Research Article

A Step-wise Approach for Transfer of Immunogenicity Assays during Clinical Drug Development

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Abstract. We designed a three-step statistical approach to transfer bioanalytical assays (ELISA and Biacore) which evaluates the (1) average equivalence between the two labs (2) concordance in individual sample results between the two labs, and (3) long-term stability of assay performance. Each experimental design evaluated the contribution of four critical variables to the overall variability. Two lots of each variable were examined in a controlled experiment. The variables tested for ELISA were analyst, plate washer, biotinylated-therapeutic protein, and streptavidin-horseradish peroxidase; and for Biacore were analyst, instrument, chip lot, and conjugation chemistry reagent lots. Equivalence in the mean signal to noise (S/N) or mean relative units (RU) between the two labs was established through statistical evaluation of the assay performance characteristics across multiple assay variables. Concordance between the two labs in the individual sample results was subsequently verified both quantitatively and qualitatively. The long-term maintenance of assay stability was monitored by performance testing of a predefined set of samples which were prepared in sufficient quantities to last several years. The process of method validation for biomarker testing in clinical trials is to analyze the variability of the assay performance. However, different factors contribute to this variability and need to be evaluated when the method is transferred to another site/lab. Lack of understanding the critical variables can potentially result in unexpected problems and delays. The three-step statistical approach of assay transfer provides a robust process for transferring complex biological assays.

KEY WORDS: biacore; bioanalytical; concordance; ELISA; method transfer; robustness.

INTRODUCTION

Transfer of complex validated immunogenicity assays across laboratories is an essential component of the clinical drug development process. The lack of a robust method for transfer of assays, and lack of appropriate guidelines creates a significant impact on the interpretation of data, cost, timelines, and overall burden of managing the performance of clinical assays over the long period of drug development. In this respect, the regulatory agencies have requested drug development companies to provide evidence of clinical impact on performance characteristics of bioanalytical assays when performed at various sites (4). Furthermore, lack of apparent control processes to manage the long-term performance of the same assays across sites has been pointed out during review of biological license applications. In order to

understand the performance of bioanalytical assays, we have developed a statistically robust and practical method for transferring validated assays with a high probability of success.

The aim of an assay transfer is to achieve efficient transition of assays to support multi-center clinical studies, to optimize resource utilization, and to improve overall efficiencies in the clinical laboratories. The transfer process should involve a team of representatives from various groups in order to review desirable attributes and activities of the groups involved in the transfer of clinical assays. The assay transfer process results in the following key needs: (1) ensure transparent transfer of assays by involving key areas, (2) address the contribution of the significant variables in an assay that contribute to the overall variability of an assay; (3) maintain assay "biology" throughout transfer and assay implementation, and (4) guarantee continuity of scale throughout a clinical program. The following major questions are addressed as part of the evaluation for the transfer of assays: (a) has the performance of the assay from the source lab been carefully understood and documented? (b) Have the key contributors to the variability of the assay been defined during assay validation and assay performance? (c) Has the instrumentation for the assay been qualified at the both the source and transfer facility? (d) Are procedures and plans in place for the transfer/shipping/delivery of the samples needed

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ABBREVIATIONS B-panitumumab, biotin-conjugated panitumumab; CCC, coefficient of concordance correlation; RU, relative units; SAHRP, streptavidin-conjugated horse radish peroxidase; S/N , signal to noise ratio

for the transfer experiments? (e) Is the performance of the assay at the new lab/facility acceptably similar to its current location? (e) Has the clinical impact of the potential difference been evaluated through simulation? (f) Is the version of the assay-processing system software, used for calculations, the same? And (g) is the software application performance acceptable at the new lab/facility?

In this manuscript, we have provided a step-wise method for transferring bioanalytical assays which is strategically designed to address the aforementioned questions. The primary hypothesis of an assay transfer is that the assay performance between the source and transfer lab is equivalent.

METHODS

Source lab indicates the laboratory where the assay was developed/validated; *transfer lab* indicates the laboratory at the new location where the assay will be performed

Immunogenicity Assay Methods

All transfer experiments were performed according to the validated method. The ELISA and Biacore assay methods used in this assay transfer have been previously described (7). The assay sensitivity of the ELISA was 10 ng/mL in neat serum and that of the Biacore was 1 µg/mL in neat serum based on titration of the anti-panitumumab positive control Ab.

