A PRACTICAL ATTEMPT FOR INTERPRETATION OF SYSMEX KX-21 BLOOD ANALYZER HISTOGRAMS AND FLAGS RESOLVING

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Abstract

The main principle of the automated blood analyzers is to get the quantity (count) of different blood components (parameters) in the peripheral blood samples. Additionally, in case of Sysmex KX-21 blood analyzer, the work performance includes also 3-part differential white blood cells (WBC) count represents eight parameters of the complete blood count (CBC). Extremely important to mention here that many of the analyzed information about the main blood corpuscular are provided in graphical forms called histograms. Remarkably, any abnormal results in the investigated blood parameters due to multi different factors such as red blood cell fragments, platelets clumps, cold agglutinins, giant platelets and many other factors will appear in an abnormal histograms and flags. These abnormalities in values and histograms or flags appeared are due to be interpreted and adjusted depending on extensive knowledge of the analytic performance of the analyser and the clinical significance of the results they provide.

As the KX-21 is a fully automated haematology analyser, easily fitting into a lot of medical laboratories in our country and ideal as a backup analyser to the Sysmex full differential analyser system, we tried to create a scientific method (it can be in a hard or soft copy) to avoid automating bad work processes. This is carried out in order to get accurate results, to interpret different histograms in a good way and to discover the reasons lead to appearance of the flags during analysing.

Keywords: Complete blood count, Blood analysers, Sysmex, Histograms, Flags

Introduction

The automated haematology analysers with complete blood count (CBC) results have replaced the traditional manual or individual assay methods for haematological parameters and the eye count leukocyte differential as the initial screening and detection system for haematological abnormalities in modern hospitals and clinics (Lantis et al. 2003).

During recent years, there has been increasing demand for hematologic tests with clearly defined turnaround times and within the context of cuts to laboratory budgets. Staff members represent the major expenditure, and, in many laboratories in which staff numbers have even been reduced, there is now more work for fewer laboratory scientists. Automated blood cell counters offer leukocyte, red blood cells (RBC) and platelet (PLT) counts 3-Part and a 5-part (some 6-part) leukocyte differential count (Fig. 1).

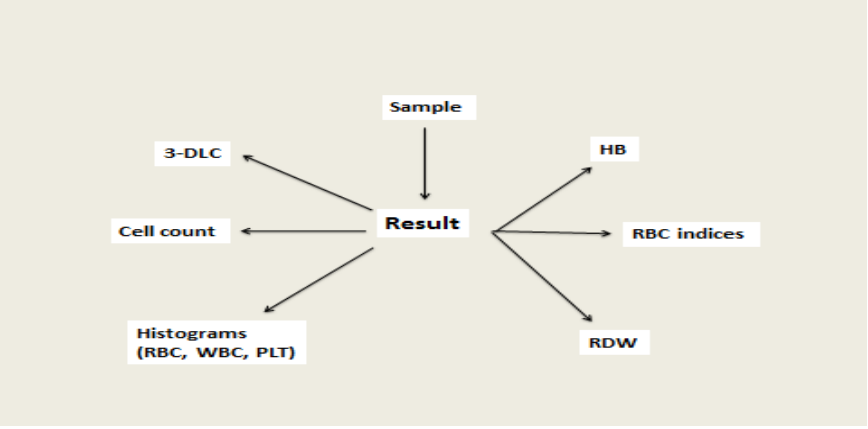


Figure (1): Major outputs of 3-Part differential analyser (KX-21N).

Additionally, some instruments provide a nucleated RBC (NRBC) count. Haematology instrument differentials provide only limited information about cell morphologic features using various algorithms to generate abnormal cell flags and are often unable to reliably classify abnormal and immature cells. The usefulness of instrument-generated flags depends on their sensitivity and specificity (Carol et al., 2005). Clinical laboratories routinely use haematology analysers to perform the so-called complete blood counts (CBCs) on patient blood samples whereas flagging is defined as a signalling or communicating a message with a flag". In the haematology laboratory, a flag is the signal to the operator that the analysed may have a significant abnormality during analysing blood samples. Haematology analysers generate suspect flags in the presence of abnormal cells. Actually, false-positive rates for flags are high on all analysers (Zandecki et al. 2007). Furthermore, haematology analysers provide quick and accurate results in most situations. However, false results related either to platelets or other parameters from complete blood count may be observed in several instances (Zandecki et al. 2007).

