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Performance evaluation using the "Decision Chart" and "Six Sigma Metric" concept for measurement of haematological parameters for Athlete Biological Passport

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Abstract

Determination of analytical method performance is critical in quality assurance, in providing precise and accurate results. A dual reference system for managing the analytical accuracy for determination of haematological parameters was introduced. This system is based on two practical application tools: "Decision Chart" and "Six Sigma Metric", developed by James O. Westgard. The assessment criterion is based on experimental Total Error value (TE%) calculus versus the Allowable Total Error (TEa%) value, specified in the "Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation", a database of biologic goals, updated and compiled by Dr. Carmen Ricos et al. and updated in 2014. As an example, industrial guidelines recommend a minimum of 3-sigma for a routine production process. The value of the sigma metric applied to the analytical method, higher than 6 (six), proves the world class quality of the testing process, which is our set goal. Our results performance level, using "Method Decision Chart" and "Six Sigma Metric" concepts was: 50% World Class, 16.6% Excellent, 16.6% Good and 16.6% Limit. This proves the applied method for measuring haematological parameters for Athlete Biological Passport is highly reliable and performant. This quality control system allows for quick detection of a measurement error by monitoring over time, the accuracy and precision of test performance. These attributes may be influenced by various factors such as: analyzer performance, environmental conditions and operator's precision.

Introduction

The main objective of doping control laboratories is obtaining accurate and reliable results for the analyzed samples. Laboratories are better assisted in achieving this goal when using additional quality control systems for validating results's accuracy. A dual reference system used as internal quality control for determination of haematological parameters method for the Athlete Biological Passport was introduced. The system is based on the concept of Total Error (TE%) which describes the combined effects of random and systematic errors, including practical application tools such as: "Method Decision Chart" and "Six Sigma Metric" developed by Westgard JO [1]. The evaluated parameters were: RBC, HGB, HCT, RET #, WBC and PLT, directly measured, TEa specified in Dr. Ricos C. biodatabase.

Experimental

Sysmex XT-2000*i* (Sysmex Corporation, Kobe, Japan) - an automated haematology analyzer, capable of providing a 16-parameter hemogram, a 5-part differential, and RET including IRF information.
The analyzer Sysmex XT-2000*i* was *calibrated* by Sysmex service engineers according to the manufacturer's guidelines, using Sysmex recommended calibrator SCS-1000 lot number 3148 0525.
The *reagents* used in the study were those recommended by the manufacturer.
Three levels of quality control material *e*-Check (Level 1, 2, and 3) were used for this study.

The *precision* (CV%) and *accuracy* (Bias%) for both, closed and open mode, were evaluated by performing 15 measurements in each mode, over a period of 3 consecutive days on the *e*-Check materials (Level 1, 2, and 3).
Total error (TE%) was obtained from combined of total random component (CV%), and systematic component (Bias%), and must be less than TEa, (eq.1):
TE% = 2XCV% + Bias% (eq.1)
For the "Six Sigma Metric" method, the *Sigma metric* parameter is calculated with (eq.2):
Sigma metric = [TEa% – Bias%]/CV% (eq.2)
For the "Decision Chart" method, the performance limit for TE% were established by following relations, (eq.3a-e), [3]:
Bias% + 2 CV% ≤ TEa% (eq.3a)
Bias% + 3 CV% ≤ TEa% (eq.3b)
Bias% + 4 CV% ≤ TEa% (eq.3c)
Bias% + 5 CV% ≤ TEa% (eq.3d)
Bias% + 6 CV% ≤ TEa% (eq.3e)
On the *Decision Chart,* illustrating these five inequalities, an "operating point" is plotted to represent the observed Bias% as the y-coordinate and the observed CV% as the x-coordinate. Diagonal lines are area-limiting, from left to right: 6-sigma, 5-sigma, 4-sigma, 3-sigma, and 2-sigma areas.

