Lina Khorovskaya[1], Anders Kallner[2], Svetlana Kovalevskaya[3], Inna Schmidt[4]

[1]North-Western State Medical University, Saint-Petersburg, Russian Federation
[2]Kalorinsla Hospital, Stockholm, Sweden
[3]Institution of Laboratory Medicine, Moscow, Russian Federation
[4]St. Luka Hospital, Saint-Petersburg, Russian Federation

Corresponding author Lina Khorovskaya: Lina.khorov@gmail.com, Lina.Khorovskaya@szgmu.ru

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

Abstract

Validation and verification of blood collection tubes became procedures that medical laboratories need since they are using different brand of IVD technologies for preanalytical phase. Common principles of comparisons of tested and reference tubes from analytical point of view are explained with evaluation of precision from duplicates, trueness, ordinal linear regression analysis with indication of risk in clinical interpretation, estimation of difference and normality of distribution. Visual techniques in addition to numerical procedures are efficient tool to analyze distribution of the data that is helpful for Uncertainty estimation following ISO 15 189:2012. Applying CLSI protocols for analytical validation of evacuated tubes optimizes harmonization and standardization of preanalytical phase and helps to implement preferable analytical performance specifications based on the effect of analytical performance on clinical outcomes according Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine in November 2013.

Introduction

Blood collection tubes are one of the most crucial technologies of preanalytical phase that maintain safety and quality of laboratory investigations. Evolution of blood collection devises had a way from routine glass tubes and different types of springs to closed vacuum systems [1]. Modern technologies of preanalitical phase includes evacuated blood collection tubes that belongs to in vitro diagnostic (IVD) devices and needles with sets for specimen collection that are medical devises [2]. Acceptable quality of all types of equipment for preanalytical phase is a starting point for effective diagnosis. Evacuated tubes became a multicomponent technology that helps to receive suitable specimen (blood, serum, plasma) without damage of biological properties for analytical stage to measure certain analyte and give a result of investigation. Historical development of blood collection tubes includes replacement of plastic to glass and implementation of polymer gel separating plasma or serum from blood cells. It required insertion of additional components to evacuated tubes such as clot activator particles for serum formation, surfactant to internal surface of the tube to prevent cell adhesion to the plastic wall and avoid hemolysis that also helps for better distribution of clot activator particles. New blood collection devices improve a safety for medical staffs and patient because of decreasing of breakage hazard and using of stopper that coated by lubricant for keeping vacuum and convenience of removal [3]. Quality of evacuated tubes should be standardized and confirmed by manufacturers and clinical laboratories according ISO 15189 and Directive 98/79/EC that is essential for market and accredited clinical laboratory [4].

Manufactures follow requirements for evacuated tubes for venous and capillary blood including additives, material, construction, labeling recommended by Clinical and Laboratory Standards Institute (CLSI) that helps to produce devices with a reliable properties [5]. To assess impact of blood collection tubes on test performance and influence of certain type of evacuated tube to accuracy of the results of laboratory investigations CLSI GP-34A guideline proposes validation and verification procedures for manufacturers and for clinical laboratories. This document recommends assessing components and additives of collection tube (material of tube wall, closures, closure lubricant, surfactants, clot activators, anticoagulants, separator gel, trace metals and evaluation) and validation and verification procedure by tube comparisons. Validation procedure for manufactures estimates evaluation and tubes comparative in different instrument platforms, different lots including stability study according recommended serious of steps. Clinical laboratory validate evaluation tube per each manufacturer by comparison with

compared tube in duplicates using specimens from minimum 20 patients to estimate accuracy according CLSI EP9-A guideline [6].

Clinical laboratories use blood collection tubes from different manufactures according their demands and financial requirements. Laboratory should evaluate a new brand of tube before practical use in laboratory process. The Working Group for Preanalytical Phase (WG-PRE) of European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) proposed "local validation" of blood collection tubes in clinical laboratories before its routine implementation. Implementation of blood collection device validation is useful for certification and accreditation of medical laboratories according to ISO 9000:2005 and ISO 15189:2012 for quality management of preanalytical phase [7]. EFLM WG-PRE outlined essential requisites of manufactures for local technical validation of evacuated tubes that should be fulfill in local practice and recommended to estimate 11 quality indicators for technical validation of evacuated tubes for quality assessment of preanalytical phase in clinical laboratories. Local validation should include comparison of control and comparative blood system tubes in paired measurement with estimation allowable deviation, bias and regression analysis. Local validation of blood collection tubes should be beneficial for clinical laboratories and detect failure of tested devises [8].