Most of the automated haematology analysers are programmed in a variety of ways to "flag" or otherwise identify samples that may have abnormalities. The warning messages created by the automatic blood analyser enables the user (Technician, Nurse or physician) to detect positive samples and to react with the follow-up actions because of the warnings. Therefore, working time can thus be saved without loss of

quality. Performance of the most popular hematology instrumentation reveals reasonably good outcome for quantitative abnormalities. According to the experience, a laboratory may safely broaden the flagging limits beyond the narrow limits of the quantitative reference ranges. The flagging performances for qualitative abnormalities have had false positive rates of up to 30% or more and false negative rates of up to 15%.

Technically, the counting theory of the cellular components of the blood (erythrocytes, leukocytes, and thrombocytes) is based on the classic method of electrical impedance. The methods presented are relatively inexpensive and easy to use in a number of medical applications, which constitute the main arguments for employing EI in clinical practice (Nowakowski et al. 2005). The aspirated whole blood specimen is divided into two aliquots and mixed with an isotonic diluent. The first dilution is delivered to the RBC aperture bath, and the second is delivered to the WBC aperture bath. Cell counting and sizing is based on the detection and measurement of changes in electrical impedance (resistance) produced by a particle as it passes through a small aperture. Particles such as blood cells are nonconductive but are suspended in an electrically conductive diluents. As a dilute suspension of cells is drawn through the aperture, the passage of each individual cell momentarily increases the impedance (resistance) of the electrical path between two submerged electrodes that are located on each side of the aperture (Segal et al. 2005) and (Buttarello et al. 2001).

During the last two decades, automated blood cell counters have undergone a formidable technological evolution owing to the introduction of new physical principles for cellular analysis and the progressive evolution of software. The results have been an improvement in analytic efficiency and an increase in information provided, which require specialized knowledge to best discern the possible clinical applications (Buttarello, 2008). In addition to the traditional parameters of the CBC count and leukocyte differential count (LDC), the more complete analyzers are able to provide much more information, both quantitative, such as the extended differential count (EDC), and qualitative. The latter is represented by flags that indicate technical problems (e g, malfunction, analytic interference) and, above all, cells that are normally absent from peripheral blood such as blasts, atypical lymphocytes, immature granulocytes (IGs), and nucleated RBCs (NRBCs) (carol et al. 2005) and (Zandecki et al. (2007). Remarkably, blood cell histograms which are produced by the modern automated haematology analysers are very important in result interpretation. A good interpretation of this histogram provides a wealth of information on many haematological conditions than mere cell counts, helping to narrow down the differential diagnosis at a very early stage even before higher level investigations are ordered (Thomas et al. 2017). These histograms are used also to understand some special properties of different blood cells (RBCs, WBCs and PLT)

by careful analysis of curves produced. These curves also known as complete blood count (CBC) histogram are derived by plotting the size of each cell on X-axis and their relative number on Y-axis (Brown and Barbara 1993). Unfortunately, due to various reasons blood cell histograms have not gained popularity among clinicians (Thomas et al. (2017).

Methods

Unquestionably, the new blood analyser technology has offered good knowledge about how to interpret different cyto-grams and histograms which can be used to detect different problems in samples that are likely known to produce erroneous results.

In this study, we tried to create a new approach that can be followed by the blood analyser operator (Medical laboratory technicians, Nurses and physicians) in order to give good result interpretation and to solve any other problems that may appear in form of flags during samples analysing or after result printout.

Scientific team and objectives: In order to begin the real steps to put the outlines of this program a scientific team has been composed from different scientists and different specialists. After a number of meetings we put the scientific frame of this tool which has been called educational software tool (EST) and main objectives should be discussed in the study including target groups such as technicians already working on 3-part differential blood analyser (KX-21N blood analyser), nurses and or physicians.

The scientific part should be supported with the educational tool, technical part including quality control, troubleshooting, calibration and maintenance besides basics of scientific knowledge such as normal range, delta check, samples quality, technical flag and definition of accreditation (WHO explanation).

Interpretation of histograms and understanding different errors those might be appeared during blood sample measurement in form of single or multi flags described in KX-21N automated haematology analyser instructions for use (IFU) **(Zindel (2001).**

Determination of main causes of flags and abnormal histograms

Possible actions should be taken to solve the problems of flag appearance and proper way to interpret positive histograms and the main follow up actions.

