A Central Laboratory Inter-laboratory Comparison Program to Assess the Comparability of Data of Forty-one Tests from Four Regional Laboratories Involved in Global Clinical Trials over a Twelve-Month Period

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ABSTRACT

Objective
The objective of this study was to develop and support a process for assessing the comparability of data used in global clinical trials from four individual laboratories, owned by the same central laboratory entity, and that the same tests on the same samples would be under statistical control and acceptable limits of variation.

Relevance
Data from central laboratories have been key for assessing safety, tolerability, and efficacy of new drugs in clinical trials. With the increasing complexity and global scale of many clinical trials, it is important to maintain harmonization among the regional laboratories as part of a central laboratory participating in the same study. However, continuous monitoring of the same samples run at all laboratory locations may not be common practice.

Methodology
Pooled serum, plasma, and urine samples were aliquoted, frozen at -70ºC, and distributed quarterly to each laboratory. Samples were analyzed weekly on the same day at each laboratory for twelve months. The percent and absolute bias were calculated for each result using the US laboratory as the reference laboratory. The percent bias for each week and month was also calculated. A Bland-Altman plot was created between each laboratory and the reference laboratory for the twelve-month period, and a student T-test was run using a relative bias limit for each test and a significance level of 5%.

Results
Overall, during the twelve month period, all forty-one tests had a twelve month mean bias within the acceptable bias limit for each individual test compared to the reference laboratory. When comparing the mean weekly bias for each tests from all laboratories, any week where the bias was outside acceptable limits, investigation and corrective action was undertaken to determine the source of the error. Examples include:

- One week during the six-month period, three chemistry tests from the same laboratory had a mean weekly bias outside the acceptable limit. After investigation, it was determined that a pre-analytical issue with thawing/mixing of frozen samples was the cause of the bias.
- A negative bias was observed in a US laboratory for triglyceride. However, subsequent review of CDC Lipid Standardization Program Part III data from all laboratories globally demonstrated acceptable performance.
- Inconsistencies in the reporting of results across laboratories, specifically the technical decision not to report data deemed biologically implausible, were noted after monthly review of calcium data, and all technologists were re-educated on handling repeat analysis and appropriate consultation of Laboratory Directors.

Conclusion
An inter-laboratory program where frequent monitoring of identical samples run at all laboratories involved in clinical trials can provide valuable information into the harmonization of data reported by the central laboratory and help mitigate pre-analytic, analytic, and post-analytic issues that may arise when assessing data used in the development of new therapeutics.

INTRODUCTION

- Data from central laboratories are key for assessing safety, tolerability, and efficacy of new drugs in clinical trials.
- The Inter-laboratory Comparison Program was set up among all laboratories wholly owned by Medpace Reference Laboratories (MRL), a global central laboratory, in 2006. The locations include Cincinnati, OH (US); Leuven, Belgium (BE); Singapore (SG); and Beijing, China (CN). The program involves multiple platforms to assess over 40 analytes tested globally, encompassing several therapeutic areas including Oncology, Cardiometabolic, Infectious disease, and others.
- Continuous monitoring of sample analysis at all regional laboratories is essential to ensure data harmonization within pre-defined acceptance criteria is maintained, irrespective of laboratory location.

METHODOLOGY

Samples
- Pooled serum, plasma, and urine samples were aliquoted, labeled with the appropriate sample information, frozen at -70ºC, and distributed quarterly to each laboratory for weekly analyses on the same day.
- The pooled samples were from participants in clinical trials. All samples were received de-identified of demographic information.

Analytical methods
- Over a 12-month period, sample analysis was performed on 41 tests (Table 1) at each Medpace laboratory location in weekly batches of 10 samples and were analyzed on the same day in each laboratory (N=520).
- On the day of analysis, samples were thawed and thoroughly mixed.
- Appropriate Quality Controls were analyzed with each batch and results accepted based on global SOPs.
- Acceptable results were electronically transferred into ClinTrak Lab®, an in-house developed, clinical trial management system.