Transfer Plan and Statistical Analysis

The assay acceptance criteria were used to include experiments for the statistical evaluation described in a transfer plan. The structure of this transfer plan consisted of three parts. Part A contained a design of experiments meant to evaluate the contribution of critical assay variables (e.g., chip lot, conjugated-secondary antibodies, analyst, etc.) to the overall variability of the assay performance and to statistically measure the average equivalency of the assay performance between sites. The statistical criteria and data were used to understand and define the limits of acceptable assay performance between sites.

Transfer Plan Part A (Analytical Performance)

Part A of the transfer experiments of the ELISA was designed to study the effect of four critical assay variables, their contribution to the assay variability and to evaluate the equivalency between the two labs. The critical variables for the ELISA tested were analyst, plate washer, streptavidin-conjugated horseradish peroxidase (SAHRP) and biotin-panitumumab with BSA (B-panitumumab). The critical variables for the Biacore assay tested were analyst, Biacore instrument, chip lot, and EDC/NHS. These variables were tested across four runs by two analysts at each site. The sample set included eight sets of individual donor samples, prepared and analyzed unspiked (condition 1) and spiked with various concentrations of anti-panitumumab antibody (conditions 2, 3, 4), and panitumumab (condition 5).

The two one-sided *T* test (TOST) statistical test was used to assess average equivalence between two labs. The

TOST translates to comparing the 90% confidence interval of the ratio with a predetermined equivalence interval. In this analysis plan, if the 90% confidence interval was completely contained within the equivalence interval, the equivalence between the two laboratories in average response was concluded. A determination of lab equivalence was based on the two-sided 90% confidence interval of the ratio in mean signal/noise (*S/N*) between transfer lab and source lab and a predefined equivalence interval (0.80, 1.25). To claim that the two labs are in agreement with each other with regard to mean *S/N*, the 90% confidence interval must be contained entirely within the equivalence interval (0.80, 1.25).

Transfer Plan Part B (Clinical Performance)

Part B of the transfer for the ELISA assay was designed to evaluate the assay reproducibility between source and transfer labs using Lin's coefficient of concordance correlation (CCC; 5,6). Lin's CCC is a reproducibility index which takes consideration of both the degree of precision and the degree of accuracy and is calculated as the production of the precision and accuracy. The accuracy measures how far each observation deviates from the best-fit line and the precision measures how far the best-fit line deviates from the 45° line through origin. The sample set consisted of 50 individual donor samples from healthy subjects unspiked and spiked with 60 and 250 ng/mL anti-panitumumab. These samples were randomized and assayed at both source and transfer labs on the same day. The hypothesis-testing framework was set up to conclude the alternative hypothesis that the two labs are concordant. The Lin's CCC between two labs was estimated and its 95% lower (one-tailed) confidence limit was calculated. In order to claim that the two labs are in concordance with regard to the test result (*S/N*) for a given sample, the predefined acceptance criterion was that the 95% lower (one-tailed) confidence limit must be greater than or equal to the least acceptable Lin's CCC of 0.95.

Transfer Plan Part C (Performance Testing)

The third part of the assay transfer required demonstration of successful analysis using the processes involved in testing clinical samples, such as the use of statistical analysis and samples handling in laboratory information management systems (LIMS), electronic transfer of the data, etc. This part of the plan was performed using a set of samples, spiked with appropriate concentrations of the analyte, across the range of the assay. Several aliquots of this sample set were made, for long-term proficiency testing at the transfer lab.

RESULTS

ELISA Assay Transfer

Transfer Part A Summary Results: Demonstration of Average Equivalence

All experiments were performed and included in the analysis; any deviations were documented. Run 1 of Day 3

had a high positive control with a coefficient of variation greater than 20% due to an unknown cause. Both replicates independently met the signal to noise criteria (>1.19) described in the analytical method. It was determined to accept the run in the statistical analysis. The statistical analysis did not show the need of reanalysis.

A mixed effect model which utilizes mixed effect analysis of variance was fit to the log-transformed S/N ratios to study the effects of the four assay variables on the assay performance. In the mixed effect model, Site, SAHRP, B-Panitumumab, Subject and their two-way interactions SAHRP $\times$ Site, B-panitumumab $\times$ Site, Site $\times$ Subject, SAHRP $\times$ Subject, B-panitumumab $\times$ Subject were treated as fixed effects and Analyst(Site), Washer(Site) and Day(Site) were treated as random effects. The observations with larger than 3SD or less than -3SD residuals from the mixed effect model were removed from the analysis.