Results and Discussion

Decision Charts for each parameter following *e*-Check Level 2 analysis for closed mode vs. open mode are represented in Figure 1a-f.
The method shows World class performances for RBC-closed mode, HGB-closed and open mode, WBC-closed and open mode, PLT and RET#-open mode. The World class performance area is situated bellow the line corresponding to equation 3e. For example, in the Decision chart for HGB-closed mode, the method has a Bias of -0.06% and a CV of 0.38% and the operating point falls in the area corresponding to 6-sigma. The calculated Sigma metric value is 10.87; the Sigma metric > 6 is confirming 6-sigma Decision chart [fig1b].
For RBC-open mode and HCT-closed mode the method shows Excellent performances; the operating points are plotted bellow the line corresponding to eq.3d in the area corresponding to 5-sigma, confirming the 5-sigma quality.
For HCT-open mode the method shows Good performance. The operating point, bellow the line of the eq.3c, indicates 4-sigma quality. The Bias is 0.78%, the CV is 0.66%, Sigma metric is 4.83.
The method shows Limit performance for PLT and RET#-closed mode, with Bias of -5.68%, CV of 1.94% and Sigma metric of 3.98. The operating point falls close to the line that separates 4-sigma from 3-sigma (eq.3c).
Similar performance classification of the closed mode vs. open mode analysis for *e*-Check Level 1 and 3 was obtained (Tables 1, 2).
Overall performance: 50% World Class, 16.6% Excellent, 16.6% Good and 16.6% Limit.
For values below 3-sigma metric, quality control check list for the method is absolutely mandatory. Any decalibration of the analyzer can result in errors, leading to unacceptable results. For this reason, there should be paid more attention to pre-analytical process (samples condition, homogenization of samples, quality of reagents used, ambient temperature, etc.) to improve the quality and accuracy of measurements.

