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Peykan check, a simple continuous quality control method for hematology analyzers

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Abstract

A simple inexpensive continuous quality control method, by means of eight basic blood counting parameters, obtained from automated hematology analyzers, using patient samples, is described.

A few samples with low, normal and high values were selected and introduced to the instrument early in the morning as the first count, the results of which are plotted on the appropriate chart as dots. The same samples were again given to the instrument at noon and the results were plotted on the same chart as the arrow heads. An arrow is then drawn from each pair of consecutive counts. The same procedure is repeated every 6 hours, using a newly selected set of samples, and the last sample which is selected in late evening on each day, is introduced to the instrument on the next morning. Hence patient samples are run at the beginning and the end of three daily work shifts. An explanation is given to use the direction of the arrows as the main factor to assess the quality of the instrument performance. This method can be easily applied to any hematological laboratory and can simply be performed even by laboratory technicians.

Keywords: Quality control; blood, cell count

Introduction

The principles of quality control (QC) methods in biochemical analyses were first described by Levey and Jennings in 1950 [1], and then by Copeland [2] in 1957. The issue then gained favor in Hematology. [3] The aim of most control methods is increasing the accuracy and precision and in general reducing the errors, which could happen in all medical practices. Some studies consider the errors occurring in hospital services [4,5], and some are focused on problems across the clinical laboratory practice [6-8], while others investigate problems within specific laboratory areas, such as biochemistry [9,10], blood banking [11,12] and genetic testing. [13] The appearance of a large variety of sophisticated instruments demands that the QC be progressed to even more advanced and rather complicated methods. However some researchers consider these "control" methods to be time consuming, expensive and "out of control" . [14]

Methods to perform QC on laboratory instruments are based on preparation of control materials. Control samples of serum which are used for biochemical methods are relatively stable even after different manipulations such as freezing, storage, lyophylization etc. Hence a large variety of commercial serum controls are now available, though they have some inherent difficulties.

The subject is more complicated for hematological counting instruments. These instruments use whole blood as the control samples. Whole blood can hardly be stored for 24 hours, during which it may change either qualitatively or quantitatively. Commercially prepared control blood samples, which are derived from the whole blood, are not as stable as serum. Moreover they are expensive [15], and do not exactly represent the test performance in the manner of fresh whole blood samples [16, 17]. They also fail to take into account the errors in blood collection, sample transportation and specimen preparation.[16] The above and many other weaknesses reveal that the clinical laboratories need control materials with

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"ideal properties" . [17]

To overcome the above problems some control methods based on patient's own samples have been described, such as using the average of normal samples [18, 19], Delta Check [20] , Pattern Recognition[21], Randomized Duplicate Samples[22], XB-Calibration [15] and using the average difference of five specimens analyzed on two consecutive days[23]. These methods though appearing to be headed in the right direction are not exempt from complications.

. The aim of this study is to establish an accurate, simple and inexpensive QC method called Peykan (means arrow in Persian Language) Check. In this method patients' samples are used for QC and a part of the control procedure from each day will be linked to the next day. Therefore, this method is planned to be used as a continuous QC method to monitor the whole procedure of the test performance and to become aware of the daily, weekly and monthly variations.

Materials and Methods

In this study, whole blood samples were selected from patients referred to Central Laboratory of Imam Khomeini Hospital, Cancer Institute, and Milad Hospital Tehran, Iran. The work was carried out over two years.

The only prerequisite action before starting this method is to calibrate the instrument, by any usual method available in the laboratory. In our work, the instrument was calibrated a few times by introduction of the laboratory made control sample as well as commercial blood control specimens (Eightcheck-3WP, Sysmex Corporation, Japan). The data was then entered into a statistical analysis program to ensure the accuracy and precision of the instrument. Once verified, the Peykan Check was started.

Patients' whole blood samples were collected for routine work, in ethylenediamine tetra-acetate (EDTA), dipotassium salt (Merck Chemical Co. Germany), at a concentration of 1.5mg/ml of blood [24], to determine complete blood count (CBC). The procedures of sample collection, specimen preparation and transportation were closely monitored. Eight basic parameters that are determined by most electronic counting machines were employed here: white blood count (WBC), red blood count (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), MCH concentration (MCHC) and platelet count (PLT).