Table I shows the proportion of the total sum of squares (measuring total amount of variation around overall mean) contributed by different effects and the proportion that was not explained by the fixed effects was further portioned into the contribution of different random effects and residual. For an assay to be robust enough to transfer, a minor contribution to the total sum of squares from the assay factors and their interactions and large contribution from subject to subject variability would be expected. Table I helps to identify the factors that have relatively larger influence on the performance of the assay. For example, analyst(Site) and B-panitumumab contributed significantly to total variation of the observed data in comparison to other assay factors.

In order to evaluate the average equivalence between the two labs, the ratio of S/N between source lab and transfer lab and its 90% confidence interval were estimated for each subject at each B-panitumumab level (results not shown). Consideration was given to the statistically significant subject $\times$ site interaction which indicated that the difference between the two sites changes across samples. In addition, consideration was given to the statistically significant B-panitumumab $\times$ site interaction which indicated that the difference between the two sites is different for the two B-panitumumab lots. The results show an association between the failure to conclude equivalence and the B-panitumumab lot 2.

Transfer Plan Part B Summary Results—Demonstration of Lab Concordance

Following the analysis of variability in transfer plan part A, one lot of the B-panitumumab reagent and an analyst were considered outliers, and therefore not used as the variables in the concordance analysis in part B. The specific reagents used in the assay run in transfer plan B were prespecified. All experiments passed assay acceptance criteria for the controls. However, the amount of antibody added to each donor to prepare the 60 ng/ml and 250 ng/ml spiked samples was systematically less than required. This was equally apparent for samples analyzed at source and transfer labs. Since an assignable cause was identified and contributed to the sample analysis in both sites, it was determined that the data were appropriate for use in the statistical analysis to determine the concordance between the two sites.

Table I. For ELISA Data and for Each Condition (Different Spike-in Concentration of Drug and Antibody), Total Variability in the Observations was Partitioned into Components that Measure the Variation Caused by Different Fixed Effects as Measured by the Percentage of the Total Sum of Squares (Measuring Total Amount of Variation Around Overall Mean), the Variability that was Not Explained by Fixed Effects was Further Partitioned to the Contribution of Different Random Effects and Residual as Measured by the Percentage of the Total Unexplained Variability

Source	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5
Percentage of total sum of squares explained by fixed effects					
Site	0%	3%	5%	3%	2%
SAHRP lot	2%	2%	1%	1%	5%
B-panitumumab	16%	8%	27%	34%	2%
SAHRP lot $\times$ site	0%	0%	0%	0%	0%
B-panitumumab $\times$ site	0%	3%	4%	3%	2%
Donor	40%	25%	2%	4%	19%
Site $\times$ donor	2%	3%	1%	2%	2%
SAHRP lot $\times$ donor	0%	0%	0%	0%	1%
B-panitumumab $\times$ donor	16%	5%	1%	1%	15%
Percentage of total assay variability ^a contributed by random effects and random noise:					
Analyst (site)	6%	28%	34%	36%	43%
Washer (site)	2%	5%	8%	3%	0%
Day (site)	6%	5%	9%	8%	8%
Random noise	87%	62%	49%	54%	49%

The critical variables for the ELISA tested were analyst, plate washer, streptavidin-conjugated Horseradish peroxidase (SAHRP) and biotin-panitumumab with BSA (B-panitumumab). These variables were tested across four runs by two analysts at each site. The sample set included eight sets of individual donor samples, prepared, and analyzed unspiked (condition 1) and spiked with various concentrations of anti-panitumumab antibody (conditions 2, 3, 4) and panitumumab (condition 5)

^aTotal assay variability is the sum of the variability contributed by the variance components of the assay, such as analyst, washer and day, and random noise

The Part B experiments concluded the concordance between source lab and transfer lab based on the predefined acceptance criterion. Tables II and III shows a summary of the estimated Lin's CCC, precision, accuracy, and their one-sided 95% lower confidence limits. The one-sided 95% lower confidence limit of Lin's CCC was 0.9556 which is greater than the least acceptable Lin's CCC of 0.9500. Thus, the acceptance criteria for concordance between the two labs were met. In addition, Tables IV and V also includes a 2×2 table which summarizes the frequency of results for the samples tested in Part B based on the assay cut point established during the assay validation. The Kappa coefficient was used to evaluate the degree of agreement in result classification between the two labs. Kappa coefficient is a descriptive statistic indicating the degree of agreement beyond chance, between two ratings per subject, based on a dichotomous response. The Kappa coefficient was 0.9035 with the one-sided 95% confidence bound (L95) 0.8450. A plot of the *S/N* ratios of transfer lab vs. *S/N* ratios of source lab was shown in Fig. 1. Closeness of the regression line and the concordance line shows good accuracy and the tightness of scatters around the regression line shows good precision.