Laboratory specialists are developing and implementing different approaches of local validation of evacuated tubes for medical laboratories improving the quality of preanalytical phase. Method comparisons of different brands of tubes is the most efficient method to analyze analytical quality characteristics for testing in routine hematology, coagulation biochemistry and other types of laboratory investigations preventing preanalytical variability of laboratory process [9,10].

Materials and Methods

Validation and verification of tubes for venous and capillary blood specimen collection is multicomponent process with estimation of clinical and analytical suitableness of the devises that includes manufacturer's validation studies and end-user or medical laboratory's verification studies. Method analysis of trueness includes analytical comparability of the results receives from two different manufactures. Blood collection tube currently used by the clinical laboratory according terminology is comparative/tested and another one is control/reference that means any reference tube with reliable validated quality characteristics for comparison with a new or substantially modified tube according recommendations of CLSI GP-34A [11]. Investigated

brands of tubes should have similar composition according labeling data and proposed comparison did not assume to analyze of these technologies in details.

The aim of this study was to apply analytical approach for blood collection tube validation using comparison procedure with estimation the trueness and precision from different brands of tubes with clot activator by method describes in EP9-A [6] with additional use of graphical design for comprehensive analysis of comparisons including analysis of regression function, Uncertainty from duplicates, difference graphs, empirical evaluation of a comparison using the error grid, graphical representation of distribution of input data [12].

Sample collections were made in 40 patients from St. Luka Hospital, Saint-Petersburg (Russian Federation) to two tubes of Lind-Vac and Vacuette per each using CLSI H3-A6 and analyzed in biochemistry analyzer RX Imola Randox (Ireland) on 13 analytes: Alanine Aminotrasferase (AST), Aspartat Aminotrasferase (AST), Alkaline Phosphatase (ALP), Amylase (Amy), Total Calcium (T. Calcium), Creatine kinase (CK), Creainine (Cre), Iron, Total Protein (T. Prot), Triglycerides (Tri), Total Bilirubin (T. Bil), Urea, Uric Acid. The study was submitted by Ethics Committee and all patients signed informed concern.

Comparisons of results received from 2 vacuum tubes with clot activator of 2 different manufactures (of Lind-Vac (Estonia) and Vacuette (Austria)) were performed using measurement of patient samples (split sample) in duplicates from comparative and control tubes in medical health care organization in analogy with established procedure in laboratories to verify measurement methods.

For comparison procedure of analytical methods CLSI EP9-A2-IR recommends to make comparisons of 40 patient samples otherwise CLSI GP-34A allows to reduce the number of samples up to 20 pairs for economic reasons to decrease a cost of evacuated tube validation and verification procedure. Pairwise measurements expand possibilities of applied statistics for regression analysis, relative and absolute difference graphs for distribution analysis using histograms and box plots.

Pooling patient material could be used for regression from repeated measurements of two samples providing representative distribution [13]. Regression analysis allows estimating comparability of the results from different tubes using regression graphs of independent and dependent variables to receive linear relationship between the results from both typed of preanalytical IVD devices. Independent variables assume to be the results of measurements received from a reference or control tube and are plotted on the X-axis. Depended variables are

received from comparative or tested tube 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. The main characteristics of OLR are the regression coefficient “slope” (b) and intercept (a) that are received by inserting the values of the averages form duplicates of the independent and depended quantities in the regression function

$$Y = bX + a \qquad (1)$$

where Y – depended variables received from comparative or tested tube;

X – independent variables receives from reference or control tube;

b – slope, tangent of an angle of regression graph and X-axis;

a – intercept, interval between the beginning of the regression graph and “zero” coordinate on the Y-axis.

Comparability results could be accepted in conditions of slope is closed to one (1) and the intercept is closed to zero (0).

