Analytical Approach for vacuum tube validation: Comparison procedure of blood collection tubes as a part of local validation in pre-analytical phase

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↓ BACKGROUND:

Validation and verification of blood collection tubes have become demand procedures in medical laboratories, since they use various brands of IVD technologies for preanalytical phase. Common principles of comparisons of tested and reference tubes are explained from analytical point of view with evaluation of precision from duplicates, trueness, and ordinal linear regression analysis with indication of risk in clinical interpretation, estimation of difference and normality of distribution.

↓ OBJECTIVES:

To apply analytical validation approach for different brands of tubes with clot activator by method describes in CLSI protocols EP9-A. EXCEL spreadsheet program developed by Kallner A. was used for calculation quality specification, regression analysis and visualization graphs of comparisons

↓ METHODS:

Sample collections were made in 40 patients from St. Luka Hospital to two tubes of Lind-Vac (Estonia) and Vacuette(Austria)pereachusingCLSIH3-A6andanalyzed in biochemistry analyzer RX Imola Randox (Ireland) on 13 analytes: AST, ALT, ALP, Amy, Total Calcium, CK, Cre, Iron, Total Protein, Triglycerides, T. Bil, Urea, Uric Acid. Independent variables assume to be the results of measurements received from a reference Vacuette (Austria) or control tube and are plotted on the X-axis. Depended variables are received from comparative or tested tube Lind-Vac (Estonia) and take up position on Y-axis. Comparison procedure assumes that there is no measurement uncertainty in the independent variable therefore use of the ordinary lest square regression (OLR) seems one of the most acceptable practical approaches for this purpose. Error grid estimated patient risk depending on Allowable Total Error (ATE) that is equivalent to Total Error (TE). ATE assumes allowable variability that leads to correct test interpretation and has a status of A-Zone. C-zone indicates a risk for patient.

↓ RESULTS:

Results of comparisons of tubes with clot activator (tubes with red cup) did not revealed any significant difference between samples from Lind-Vac and Vacuette tubes (p>0.05). Imprecision from duplicated (CV%) significantly differed on the results of 7 analytes for tubes with clot activator and clot activator and gel (p<0.05). Nevertheless values of CV% were in frame of international quality goals based on biological variation for imprecision and had no any influence to test interpretation.

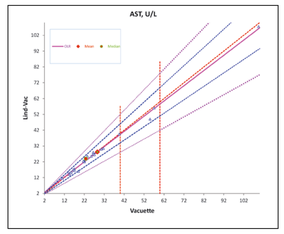
Table 1. Quality characteristics of blood samples measured in RX Imola Randox (Ireland) from biochemistry tubes with gel from of different manufactures Greiner (Austria) и Lind-Vac (Estonia)

Analyt. Units	Bias between Lind-Vac and Ref Greiner	Mean value		SD / CV%		Quality specification (Ricos et al., 2014)			p-probability of differnces between the results
	Bi%±SEM	Lind-Vac	Ref Greiner	Lind-Vac	Ref Greiner	9.7	11.5	27.5	
ALT U/L	−0.5±0.9	28.8	29.1	1.1 (3.7%)	1.0 (3.0%)	6.2	6.5	16.7	0.4
AST U/L	−0.1±1.2	28.3	28.8	0.8* (2.9%)	1.4* (4.8%)	4.4	7.4	14.6	0.3
Amilase U/L	1.0±0.5	87.3	86.2	3.7 (4.9%)	2.45 (2.85)	3.1	9.5	14.6	0.2
ALP U/L	−01±0.5	192.8	192.4	3.1* (1.6%)	2.5* (1.3%)	10.9	8.9	26.9	0.6
T. Bil µmol/L	0.4±0.4	21.3	21.3	0.8* (4.4%)	0.5* (2.7%)	2.7	1.7	6.1	0.4
T. Calcium mmol/L	0.2±0.4	2.1	2.1	0.04 (1.9%)	0.1 (2.9%)	11.4	11.5	30.3	0.8
CK U/L	−1.5±1.4	171.8	173.6	2.4* (1.4%)	10.3* (5.9%)	3.0	4.0	8.9	0.8
Creatinine µmol/L	−0.8±0.5	116.2	117.3	2.8 (2.4%)	2.6 (2.9%)	13.3	8.8	30.7	1.0
Iron µmol/L	−0.8±1.1	17.4	17.4	0.2 (1.3%)	0.3 (1.9%)	1.38	1.36	3.6	0.4
T. Protein g/L	−0.0±0.2	70.8	70.83	0.7 (0.9%)	0.7 (2.0%)	9.9	9.6	25.9	8.9
Triglicerides µmol/L	0.7±0.3	1.5	1.5	0.03* (2.0%)	0.03* (1.3%)	6.0	5.57	15.5	0.4
Urea mmol/L	−3.1±1.2	6.4	6.6	0.2* (3.0%)	0.3* (4.7%)	4.3	4.87	11.9	0.05
Uric acid µmol/L	-4.5±4.8	317.0	321.4	16.2* (5.1%)	10.85* (3.4%)	4.3	4.9	12.0	0.08