Additionally, depending on the results obtained and different reports prepared or concluded during the scientific team meetings all over a year, many illustrative schemes and tables of comparison has been prepared explains different relations between different parameters and variables.

Results

Automated haematology analysers are very complex and incorporate multiple technologies within one instrument due to many effective factors. Such factors lead to many benefits achieved by different blood analysers. Those benefits as more functions, operate more efficiently, incorporate more automation of what are presently manual processes and improved accuracy. At the same time depending on the results obtained through this study, we found that many of the KX-21N blood analyser results might be misinterpreted due to many causes starting from the pre-analytical mistakes, peri-analytical or post-analytical process besides many other causes usually lead to different flags and abnormal histograms (Table 1).

It is obvious that the Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 17 hematological parameters. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM#, MIXED# and NEUT#. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT% (Sysmex KX-21N Operator's Manual, 2000) and (Koepke (1991).

Table (1): Frequent causes expected to produce flags during blood sample measurement by KX-21N blood analyser.

No	Frequent causes	RL	RU	MP	DW	PL	PU	MP	DW	WL	AG	WU	T1,T2	F1,2,3
1	Giant PLT	+			+		+		+					
2	Microerythocytosis	+			+		+		+					
3	RBC fragment	+			+		+		+					
4	PLT clumps	+			+		+		+	+				
5	Cold agglutination of RBC		+		+					+				
6	Roulex formation		+		+									
7	IDA under therapy			+										
8	Infection/tumor anemia			+										
9	RBC transfusion			+										
10	Extreme leukocytosis			+								+		
11	High blank value					+			+					
12	Cell fragment					+			+					
13	High no. Of bacteria					+			+					

14	Contaminated reagent					+			+					
15	PLT aggregation					+	+	+	+		+			
16	EDTA incompatibility						+	+		+	+			
17	Clotted sample		+		+		+	+		+				
18	PLT anisocytosis							+						
19	After chemotherapy							+						
20	RBC lyse resistance									+	+			
21	NRBC									+	+			
22	WBC agglutination											+		
23	Abnormal leukocytosis												+	+

In order to describe the actions required to be followed to interpret the blood analyser results in the mentioned cases (flags) or to explain abnormal histograms appeared during samples measurement we correlated those causes with the possible actions should be applied to correct possible mistakes that might occur before sending the result as a final result to the physicians.

Table (2) shows the relation between causes of flags or abnormal histograms and required actions expected to be followed by the technicians or nurses in order to correct the wrong result depending on the scientific knowledge supported with the analyser in the form of integrated software. Additionally, as shown in table (3) we collected the frequent causes lead to produce different flags. We have also considered the possible actions that must be taken to solve the problem of misinterpretation of the result and the follow up action if possible and the impact procedures that should be done regarding other parameters or about the final report being printed out in a final form.

Table (2): The relation between causes of flags or abnormal histograms and required actions expected to be followed to correct the wrong result.

No	Frequent causes	New sample	Warm up	Cell wash	Remove clot	Manual count	Count correct	Blood smear	Change reag.	Repeat analysis	Sample dilut
1	Giant PLT					+		+			
2	Microerythocytosis					+		+			
3	RBC fragment	+				+		+			
4	PLT clumps					+		+			
5	Cold agglutination of RBC		+								
6	Roulex formation							+			+
7	IDA under therapy										
8	Infection/tumor anemia					+		+			

9	RBC transfusion							+			
10	Extreme leukocytosis					+					+
11	High blank value										
12	Cell fragment	+						+			
13	High no. Of bacteria					+					
14	Contaminated reagent								+		
15	PLT aggregation	+						+			+
16	EDTA incompatibility										
17	Clotted sample	+			+						
18	PLT anisocytosis	+				+		+			
19	After chemotherapy							+			
20	RBC lyse resistance					+			+		+
21	NRBC					+	+	+			
22	WBC agglutination	+	+	+							+
23	Abnormal leukocytosis					+		+			
24	Hemolysed sample	+						+			
25	Old sample	+									
26	confirmation									+	

Table (3): Frequent causes of different flags and the possible actions to solve the problem of miss interpretation of the results and the follow up action and the impact procedures.