Verification of tested vacuum tube comparing with previous or reference assumes to analyze analytical quality specifications like precision and trueness. Precision is established by repeated measurements of the same patient sample that was calculated from duplicate and characterizes the Uncertainty from duplicates using Dahlberg formula [14].

$$s = \sqrt{\frac{\sum_{l=1}^{N}(x_{1l} - x_{2l})^2}{2 \times N}} = \sqrt{\frac{\sum_{l=1}^{i=N} d_i^2}{2 \times N}} \qquad (2)$$

where is d - difference between 2nd and 1st measurement;

X - numerical result of the measurement

N - numbers of the paried measurements

Distribution of measured replicates should cover different levels of concentration to reduce the standard error.

Statistical difference between averages of duplicates between different types of tubes was estimated with t-dependent Student criterium (t_{dep}).

$$t_{dep} = \frac{\bar{d}}{\frac{SD}{\sqrt{n}}} \quad (3)$$

where $\bar{d}$ – mean difference between observations;

SD – standard deviation from duplicated;

n – number of measurements.

Measurement interval was partitioned to 3 parts significant for clinical tests interpretation for assessment of trueness in different levels of concentrations. Absolute Bias (4) and relative Bias was estimated in low, middle and high concentration of investigated analytes like the difference between the mean ($\bar{X}$) of the comparative/test results and the control/reference value (Y_0):

$$Bias = \frac{\bar{X} - Y_0}{Y_0} \quad (4)$$

$$Bias = \frac{\bar{X} - Y_0}{Y_0} \text{ x } 100\% \quad (5)$$

The significance of the differences of the results of evacuated tube brands was assessed by paired Student's t-test and of Imprecision by Fisher test (F-test).

Difference graphs help to visualize the differences between the results received from tested and reference evacuated tube and the trend of the size of it. Method comparisons using difference graphs was described by Bland and Altman [15] with possible modifications of Krouwer [16] .

Graphical representation of the distribution of the input data and difference between the results received form testes and reference tube allows analyzing normality of the distribution [10]. Histograms and Quantile-Quantile plots (Q-Q plots) demonstrably helps to visualize distributions. Histogram is a graphical display of the sorted data according to size and grouped in suitable intervals displayed on X-axis. The frequency or number in each group called "bins" is displayed along the vertical (value) axis (Y-axis). For comparison purpose distribution of the input data of vacuum tube per each manufacture and difference between the results are visually presenting the values and it's differences. For Q-Q plots distribution of the quantiles of received

results of tested tube is compared with the reference tube using regression analysis. If the distribution of the data coincides with the assumed distribution, the data will follow a linear regression. Thus Q-Q plots demonstrate overall imprecision of the results from tested tube in relation to reference tube.

Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine in November 2013 defined preferable analytical performance specifications based on the effect of analytical performance on clinical outcomes [17]. From clinical point of view analytical difference between the results should not have any influence to test interpretation, i.e. results by either of the tubes should lead to the same diagnosis or treatment that conventionally illustrated in an error grid [18]. Error grid approach divides all patients' results on comparisons to several zones with borders that are defined on both sides of the regression line to indicate a risk for patient. 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 because of big deviation of the results that could lead to errors in diagnosis and clinical decision making and could not be acceptable in clinical and laboratory practice. B-zones are situated between the A- and the C-zones and includes not ideal results, but those that have not big risk for patient and may not jeopardize diagnosis or treatment of disease. The zones are created from clinical point of view individually for each analyte. Error grid approach is well-known and widely used in clinical practice in defining the allowable variability for blood-glucose monitoring systems for self-testing in managing diabetes mellitus [19].

EXCEL spreadsheet program developed by Kallner A. are used for calculation quality specification, regression analysis and visualization graphs of comparisons [12, 20]

Results and discussion

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 tubes ($p>0,05$). Imprecisopn 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 biopogical variatin for imprecision and had no any influence to test interpretation (Table 1).

