Transfusion and Apheresis Science 57 (2018) 132–142

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Review

Statistical methods to the control of the production of blood components: principles and control charts for variables

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ARTICLE INFO

Article history:

Keywords:
Control charts
Rational subgroup
SPC
Specifications
Variables

ABSTRACT

General quality control good practices require the control of the production of blood components using statistical techniques, such as mandatory by the European Commission Directives and the American Association of Blood Banks standards. Sometimes, the control procedure is exclusively in favor of the compliance verification with specifications per individual component or to compute the number of defective parts usually on a monthly basis. However, this is a critical restriction to detect unnatural patterns such as to guarantee that the production has a non-significance chance to manufacturing nonconforming components. Therefore, a crucial issue in Blood Establishments is the application of a reliable statistical process control methodology to assure products reliable and consistent to specifications. Statistical principles and control charts for variables are reviewed, discussed and recommended, based on current good practices. The empirical data demonstrate the consistency of these models on blood establishment routine. A flowchart to select the type of control chart is suggested.

Contents

1. Introduction

1.1. General principles of statistical process control in the production of blood components

The European Directive 2002/98/EC [1] and The European Committee (Partial Agreement) on Blood Transfusion (CD-P-TS) TS111

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https://doi.org/10.1016/j.transci.2018.02.022
1473-0502/

"Good Practices Guideline" [2] requires the control of the production of blood components and in the United States is mandatory by the standards for blood banks and transfusion services [3]. The CD-P-TS is hosted by The European Directorate for the Quality of Medicines & HealthCare (EDQM) of the Council of Europe. The EDQM publishes the "Guide to the preparation, use and quality assurance of blood components." This guideline includes the TS111 specifications on its 19th edition [4]. Appendix D refers a policy for statistical sampling on a statistical process control (SPC) methodology viewpoint. Such as happens in other blood components specifications [5,6], there is a lack of standardized practices in the European Union.

The SPC is the standard statistical method to monitor the production processes [7], built on objective evidence to assure the control of individual or lot of products. Mostly, this method is intended to enhance the processes continuously. Consequently, it is oriented to decrease the variance, to control and report the stability of the operations, to follow-up the methods, to determine the procedure performance, to identify for non-conformities about lots specifications on an early stage, and to process documentation fulfilling the law such as additional requests. Hypothetically, two central tendency measures indicate a failure in a process with an infinite number of samples: random error (2.19 of [7]) and systematic error (2.17 of [7]). Accordingly, a possible variable from a normal distribution x with known σ and μ can be controlled. Though, the σ and the μ are unknown, for what the variance and average of the results are determined. If a normal distribution is unavailable, the data is normally transformed, or it is considered an attribute method instead of the variables method use. The common/natural/expected sources of error are expected causes of failure common to any production process. In the scenario of the identified error to be higher than what is expected, special causes of failure are supposed to exist. On a short-term strategy are considered the variance and out-of-specification data. Otherwise, on a long-term plan, the common and special causes are regarded. The long-term approach is also oriented to the continuous satisfaction of high-quality requirements with a significant decrease in the products' cost and preventive measures. The short-term is related principally to the corrective actions [8]. Commonly, a manufacturing process is classified as "world class quality" when the average results are "on target" with "minimum variance." Working with "minimum variance" is achievable since the process demonstrates a reasonable grade of statistical control.

The specification limits should be selected according to the requirements applied to blood components. Control charts are used to identify nonconforming lots or products. Capability indexes are used to classify the level of production. These charts include control limits informing if the process is operating correctly. It allows to understood if a specific process operation assures a low probability of manufacturing non-conforming products. In theory, an in-control process could not meet the individual specification or vice-versa. Samples are verified according to specifications, not according to control limits. For further details on SPC on the production of blood components see [9].

The conformity of the blood components according to the specifications should be classified. The goal is to detect trends that could affect the components' compliance and take actions on a correction, control of nonconforming products, and corrective-action/preventive-action(CAPA) perspective.

The consistency of the SPC outcomes is directly dependent on the sampling plan. Please, refer to [10] for the implementation of reliable sampling practice.

This article is focused on the selection and implementation of a suitable SPC methodology in a Blood Establishment based on control charts for variables. The text is based on theoretical principles on a blood establishment perspective using real-world examples to support the quality laboratory staff on the understanding of the basic statistical concepts and the application of an SPC procedure.

2. Material and methods

2.1. Implementation of an SPC methodology in a blood establishment

A potential roadmap for the implementation is established. The problem, the scope, and the stakeholders (interested parties) are identified (3.2.3 of [9]). The success of the implementation should be recognized, and any failure understood. Real or theoretical examples of the problems could be taken on the training stage. When applicable, the staff involved in the training should be evaluated and the evaluation reported (7.2 of [9]). The roles of each of the individuals working with SPC activities is described. An individual has to verify on a lot basis that the operators fill in the chart correctly. This same person is the first to be contacted if a statistical rule is violated, and also has the role in reviewing the control charts to the process owner on a periodic basis (e.g., weekly) including observations, special causes, and actions. Habitually, these individuals are the process owner and the leadership team.

2.2. Understanding the causes of variation

The blood components production process $f(x)$ has a repeatable value generating a sequence of activities transforming inputs x into reproducible outputs y. Process output is always affected by a variation, including common causes of variation, recognized as "background noise." The biological variation of the testing parameters of human blood is a significant variation component that cannot be corrected, such as others factors arising predominantly from people, environment, methods, materials, measures, and equipment. The biological variation is related to inter-individual (between-subject) and to intra-individual (within-subject) sources [11]. As suitable, this type of variation could be considered on the interpretation of the capability of the production process, principally when the capability is low in stable process conditions.

Reducing the common causes of variation is a challenge that should be understood as possible, but it can never be removed. Special causes are identified using a root-cause analysis and a correction, and CAPA is applicable (10.2 of [9]). The effort to determine the root-cause for special causes increases with the complexity of the process. The three-sigma limits are referred as Natural Process Limits, where the data arises from an undisturbed process. Out-of-control results indicate that a variation is due to a particular cause. If the special causes are not detected or if there are a false rejection of lots due to unreliable SPC (false alarms), the consistency of the production and waste on the budget could happen, respectively.

2.3. Data reliability and the importance of the normal distribution

An observation that seems to diverge noticeably from others in sampling is referred to as an outlier (false result) [12–14]. A particular outlier could increase the variation, changing the average and could even alter the acceptance of a lot. The "common-sense" suggest the use of a pair of analysts to review the SPC inputs. But, on a large number of observations n should be considered the use of statistical tests such as the Grubbs test to detected a single outlier [12] or the Generalized Extreme Studentized Deviate (ESD) test [13] or the Tukey test [14] if it is suspected more than one. Let consider the hematocrit results (ratio) in a normally distributed sampling with 87 *red cells, leucocyte depleted in additive solution* components. In this example the maximum and minimum values are 0.54 and 0.65. The normality is tested using three tests in MedCalc® software

Table 1
Table of control charts constants.