Common causes	Action to identify cause	Follow up action	Impact
Giant PLT	-Low PLT-PU, RU -Blood smear	PLT manual count -RBC correction	Report other parameters
Microerythrocytosis	-High PLT- PU -Blood smear(RBC size)	-PLT manual count	Report other parameters
PLT clumps	-Low or high PLT-PU, high WBC-WL and high RBC-RL -Blood smear (PLT clumps)	-Sample dilution (NaCl) -Re-measure -PLT manual count	RBC indices not effected
RBC cold agglutinin	- Low RBC-RU - Blood smear	- Incubation at 37C° - Re-measure *	RBC indices effected
Roulex formation	-Low RBC-RU -Blood smear (Roulex)	-RBC manual count	RBC indices effected

Extreme leucocytosis	-High RBC-MP -High WBC-WL	-RBC indices should be controlled -Sample pre-dilution	WBC correction
RBC cell fragment	-High PLT-PU & PL	-PLT manual count -PLT correction	RBC slightly interfered
PLT aggregation	-Low PLT-PU or PL & MP -AG in WBC histogram -Blood smear (PLT aggregat.)	-New sample (Na-citrate) -PLT count correction	RBC indices reported
RBC lyse resistance	-high WBC-WL & AG -Blood smear-(acanthocytes)	-Sample dilution (NaCl) - Dilution with lyse reagent	-only WBC reported -PLT correction
NRBC	- High Lymph.-WL - Blood smear	NRBC microscopic count/ WBC count	WBC correction
WBC agglutination (rare)	- Low WBC-WU - Blood smear (PMNs)	- Incubation at 37C° - New sample in Na-citrate	Lymphocytes count correction
Abnormal leucocytosis	-T1, T2 -F1, F2, F3 Blood smear (imm. Leuk.)	Microscopic DLC	If no WL or WU WBC count reported
Clotted sample	Remove clot(PLT not ordered)	measurement	WBC, RBC indices reported
Clotted sample (Full CBC)	New sample	measurement	

Depending on aforementioned relations it was clear the importance of more explanation of the sources of possible causes of flags or abnormal histograms and the expected actions. As a consequence of above mentioned points we put most common causes in a table 4 to be described scientifically correlated with the related flags as shown previously in table 1.

Table (4): Causes description and their relation with flags.

Causes and description			
No	Cause (disease)	Description	Flag No from flags & causes
1	Cell fragments contaminated reagent high number of	In rare cases, bacteria present in septic patients, might influence the PLT result as well as high numbers of bacteria. e. g. due to contamination) and presence of small particles due to fragmented cells. A background check is necessary to exclude a contaminated	1 (PL)

	bacteria	reagent.	
2	RBC fragments	Fragmented red cells are also called schistocytes. They are shaped irregularly and sometimes referred to as "helmet cells". They are usually present in combination with a poikilocytosis. The presence of fragmented RBC may be an indication for a haemolytic anaemia.	2 (PU) 3 (RL) 5 (PDW) 6 (RDW)
3	Microcytes	The presence of microcytes usually results from a defect in haemoglobin formation. Microcytosis related to defect in haemoglobin synthesis should be distinguished from red cell fragmentation or schistocytosis. In these cases checking the MCV value is very important. Microcytes are very common in a diseases such as IDA, thalassemia & some chronic diseases.	2 (PU) 3 (RL) 5 (PDW) 6 (RDW)
4	Giant platelets	Very large platelets are called giant platelets. Giant platelets can occur in congenital diseases, like the Bernard-Soulier syndrome, but they can also be »concomitant symptoms« of acquired diseases, such as the essential thrombocythaemia .Giant platelets are occasionally observed as an incidental finding in routine blood smear examinations. Most of them are due to acquired disorders such as idiopathic thrombocytopenic purpura (ITP) and myelodysplastic syndrome (MDS).	2 (PU) 3 (RL) 5 (PDW) 6 (RDW)
5	Platelet aggregation (clotted sample, ..? EDTA incompatibility)	Pseudothrombocytopenia is one of false diagnosis of thrombocytopenia due to platelet clumping caused by a reaction to chemicals in the collecting tubes. The most common chemical that causes this problem is EDTA. PLT aggregation may be seen in some pathological disorders such as myeloproliferative disorder.	2 (PU) 3 (RL) 5 (PDW) 6 (RDW) 8 (WL) 13 (AG)
6	RBC agglutination/ Rouleaux formation	A state where RBC agglutinate for example due to "cold agglutinin disease" or A cold auto-immune mechanism (Chronic cold agglutinin disease) produces a special type of hemolytic anemia. (Check again how detailed the explanation should be (UOS)) The RBC's here have stacked together in long chains. This is known as "rouleaux formation (Rouleaux is clinically significant when increased proteins are present, as in multiple myeloma.(this is enough) " and it happens with increased serum proteins, particularly fibrinogen and globulins.(delete..) Such long chains of RBC's sediment more readily. This is the mechanism for the sedimentation rate, which increases non-specifically with inflammation and increased "acute phase" serum proteins. (Check UOS	4 (RU) 8 (WL)
7	lyse-resistant RBC	In rare cases red blood cells may not completely lysed and interfere the white blood cells count (incorrect high results). Lyse resistant RBCs can be seen in some patients with severe liver dysfunction or in early newborns. There is a correlation between lyse resistant RBC and reduced osmotic fragility due to the defect in haemoglobin composition.	8 (WL) 13 (AG)
8	extreme leucocytosis	Leukocytosis means an increase in the total number of WBC dut to any cause. It should be considered that there is no clear differentiation between different types of leukocytes due to	9 (WU)