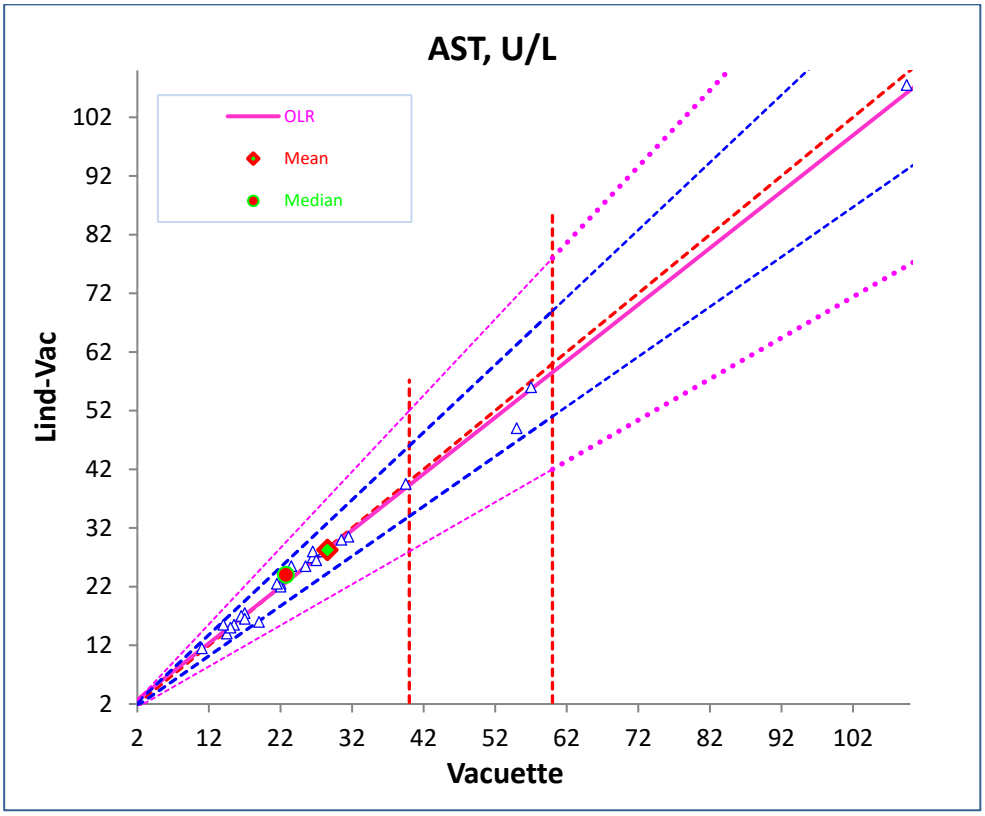
Table 1. Quality characteristics of blood samples measured in RX Imola Randox (Ireland) from biochemistry tubes with gel from of different manufactures Greiner (Austria) *и* Lind-Vac (Estonia)

Analyt, Units	Bias between Lind-Vac and Ref Greiner	Mean value		SD / CV%		Quality specification (Ricos et al.. 2014)			p-probability of differences between the results
	B%±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 (2.8%)	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%)	0.02* (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)*