Table 1: List of Analytes and Platforms

Beckman Coulter AU Series Chemistry Analyzers

ALP (U/L)	Lipoprotein (a) (mmol/L)
ALT (U/L)	Iron (µg/dL)
AST (U/L)	BUN (mg/dL)
Amylase (U/L)	Creatinine (mg/dL)
Chloride (mmol/L)	UA (mg/dL)
Creatinine Kinase (U/L)	PO4 (mg/dL)
LDH (U/L)	Sodium (mmol/L)
GGT (U/L)	Triglyceride (mg/dL)
TBil (mg/dL)	Total Cholesterol (mg/dL)
Albumin (g/dL)	HDL-Cholesterol (HDL-C) (mg/dL)
Total Protein (g/dL)	Magnesium (mg/dL)
Calcium (mg/dL)	Urine Creatinine (mg/dL)
Glucose (mg/dL)	Urine Protein (mg/dL)
Potassium (mmol/L)	

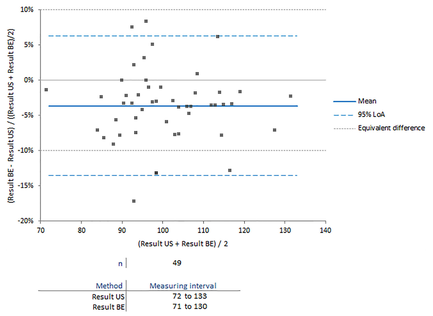
Roche Immunoanalyzers	Siemens BNII Nephelometer
Insulin (µIU/mL)	Apo AI (mg/dL)
TSH (µIU/mL)	Apo B (mg/dL)
T4 (ug/dL)	hsCRP (mg/L)
C-peptide (ng/mL)	Urine Albumin (mg/dL)
Stago Compact	**Tosoh G7/G8**
PT (sec)	HbA1C (%)
aPTT (sec)	

Preparative Ultracentrifugation (PUC)

LDL Cholesterol (LDL-C) (mg/dL)
VLDL Cholesterol (VLDL-C) (mg/dL)
VLDL-C/Trig ratio

Statistical methods
- Summary statistics listing the bias criteria, number of samples, and mean difference (absolute or relative) compared to the reference laboratory (MRL-US) were included. The P-values are from TOST t-test performed on absolute or relative difference.
- A Bland-Altman plot was created between each laboratory and reference laboratory for the 12-month period (Figure 1).
- Bias criteria were based on criteria from the College of American Pathologists, Westgard Biological Variation Database, and Royal College of Pathologists of Australasia.
- Some test and laboratory locations have an N of <520 for a given analyte due to insufficient quantity, instrument error, etc.

Figure 1: Bland-Altman difference plot of LDL-C



RESULTS

All tests run on Beckman Coulter AU Series Chemistry Analyzers, Roche Immunoanalyzers, Siemens BNII Nephelometers, Stago Compact Coagulation Analyzers, Tosoh G7/G8 HPLC Analyzers, or by preparative ultracentrifugation demonstrated acceptable equivalence when compared to the reference laboratory over a 12-month period (Table 2). Representative examples of pre-analytic, analytic, and post-analytic issues detected over the twelve-month period include:

- Monthly review of BUN data (Table 3) showed a mean bias for BE laboratory of -14.28%. Upon further review of other tests run on the same sample, a similar negative bias was seen (Figures 2, 3, 4). After investigation, it was determined the cause of bias was due to a pre-analytical error, specifically improper mixing during the freeze/thaw process.

  Technologists were re-educated on the proper freeze/thaw process when analyzing samples that have been stored at -70ºC.
- A negative bias of ~4.5% for triglyceride was observed in the US laboratory compared to the other laboratory locations (Figures 5, 6, 7). The CDC Lipid Standardization program results for 2016 were reviewed for all four laboratories and indicated good performance, with the bias averaging -2.29%, -0.51%, -0.57%, and -1.76% for the US, BE, SG, and CN labs, respectively, when compared to CDC targets (Table 4).
- Reporting inconsistencies were noted between laboratories for calcium during the review period, with some labs reporting a numeric value, and other labs electing to not report a numeric value due to concerns the calcium values were biologically implausible, indicating a pre-analytic error affecting sample integrity (data not shown).

  Corrective actions included re-education on the process to determine the validity of results, including, but not limited to, repeat analysis, delta checking, and consultation with the Laboratory Director as to clinical validity.

CONCLUSION

An inter-laboratory comparison program where frequent and continuous monitoring of identical samples analyzed at all laboratories involved in clinical trials is conducted can provide valuable information into the harmonization of data reported by the central laboratory and help mitigate pre-analytic, analytic, and post-analytic issues that may arise when assessing data used in the development of new therapeutics.