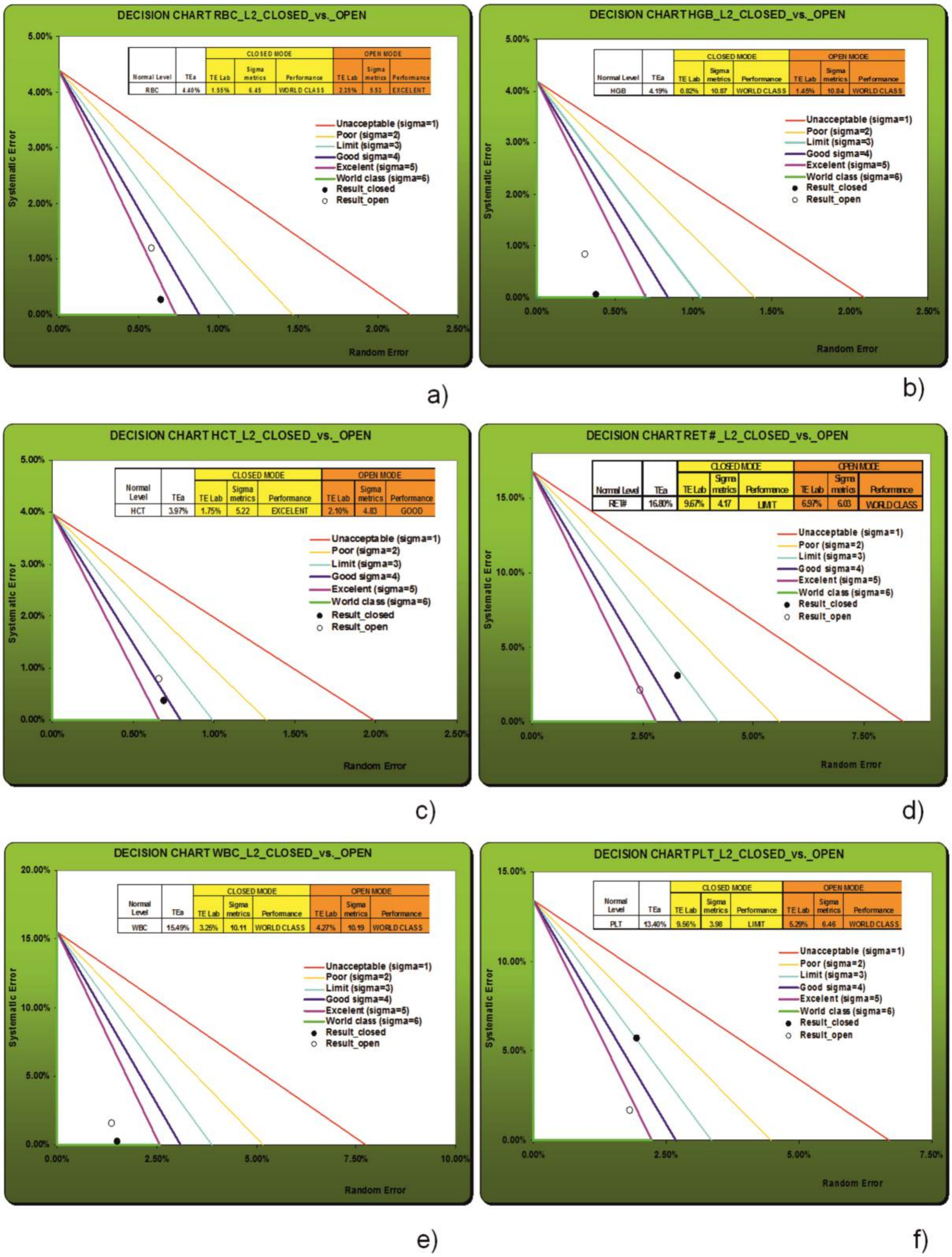

Figure 1. Decision charts of qualitative performance areas, closed and open mode analysis of *e*-Check Level-2 for: a) RBC; b) HGB; c) HCT; d) RET #; e) WBC; f) PLT

Low Level	TEa (%)	OPEN MODE TE Lab (%)	OPEN MODE Sigma metrics	OPEN MODE Performance	CLOSED MODE TE Lab (%)	CLOSED MODE Sigma metrics	CLOSED MODE Performance
RBC *10^6/uL	4.4	1.43	7.82	World class	2.19	7.02	World class
HGB g/dL	4.1	1.79	>15	World class	1.81	5.26	Excellent
HCT %	3.97	1.69	5.21	Excellent	3.17	3.6	Limit
RET # *10^6/uL	16.8	12.63	3.6	Limit	9.02	4.32	Good
WBC *10^3/uL	15.49	6.81	5.69	Excellent	5.70	6.06	World class
PLT *10^3/uL	13.4	9.00	3.26	Limit	8.10	4.86	Good

Table 1. Closed mode vs. open mode Low Level (e-Check Level 1) results

High Level	TEa (%)	OPEN MODE TE Lab (%)	OPEN MODE Sigma metrics	OPEN MODE Performance	CLOSED MODE TE Lab (%)	CLOSED MODE Sigma metrics	CLOSED MODE Performance
RBC *10^6/uL	4.4	2.5	6.20	World class	1.37	9.21	World class
HGB g/dL	4.1	1.96	8.03	World class	1.74	12.21	World class
HCT %	3.97	2.09	5.3	Excellent	2.45	4.87	Good
RET # *10^6/uL	16.8	9.04	4	Good	7.79	4.36	Good
WBC *10^3/uL	15.49	3.71	12.91	World class	4.24	11.00	World class
PLT *10^3/uL	13.4	3.36	9.72	World class	10.79	3.92	Limit

Table 2. Closed mode vs. open mode High Level (e-Check Level 3) results

Conclusions

Using “Decision Chart” and “Six Sigma Metric” system as additional refining validation tools in methods lacking reference point for estimation of measurement uncertainty, shows that the measurement of haematological parameters method for Athlete Biological Passport, support in the highest degree the analytical performance. The main benefits of this quality control system are: quick visualization of measurement errors, straight monitoring of accuracy and precision of the test performance; valuable, immediate support tool for re-checking the pre-analytical steps where test parameters may be influenced by alterations in analyzer’s performance, in environmental conditions and variance in operator’s performance.
Our goal of reaching World class performance by using these additional validation tools, opened the way to refining the pre-analitical procedures and documenting them accordingly.

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