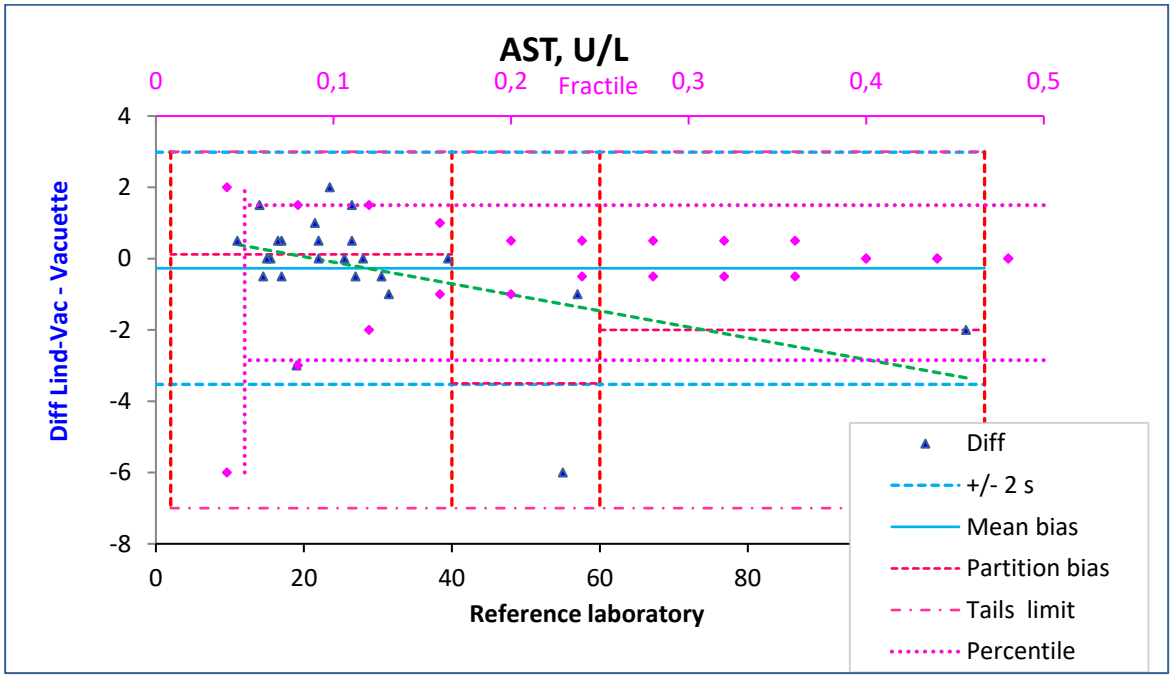
Graphical design is presented for tubes comparison for AST. Ordinary linear regression graph demonstrates results with slope 0,96±0,01 and intercept 0,81±0,51. 95,8 % of the observations are within zone A (±14,6 % ATE) from the OLR and 14,2 % fall in the B-Zone and no results are found in the C-zone.

Fig.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.



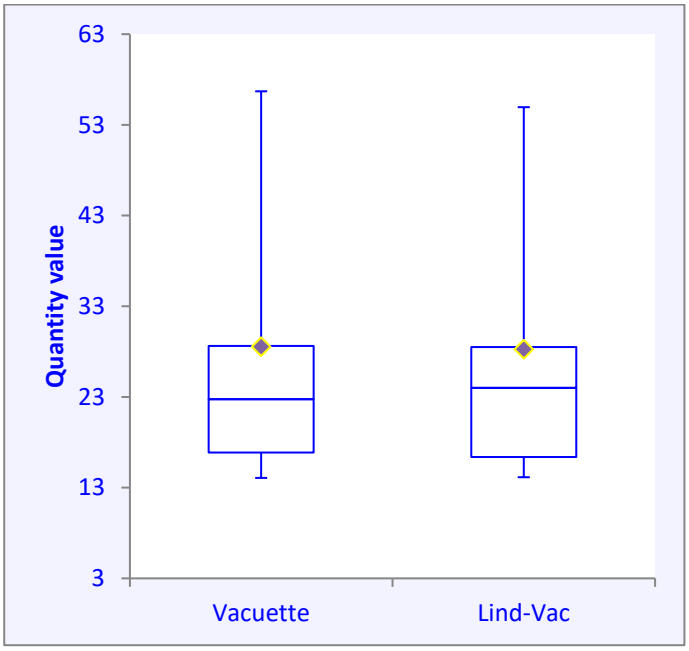
Difference graph shows that all results are in "limits of agreement" (fig.2).

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



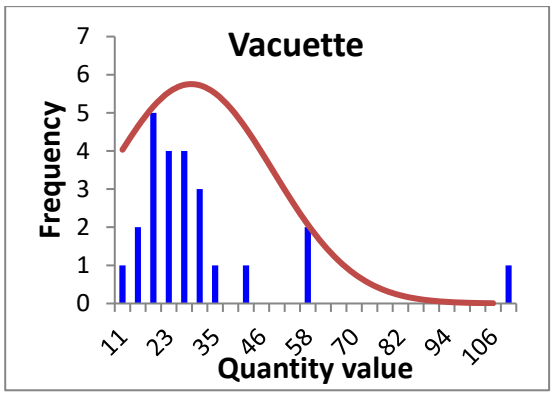
Distribution of averages for AST received from Vacuette and Lind-Vac visually demonstrates that values are most likely identical (fig.3).