Table 2: Summary Statistics of Analytes

	Test	Bias Criteria	BE N	BE Mean Diff. (Abs or %)	CN N	CN Mean Diff. (Abs or %)	SG N	SG Mean Diff. (Abs or %)
Beckman Coulter Chemistry Analyzers	ALP	12%; Abs (15) if ≤125	512	2	513	-3	509	-2
	ALT	10%	510	-2.3%	520	-3.9%	516	5.7%
	AST	10%	510	-1.0%	520	-1.9%	516	4.2%
	Albumin	6%; Abs (0.2) if ≤3.3	510	0.4%	520	2.1%	516	2.3%
	Amylase	10%	510	1.8%	520	-0.1%	516	2.2%
	BUN	10%	510	0.1%	520	-1.8%	516	2.2%
	Calcium	4%	499	0.8%	510	2.5%	508	0.2%
	Chloride	2.5%	510	0.5%	520	0.2%	516	0.1%
	Creatinine	10%	510	0.9%	520	5.0%	516	4.0%
	Creatine Kinase	8%	510	-0.4%	520	0.7%	516	-0.5%
	Gamma-Glutamyl Transferase	8%	510	2.4%	519	1.5%	516	-1.0%
	Glucose	5%	510	0.8%	520	1.5%	516	1.9%
	Iron	6%	498	1.0%	507	-0.1%	507	1.3%
	LDH	7.5%	510	-3.0%	520	0.3%	516	-0.7%
	Magnesium	10%	502	1.6%	512	-0.2%	508	1.0
	PO_4	8%; Abs (0.2) if ≤2.3	510	0.1	520	0.1	516	0.1
	Potassium	3%	498	0.9%	509	1.6%	507	0.2%
	Sodium	2%; Abs (3) if ≤150	510	1.2%	520	0.9%	516	0.7%
	Total Bilirubin	12%; Abs (0.18) if ≤1.46	520	0.0%	520	-2.9%	516	5.9%
	Total Protein	5%	510	5.0%	520	1.3%	516	0.6%
	Triglyceride	8%	510	4.2%	520	2.1%	516	3.9%
	Total Cholesterol	6%	175	0.1%	176	0.8%	173	1.7%
		Abs (11.5) if ≤193	337	0.6	346	1.3	343	2.7
	HDL-Cholesterol	9.24%	510	0.1%	520	2.9%	516	5.5%
	Lipoprotein (a)	15%	518	1.9%	517	3.4%	514	5.1%
	Uric Acid	6%	510	2.5%	520	0.5%	516	3.1%
	Urine Creatinine	14%	520	-0.5%	520	6.5%	520	-1.1%
	Urine Protein	20%	518	-5.2%	518	5.3%	518	0.0%
Roche Immunoanalyzers	Insulin	12%	520	-0.2%	520	1.0%	516	3.6%
	Thyroid Stimulating Hormone	10%	510	-1.2%	519	1.5%	516	2.6%
	T4	10%; Abs (0.93) if ≤9.32	510	-1.9%	*CN laboratory did not perform T4 Analysis*		516	-3.6%
	C-Peptide	10%	513	0.6%	519	-0.6%	516	-2.3%
Siemens BNII Nephelometers	Apolipoprotein AI	10%; Abs (20) if ≤200	510	-3	520	10	516	-3
	Apolipoprotein B	10%; Abs (20) if ≤200	510	-3	520	4	516	2
	High Sensitivity CRP	10%	430	-0.9%	437	4%	435	-4.0%
		Abs (0.2) if ≤2	79	-0.03	81	0.05	81	-0.06
	Urine Albumin	20%	153	-2.7%	154	7%	153	2.3%
		Abs (0.4) if ≤2	324	0.063	154	2.34	335	0.06
Preparative Ultracentrifugation (PUC)	LDL-C	10%	49	-3.6%	49	-1.2%	*SG laboratory did not perform PUC*	
	VLDL	40%	49	10.0%	49	2.6%		
	VLDL/Trig Ratio	40%	49	5.3%	49	-0.9%		
Stago Compact Coagulation Analyzers	PT	10%	108	4.9%	*CN laboratory did not perform PT or PTT analysis*		107	3.4%
	PTT	10%	109	7.9%			110	-0.4%
Tosoh G7/G8	HbA1C	6%	120	-0.9%	100	3.3%	120	1.8%
	All P-values are from TOST t-test performed on absolute or relative difference: <0.0001							

