Multisite study of Enzyme analytical performance reveals opportunity to tighten global performance specifications

S. Westgard *Westgard QC, Madison, WI, corresponding author: westgard@westgard.com.*

We gratefully acknowledge the contribution of data from the following Sigma VP laboratories: Ara Damansara, Kuala Lumpur, Malaysia; Sunway Medical Centre, Selangor, Malaysia; INVITRO Laboratories, Moscow, Russian Federation; Guizhou Provincial Hospital, Guiyang, China; Winchester Medical Center, Winchester, VA, USA; Multicare healthcare system (6 branches: Allenmore, Covington, AMCH, GSH, GH, Tacoma core lab); Chi Mei Hospital System(2 branches: Liou Yong, Yong Kang), Tainan, Taiwan; Quality Healthcare Medical Service (QHMS), Hong Kong; PPUKM, Kuala Lumpur, Malaysia; Praram 9 Hospital, Bangkok, Thailand; Innovative Diagnostics (2 branches, main core lab and camden), Singapore; Loh Guan Lye Specialists Centre, Penang, Malaysia; Penang Pantai Premier Pathology, Penang, Malaysia; Prodia Laboratories (5 branches: Semarang, Bandung, Bekasi, Malang, Surabaya), Jakarta, Indonesia; Ramathibodi Laboratory (2 branches: SDMC, and Lean Lab), Bangkok, Thailand; Siriraj Piyamaharajkarun Hospital (SiPH), Selangor, Malaysia; Serdang Hospital, Kajang, Malaysia; National Guard Health Affairs Laboratory, Riyadh, Saudi Arabia; Wuhan Union Hospital, Jinghan, Wuhan, China; Shandong Hospital, Jinan, China; Zhongshan Hospital, Shanghai, China; People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi, China; Women's Hospital of Zhejiang University, Hangzhou, China; Sheng Jing Hospital of China Medical University, Shenyang, China.

♦ Abstract

This study evaluated the practical capability of labs around the world to achieve different allowable total error (TEa) goals from CLIA, the older and newly revised "Ricos Goals", the Chinese NCCL goals, and the RCPA goals. Five enzymes assays were evaluated: AST, ALT, Alkaline Phosphatase, Amylase, and Creatinine Kinase (CK). 32 laboratories from 9 different countries contributed imprecision data from controls and bias data from EQA or peer group assessment on a total of 62 instruments. Sigma-metrics were calculated using different TEa goals. The percentage of laboratories able to achieve a given TEa at a specific Sigma-metric was tabulated, with 80% success in compliance set as the goal for practical implementation.

♦ Introduction

Analytical Performance specifications (also known as allowable total error goals, TEa) for enzyme assays are not harmonized throughout the world. On just AST, allowable total error specifications range from 12% to 21% depending on the country or EQA/PT program. Since the clinical use and interpretation of most enzyme assays *are* standardized, the goals to judge method acceptability would also benefit from standardization. However, standardizing on an analytical performance specification that no current methods on the market can achieve would be counter-productive; it would require all methods to drastically increase their use of control materials, rules, and QC frequency without necessarily providing any tangible benefit to patient care.

It would be beneficial to compare analytical performance specifications with current analytical performance of enzyme methods. If current methods cannot achieve today's TEa goals with high reliability, that may indicate that some performance specifications aren't realistic or practical for today's laboratories.

♦ Methods

62 instruments (Abbott ARCHITECT instruments, ranging from the c16000, c8000, to the c4000 model, as well as Roche and Olympus) from 32 laboratories in 9 countries (China, Hong Kong, Indonesia, Malaysia, Russia, Saudi Arabia, Taiwan, Thailand, USA) participated in a Sigma Verification program where they reported their analytical performance data. Imprecision was estimated from routine controls (typically Bio-Rad), with 1 to 3 months of data. Bias or inaccuracy was estimated from EQA/PT programs, peer group comparisons, or comparisons of the observed mean vs. the assayed/target means of the controls. Using the data from these laboratories, their ability to achieve the allowable total errors from CLIA proficiency testing criteria, the existing biological variation-based "Old Ricos" goals, the 2017 revised "New Ricos", the NCCL (national Chinese EQA), and the Australian RCPA goals were assessed. The method of evaluation the appropriateness of performance specifications was assessed by the calculation of analytical Sigma-metrics.