	$\bar{x}$-chartconstants		*R*-chart constants		For sigma estimate	*s*-chart constants		
Sample size *m*	A_2	A_3	D_3	D_4	d_2	B_3	B_4	C_4
2	1.880	2.659	0	3.267	1.128	0	3.267	0.7979
3	1.023	1.954	0	2.574	1.693	0	2.568	0.8862
4	0.729	1.628	0	2.282	2.059	0	2.266	0.9213
5	0.577	1.427	0	2.114	2.326	0	2.089	0.9400
6	0.483	1.287	0	2.004	2.534	0.030	1.970	0.9515
7	0.419	1.182	0.076	1.924	2.704	0.118	1.882	0.9594
8	0.373	1.099	0.136	1.864	2.847	0.185	1.815	0.9650
9	0.337	1.032	0.184	1.816	2.970	0.239	1.761	0.9693
10	0.308	0.975	0.223	1.777	3.078	0.284	1.716	0.9727
11	0.285	0.927	0.256	1.774	3.173	0.321	1.679	0.9754
12	0.266	0.886	0.284	1.716	3.258	0.354	1.646	0.9776
13	0.249	0.850	0.308	1.692	3.336	0.382	1.618	0.9794
14	0.235	0.817	0.329	1.671	3.407	0.406	1.594	0.9810
15	0.223	0.789	0.348	1.652	3.472	0.428	1.572	0.9823
16	0.212	0.763	0.364	1.636	3.532	0.448	1.552	0.9835
17	0.203	0.739	0.379	1.621	3.588	0.466	1.534	0.9845
18	0.194	0.718	0.392	1.608	3.640	0.482	1.518	0.9854
19	0.187	0.698	0.404	1.596	3.689	0.497	1.503	0.9862
20	0.180	0.680	0.414	1.586	3.735	0.510	1.490	0.9869
21	0.173	0.663	0.425	1.575	3.778	0.523	1.477	0.9876
22	0.167	0.647	0.434	1.566	3.819	0.534	1.466	0.9882
23	0.162	0.633	0.443	1.557	3.858	0.545	1.455	0.9887
24	0.157	0.619	0.452	1.548	3.895	0.555	1.445	0.9892
25	0.153	0.606	0.459	1.541	3.9	0.565	1.435	0.9896

(Medcalc Software bvba, Ostend, Belgium): The *p*-value is equal or higher than to 0.05 to the Grubbs double-sided and ESD. Therefore none outlier is identified such is also suggested by the Tukey's test ($p = 0.05$). The *p*-value value is understood as the chance of a sample in the rational subgroup to be an outlier is not significant at the 0.05 significance level.

The data normality should be verified before to apply a sampling methodology. We suggest the D'Agostino's K-squared test [15] intended for the assessment that the underlying distribution of a random variable is normally distributed. For testing that the distribution is normal, the skewness and kurtosis statistics are combined. The test first calculates the skewness and kurtosis to compute how far from the normal distribution is regarding the asymmetry and shape. It is calculated how considerably each of these values differs from the value expected for a normal distribution, computing a single *p*-value from the sum of these differences. The test should apply to an $n > 20$. In the previous example, and using the same software, the *p*-value is equal or higher than 0.05 to the D'Agostino's K-squared test ($p = 0.6201$) whereby the normality distribution is not rejected.

2.4. Principles of control charts for variables

The application of quality control charts for variables illustrates the state of the process. Quality control rules per individual sample or lot are applied. These type of charts cannot involved when a parameter of a product is not conveyed in a numerical quantity, or the data distribution is inapplicable (considering no data transformation). For instance, leucocytes have a nonnormal distribution for what charts requiring normally distributed data cannot be considered. One option is to transform the data, and another is to use attributes charts. These charts use the number of nominal observations, e.g., no/yes, non-conforming/conforming (1.30 of [7]). For instance, *n* of nonconforming results (defects) or *n* of defective units. Its use should be a secondary option since the information given using attributes is reduced when compared to the use of numerical measurements.

a) Individual and moving ranges charts

Individual and moving range charts(x-$\bar{R}$ charts)are used to verify changes in significant components of random and systematic error in$\bar{R}$-chart and x-chart, respectively. For small n, the individual sample results chart area used, such as when a limited number of products are preferable to be fully tested instead sampled due to retrospective analysis limitations. The x-chart evaluates the systematic variation over time ("long-term" variation). The average of the individual results is close to the mean of the full production if the data is normally distributed. Supposedly, the standard deviation determination using cumulative results from the accepted lot is complementary to the moving average. Nevertheless, in the blood components production, the number of samples per rationale subgrouping is typically no more than 10. Therefore the moving range is used instead of the "moving standard deviation." $\bar{R}$-chartis used to evaluate the "short-term" variation in ranges between consecutive samples, complementing the x-chart estimation. The moving average is expressed, as follows:

$$MR_i = |x_i - x_{i-1}| \tag{1}$$

where x_i is the observation i, and $x_{i\text{-}1}$ is the previous observation.

The center line of the x-chart is the result of the average of the individual results in-control conditions, computed as follows:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \tag{2}$$

where x_i is the observation i, $i = 1, \ldots, n$, and n is the number of observations.

The control limits are determined according to the mathematical model:

$$UCL_x = \bar{x} + 3\frac{\overline{MR}}{d_2} \tag{3}$$

where$\bar{x}$is the average of the individual results, $\overline{MR}$ is the average of the moving ranges (see Eq. (5)), and d_2 is the constant for a number of samplings n (see Table 1 for sigma estimate).

$$LCL_x = \bar{x} - 3\frac{\overline{MR}}{d_2} \tag{4}$$

The center line of the $\overline{R}$-chartis the result of the average of the moving ranges in-control conditions, determined as follows:

$$\overline{MR} = \frac{\sum_{i=1}^{n} MR_i}{n-1} \quad (5)$$

where $MR_i = |x_i\text{-}x_{i\text{-}1}|$, where x_i is the data point and $x_{i\text{-}1}$ it is the previous data point, and for n individual values there are n-1 ranges. Consequently, $\overline{MR}$ expresses the average of the ranges in a number of lots during a particular period. Uniquely the combination of the moving average and the moving range able to comprehend the overall level of confidence in the production, caused by their complementarity.

The control limits in the $\overline{R}$-chart are computed by the model:

$$UCL_x = D_4\overline{MR} \quad (6)$$

where D_4 is the constant for a number of samplings m (see Table 1 for R-chart constants).

$$LCL_x = 0$$

The computation of the control limits should be based on 20 to 30 data points in reproducibility conditions. The use of a lower sampling n or repeatability conditions produces unrealistic estimates (inaccurate), causing the incorrect use of the statistical rules (i.e., "false alarms") due to the control limits to be wide. Note that special causes can affect the process wide control limits, for what the process must be in stable conditions to avoid the use of unrealistic borders. If the number of samples to determine the control limit is very small, the control limits could also be unreal due to a nonrepresentative sampling. Preferably, the control limit should be computed (updated) every time a new data point is registered. The calculating conditions of limits should be rigorously checked. Out-of-control results are excluded from this computation.