Table 3: Weekly Bias for BUN

Week of	% Bias Weekly Avg (BE)			% Bias Weekly Avg (CN)			% Bias Weekly Avg (SG)		
	BUN	K^+	Cl^-	BUN	K^+	Cl^-	BUN	K^+	Cl^-
31-Jan-2016	-2.89	2.28	1.81	-5.31	1.39	0.49	-0.04	0.22	-0.10
07-Feb-2016	-0.76	3.74	-0.46	-4.19	1.35	-2.54	2.84	0.66	-3.02
14-Feb-2016	-14.28	-11.95	-10.61	0.51	1.80	1.14	2.52	0.93	0.10
21-Feb-2016	-2.20	-4.77	-5.01	-0.69	0.92	0.67	5.87	0.87	0.29
28-Feb-2016	-2.88	1.81	0.57	0.05	1.37	0.19	0.54	0.91	-0.29

Table 4: CDC Lipid Standardization Program 2016

Triglycerides									
Pool Series		US		BE		SG		CN	
		mg/dL	% Bias	mg/dL	% Bias	mg/dL	% Bias	mg/dL	% Bias
Q1 136	141	116.35	-0.89	119.00	1.37	117.75	0.31	116.75	-0.55
	471	105.73	-2.34	109.00	0.68	108.00	-0.24	92.50	1.38
	472	93.68	-2.67	96.25	0.01	95.50	-0.77	79.75	-3.89
Q2 137	144	78.40	-4.76	81.50	-1.00	80.75	-1.91	107.75	-3.12
	471	106.35	-1.76	108.25	-0.01	107.75	-0.47	107.75	-0.47
	473	227.93	2.84	229.75	3.66	226.00	1.97	230.50	4.00
Q3 138	474	78.03	-4.87	79.25	-3.38	79.50	-3.07	76.50	-6.73
	571	87.15	-2.55	87.00	-2.72	87.50	-2.16	85.50	-4.39
	801	112.95	-3.02	116.75	0.24	116.50	0.03	115.00	-1.26
Q4 139	476	122.63	-3.12	126.25	-0.25	126.50	-0.06	123.50	-2.43
	477	262.18	0.01	260.00	-0.82	267.25	1.95	264.50	0.90
	571	85.58	-4.31	86.00	-3.84	87.25	-2.44	87.75	-1.88
Average Bias %			-2.29		-0.51		-0.57		-1.76

Figure 2: Weekly bias for BUN

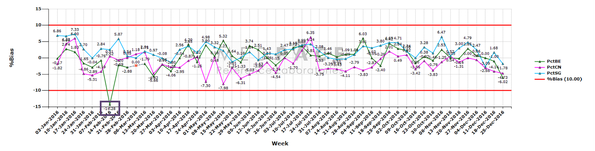


Figure 3: Weekly bias for chloride

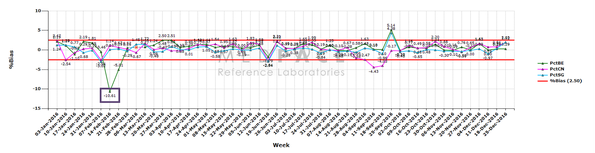


Figure 4: Weekly bias for potassium

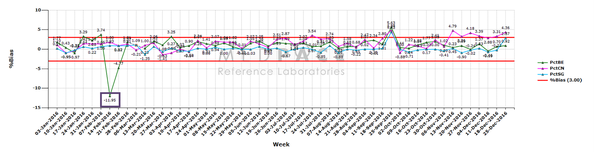


Figure 5: Triglyceride samples during a 12-month period

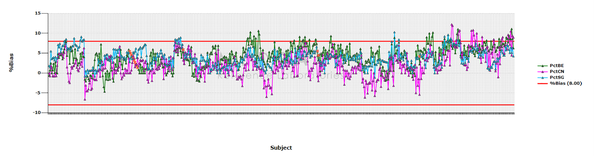


Figure 6: Weekly bias for Triglyceride during a 12-month period

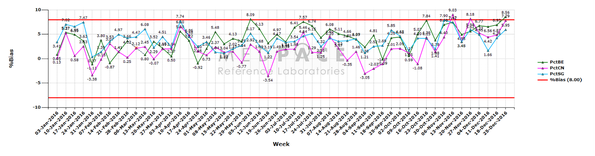
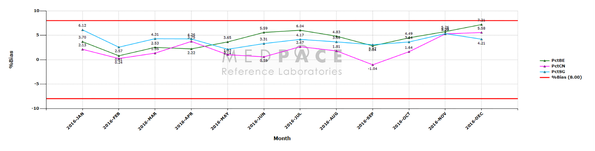


Figure 7: Monthly bias for Triglyceride during a 12-month period



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ACKNOWLEDGMENTS

The authors gratefully acknowledge Megan Coffman for her assistance in the design and layout of this presentation.

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