* – The significance of the differences of Imprecision by F- criterium (p<0.05)

Implementation of CLSI protocols for complex analytical validation of evacuated tubes optimizes harmonization and standardization of verification and validation procedures of preanalytical phase of the laboratory process. Spreadsheet program in Excel simplifies analytical validation of blood collection tubes and could be used in routine laboratories.

Fig.1. Ordinary linear regression of AST comparison (solid) and the lines delineating the A (dotted) and B (hatched) zones. The average and median are indicated in the graph.



Ordinary linear regression graph demonstrates results with slope 0.96±0.01 and intercept 0.81±0.51. 95.8 % of the observations are within zone A (±14.6 % ATE) from the OLR and 14.2 % fall in the B-Zone and no results are found in the C-zone.

Fig.2. Difference graph for tubes comparison results for AST.

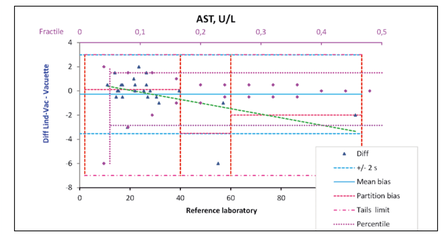


Fig. 3. Distribution of averages for AST comparison

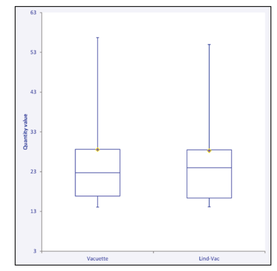


Fig. 4. Diagrams illustrating the distribution of results for AST measurements from evacuated tubes Vacuette (a) and Lind-Vac (b) compared to superimposed Gaussian distributions calculated from the average and standard deviation of the data.

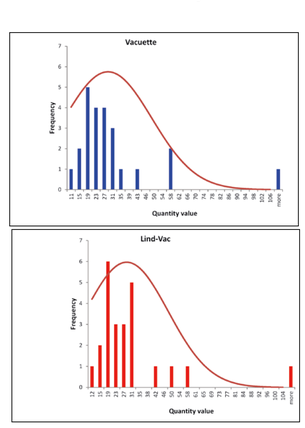


Fig. 5. Diagrams illustrating the distribution of difference of AST results received from tubes Vacuette and Lind-Vac compared to superimposed Gaussian distributions.

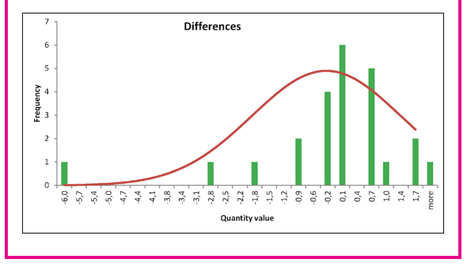
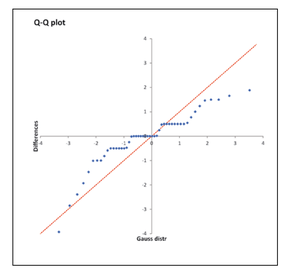


Fig. 6. Q-Q plot of AST results of measurements from Vacuette and Lind-Vac



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