All patient samples were mixed well and introduced to the instrument (Sysmex K-800, K-1000, KX-21, Advance Medtronics Co., Shiraz Avenue, Tehran, Iran), in the usual manner and the results were obtained by collecting the instrument print-out, for daily patient reports.

For this study, at least 3 samples were selected, early in the morning (6 AM), from the routine patient sample tray, preferably from those containing low, normal and high values of above parameters.

For the purpose of this research a second print-out from the results of the chosen samples were taken from the instrument. These results were considered as the first count and were plotted on the appropriate chart as dots (figure 1). The samples were then kept in refrigerator (4ºC), separate from other patient samples.

At noon (12 AM), the selected samples were brought to room temperature, mixed well and introduced again to the instrument in the same manner as done for the first count, and another set of print-out was taken from the instrument representing the second count. The result of the second count was plotted on the appropriate chart as arrow heads (figure 1).

The dot (from the first count) was then joined to the arrow head (from the second count); hence an arrow was drawn from the results of two consecutive counts (figure 1). This procedure was followed for the eight parameters and for all selected samples. These samples which were not used for this research any more were returned to the routine patient sample tray.

A second set of 3 routine samples was again selected at noon and analyzed by the same procedure to obtain the results of their first counts and left in 4ºC to be used again at late afternoon (6 PM), to obtain the results of the second count. After re-counting the second set of samples, and tracing the arrows, the third set was selected at (6 PM) and counted. These samples were then kept in 4ºC overnight to be counted again early next morning (6 AM), when the third set arrows were also drawn and the QC procedure for the day was finished. The next day samples were then selected at 6 AM and the same procedure was followed.

The same procedure was carried out every day

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analyzing a set of three selected samples, every 6 hours, and keeping the last set to be checked on the next day.

The net differences of the data obtained from patient samples in this work were compared with the results obtained from the commercial control sample (Eightcheck-3WP).

Results

Separate charts were prepared for eight blood parameters (figure 2). The values for each parameter are shown on the left column of the charts. The range of values for WBC is from 3 to 13 (x109) white blood cells/L. For RBC from 2 to 8 (x1012) red blood cells/L. Values for HGB, were from 5 to 20 g/100 mL of blood, for HCT from 20% to 60%, for MCV from 70 to 110 fL, for MCH from 20 to 38 pg, for MCHC from 25to 40% and for PLT from 80 to 480 (x109) platelets/L.