		presence of abnormal or immature leukocytes.	
9	WBC aggregation	WBC aggregation predates the appearance of clinical symptoms of macrovascular disease in diabetes. (UOS check: very seldom - better delete?) Agglutination of white blood cells is a rare only in vitro phenomenon with usually little clinical correlation. In some very rare conditions WBC agglutination can be seen in combination with EDTA incompatibility.	9 (WU)
10	PLT satellitism	Platelets rosetting around polymorphnuclear neutrophils and thus won't be counted as PLT. This may lead to a falsly wrong dignosed thrombocytopenia. This is a rare in vitro phenomenon seen in blood treated with EDTA as an anticoagulant.	9 (WU)
11	abnormal/ immature WBC	Cancer. Presence of one or more of immature stages of leukocytes (left shift) which is lead to an abnormal distribution of leukocyte population and then can not be clearly differentiated. This can be seen in different leukemic disorders.	10 (T1) 11 (T2) 12 (F1,2,3)
12	NRBC	Nucleated RBC (NRBC) are immature red blood cells normally not seen in the peripheral blood beyond the neonatal period. Their appearance in peripheral blood of children and adults signifies bone marrow damage or stress and potentially serious underlying disease.	8 (WL) 13 (AG)
13	Anemia RBC transfusions Iron deficiency under therapy	The RBC's here have stacked together in long chains. This is known as "rouleaux formation" and it happens with increased serum proteins, particularly fibrinogen and globulins. Such long chains of RBC's sediment more readily. This is the mechanism for the sedimentation rate, which increases non-specifically with inflammation and increased "acute phase" serum proteins. (Check UOS copied from internet)	7 (MP)
14	Anisocytosis of RBC	RBC anisocytosis means increased variation in size of the red cell population. On the blood smear, normal, small and large cells can be seen in one field. The main features of RBC anisocytosis are normal MCV and high RDW. As the severity of the anemia increases, the amount of significant anisocytosis present also increases.	6 (RDW)
15	Anisocytosis of PLT	An increased variability in platelet size is called platelet anisocytosis. Small size of PLT is associated with thrombocytopenia due to bone marrow failure whereas the large size in case of thrombocytosis is due to a myeloproliferative disorders. In such cases a number of PLT will partly reach the RBC size and will counted as RBC.	7 (PDW)
16	Fragmentocytes	torn appart PLT aggregates (due to sheath flow method) are fragmentocytes and will be counted as PLT. False results of PLT count can be occur in presence of small particles due to fragmented cells, microcytes or any other particles their size is equal to PLT size or smaller than 25 fL. In such cases, those small particles will counted as PLT due to difficult of separation lead to appearance of PDW flag.	7(PDW)
17	Rouleaux formation	The RBC's here have stacked together in long chains. This is known as "rouleaux formation" and it happens with increased serum proteins, particularly fibrinogen and globulins. Such long chains of RBC's sediment more readily. This is the mechanism for	Should be deleted. Mention

		the sedimentation rate, which increases non-specifically with inflammation and increased "acute phase" serum proteins. (Check UOS copied from internet)	ed above

Discussion

From the deep discussion throughout the scientific team meetings, many questions were highlighted such as: what should the EST exactly be? What kind of workflow is in the lab? How does the idea of such a tool improve the lab workflow and how? What kind of information should be included in such a tool?

