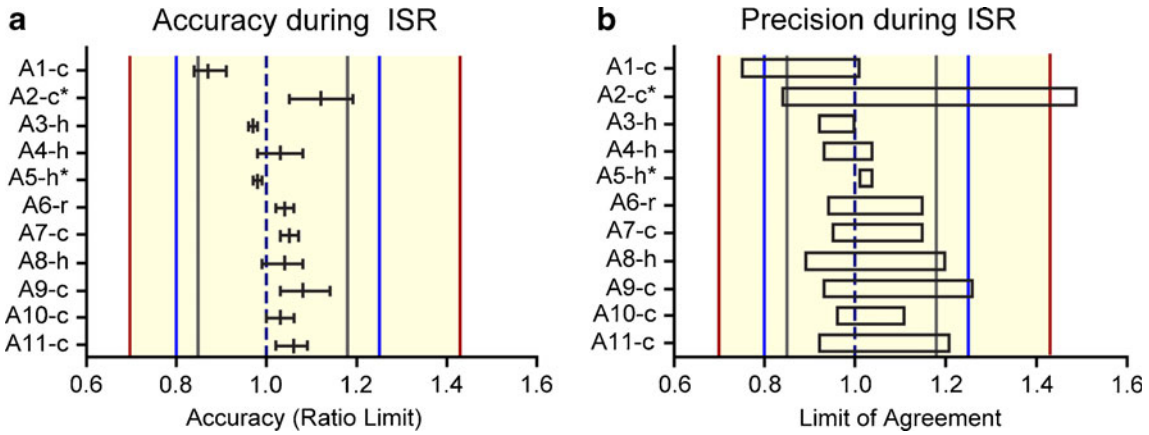

Fig. 2. Accuracy (**a**) and precision (**b**) of ISRs in the 15% TE method category. *Alphabetical initials* indicate the species used in each study (*c* cynomolgus monkey, *h* human, *r* rat). *Asterisk* indicates the studies conducted at CROs. Each *colored line* represents the limit for 15% (*gray*), 20% (*blue*), and 30% (*red*). **a** 100% of ISRs had the accuracy limit, including the 95% confidence interval, within 30% agreement. **b** 90% of ISRs had the precision limit within 30% agreement. The method used in study *A1-c* was developed and validated at Amgen Inc. and transferred to a CRO, where partial validation was performed to support the study A2-C

required to maintain a greater probability of passing ISR when using the mBA as the ISR acceptance criteria.

Probability of Original and Reanalysis Results Being Within Percent CV

To evaluate whether percent CV is appropriate for use as the acceptance criterion or threshold for ISR, the probability of the original and reanalysis results being within the method percent CV was estimated by using simulations. Figure 6 illustrates the probability of all sample pairs being within the percent CV (1.0× CV) of the mean of the pair. The figure also illustrates the probability of all sample pairs being within 1.5 times the percent CV (1.5× CV) of the mean and within two times the percent CV (2.0× CV) of the mean. On the basis of simulation, approximately 52%, 71%, and 84% of sample pairs will be within the method CV, within 1.5 times the method CV, and within two times the method CV, respectively.

DISCUSSION

LBAs typically have greater variability than chromatographic and liquid chromatography–mass spectrometry methods. These methods, however, are commonly used to quantitatively measure macromolecules in support of PK and TK assessments. The regulatory recommendations to assess the accuracy of ISR results raised concerns for the LBA community since imprecision can also contribute to the variability of the LBA. Data from 37 ISR studies that used LBA methods show that these methods can be reproducible when assays with robust prestudy validation designs are performed. The results indicate that the mBA approach has more comprehensive criteria than the current ISR recommendations. When a method TE of 15% was used for