Biacore Assay Transfer

Part A Results Summary: Demonstration of Average Equivalence

Part A of the transfer experiments was designed to study the effect of four critical assay variables, their contribution to the assay variability and to evaluate the equivalency between the two labs. The critical variables tested were analyst, Biacore instrument, chip lot, and EDC/NHS. The sample set included eight sets of individual donor samples, prepared and analyzed unspiked and spiked with various concentrations of anti-panitumumab antibody and panitumumab. The statistical one-sided *T* test was used to assess average equivalence between two labs. A determination of lab equivalence was based on the two-sided 90% confidence interval of the ratio in response units (RU) between transfer lab and source lab and a predefined equivalence interval (0.80, 1.25). To claim that the two labs were in agreement with each other with regard to RU, the 90% confidence interval was contained entirely within the equivalence interval (0.80, 1.25).

A mixed effect model which utilizes mixed effect analysis of variance was fit to the log-transformed RU values to study the effects of the four assay variables on the assay performance. In the mixed effect model, Site, Chip, EDC/NHS, Subject and their two-way interactions Chip × Site, EDC/NHS × Site, Site × Subject, Chip × Subject, and EDC/NHS ×

Table III. Lin's Concordance and Kappa Statistics for the ELISA Assay (Concordance Table)

Frequency	Transfer lab	
	Negative	Positive
Source lab		
Negative	85	3
Positive	4	58

Kappa=0.9035 with one-sided lower 95% confidence bound 0.8450

Subject were treated as fixed effects and Analyst(Site), Biacore(Site), and Day(Site) were treated as random effects. The observations with larger than 3SD or less than -3SD residuals from the mixed effect model were removed from the analysis.

Table VI describes the effect of critical assay factors on the variability of the assay performance in Part A. For example, in some conditions, analyst(Site), chip lot, and Biacore(Site) have a bigger impact in the variation observed in the data in comparison to the effects of other assay factors. In order to evaluate the average equivalence between the two labs, the ratio of RU between source lab and transfer lab and its 90% confidence interval were estimated for each condition. For condition 4 (samples spiked with 5.0 µg/mL of anti-panitumumab), consideration was given to the statistically significant chip lot × site and EDC/NHS × site interactions which indicated that the difference between the two sites changes across different chip lots and different EDC/NHC lots. These results show that equivalency could only be demonstrated in donor samples spiked with 5.0 µg/mL anti-panitumumab antibody and then only when chip lot 1 was used with NHS/EDC lot 1 and when chip lot 2 was used with NHS/EDC lot 2. In addition, these results indicated that the transfer lab ran systematically higher compared to source lab for all five conditions by as much as 20%.

The concordance panel samples were tested at both the source and transfer labs to confirm the differences observed in experiments in transfer plan A. The results, shown in Tables IV and V and Fig. 2 show that although the experiments passed the predefined acceptance criteria of concordance correlation, there was a significant discordance of final results of set of samples tested at the two labs. The transfer process, until this stage, had not defined acceptance criteria for kappa statistics, and post-hoc analysis demonstrated that the bias at the transfer lab contributed to the disagreement of results between the labs. The transfer at this stage was considered not successful, and investigation undertaken.

Investigation of the Root Cause of the Difference in Assay Performance between Source and Transfer Labs

Since the transfer labs demonstrated a bias, a detailed investigation of all the assay performance steps and reagents was conducted prior to establishing concordance between the labs. Based on these results, the factors that contributed significantly to the variability of the assay (determined by the transfer plan Part A data) were used to assess assay performance. The investigation suggested a discrepancy in the instrument maintenance between sites and potential

Table II. Lin's Concordance and Kappa Statistics for the ELISA Assay (Lin's Concordance Results)

Statistics	CCC	Precision	Accuracy
Estimate	0.9642	0.9777	0.9861
95% Conf. Limit	0.9556	0.9709	0.9804
Criterion	0.9500		