To simplify the steps in order to be understood and done by the Sysmex KX-21N operator, we determined the main parts of the problem detection as it is shown previously in (fig.1).

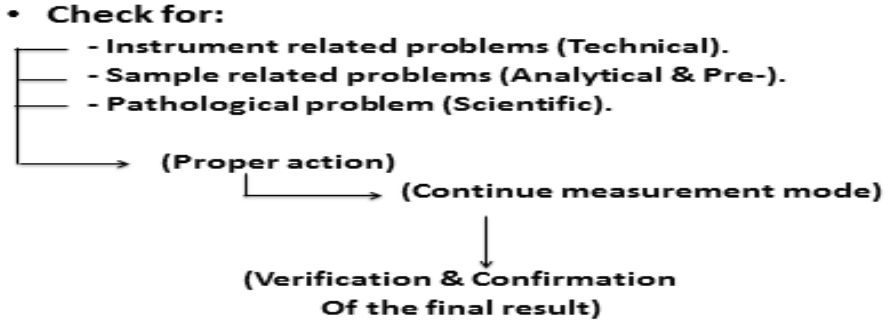


Figure (2): The main problems should be taken in consideration before, throughout and after measurement.

To put an answer for all the above mentioned questions, we created a lot of illustrated schemes to explain different scientific procedures achieved in the laboratory beginning from sample receiving, sample measurement, result printout and interpretation (Fig. 2).

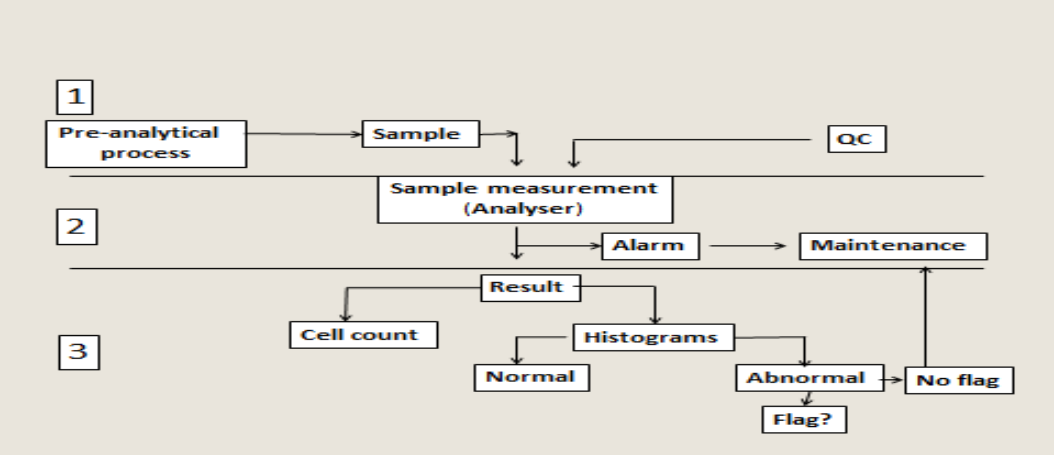


Figure (3): Steps of problem detection throughout pre and post blood sample measurement using KX-21N blood analyser.

Theoretically, taking into consideration the main technical check points such as application of quality control, calibration, troubleshooting and maintenance, figure (3) explains how to detect whether the problem is technically related or sample related. Consequently, if after quality control application the problem still appear, then the operator must go directly to the troubleshooting procedures. But if the quality control result is within the acceptable range then the operator should continue the sample measurement taking into account the abnormality results of cell count, normality of histograms, delta check and of course the appearance of different flags (Fig. 4 & 5).