Sigma-metric Assessment

The method of evaluation of the appropriateness of performance specifications was assessed by the calculation of analytical Sigma-metrics. The standard analytical Sigma-metric equation was used:

Sigma-metric = (TEa - bias) / CV

The percentage of laboratories able to achieve 5 Sigma (excellent) performance or better based on these goals was determined. A target of achieving 80% or better was considered successful, as this mirrors the Spanish EQA approach for determining minimum acceptable performance criteria for External Quality Assessment programs (see references).

A further refinement of the assessment approach was to focus key decision levels, usually the low end of performance. Since performance can vary around the entire reportable range of an assay, using a broad sweep of the data may miss the specific clinical needs at medical decision levels. Therefore, we narrowed our gaze to the performance closest to a key medical decision level for each test.

TEa source	AST	ALT	Alkaline Phosphatase	Amylase	Creatinine Kinase
CLIA	20%	20%	30%	30%	30%
NCCL	15%	16%	18%	15%	15%
"Old" Ricos	16.69%	27.48%	12.04%	14.6%	30.3%
"New" Ricos	13.4%	14.4%	10.7%	13.4%	20.4%
RCPA	5 U/L < 40 U/L 12% > 40 U/L	5 U/L < 40 U/L 12% > 40 U/L	15 U/L <25 U/L 12% > 25 U/L	10 U/L < 100 U/L 10% > 100 U/L	15 U/L > 125 U/L 12% > 125 U/L

Table 1. Review of Global Analytical Performance Specifications for enzymes

Assay	Critical Medical Decision Level
AST	40 U/L
ALT	95 U/L
Alkaline Phosphatase	150 U/L
Amylase	145 U/L
Creatinine Kinase (CK)	275 U/L

Table 2. Critical Medical Decisions Levels for enzymes

♦ Results

AST % of levels achieving Sigma	CLIA	NCCL	Old Ricos	New Ricos	RCPA
≥6 Sigma	88.52%	45.90%	63.93%	32.71%	36.07%
5 Sigma	6.56	24.59	21.31	21.31	9.84
4 Sigma	4.92	18.03	9.84	27.87	22.95
3 Sigma and below	0	11.48	4.92	18.03	31.15
Achieved 4,5,6 Sigma	**100%**	**88.52%**	**95.08%**	**81.97%**	**68.85%**

Table 3. Ability of AST assays to achieve different TEa performance specifications

ALT % of levels achieving Sigma	CLIA	NCCL	Old Ricos	New Ricos	RCPA
≥6 Sigma	87.1%	64.52%	93.55%	56.45%	40.32%
5 Sigma	6.45	19.35	4.84	19.35	12.9
4 Sigma	0	9.68	1.61	12.9	19.35
3 Sigma and below	6.45	6.45	0	11.29	27.42
Achieved 4,5,6 Sigma	**93.55%**	**93.55%**	**100%**	**88.71%**	**72.58%**

Table 4. Ability of ALT assays to achieve different TEa performance specifications

♦ Results continued.

Alk Phos % of levels achieving Sigma	CLIA	NCCL	Old Ricos	New Ricos	RCPA
≥6 Sigma	89.83%	54.24%	15.25%	3.39%	11.86%
5 Sigma	8.47	8.47	22.03	15.25	22.03
4 Sigma	0	10.17	15.25	23.73	16.95
3 Sigma and below	1.69	27.22	47.46	57.63	49.15
Achieved 4,5,6 Sigma	**98.31%**	**72.88%**	**52.54%**	**42.37%**	**50.85%**

Table 5. Ability of Alk Phos assays to achieve different TEa performance specifications

Amylase % of levels achieving Sigma	CLIA	NCCL	Old Ricos	New Ricos	RCPA
≥6 Sigma and higher	97.14%	62.86%	62.86%	57.14%	40.00%
5 Sigma	0	20	22.86	17.14	11.43
4 Sigma	0	5.71	5.71	14.29	8.57
3 Sigma and lower	2.86	11.43	8.57	11.43	22.86
Achieved 4,5,6 Sigma	**97.14%**	**88.57%**	**91.43%**	**88.57%**	**77.14%**

Table 6. Ability of Amylase assays to achieve different TEa performance specifications

Creatinine Kinase (CK) % of levels achieving Sigma	CLIA	NCCL	Old Ricos	New Ricos	RCPA
≥6 Sigma and higher	98%	74%	98%	92%	46%
5 Sigma	2	12	2	2	18
4 Sigma	0	6	0	0	20
3 Sigma and lower	0	8	0	6	16
Achieved 4,5,6 Sigma	**100%**	**92%**	**100%**	**94%**	**84%**

Table 7. Ability of CK assays to achieve different TEa performance specifications

Assay	Recommended TEa goal
AST	"New Ricos" 13.4%
ALT	"New Ricos" 14.4%
Alkaline Phosphatase	CLIA 30%
Amylase	"New Ricos" 13.4%
Creatinine Kinase (CK)	RCPA 15 U/L > 125 U/L 12% > 125 U/L

Table 8. Final recommendation of most stringent but practical TEa goals.