Each new point is checked after the establishment of a chart to very if any rule is violated (see 2.5). If a statistical rule is violated, a special cause of variation is associated (6.4 of [16]). The use of a root-cause thinking is suggested to the control of changes in the production. There is a guarantee that the change is going to be permanent if at least 20 samples confirm the new process, e.g., conforming to the Western Electric rules (see 2.5), and the control limits are recalculated.

b) Average and range charts

Average and range charts($\bar{x}$-R charts)are used when there are frequently lots/sampling, e.g., day-to-day lots. The sampling or rational subgroup is collected according to a statistical method [17]. The number of observations per sampling allows the computation of the average, and range, as well as to plot these points to verify if the lot is in or out-of-control. If possible, the sampling should not occur in more than one day to decrease the risk to accept non-complaint blood components, i.e., real-time sampling. The $\bar{x}$-R charts are a useful tool to identify the variation between lots. The$\bar{x}$-chartis used to verify the variation between lots (process changes). Otherwise, the R-chart is oriented to verify the variation within a lot (short-term variation).

The average is a measure of central tendency. It is also referred as the arithmetic mean or means. It expresses the sum of a group of numbers divided by the count of numbers in the group, as follows:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \quad (7)$$

where x_i is the observation i, $i = 1, \ldots, n$, and n is the number of observations.

The range is the difference between the largest and smallest of the values. On the SPC, it is the difference between two consecutive measurements:

$$R = x_{\max} - x_{\min} \quad (8)$$

where $x_{\max}$ is the maximum and $x_{\min}$ is the minimum value of sampling. Once more, the extreme values critically influence this estimate. As it is already mentioned, this model is an alternative to the use of variance when the sampling is equal or less than 10 due to expresses a more realistic estimate of the random error. The range through a lot is not suggested be used to assess the variance of a parameter determined between lots since within lot is not equal from between lot statistics.

Extreme values influence the average. So, it could be unrealistic estimated in the case that outliers are not detected (see 2.3). The accuracy of average is affected by the standard deviation since it is directly related to the chance of the average to happen. Larger standard deviation minimizes the frequency of a certain average. Larger standard deviation minimizes the frequency of a particular average. On the other hand, minor standard deviation means that the average has a high chance to occur. Bias quantifies the distance between the average and the reference value (an approximation to the truth). Averages with significant bias could be corrected.

The center line measurement of the $\bar{x}$ chart requires the determination of the average of the sampling using the following model equation:

$$\bar{\bar{x}} = \frac{\sum_{i=1}^{m} \bar{x}_i}{m} \quad (9)$$

where x_i is the observation i, $i = 1, \ldots, m$, and m is the number of samplings.

The average control limits, the upper control limit $UCL_{\bar{x}}$and the lower control limit$LCL_{\bar{x}}$, are computing using the following models:

$$UCL_{\bar{x}} = \bar{\bar{x}} + A_2\overline{R} \quad (10)$$

where$\bar{\bar{x}}$is the average of the sample means, A_2 is the$\bar{x}$-chartconstant for a number of samplings m (see Table 1), and;

$$LCL_{\bar{x}} = \bar{\bar{x}} - A_2\overline{R} \quad (11)$$

These control limits are equivalent to three-sigma control with $\alpha = 0.0027$, and the same is considered in the R-chart.

To determine the center line of the R-chart is computed the average range of the samples as follows:

$$\overline{R} = \frac{\sum_{i=1}^{n} R_i}{m-1} \quad (12)$$

where R_i is the range i, $i = 1, \ldots, m$, and m is the number of samplings. To accentuate that R is related to the sigma regarding a constant (depending on n) listed in statistical tables, where sigma is equal to the$\overline{R}$divided by the constant d_2 (see Table 1 for sigma estimate). Accordingly, when the R-chart is in-control, it could be computed the standard deviation s_R using the model (unbiased estimator):

$$\hat{\sigma} = \frac{\overline{R}}{d_2} \quad (13)$$

where$\overline{R}$ is the average of ranges and d_2 is the constant for sigma estimate for a number of samplings m (see Table 1).

The R-chart control limits, the upper control limit UCL_R and the lower control limit LCL_R, are computed using the following models:

$$UCL_R = D_4\overline{R} \quad (14)$$

where $\overline{R}_2$ is the average of ranges and D_4 is the R-chart constant for a number of samplings m (see Table 1).

The LCL range is determined using the model:

$$LCL_R = D_3\overline{R} \tag{15}$$

where $\overline{R}$ is the average of ranges and D_3 is the R-chart constant for a number of samplings m (see Table 1).

In the development of Eqs. (10) and (11) the σ_R substitutes the $\overline{R}$ component in the determination of the R-chart control limits to enhance the realism of estimates. Therefore, the model equations is rewritten considering A_2 equal to the division of three by the product of the multiplication of d_2 (see Table 1 for sigma estimate) by the square root of n:

$$UCL_{\overline{x}} = \overline{\overline{x}} + \frac{3}{d_2\sqrt{n}}\overline{R} \tag{16}$$

$$LCL_{\overline{x}} = \overline{\overline{x}} - \frac{3}{d_2\sqrt{n}}\overline{R} \tag{17}$$

The development of Eqs. (14) and (15) considers that the standard deviation of $R\hat{\sigma}_R$is the product of the multiplication of d_3 by$\hat{\sigma}$, D_4 (see Table 1 for R-chart constants) which is equal to the sum of one with the product of the multiplication of three by the result of the division of d_3 by d_2 (see Table 1 for sigma estimate). D_3 (see Table 1 for R-chart constants) is the difference between one and the product of the multiplication of three by the result of the division of d_3 by d_2 as follows:

$$UCL_R = \overline{R} + 3d_3\frac{\overline{R}}{d_2} \tag{18}$$

$$LCL_R = \overline{R} - 3d_3\frac{\overline{R}}{d_2} \tag{19}$$

A rule of thumb in $\overline{x}$-Rcharts is if a R-chart point/points is/are out-of-control, then the $\overline{x}$-chart control limits cannot be reliably estimated, for what they are unacceptable for this computation. Note that the R-chart has LCL equal to zero when the sampling size is equal or less than six (6.2 of [16]).

c) Average and standard deviation charts

Creation and operation of average and standard deviation charts ($\overline{x}$-s charts) are close to those of$\overline{x}$-Rcharts. They differ given that for one is computed the sample standard deviation and for other the range. If fixed samplings are used, then the$\overline{x}$-Rcharts are commonly preferred when compared to the$\overline{x}$-$s$charts. This paper focuses on the sampling when is variable. The $\overline{x}$-R charts are generally not used in this situation since they lead to a changing center line on the R-chart. In the condition with variable selection, it is used a weighted average method to calculate the center lines of both charts.