Kappa=0.9035 with one-sided lower 95% confidence bound 0.8450

Fig. 1. Concordance plots of the performance of the ELISA assay at the source and transfer labs in the investigation. The *vertical* and *horizontal* lines represent the assay cut point (ACP). There were two lines going through the scatter of the data with the *gray solid* one representing the concordance line (45° and passing through (1,1)) and the *dotted blue* one representing the regression line of *S/N* at source lab against *S/N* at transfer lab. Three different spike-in conditions were represented by *symbols* in different colors and shapes

stability of the spiked positive control antibody used in the experiments. However after testing various use and maintenance processes, and reagent stability, the bias at the transfer lab could not be resolved. The influence of the bias was more significant for the unspiked samples (with lower RU values), rather than spiked samples.

In order to assess the results of the investigation, the investigation plan was executed, which included a set of 16 samples, comprising of four donors, spiked each with 0.0, 1.8, 3.6, and 7.2 $\mu\text{g/mL}$ of anti-panitumumab antibody. The experiment was conducted at both source (one time) and transfer labs (four times; one time with the analyst from the source lab) using conditions which showed minimal variability in transfer plan A. The results showed that the 90% concordance limits were contained within the predetermined equivalence interval (0.80, 1.25) for all the spiked samples. Equivalence for the unspiked samples was not achieved due to the overall bias at the transfer lab. Thus, in order to assess the concordance of the labs, the experiments were repeated using redefined acceptance criteria, which reflected the performance of the assay at the two labs (see below). This

was done prior to repeating the Part B experiments. The clinical impact of this change was considered minimal, due to the qualitative nature of the assay results.

Transfer Plan B: Re-execution Following Modified Acceptance Criteria

A set of samples from 20 donors were used for the formal concordance analysis in the transfer process. Each donor sample was analyzed in three different conditions; unspiked, 1.8 $\mu\text{g/mL}$ and 3.6 $\mu\text{g/mL}$ of anti-panitumumab antibodies spiked. The variables that were outliers were not used in the assay run in transfer plan B. The critical assay factors between sites were chip lot 2; NHS/EDC lot 2; Analyst 2 (source lab and transfer lab); Biacore 2 at source lab and Biacore 1 at transfer lab. The acceptance criteria were redefined based on the information obtained from results in transfer plan A and the investigation. In this respect, a new least acceptable Lin's CCC (0.9457) was determined by expanding the assumed location shift to 20% (previously at

Table IV. Concordance Plots of the Performance of the Biacore Assay at the Source and Transfer Labs, Prior to the Investigation of Bias (Lin's Concordance Results)

Statistics	CCC	Precision	Accuracy
Estimate	0.9837	0.9890	0.9946
95% Conf. Limit	0.9791	0.9856	0.9917
Criterion	0.9457		

Kappa=0.6952 with one-sided lower 95% confidence bound 0.6021

Table V. Concordance Plots of the Performance of the Biacore Assay at the Source and Transfer Labs, Prior to the Investigation of Bias (Kappa Statistics Table)

Frequency	Transfer lab	
	Negative	Positive
Source lab		
Negative	56	21
Positive	2	71

Kappa=0.6952 with one-sided lower 95% confidence bound 0.6021

Table VI. For BIACORE Assay Data and for Each Condition (Different Spike-in Concentration of Drug and Antibody), Total Variability in the Observations was Partitioned into Components that Measure the Variation Caused by Different Fixed Effects as Measured by the Percentage of the Total Sum of Squares (Measuring Total Amount of Variation Around Overall Mean), the Variability that was not Explained by Fixed Effects was Further Partitioned to the Contribution of Different Random Effects and Residual as Measured by the Percentage of the Total Unexplained Variability

Source	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5
Percentage of total sum of squares explained by fixed effects					
Site	3%	6%	7%	17%	3%
Chip Lot	18%	10%	4%	1%	21%
EDC/NHS Lot	0%	0%	0%	1%	0%
Subject	69%	61%	48%	31%	67%
Chip Lot $\times$ site	0%	0%	0%	3%	0%
EDC/NHS lot $\times$ site	0%	0%	0%	1%	0%
Site $\times$ subject	0%	0%	1%	1%	0%
Chip lot $\times$ subject	6%	7%	6%	8%	6%
EDC/NHS lot $\times$ subject	0%	0%	1%	2%	0%
Percentage of total assay variability ^a contributed by random effects and random noise:					
Analyst (site)	64%	52%	51%	41%	59%
Biacore (site)	3%	17%	19%	21%	0%
Day (site)	9%	3%	3%	7%	19%
Error	24%	28%	27%	31%	22%