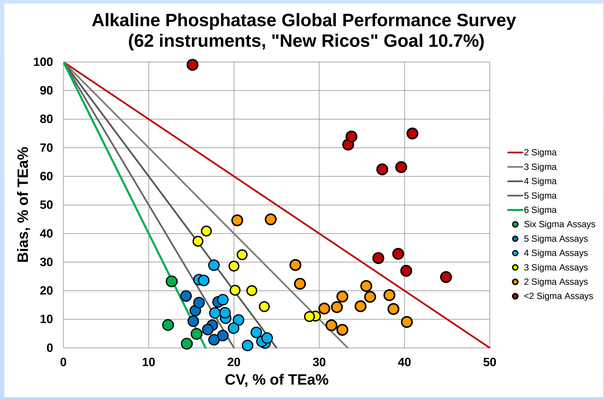

Figure 1. Normalized Method Decision Chart (MEDx) display of Sigma performance of 62 instruments running an Alkaline Phosphatase assay, benchmarked to a "New Ricos goal" of 10.7% allowable total error.

♦ Limitations

Mainly this study included data from just one instrument system, so the findings may not reflect the capability of other diagnostic manufacturers to achieve enzyme analytical performance specifications. Other studies should be done to evaluate the other manufacturers.

The heterogeneous nature of data collection (i.e. different controls, different ways to determine bias) may have injected too much variation in the study results. It also would be preferable to assess bias against a reference method or material. Typically, however, laboratories cannot perform this type of bias assessment due to the impractical expense and general unavailability of such methods and materials. While the laboratories included in this study may not conduct ideal bias assessments, they nevertheless represent a global sample of actual analytical performance, provide a true "real world" snapshot of routine operation.

Thus the findings of this study are more realistic for practical implementation of analytical performance specifications.

♦ Conclusion

All labs were easily able to achieve CLIA TEa goals. With the exception of Alkaline Phosphatase, more stringent analytical goals are possible for laboratories to achieve for AST, ALT, and Amylase. For CK, the RCPA goal for performance can be adopted. This evaluation revealed that *there is no single set of goals that is uniformly practical* for all analytes. Sometimes the Australian RCPA goals are appropriate. Other times, the older or newer "Ricos goals" are a better choice.

There is ample evidence that CLIA goals and the "Old Ricos" goals can be discarded and newer, tighter goals can be adopted *for most enzyme assays*. Even as these more challenging goals are applied, the evidence shows that laboratories will not have to significantly increase their QC monitoring, striking a balance between the pragmatic financial operations and the scientific mission to improve quality.

♦ References

CLIA proficiency testing criteria for acceptable analytical performance, Federal Register February 28, 1992;57(40):7002-186. Also unofficially posted at https://www.westgard.com/clia.htm

College of American Pathologists Proficiency Testing. http://www.cap.org/web/home/lab/proficiency-testing Participation is required to access the acceptability criteria.

Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation, **Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500. Updated in 2014 and posted at** https://www.westgard.com/biodatabase1.htm

RCPA Allowable Limits of Performance for Biochemistry. http://www.rcpaqap.com.au/docs/2014/chempath/ALP.pdf accessed5/23/2017. Also unofficially posted at https://www.westgard.com/rcpa-biochemistry.htm

Minimum analytical quality specifications of inter-laboratory comparisons: agreement among Spanish EQAP organizers, Carmen Ricos, Francisco Ramon, Angel Salas, Antonio Buno, Rafael Calafell, Jorge Morancho, Gabriella Gutierrez-Bassini and Josep M Jou, Interdisciplinary Expert Committee for Quality Specifications in the Clinical Laboratory, Clin Chem Lab Med 2012;50(3):485-461. Also unofficially posted at https://www.westgard.com/minimum-requirements.htm

Revision of the "Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations – RIli-BAEK" (unauthorized translation) J Lab Med 2015; 39(1): 26–69. Also unofficially posted at https://www.westgard.com/rilibak.htm

Biological Variation Estimates Obtained from 91 Healthy Study Participants for 9 Enzymes in Serum. Carobene A, Røraas T, Sølvik UØ et al Clin Chem. 2017 Jun;63(6):1141-1150.