The center line measurement of the $\overline{x}$-chart when sampling is variable is expressed by the average of the sampling, as follows:

$$\overline{\overline{x}} = \frac{\sum_{i=1}^{m} n_i\overline{x}_i}{\sum_{i=1}^{m} n_i} \tag{20}$$

where$\overline{x}_i$is the average i, $i = 1, \ldots, m$, n_i is the number of averages, and m is the number of rational subgroups.

Though the variance is an unbiased estimator of sigma, the standard deviation is a biased estimator, for what a correction factor as a function of n is found in Table 1 (s-chart constants). The average control limits, the upper control limit $UCL_{\overline{x}}$ and the lower control limit$LCL_{\overline{x}}$, are computed using the next models for an unbiased estimate:

$$UCL_{\overline{x}} = \overline{\overline{x}} + A_3\overline{s} \tag{21}$$

where $\overline{\overline{x}}$ is the average of the sample means, A_3 is the $\overline{x}$-chart constant for a number of samplings m (see Table 1), and;

$$LCL_{\overline{x}} = \overline{\overline{x}} - A_3\overline{s} \tag{22}$$

A_3 is according to the sample size of each rational subgroup.

The center line computation of the s-chart is equivalent to the average range of the sample, according to the mathematical model:

$$\overline{s} = \sqrt{\frac{\sum_{i=1}^{m}(n_i - 1)s_i^2}{\sum_{i=1}^{m} n_i - m}} \tag{23}$$

where s_i is the standard deviation i, $i = 1, \ldots, m$, n_i is the number of standard deviations, and m is the number of samplings.

The average control limits, the upper control limit UCL_s and the lower control limit LCL_s, are determined in the same condition using the next models for an unbiased estimate:

$$UCL_s = B_4\overline{s} \tag{24}$$

where B_4 is the $\overline{x}$-chart constant for a number of samplings m (see Table 1), and$\overline{s}$is the average of standard deviation.

$$LCL_s = B_3\overline{s} \tag{25}$$

where B_3 is the $\overline{x}$-chart constant for a number of samplings m (see Table 1), and $\overline{s}$ is the average of the standard deviation. The constant B_3 and B_4 are according to the sample size of each subgroup (6.3 of [16]).

2.5. Interpreting control charts for variables

A rule of thumb on the interpretation of control charts for variables is that the x-chart is exclusively valid if the variability in the R or $\overline{R}$-chart is normal, such as is related in the s-chart and the x-chart. Then, the R or $\overline{R}$-chart and the s-chart are analyzed using decision rules. Results are nonconforming in x-chart if one of the R or the s-chart is out-of-control. In this situation,the average estimates are doubtful. In this situation, the average estimates are doubtful. A result outside the control limits is an indicator of non-randomness,generally associated with a special cause of variation. Other unnatural patterns are possible to happen. For instance, a cyclic pattern with a periodic signal, a mixtures pattern caused by two or more sources, shifts indicating an abrupt change, trends representing a gradual change, and stratification indicating the variability is too minor (6.2.4 of [16]). Different groups of decision rules are available, and their application is not uniform in blood establishments, identical to what is happening in the industry. Suitable rules are intended to detect special patterns. The use of inadequate statistical rules can lead to an increase in the false alarms, or an increment in the sensitivity to identify unnatural variation. An example of a false alarm is that related to the probability close to one false alarm per 370 samples for the three-sigma control limits in stable production conditions.

Usually, 20 to 30 results are required complying with individual requirement's limits to establish control limits. The classification of points out-of-control on a scenario where control limits are not defined is a challenge to the quality control decision maker. On this phase, a sampling with larger n is proposed, for instance, using sampling based on the inspection level such as a tightened inspection with the s-method [17]. The quality control personnel should recognize that the control limits should not be easily reviewed.

A simple condition/single rule could be used to interpret one or more points outside of the three-sigma control limits. However, it cannot detect other unnatural patterns that are important to

Table 2
Data for the case of sampling with 30 *red cells, leucocyte depleted in additive solution* components.

No.	Hematocrit (ratio) x	MR	No.	Hematocrit (ratio) x	MR	No.	Hematocrit (ratio) x	MR
1	0.64	0.00	11	0.60	0.03	21	0.61	0.01
2	0.63	0.01	12	0.58	0.02	22	0.56	0.05
3	0.60	0.03	13	0.56	0.02	23	0.56	0.00
4	0.64	0.04	14	0.57	0.01	24	0.63	0.07
5	0.57	0.07	15	0.58	0.01	25	0.60	0.03
6	0.58	0.01	16	0.62	0.04	26	0.60	0.00
7	0.58	0.00	17	0.56	0.06	27	0.63	0.03
8	0.63	0.05	18	0.64	0.08	28	0.60	0.03
9	0.60	0.03	19	0.61	0.03	29	0.62	0.02
10	0.57	0.03	20	0.62	0.01	30	0.64	0.02
							$\bar{x} = 0.60$	$\overline{MR} = 0.03$

be recognized to take principally preventive actions. After 1956 a well-established set of rules are applied in the industry: the Western Electric rules [18]. They are a type of decision rules suited to identify a series of unnatural patterns, increasing the sensitivity of detection when compared to the single use of the three-sigma rule. Three zones are considered: A, B, and C recognized as the three-sigma, two-sigma, and one-sigma zones, respectively. The zone A is between the two-sigma and the three-sigma limit, the B is between the two-sigma and one-sigma limit, and the C is between the one-sigma and the center line.

A group of rules based on the Western Electric text is presented in Table 5. The first four rules are well-known as the "Zone rules," and are recognized as the most important rules for symmetric control limits. They are also applicable to nonsymmetric limits with n equal or higher than five. Let's focus on these rules: they are intended to detect the process instability including the identification of unnatural patterns. The probability of each of the "Zone rules" to happen in stable production conditions/residual risk is possibly the easiest way to understand the statistical principles associated with each one. Fig. 1a. displays a probability of a point equal to 0.0135% to occur in stable production conditions if it is out of the three-sigma limit. This is a case of the rule 1 violation. Fig. 1b. demonstrates the case of the probability for one pointout of two-sigma from the center line is computed adding the probability equal to 2.14% with 0.135%, which is equal to a probability of 2.28%. For the probability of two points out of one-sigma from the center line is the square of 2.28% = 0.0518%. The chance for the third point is calculated subtracting 2.28% from 100% = 97.72%. Therefore, the likelihood for two out of three is determined according to $2.28^2\% \cdot 97.72\% \cdot 3 = 0.152\%$ (infringement of rule 2. Fig. 1c. elucidates the case where rule 3 is infringed. The probability for one point to be higher than one-sigma from the center line is the sum of 13.60%, 2.14%, and 0.135%, which is equal to 15.88%. The chance for four points to be out of one-sigma from the center line is $15.88^4\% = 0.0635\%$. The likelihood for the fifth point is calculated summing 2·34.13%, 13.60%, 2.14%, and 0.135%, which is equal to 84.14% or subtracting 15.88% from 100%. The possibility of the four out of five is the calculated multiplying $15.88^4\%$, 84.12%, and five; the chance is of 0.267%. The case of rule 4 violation is comprehended in Fig. 1d. The probability for one point is equal to the sum of 34.13%, 13.60%, 2.14%, and 0.135% = 50%. The likelihood for two points is the square of 50% = 25%. The sample square criteria sequence is considered to the other points, where the eighth point is equal to $50^8\% = 0.36\%$. Rules 5 to 10 could be used to identify other patterns. Its use could happen in processes where it is assumed a very low risk to manufacture nonconforming products. Therefore a more complex control is necessary.