The critical variables for the Biacore assay tested were analyst, Biacore instrument, chip lot, and EDC/NHS. These variables were tested across four runs by two analysts at each site. The sample set included eight sets of individual donor samples, prepared and analyzed unspiked (condition 1) and spiked with various concentrations of anti-panitumumab antibody (conditions 2, 3, 4) and panitumumab (condition 5)

^aTotal assay variability is the sum of the variability contributed by the variance components of the assay, such as analyst, washer and day, and random noise

12.5%). The criteria also included the qualitative requirement of >75% and >95% of the samples spiked at the intermediate concentrations, and higher concentrations have equivalent final results. In addition, a kappa coefficient criteria of >0.800 was established for the equivalence of sample results across both sites. All experiments passed assay acceptance criteria for the controls as stated in the analytical procedure.

All samples were tested using the specificity assay in order to generate a final result (positive or negative) similar to actual sample analysis. Twenty out of 20 of the unspiked samples had a final negative result at both sites. Seventeen out of 20 (transfer lab) and 18 out of 20 (source lab) samples spiked with 1.8 $\mu\text{g/mL}$ antibody had a final positive result. This met the criteria of >75% with 3.6 $\mu\text{g/mL}$ antibody had a final positive result. This met the criteria of >95%. The Kappa coefficient was determined to be 0.9645. This met the criteria of >0.8000 (Tables VII and VIII). The Lin's CCC estimate is 0.9913 with the one-sided 95% confidence (L95) 0.9870 (Fig. 3). The L95 estimate was larger than the predetermined criteria 0.9547 and thus met the redefined acceptance criteria.

Thus, the above transfer experiments conclude the concordance between source and transfer labs.

Transfer Plan C. (Proficiency Testing)

The purpose of transfer plan C was to establish a baseline set of data in the transfer lab, in order to monitor the proficiency of the immunoassays. Blinded negative and positive proficiency testing serum samples were generated by the source lab and sent to designated transfer lab for testing

purposes. The proficiency testing samples were analyzed by the validated transferred analytical procedure, using the all the processes of sample receipt, data analysis by LIMS systems, and reporting. For this purpose, 20 blinded samples were submitted for analysis and shipped. The performance of the ELISA and Biacore assays over time at the transfer lab is managed through trending process (Barger et al. manuscript in preparation). During the maintenance of the assay, critical reagents are qualified by at least by one independent process and assay runs (which include controls and a sample set, spiked at the lower limit of reliable determination); e.g. biotin-conjugated reagents are qualified using an HPLC profile, and specificity reactivity on the Biacore, before testing them in assay qualification runs.

DISCUSSION

The gold standard for a successful assay transfer is the equivalent performance of the bioanalytical assay at both labs, i.e., the range of the variability of the assay across the labs is acceptable. We have assessed the advantages of a step-wise method for transferring complex bioanalytical assays by understanding the factorial interactions between critical reagents across labs. This method has the benefit of a high probability of success, with a detailed understanding of the contribution of variability of critical variables.

The statistical methods for determining equivalent assay performance across laboratories have been reported based on specific requirements of assay transfer processes. In this respect, several investigators have utilized the traditional testing approach for bioanalytical assay transfers, which

Fig. 2. Concordance plots of the performance of the BIACORE assay at the source and transfer labs prior to investigation. The vertical and horizontal lines represent the assay cut point (ACP). There were two lines going through the scatter of the data with the gray solid one representing the concordance line (45° and passing through (1,1)) and the dotted blue one representing the regression line of RU at SOURCE LAB against RU at TRANSFER LAB. Three different spike-in conditions were represented by symbols in different colors and shapes

utilize the null-hypothesis of no difference between laboratories. The statistical criteria are based on the equivalence of the means between the two laboratories (8,9). Lin has proposed an approach using concordance correlation coefficient, which assess reproducibility of an assay compared to the gold standard assay (5). Bland and Altman (2) have indicated the requirement of equivalency of individual sample reading to be equivalent across two laboratories, and have proposed establishment of limit of agreements in transfer methods. Zhong *et al.* (10) have examined the tolerance interval approach, which provides boundaries with confidence intervals and coverage levels, and also an individual equivalence criterion that is consistent to the mean equivalence criterion. De Fontenay (3) has evaluated assay transfer using 17 different analytical transfers, using 10,000 simulations. In these analyses, the influence of analytical runs, method capability, acceptance criteria was analyzed on probability

of success of transfer of assays. The authors recommend use of a new descriptive approach and use of acceptance criteria that best suits the purpose of the transfer, depending on variability of the analytical method. Thus, the application of the various approaches for assay transfer varies on a case-to-case dependent. We have evaluated a process for transferring bioanalytical assays during clinical development of bio-pharmaceuticals.