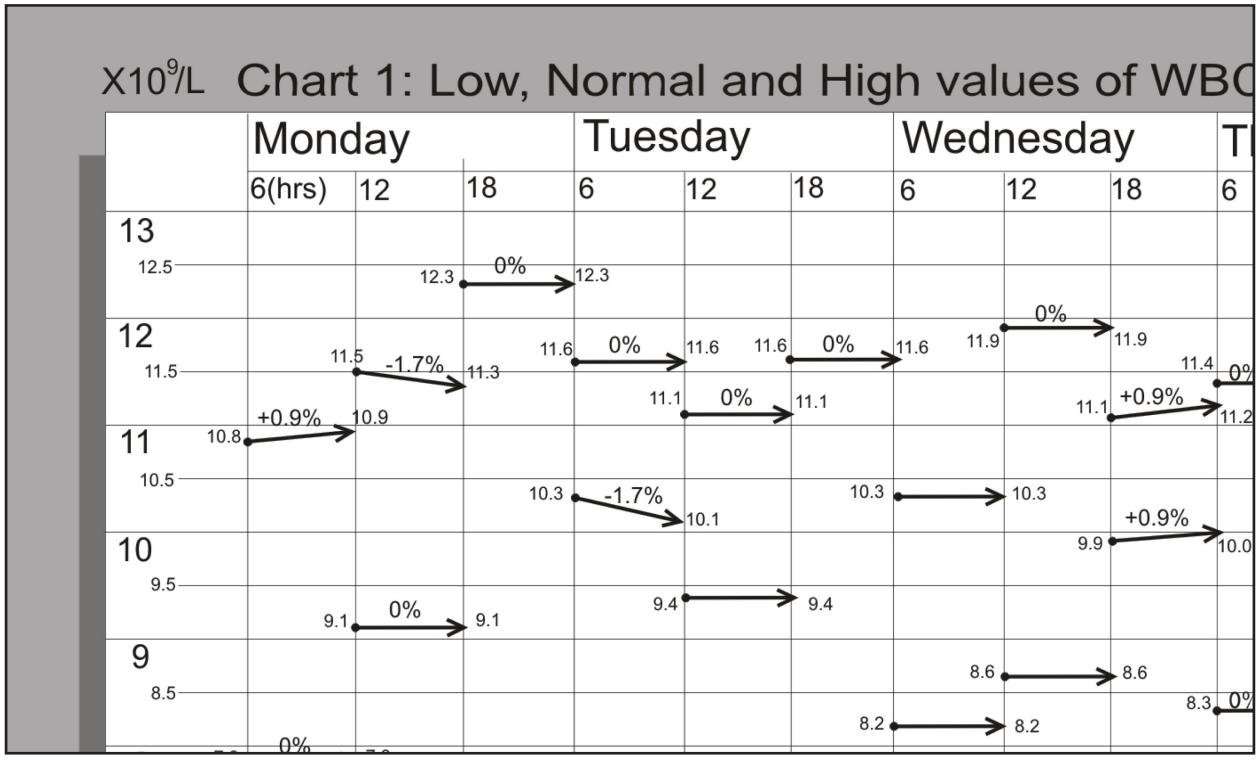
The horizontal row of all charts was divided in 21 parts and numbered, representing every 6 hours of each day of the week (see figure 1, a part of WBC chart is shown as an example). An extra row and column were added in order to insert the calculated net differences (ND). Sum of the net differences (SND) can also be calculated from NDs which should usually be equal for columns and rows. Since each chart represents one week, 4 such charts can be used consecutively to show the monthly variation of the results.

The results of all first counts were plotted on the appropriate chart as dots and the results of all second counts were shown as the arrow heads (figure 1). Hence a complete arrow was drawn from the results obtained from each pair of counts, for each parameter.

To avoid confusion, it is advised to show the results of not more than three samples with low, normal and high values on each chart.

The absolute values of the net differences of the data in this work were compared with the results obtained from the commercial control sample (Eightcheck-3WP). In table 1 hemoglobin results are shown as an example. The first column of this table shows the results of the first count of HGB of 50 patients. Only the sum, average, standard deviation (SD) and coefficient of variation (CV) are provided. The second count results are shown in the second column. The absolute value of the difference between the first and the second count is shown in the third column. The next three columns show the same results for the commercial control sample.

Figure1. A part of WBC chart, shown as an example



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As shown in table 1, similar figures are derived for the mean, SD and CV. The pattern in this part of the study shows a close agreement between the results of HGB concentration obtained by the Peykan Check and conventional run (commercial control sample).

The interpretation of the results can be carried out in various ways. For instance, by comparing the net difference of the data, the slope of the arrows as compared to horizontal line or preferably, as followed in this work, the percentage obtained from the net differences. However to prevent over extending of this article the counting data are not shown.

In another experiment, the patient samples were introduced to the instruments twice at a same time (i.e.: in duplicate). The results of this experiment show that even without any time intervals the data obtained by both counts are in most cases different. The results show that the second count can show an increase or decrease of at most 5% (i.e.: +/- 5%). For instance, the first count of a sample with a WBC of 12(X109/L) could have a second count from 11.4 (i.e.: - 0.6 or -5%) to 12.6 (i.e.:+ 0.6 or + 5%). See arrows on figure 1. Hence a minor difference between the 1st and the 2nd count, after a period of 6 hours, is quite reasonable and reflects no defect in instrument performance.