Otherwise, rules are proposed in Table 6 for the case of nonsymmetric control limits, such the case of x-$\bar{R}$ charts when n is less than five.

3. Results

3.1. Control charts for variables

a) Individual and moving ranges charts

All the charts and calculus in following examples are computed with Minitab® v.18.1 (Minitab Inc., State College, Pennsylvania, USA) software. Let consider the hematocrit results (ratio) in a sampling of 30 *red cells, leucocyte depleted in additive solution* components. The group is the first 30 components manufactured on a small blood establishment. Fig. 2 shows the$x$-$\bar{R}$charts. This case data is displayed in Table 2. Eq. (2) is applied to determine the center line and Eqs. (3) and (4) to determine the x-chart control limits as follows:

$$\bar{x} = \frac{\sum_{i=1}^{30} x_i}{30} = \frac{17.84}{30} = 0.60, \quad UCL_x = 0.601 + 3\frac{0.02897}{1.128} = 0.60, \quad \text{and} \quad LCL_x = 0.52$$

Eq. (5) is applied to determine the center line and Eq. (6) to determine the$\bar{R}$-chartcontrol limits as follows:

$$\overline{MR} = \frac{\sum_{i=1}^{30} MR_i}{29} = \frac{0.84}{29} = 0.03, \quad UCL_x = 3.267\,(0.02897) = 0.10\,, \quad \text{and} \quad LCL_x = 0$$

b) Average and range charts

Considering the hematocrit results (ratio) in a sampling with 30 lots with a constant sampling equal to three *red cells, leucocyte depleted in additive solution* components. This is a collection of a blood establishment where the production per daily lot does not significantly vary. Fig. 3 shows the$\bar{x}$-Rcharts. Table 3 lists the data of this case. Eq. (10) is applied to determine the center line, and Eqs. (10) and (11) to the control limits of the$\bar{x}$-chart as follows:

Eq. (12) is applied to determine the center line and Eqs. (14) and (15) to the control limits of the R-chart as follows:

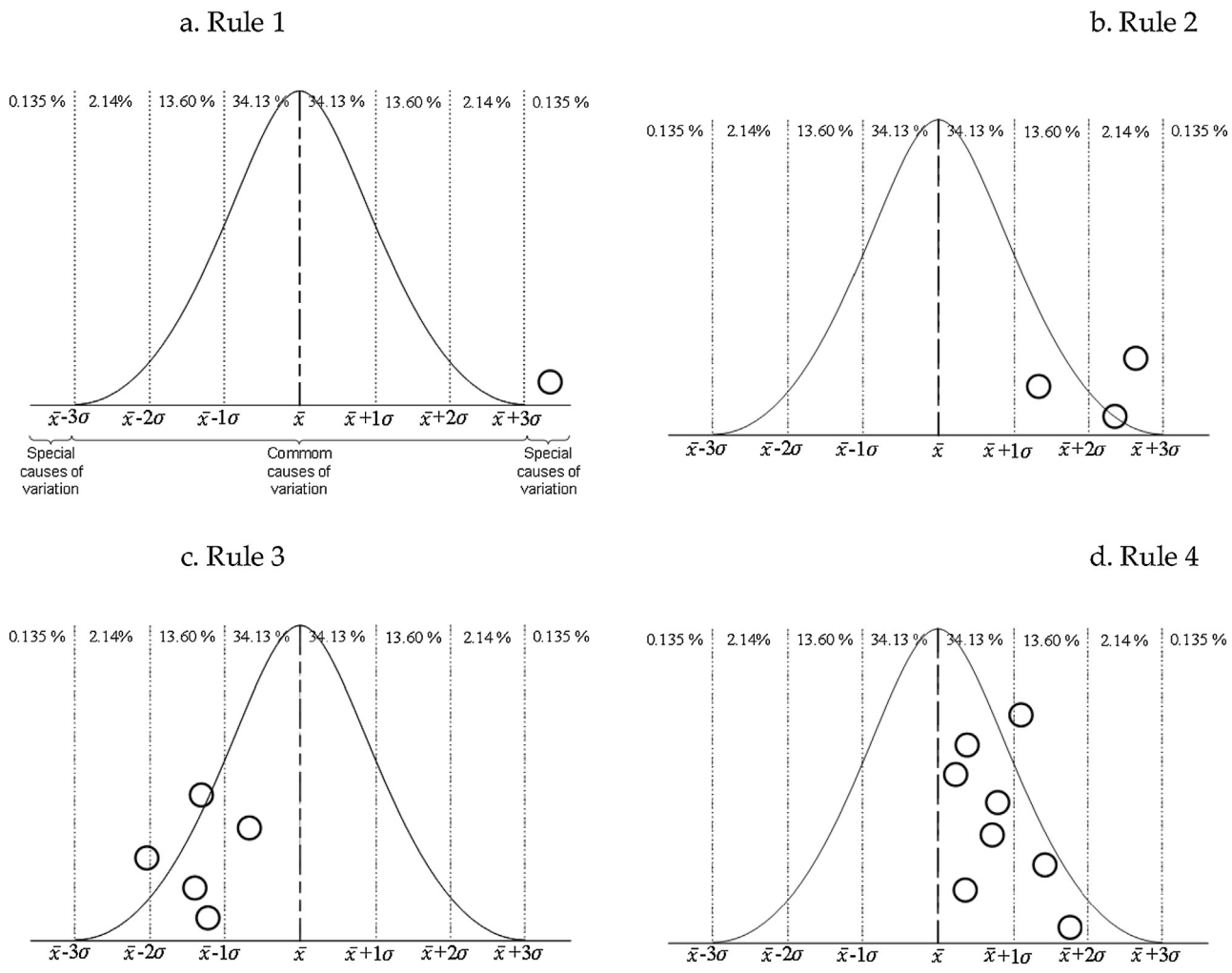

Fig. 1. Four examples of the application on the "Zone rules".

$$\overline{R} = \frac{\sum_{i=1}^{30} R_i}{30} = \frac{1.40}{30} = 0.05, \quad UCL_R = 2.574 \cdot 0.04667 = 0.12,$$

and $LCL_R = 0 \cdot 0..04667$

c) Average and standard deviation charts

Let consider the hematocrit results (ratio) in a sampling with 30 lots with a variable number of samples per sampling of *red cells, leucocyte depleted in additive solution* components. This rational subgroup is from a blood establishment where it is manufactured

Table 3
Data for the case of sampling with 30 lots with a constant sampling equal to three *red cells, leucocyte depleted in additive solution* components.