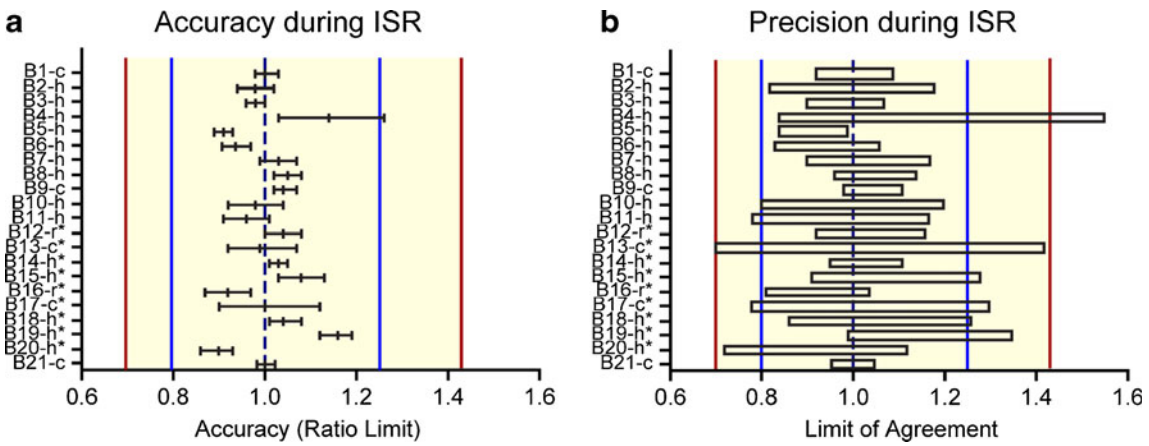

Fig. 3. Accuracy (**a**) and precision (**b**) of ISRs in the 20% TE method category. *Alphabetical initials* indicate the species used in each study (*c* cynomolgus monkey, *h* human, *r* rat). *Asterisk* indicates the studies conducted at CROs. Each *colored line* represents the limit for 15% (*gray*), 20% (*blue*), and 30% (*red*). **a** 100% of ISRs had the accuracy limit, including the 95% confidence interval, well within 30% agreement. **b** Only 85% of ISRs had the precision limit well within 30% agreement. The same analytical method was applied in studies *B2–B5*

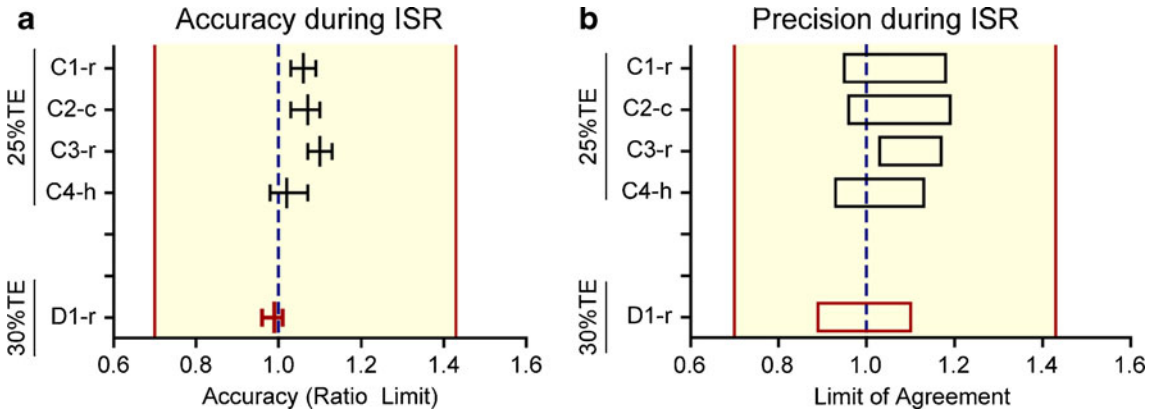

Fig. 4. Accuracy (**a**) and precision (**b**) of ISRs in the 25% (*C1–C4*) or 30% (*D1*) TE method categories. *Alphabetical initials* indicate the species used in each study (*c* cynomolgus monkey, *h* human, *r* rat). *Asterisk* indicates the studies conducted at CROs. Each *colored line* represents the limit for 15% (*gray*), 20% (*blue*), and 30% (*red*). **a** 100% of ISRs had the accuracy limit, including the 95% confidence interval, within 30% agreement. **b** 100% of ISRs had precision limits within 30% agreement

accuracy and precision assessments, our data indicated that five studies would not pass because their imprecision ranges extend beyond the 15% threshold (Fig. 2b). When a method TE of 20% was used for accuracy and precision assessments, our data indicated that seven studies would not pass because their imprecision ranges extend beyond the 20% threshold (Fig. 3b). These ISR results also suggest that it is not appropriate to set method TEs established during prestudy validation as ISR acceptance criteria. However, the data showed that all ISRs were successful when the current regulatory recommendations were used and the imprecision was ≤15%.

Simulation results described in this report indicate a relationship between the method CV and the likelihood of passing ISR on the basis of regulatory ISR criteria. The current recommendation on ISR for macromolecules requires that two thirds of ISR samples be within 30% of the average of original and reanalyzed values. For methods with 15% CV, it is expected that 84% of the original and reanalysis values will be within 30% of their mean values. With methods having 30% CV, it is expected that only 51% of the original and reanalysis values will be within 30% of their mean values. It is therefore less likely that ISR will pass for a method that has a 30% CV when the method TEs established during prestudy validation are used as the ISR acceptance criteria.