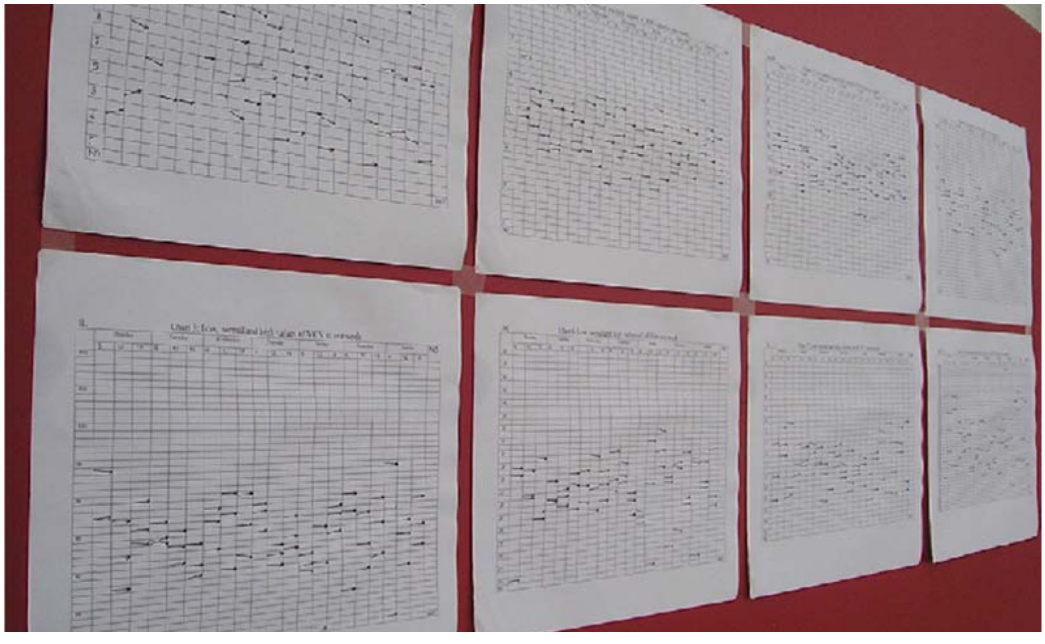
Discussion

The traditional approach to QC is commercial single-sample oriented [25], which does not cover all the aspects of a laboratory work. In a clinical laboratory setting the extension of the QC method should encompass daily, weekly and monthly sampling. The method should be efficient in monitoring all phases of laboratory performance from specimen acquisition to a complete patient laboratory report.

The whole blood which is used in the hematology laboratory cannot be stored for a long time [26] or frozen under ordinary conditions [27]. Ready made commercial cell counting control materials are expensive. [15] They are not fresh since they are fixed and are not therefore exactly simulating genuine fresh blood samples. [16, 17] They also do not take into account the possible variations existing during handling of specimens, such as sampling, transporting and specimen preparation.[16] Calibration of a laboratory apparatus, per se, is not sufficient to ensure accurate and precise results [28], unless it is followed by frequent checks. The problem of preparing control materials in hematology is therefore so great that one should assume that a ready made whole blood control sample, with all the "ideal properties"[17], overcoming the above problems cannot exist.

To overcome the above inherent problems, some

Figure2. Photograph of the charts on the wall



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control methods using patient's own material have been described. [15, 18-23] However these methods are not adapted to routine clinical laboratories because they are either practically inefficient, or need time consuming calculations and complicated computerized manipulation, and they are therefore disputed. [18, 20, 21]

In Peykan Check, the control material is the patient's own blood sample and the method is correlated with the data abtained from commercial control sample (table 1). Many advantages are yet present over commercial materials which can be explained as follows:

In this method the sample is considered to be fresh since it is stored and refrigerated for a minimum of 6 and a maximum of 12 hours. Though the blood cells may change (especially morphologically), within the first few hours of storage, the changes are retarded at 4ºC and are not significant. [26] Therefore it is assured that the fresh patient samples are handled in the same condition as the fresh control samples, rather than fixed, commercial control materials.

This method can assure the laboratory staff that they are dealing with a continuous process of QC, since the procedure of controlling the instrument, during the period of investigation, is running from one sample to another and connecting each day to the next, hence dealing with a continuous QC program at all time.

The patient sample is counted initially and stored in refrigerator and the same sample is again introduced to the instrument. This procedure of controlling does not depend to any change in the patient's situation or disease course, such as surgical procedure, bleeding, transfusion, nutrition, drug effect and so on.