	Observations						*Observations*				
No.	n_1	n_2	n_3	$\bar{x}_i$	R_i	No.	n_1	n_2	n_3	$\bar{x}_i$	R_i
1	0.59	0.59	0.64	0.61	0.05	16	0.58	0.61	0.53	0.57	0.08
2	0.61	0.62	0.60	0.61	0.02	17	0.58	0.59	0.56	0.58	0.03
3	0.65	0.58	0.61	0.61	0.07	18	0.61	0.61	0.62	0.61	0.01
4	0.57	0.59	0.58	0.58	0.02	19	0.57	0.61	0.59	0.59	0.04
5	0.64	0.62	0.56	0.61	0.08	20	0.57	0.61	0.61	0.60	0.04
6	0.59	0.63	0.60	0.61	0.04	21	0.61	0.61	0.61	0.61	0.00
7	0.60	0.56	0.58	0.58	0.04	22	0.55	0.59	0.63	0.59	0.08
8	0.57	0.61	0.59	0.59	0.04	23	0.59	0.56	0.58	0.58	0.03
9	0.61	0.57	0.56	0.58	0.05	24	0.6	0.61	0.57	0.59	0.04
10	0.57	0.60	0.62	0.60	0.05	25	0.58	0.55	0.59	0.57	0.04
11	0.64	0.60	0.57	0.60	0.07	26	0.66	0.59	0.6	0.62	0.07
12	0.60	0.62	0.59	0.60	0.03	27	0.6	0.61	0.63	0.61	0.03
13	0.53	0.57	0.62	0.57	0.09	28	0.57	0.55	0.58	0.57	0.03
14	0.55	0.52	0.62	0.56	0.10	29	0.62	0.59	0.59	0.60	0.03
15	0.60	0.61	0.59	0.60	0.02	30	0.53	0.58	0.61	0.57	0.08
								$\sum \bar{x}_i = 17.7700$		$\sum R_i = 1.40$	
								$\bar{\bar{x}} = 0.60$		$\overline{R} = 0.05$	

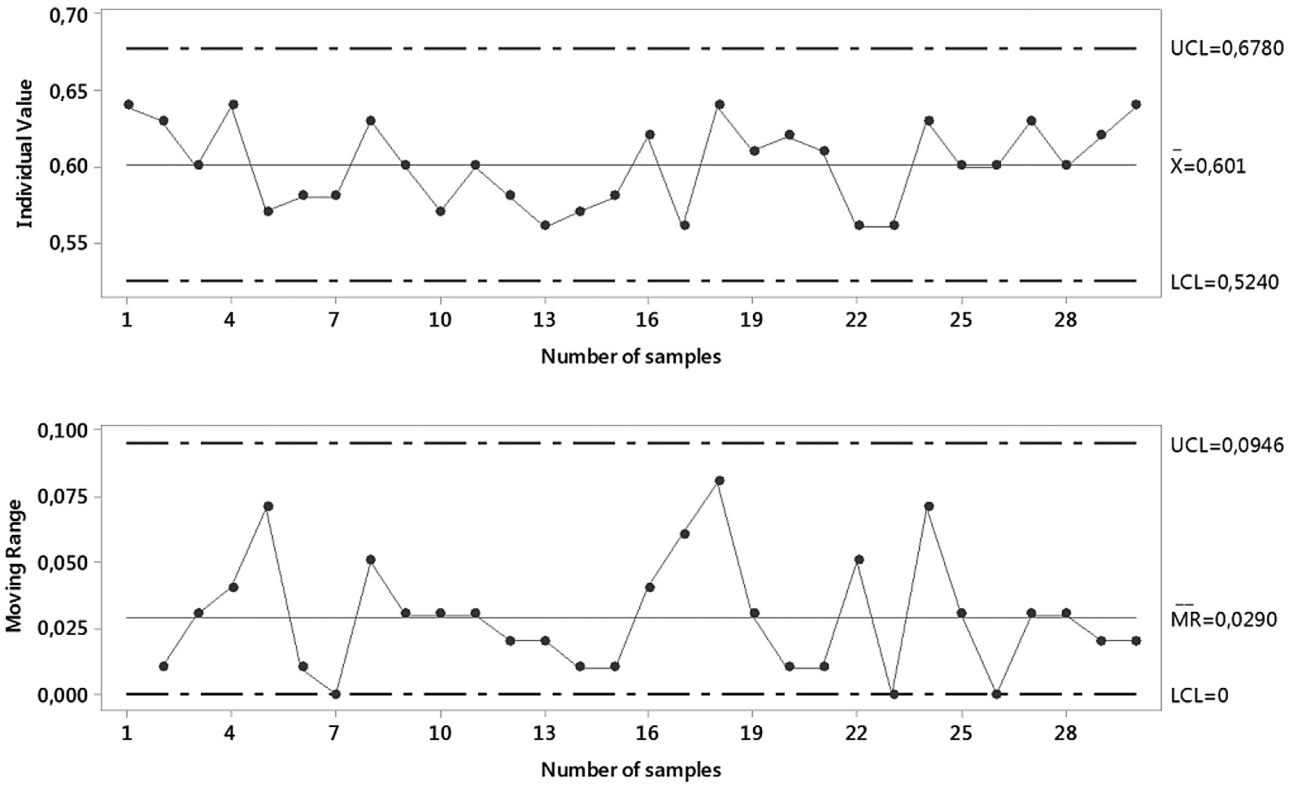

Fig. 2. x-$\bar{R}$ charts for the control of hematocrit (ratio) in a series of individual results.

Table 4
Data for the case of sampling with 30 lots with a variable number of samples per rational subgroup of *red cells, leucocyte depleted in additive solution* components.