Additional simulation data provide an evaluation of the potential relationship between method CV and ISR acceptance threshold. The data suggest that an acceptance threshold of 1.5 times the method CV may be acceptable on the basis of the recommendation that two thirds of the sample pairs be within an acceptance criteria threshold for passing ISR (currently 30% for macromolecules). When a threshold of 1.5 times the method CV is used in simulation, 71% of the sample pairs are expected to be within 1.5 times the method CV of the mean value of the two results. The threshold of 1.5 is relevant because it suggests that at least two thirds (66.7%) of ISR samples should meet the current regulatory recommendation for the number of paired samples required to meet the threshold regardless of their method CVs or sample size. Therefore, this simulation result illustrates that the method CV during prestudy validation may be indicative of the likelihood that an ISR will pass. Furthermore, these

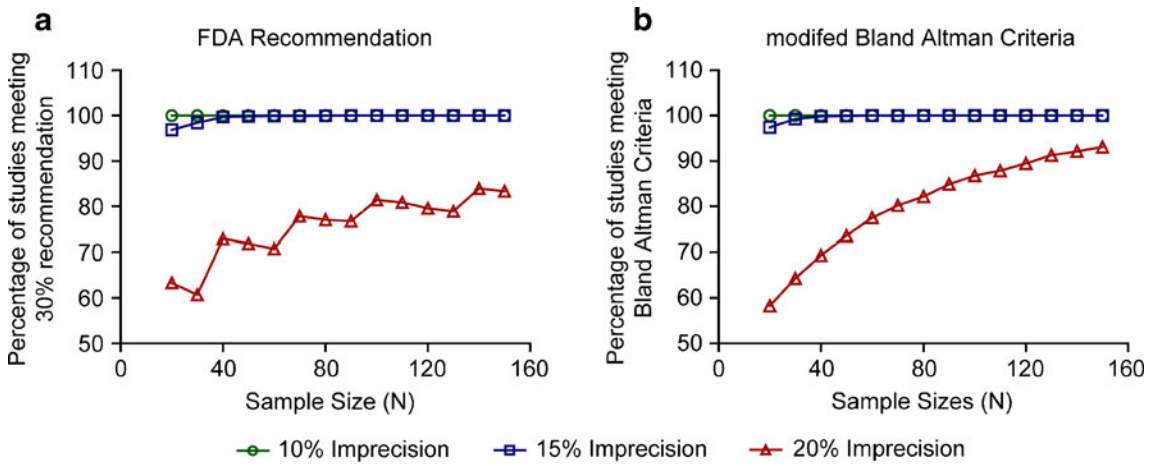

Fig. 5. Determination of acceptable imprecision level for the LBA to meet regulatory agency-recommended criteria (**a**) or mBA approach using a simulation study with various ISR sample sizes. The probability of meeting either regulatory recommendations or the mBA criteria was examined for 10%, 15%, and 20% method imprecision

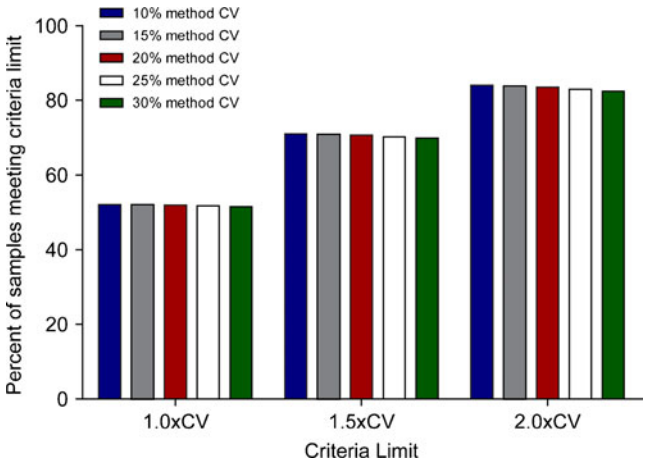

Fig. 6. Probability of original and reanalyzed results within method CV was examined using simulation study. Probability of sample pairs being within method CV (*1.0× CV*), within 1.5 times the percent CV (*1.5× CV*) of the mean, and within two times the percent CV (*2.0× CV*) of the mean

results suggest the possibility of using the method CV as a guideline for the acceptance threshold of ISR evaluation.

Previously reported simulation data (6) indicated a 95% concordance of the pass/fail conclusion in studies that used the current ISR recommendation and those that used mBA analysis. Actual macromolecule ISR data supported simulation data. Concordance was observed in 94.6% of the studies; the 5.4% discordance was due to imprecision. The first simulation data in this report suggested that bioanalytical methods should have interassay imprecision of ≤15% to have >97% probability of passing ISR. The interassay CVs for the bioanalytical methods used in the A2-c* and B4-h studies were <10% and <14%, respectively, during prestudy, showing that acceptable and wider imprecision during ISR was not expected. It is worth noting that only 21% and 16% of ISR samples had a >30% difference from averaged values for studies A2-c* and B4-h, respectively.