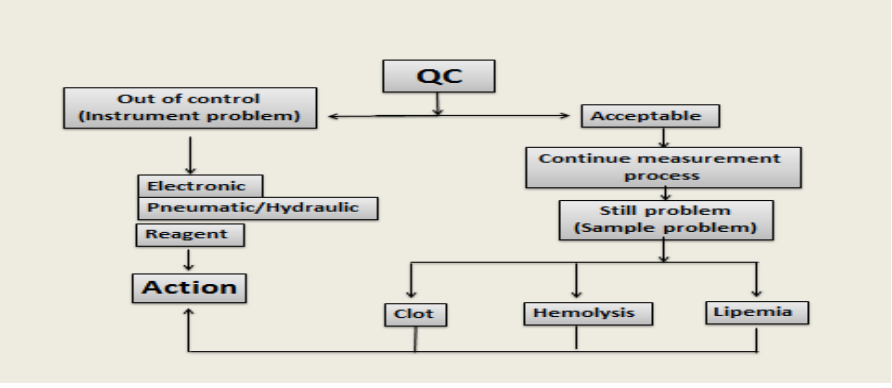


Figure (4): Detailed explanation of 1st and 2nd steps of problem detection using KX-21N blood analyser.

Accordingly, if there is any problem during measurement led to the appearance of one of the aforementioned errors, then a special action should be taken or a follow up action must be applied in order to verify and to confirm the result (Fig. 5).

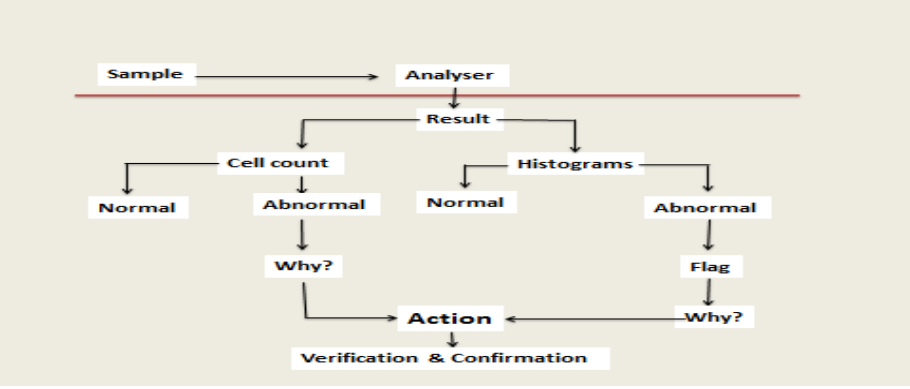
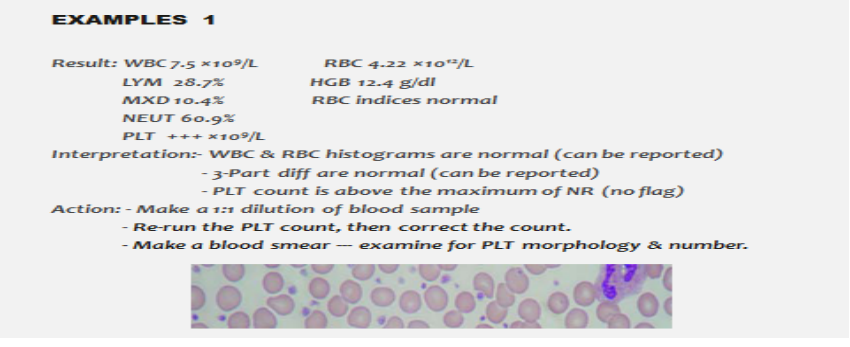


Figure (5): Detailed explanation of 3rd step of problem detection using KX-21N blood analyser.

More specifically, there are some principles that should be followed about how to understand the result determined in a few questions: What is abnormal about the result? Which parts can be reported? In addition, what procedures can be done regarding the abnormal parts? Distinctly, there are three detector blocks in an automated hematology analyzer. RBCs and platelets are counted in the same block whereas; WBCs are counted in a separate block. All the red cells in the blood directed towards the WBC counting block are lysed first using the stromatolyser solution (Brown and Barbara 1993), (Sysmex XE-2100 Main Unit Operator's Manual 2004) and (Sysmex XE-2100 IPU Operator's Manual 2004). In some special cases, it is difficult to distinguish between different sizes of different cells, fragments and some other small particles of artifacts which lead to left or right shift in low or upper discriminators of different histograms. Consequently, the result will not be accurate and reliable even when the cell count is within the normal range as shown in the examples (Fig. 6). Thereby, CBC histogram analysis is an often neglected part of automated haemograms that if well interpreted, has a good potential to provide diagnostically relevant information about many disease process even before higher level investigations are ordered.