By using a step-wise approach, we have demonstrated that the contribution of the critical reagents of the assays is not linear, but there are linked-interactions, which differ from site to site. In order to better understand the interactions of the variables, we have used the fractional factorial design of experiment approach to determine the critical reagents that contribute to the variability across sites. The critical reagents, analyst and equipment, that were considered outliers based on the analyses, were not used in the cross-site concordance

Table VII. Concordance Plots of the Performance of the ELISA Assay at the Source and Transfer Labs Following the Investigation and Adjustment of the Acceptance Criteria (Lin's Concordance Results)

Statistics	CCC	Precision	Accuracy
Estimate	0.9913	0.9936	0.9977
95% Conf. Limit	0.9870	0.9901	0.9950
Criterion	0.9457		

Kappa=0.9645 with one-sided 95% confidence bound 0.8954

Table VIII. Concordance Plots of the Performance of the ELISA Assay at the Source and Transfer Labs Following the Investigation and Adjustment of the Acceptance Criteria (Kappa Statistics Table)

Frequency	Transfer lab	
	Negative	Positive
Source lab		
Negative	22	0
Positive	1	37

Kappa=0.9645 with one-sided 95% confidence bound 0.8954

Fig. 3. Concordance plots of the performance of the BIACORE assay at the source and transfer labs in the investigation. The vertical and horizontal lines represent the assay cut point (ACP). There were two lines going through the scatter of the data with the gray solid one representing the concordance line (45° and passing through (1,1)) and the dotted blue one representing the regression line of RU at SOURCE LAB against RU at TRANSFER LAB. Three different spike-in conditions were represented by symbols in different colors and shapes

experimental run. The acceptance criteria were determined through historical experience, and were used to identify the combination of variables that have the best cross-site precision. The rationale of one assay run in the concordance experiment (transfer plan B) was to mimic the process in clinical sample testing, which is only tested once for a final result. While the ELISA performed faithfully to the assay validation at the source lab, the Biacore assays at the transfer labs had a consistent bias. Since the investigation was unable to clearly define the root cause of the bias, the acceptance criteria for the concordance analysis, in transfer plan B, was modified, and based on the reproducibility of the bias. In the Biacore assay, the assay transfer was designed, at first, only with Lin's concordance criteria, since the transfer plan was evaluating equivalent semi-quantitative RU values. The predefined concordance criteria of 0.950 were met in the assay transfer. However, the agreement of negative and positive results of the subjects was not equivalent between the sites. Due to this observation, addition acceptance criteria using kappa statistics was added, for the repeated analysis. Addition of these criteria enhanced the robustness of the assay transfer process.

Identifying the combination of variables that are outliers, provides an increased probability of success to the concordance. With the use of two separate and independent criteria, a risk-based approach could be used to make a decision on the conclusion of the data transfer. Utilizing this approach of defining critical variables and the use of independent

acceptance criteria, the high probability of success of transferring complex bioanalytical methods across sites will ultimately reduce cost, time, and anxiety over transferring and maintaining assays that is an essential component of the clinical drug development process.

Finally, we have utilized the CLIA/CAP process of proficiency testing (1), as a third step of the assay transfer, where we have defined a set of 16–20 samples, which span the range of the assay performance. Trend analysis of this sample set would provide a measure of the robustness of assay performance at the transfer lab (Barger, T *et al.* manuscript in preparation).

CONCLUSION

We have enhanced the stringency of assay transfer acceptance criteria (using precision, accuracy and concordance parameters), and improved the probability of success, by having a step-wise assay transfer design. The advantages of this assay transfer method include (1) better understanding of the contributors of variability at the source and transfer labs, at the time of transfer, (2) provides data (from the transfer plan A experiment) to establish a risk-based approach for defining acceptance criteria, (3) higher probability of success of achieving concordance (part B) and (4) a process for evaluating the long-term performance of the

assay, relative to the performance of the assay at the source lab (use of proficiency testing sample sets).

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