	Observations								*Observations*						
No.	n_1	n_2	n_3	n_4	n_5	$\bar{x}_i$	s_i	No.	n_1	n_2	n_3	n_4	n_5	$\bar{x}_i$	s_i
1	0.59	0.59	0.64	0.58		0.60	0.03	16	0.58	0.61	0.53	0.63	0.64	0.60	0.04
2	0.61	0.62	0.60	0.64	0.54	0.60	0.04	17	0.58	0.59	0.56	0.59		0.58	0.01
3	0.65	0.58	0.61	0.61		0.61	0.03	18	0.61	0.61	0.62	0.61	0.61	0.61	0.00
4	0.57	0.59	0.58	0.66		0.60	0.04	19	0.57	0.61	0.59	0.66		0.61	0.04
5	0.64	0.62	0.56	0.55		0.59	0.04	20	0.57	0.61	0.61	0.57		0.59	0.02
6	0.59	0.63	0.60	0.62		0.61	0.02	21	0.61	0.61	0.61	0.61		0.61	0.00
7	0.60	0.56	0.58	0.60		0.59	0.02	22	0.55	0.59	0.63	0.53		0.58	0.04
8	0.57	0.61	0.59	0.59		0.59	0.02	23	0.59	0.56	0.58	0.62		0.59	0.03
9	0.61	0.57	0.56	0.62		0.59	0.03	24	0.6	0.61	0.57	0.61	0.56	0.59	0.02
10	0.57	0.60	0.62	0.65		0.61	0.03	25	0.58	0.55	0.59	0.59		0.58	0.02
11	0.64	0.60	0.57	0.63		0.61	0.03	26	0.66	0.59	0.6	0.6		0.61	0.03
12	0.60	0.62	0.59	0.55		0.59	0.03	27	0.6	0.61	0.63	0.59		0.61	0.02
13	0.53	0.57	0.62	0.56		0.57	0.04	28	0.57	0.55	0.58	0.58		0.57	0.01
14	0.55	0.52	0.62	0.65		0.59	0.06	29	0.62	0.59	0.59	0.6		0.60	0.01
15	0.60	0.61	0.59	0.56		0.59	0.02	30	0.53	0.58	0.61	0.61		0.58	0.04
											$\sum \bar{x}_i = 17.8370$			$\sum s_i = 0.8273$	
											$\bar{\bar{x}} = 0.60$			$\bar{s} = 0.03$	

Table 5
The Western Electric rules for Shewart control charts.

No.	Rule
1	One point out of the three-sigma control limits*
2	Two of three successive points are out of two-sigma warning limits, but within three-sigma control limits*
3	Four of five successive points beyond the one-sigma limits*
4	Eight successive points on one side of the center line*
5	Six successive points in a row progressively increasing or decreasing (trend)
6	Fifteen points in a row in zone C (both above and below the center line)
7	Fourteen points in a row discontinuous up and down
8	Eight points in a row on both sides of the center line with none in zone C
9	An unusual or nonrandom pattern in the data
10	One or more points near a warning or control limit

Legend: * "Zone rules"

Table 6
The Western Electric rules for nonsymmetric control limits with $n < 5$.

No.	Rule
1	One point out of the center line plus three-sigma limit
2	Two successive points are out of center line plus two-sigma limit, but within three-sigma control limit
3	Three successive points out of center line plus one-sigma limit
4	Seven sucessive points above the center line
5	Ten sucessive points below the center line
6	Six sucessive points below the center line minus one-sigma limit
7	Four sucessive points below the center line minus two-sigma limit

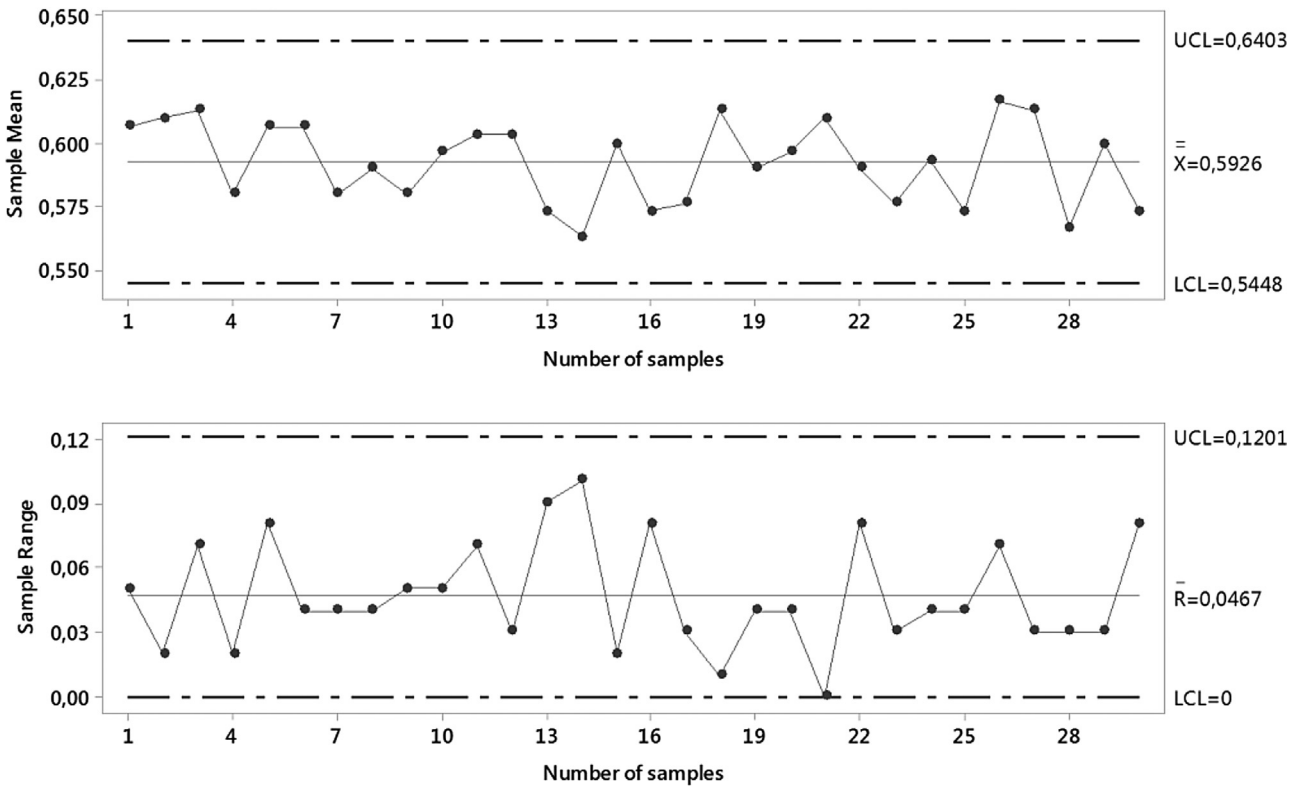

Fig. 3. $\bar{x}$-*R*charts for the control of hematocrit (ratio) in a series of rational subgroups with a constant number of samples.

a daily lot where the sampling is according to the significantly different number of blood components produced. Differently from the previous charts, in$\bar{x}$-scharts with a variable number of samples, the control limits are determined per rational subgroup. Fig. 4 shows the $\bar{x}$-*s* charts. Table 4 displays this cases' data. Eq. (20) expresses the center line and Eqs. (21) and (22) the control limits of $\bar{x}$-chart as follows:

$$\bar{\bar{x}} = \frac{\sum_{i=1}^{30} n_i \bar{x}_i}{\sum_{i=1}^{30} n_i} = \frac{4(0.6000) + 5(0.6020) + \cdots + 4(0.5825)}{4 + 5 + \cdots + 4}$$

$$= \frac{73.75}{124} = 0.60, \quad UCL_{\bar{x}} = 0.59476 + A_3\bar{s},$$

and $LCL_{\bar{x}} = \bar{\bar{x}} - A_3\bar{s}$ (note : for the first smaple n_1 , $m = 5$)