Because of their complexity, most LBAs are developed, validated, and implemented as single-analyte methods. Thus, repeat results are usually not generated or available to assess the reproducibility of reported results before the ISR recommendation. Often, the method validation guideline emphasizes the accuracy of the results rather than the precision during prestudy validation. The present results indicate that LBA should have interassay imprecision of ≤15% during prestudy validation for the method to be considered reproducible with ≥97% ISR passing rate. Once the imprecision reaches 20%, the sample size for ISR has to be increased to a level that it is no longer practical (Fig. 5). Even with a sample size of 150, it could not achieve 97% ISR passing rate. This simulation data provide useful guidelines in setting the method acceptance criteria during prestudy method validation. For regulated bioanalytical methods, it is desirable to have interassay imprecision of ≤10% for low-level QC, mid-level QC, and high-level QC. Higher percent CV may be acceptable for a QC level at the lower limit of quantification if the sensitivity of the bioanalytical method is needed to support studies that have low therapeutic doses. A bioanalytical method with TE ≤20% during prestudy validation also serves as an advantage for ISR in that less variable methods allow flexibility for technical errors during in-study validation. Assessing both accuracy and precision for ISR with an mBA analysis highlighted the potential impact on method imprecision that could affect the interpretation of method reproducibility during in-study validation. The mBA assessment on ISR might allow identification of random errors caused by the methods themselves or by human error. One can expect that percent CV during ISR should be at least within the method TE. Wider imprecision during ISR does not necessarily indicate that there are problems in methodology. Rather, the imprecision can be caused by technical or human errors. As mentioned in the White Paper from 2009 European Bioanalysis Forum (2), the practice of ISR can be considered as a way to check process adherence. However, processes that could identify a technical error such as analytical and PK repeats are put in place to ensure the quality of bioanalytical data before and after the ISR implementation. With a 100% ISR passing rate for macromolecules in-house and at CROs, the practice of ISR has provided assurance of the results and confidence that our LBA method development and validation procedures are rigorous enough to meet regulatory requirements. Although an LBA method acceptance criterion of up to 30% (15% bias and 15% CV) is allowable for prestudy validation (7), we have taken stringent method-development approaches and applied robust validation designs to reduce the TEs. The advantage of this approach has been an increase in the number of successful ISRs. Examples of these method-development approaches and prestudy validations were previously described, and the application of methods A, B, and C in ISR studies A1-c, A6-r, and A7-c (8), respectively, is described herein. Combining these approaches by well-trained individuals with the use of a fully automated sample analysis procedure should minimize technical errors that still exist with manual sample analysis processes.

CONCLUSION

The success rate for the ISR studies included in this report is 100% when the current regulatory agency-recommended criteria are applied. The results indicate that it is not appropriate to set method acceptance criteria established during prestudy validation as ISR acceptance criteria but it could be used as a guide. Precision is an important parameter in LBA reproducibility and may be useful for identifying methods that have difficulty passing ISR in the future. To meet the ISR criteria with >97% probability, the bioanalytical methods for macromolecules should have ≤15% CV during prestudy validation. Additionally, it may be beneficial to statistically evaluate the ISR precision in addition to the ISR accuracy, thereby providing additional rigor to the analysis and confirming that the bioanalytical method is performing as expected.

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REFERENCES

1. Fast DM, Kelley M, Viswanathan CT, *et al*. Workshop report and follow-up—AAPS workshop on current topics in GLP bioanalysis: assay reproducibility for incurred samples—implications of Crystal City recommendations. AAPS J. 2009;11(2):238–41.
2. Timmerman P, Luedtke S, Amsterdam P, *et al*. Incurred sample reanalysis: views and recommendations by the European Bioanalysis Forum. Bioanalysis. 2009;1(6):1049–56.
3. Ray C, DeSimone D, Thway T. Report on AAPS workshop on current topics in GLP bioanalysis: assay reproducibility for incurred samples—implication of Crystal City recommendations. Ligand Binding Assay Bioanalytical Focus Group Newsletter. 2008;2(2).
4. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307–10.
5. Rocci ML Jr, Devanarayan V, Haughey DB, *et al*. Confirmatory reanalysis of incurred bioanalytical samples. AAPS J. 2007;9(3):E336–43.
6. Thway T, Macaraeg CR, *et al*. Bioanalytical method requirements and the statistical considerations in incurred sample reanalysis for macromolecules. Bioanalysis. 2010;2(9):1587–96.
7. DeSilva B, Smith W, Weiner R, *et al*. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharm Res. 2003;20:1885–900.
8. Thway T, Macaraeg CR, *et al*. Application of a planar electrochemiluminescence platform to support regulated studies of macromolecules: benefits and limitations in assay range. J Pharm Biomed Anal. 2010;51:626–32.