Fig. 3. Distribution of averages for AST comparison

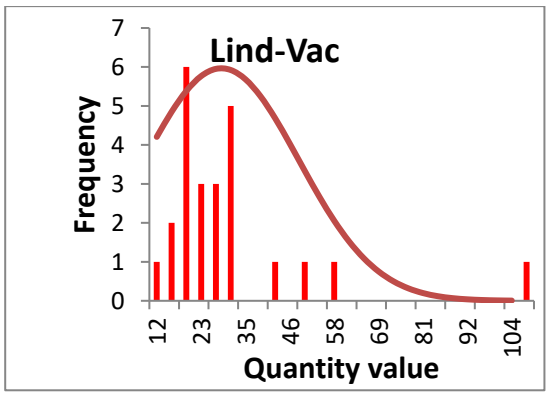


The distribution of results for AST measurements from evacuated tubes Vacuette (a, reference) and Lind-Vac (b, tested) seems to be close to normal (fig. 4).

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.



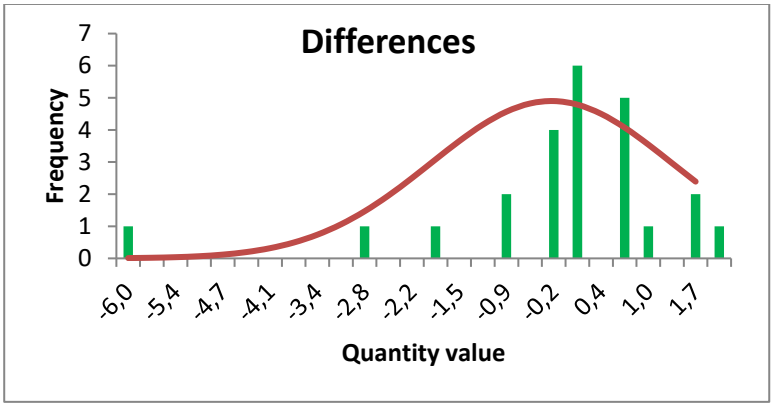
a)



b)

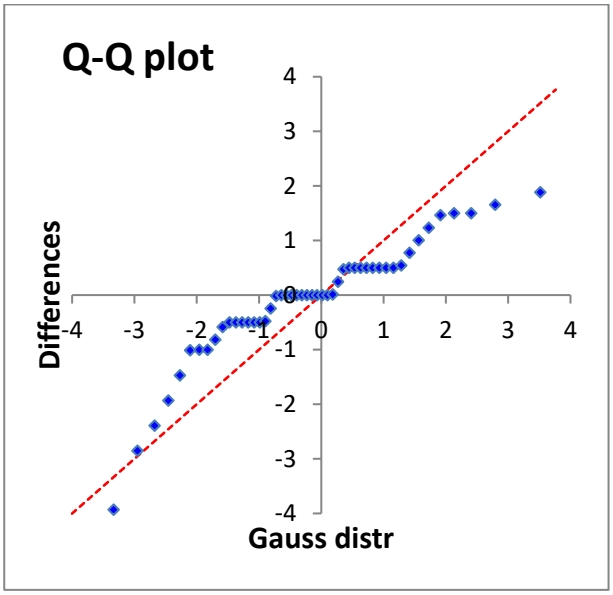
Distribution of difference of comparison AST results also is close to Gaussian (fig.5).

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



To estimate the normality of the differences, a quantile–quantile graph (Q–Q) is also displayed in which the quantiles of the tested data set are compared to those of a normal distribution (Fig. 6)

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



Validation of blood collection tubes should include analytical evaluation of comparisons of tested and reference tubes with evaluation of precision from duplicates, trueness, normality of distribution. Visual techniques in addition to numerical procedures are efficient tool to analyze distribution of the data that is helpful for Uncertainty estimation according the demands of ISO 15 189:2012 [4]. Error grid approach with analysis of risk for patient based on ATE helps to

apply defined preferable analytical performance specifications based on the effect of analytical performance on clinical outcomes following Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine in November 2013. 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 [6, 18]. Spreadsheet program in Excel simplifies analytical validation of blood collection tubes and could be used in routine laboratories.

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