Eq. (23) compute the center line and Eqs. (24) and (25) the control limits of *R*-chart as follows:

$$\bar{s} = \sqrt{\frac{\sum_{i=1}^{30} (n_i - 1)s_i^2}{\sum_{i=1}^{30} n_i - 30}} = \sqrt{\frac{3(0.00073) + 4(0.00142) + \cdots + 3(0.00143)}{4 + 5 + \cdots + 4 - 30}}$$

$$= \sqrt{\frac{0.08734}{94}} = 0.03, \quad UCL_S = 2.089\,(0.03048) = 0.06,$$

and $LCL_S = 0 \quad (0.03048) = 0$

4. Discussion

As it is referred, the emphasis of this article is the successful practice of statistical methodologies for the control of the production of blood components using charts for variables. The text is not oriented to interpret different out-of-control issues, and applying CAPA. The results show an example of a well-succeed application of these diagrams,supporting the reader on the design of an SPC for variables in the scope of blood establishments. The use of a dedicated software able the service to center the attention on the principles and control. The software used is just one of a series of statistical tools available. The advanced spreadsheet user immediately recognizes that the presented and discussed approaches are suited to be used on a conventional spreadsheet software such as Excel® (Microsoft®,Redmond, Washington, USA). See [19] for an easier practice of control chartsfor variables using Excel®.

We suggest that on cases such as the validation stage of a new process, on production runs that have $n < 1$ or when the blood components must be fully controlled, the quality control manager should consider individual and moving range charts. Moreover, they are also advantageous to control unstable processes, for diagnostic purposes, to be used with destructive tests, if very tight specifications are used or when deciding about adjustments to the process. These charts are more sensitive to the detection of short-term behaviors. Otherwise, the average and range charts are more valuable for stable production processes with $n \geq 2$. They are capable of detecting long-term issues.The type of average and range chart is depended on the sampling dimension. Typically, the individual and moving range charts are more common in the routine of small blood establishments and the average and range charts on larger establishments. See Fig. 5 for a simple flowchart to the selection of control charts for variables.

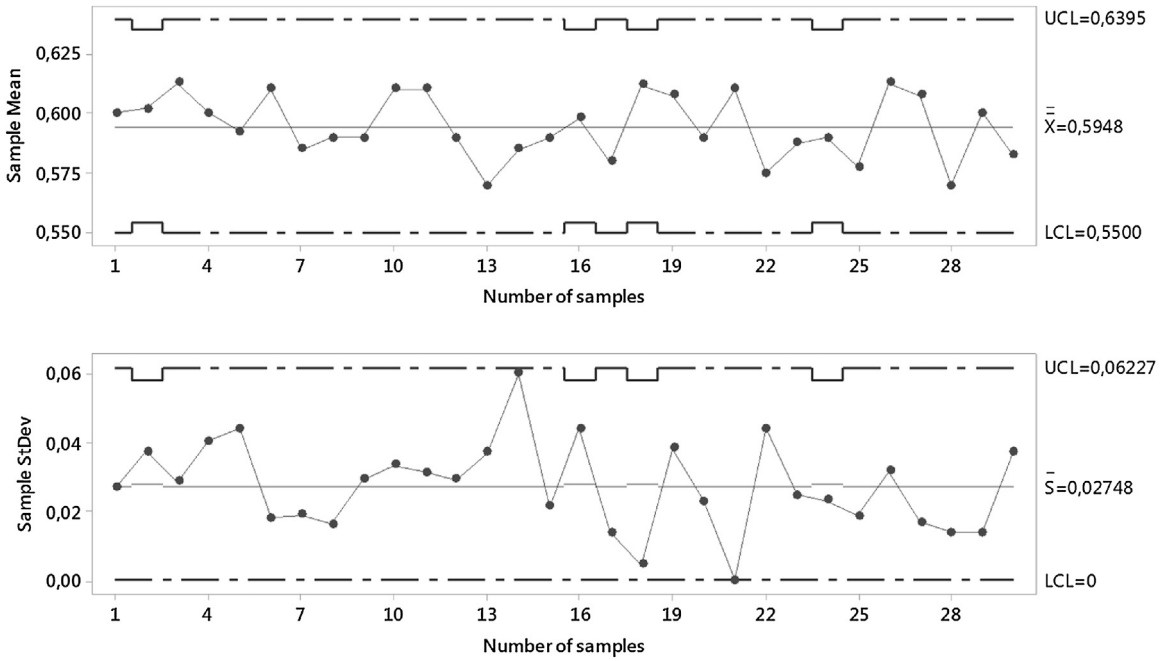

Fig. 4. $\bar{x}$-scharts for the control of hematocrit (ratio) in a series of rational subgroups with a variable number of samples.

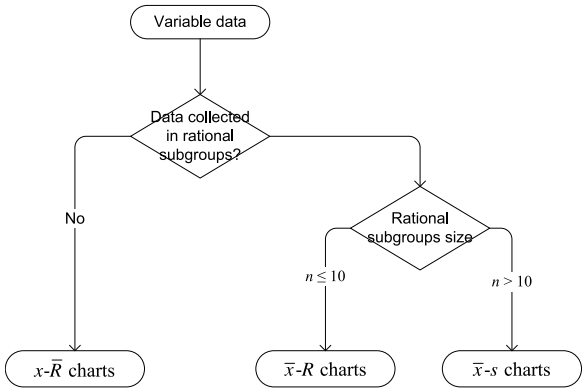

Fig. 5. Selection of the type of chart for variable.

On the other hand, on the control of the manufacturing, the laboratorian instantly places the question: so, what settings of rules are applied? There is not a single answer. The selection depends on the process stability and the case of nonsymmetric control limits, on the number of samples. The application of "Zone rules" is suitable for most of the cases (see Table 5) and for the nonsymmetric control limits with a small number of samples seven rules proposed (see Table 6). Note that the application of the "full rules" (see Table 5) in most of the cases can be a source of false alarms due to the blood components manufacturing to be usually a stable process in controlled conditions. The selection of decision rules could be seen as closely related to the capability indexes. Therefore, production processes with higher indexes require simpler rules sets and when the indexes are low a more complex rules group should be applied (Chapter3, Part 7 of [10]).

Nevertheless, the successful application of control charts for variables remains a challenge for most of the blood establishments. Skilled and successfully trained personnel are required. Furthermore, it should be recognized that an operative with established competence in blood components specifications, statistics basics, and SPC implicates a long-term formation. If it is not possible to have some operative fulfilling these function, a consultant with experience in SPC in the components production should be hired. Our blood establishment experience advises as a first step the support of a consultant and in a second phase to start the formation of someone that could be the responsible for keeping and developing the SPC methodology.

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