Instead of using a computer software to "control" the present "control" method, the arrows are traced (Figures 1 and 2), the directions of which are of considerable significance. The ideal situation is when the arrows are drawn horizontally (i.e.: the net difference equals to zero), or, at least, their directions are almost alternatively up and down. An upward arrow means the results of the second counts are increased as compared to the first counts; and a downward arrow means they are decreased.

According to the results obtained by duplicate counting, we believe that the acceptable limit for this increased or decreased value, i.e.: the difference between the 1st and the 2nd count should not exceed ±5%. Hence if an arrow is so diverted from horizontal line or if some consecutive arrows are in the same direction that the net difference is more than 5%, the instrument is not under a complete control, and may need cleaning or even recalibration. This is the case if the net difference of the values of some alternative arrows exceeds that limit. This situation happened once in our experience and we had to recalibrate the machine. Apart from that, most arrows were traced almost horizontally and no significant changes were seen in the results. The acceptable situation for any longer period of time (e.g. one month) is that the net differences of the values do not exceed 5%, though the ideal situation is being as close to zero as possible, which means the overall performance of the instrument shows no significant variation in the results.

Table1. Agreement between patient and control samples

	1st Pt	2nd Pt	Dif Pt	1st Cont	2nd Cont	Dif Cont
Sum	621.700	622.000	4.900	622.800	622.500	4.500
Average	12.434	12.440	0.098	12.456	12.450	0.090
SD	0.198	0.246	0.087	0.192	0.222	0.089
CV	1.592	1.976	88.646	1.541	1.786	98.888

1st Pt: The 1st count of patient's sample
2nd Pt: The 2nd count of patient's sample
Dif Pt: the difference between the1st and the 2nd counts of the patient's sample.
1st Cont: The 1st count of the control sample
2nd Cont: The 2nd count of the control sample
Dif Cont: The difference between the 1st and the 2nd counts of the control samples

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Most arrows traced for MCV at first were either horizontal or upward, meaning the net difference of the results were slightly increased in one week. That was due to minor swelling of erythrocytes during storage. The situation was corrected when the proportion of blood and anticoagulant was well adjusted (26). In general any parameter that may show an inherent change during storage is not suitable for this method e.g. RBC in a patient with severe hemolytic anemia. Stored erythrocytes in such a sample may lyse, the erythrocyte count decreases and the appropriate arrows will be downward. This fact should not incorrectly lead the laboratory personnel to believe that their instrument is out of control.

Depending upon the manufacturers of commercial control materials the number of parameters available to get the instrument under control can be 8 at most. In our work the available number of parameters can easily be increased. Choosing only 3 samples (i.e. one set of performance) is ascertaining that the instrument is under control, using 24 (3x8) parameters. This advantage means that there should be no concern whether an inappropriate sample or parameter (e.g. RBC in severe hemolytic anemia) is by chance, selected or not, as the particular result can be easily ignored, without any concern about controlling the machine, since many other parameters are available.

Sometimes the erroneous results only occurred when our instrument was dealing with samples of low values while it was alright with high values or vice versa. Selecting samples with low, normal and high values will enable the operator to realize if such errors are encountered.

Another advantage is that the patient samples, which are collected by the laboratory staff, are used in this procedure. Hence factors such as blood sampling, specimen preparation and transportation are also taken into account. Though the method is described for hematological laboratories it can also be adapted for other biological tests.

Newly introduced counting instruments are claimed to be stable for weeks or months, following the calibration. Although such claims are sometimes proved and accepted, they should still be monitored. For instance if the instrument is claimed to be stable for three months, to perform such monitoring, by other QC methods, every-day calculations are to be carried out and interpreted during a three months period (usually by the head of the laboratory). The instrument will then be permitted to be employed each day, if the calculation results of the previous day are acceptable. For present QC method, all that is done during that period is to draw the arrows which will be mostly horizontally situated and no further action (even by the head of the department) is needed.

In this method the charts are displayed on a board in the hematology laboratory (figure 2), where any technician can easily recognize the variation in the shape and direction of the arrows, which is an indication of the variation in the instrument performance.

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