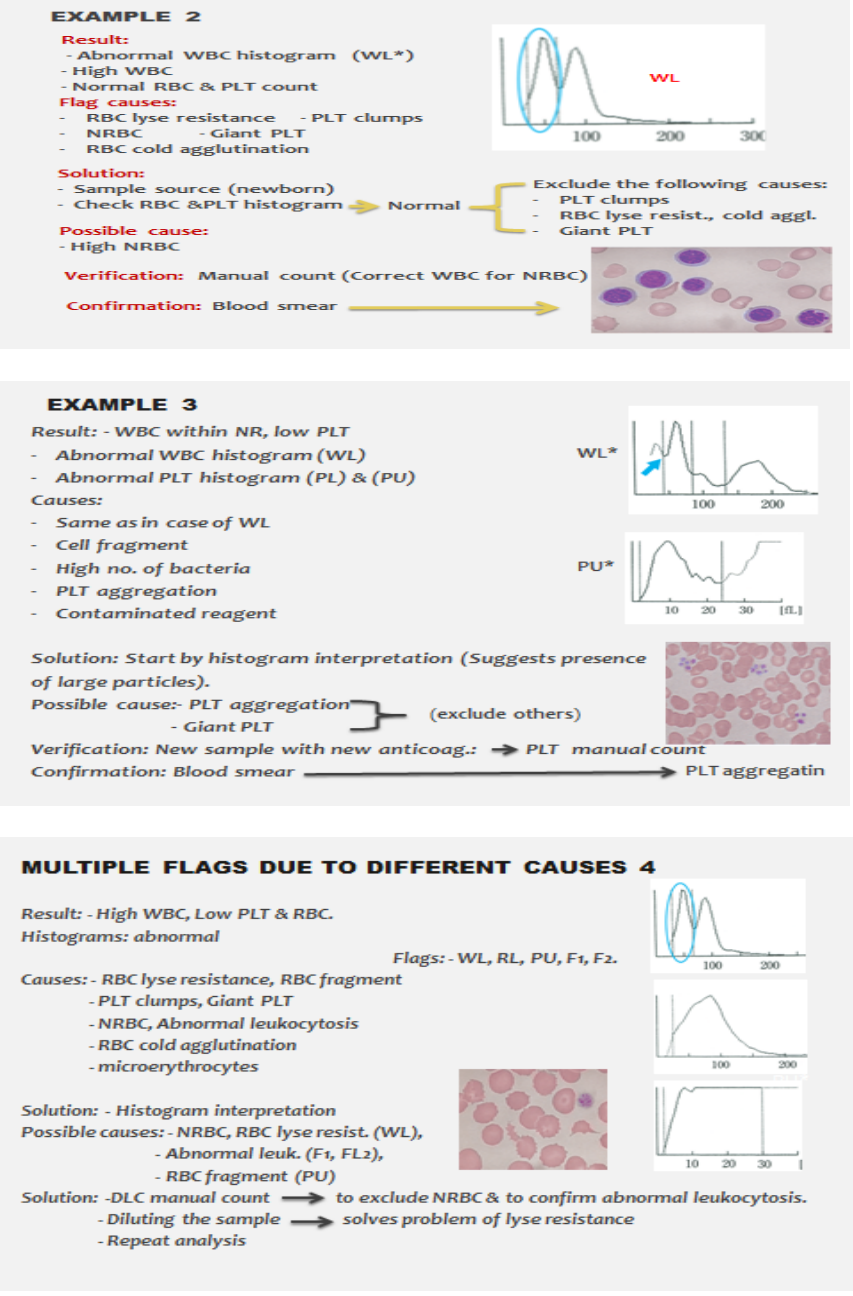


Figure (6): patterns (example 1, 2, 3 & 4) shows steps of interpretation of histograms and solving flags followed by verifacation and confirmation.

Conclusion

Reaching a clinical decision on the basis of unreliable, misunderstood, or misinterpreted results can be worse than having no results at all. Definitely, Histograms describe only cell number, size and shape but not of RBC indices and Hb. Despite the sophistication of present day instruments, this study confirmed that there is still need to depend on manual techniques for primary calibration and result confirmation specially by applying blood smear in many abnormal cases.

Additionally, good histograms interpretation significantly reduces wrong results and simultaneously let the blood analyzer operator take the proper action or follow up action to correct the possible unreliable result by determining the expected cause of wrong result. Finally, integral software tool explains how to operate the blood analyzer, how to detect the abnormal histograms and how to solve the problems of the flags remarkably became intrinsic issue.

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