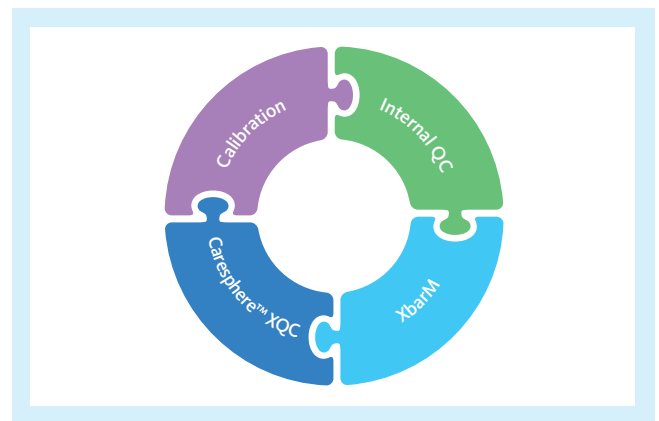# SEED Haematology

The XbarM control program – Use it wisely!

For optimal performance monitoring, Sysmex haematology analysers offer XbarM, a long-established tool on all 5-part differential analysers. XbarM offers real-time analyser monitoring based on patient samples. On a well-calibrated analyser, XbarM complements the other components that make up the comprehensive Sysmex quality concept (daily measurements of stabilised materials for internal quality control and comparison of the measurement results with a multi-national peer group via Caresphere™ XQC). Since it is based on patient samples, XbarM constitutes an additional layer of surveillance with no additional time, effort, or cost. This document explains what exactly XbarM is, how it works, how to set it up, and how to use it as a supplementing tool for monitoring analyser performance.

What exactly is XbarM control and how does it work?

The XbarM control program, also called 'moving average', is based on the calculation of weighted moving averages for all measurable parameters based on a complex algorithm developed from Bull's algorithm, which was initially designed for selected parameters of the red blood cell count [1]. In contrast to traditional quality control systems, which use stabilised control blood samples, XbarM uses measurement results of selected patient blood samples measured in the routine. Each blood test result obtained by the analyser that fulfils pre-defined inclusion criteria is automatically used to calculate these averages. Equally-sized groups of individual results are combined continuously to form so-called 'batches' and an average is calculated for every parameter from each batch.

The smoothing algorithm compares the recently measured samples with the previous moving average ($\bar{x}_M$). To reduce the effect of outlying sample results, the algorithm uses

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the square of the average root difference between the current $\bar{x}_M$ and the previous $\bar{x}_M$, whereby absolute values, as well as the difference between the previously calculated respective value, play a decisive role. This causes the averages to 'move' over time as each batch of measurements is composed of different results [1]. The newly calculated data point is automatically incorporated by the analyser into the XbarM QC program and is displayed as a further measurement point in the XbarM chart for each parameter, as displayed in figure 1.
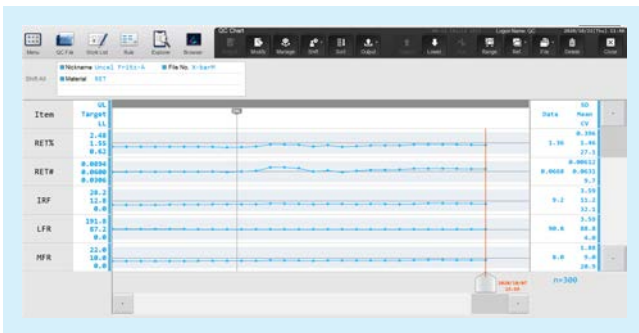

Fig. 1 XbarM QC chart on the XN-1000 analyser.

To display this chart on the XN-Series, XN-L Series and XR-Series analysers, open the QC program. The XbarM QC files are located at the bottom of the display. Select the XbarM file of interest (one for each channel) to display the data plots. Here, the data points are displayed for each parameter within parameter-specific and pre-defined limits.

Which measurements are incorporated in the XbarM calculation?

Naturally, not all patient blood samples are included in the calculation of the average. Therefore, the following inclusion and exclusion criteria for XN-Series, XN-L Series and XR-Series analysers were defined:

1. **Sample inclusion criteria:**
   - Negative results
   - Positive results including 'Anaemia', 'HGB defect?', 'Fragments?' and 'Iron deficiency?' suspect/abnormal flags but without any unreliability mark [*] on any of the parameters
   - Single parameter orders (e.g. HCT, HGB or PLT), if they fulfil other inclusion criteria

2. **Sample exclusion criteria:**
   - Analysis results analysed in a mode other than whole blood (WB)

- Analysis results with the sample number '0'
- Calibration and QC analysis results
- Blank data and background analysis results
- Analysis results including data outside the linearity range [@], low-reliability data [*], critical value data [!], and data outside the display range [++++]
- Analysis results that include masked data [- - - -]
- All other results with suspect flag

In conclusion, measurement results which do not meet any of the above inclusion criteria are excluded. This ensures the high sensitivity of this tool.

Which parameters are important for the XbarM control program?

Overall, all parameters measured on the analysers are included in XbarM but not all of them have the same significance in analyser monitoring. Sysmex suggests to divide the parameters into three groups, which should be observed and handled differently. These are named 'major', 'supporting' and 'other' parameters, differing in their significance for the analyser monitoring sensitivity.

Major parameters
The first group includes MCHC and MCV, the channel-related sensitivity parameters and delta parameters, which are considered 'major parameters' and are highly important for XbarM analyser surveillance. These parameters are mostly independent of the patient population, whereas other parameters can be influenced by population-dependent factors, for example by gender and age. Furthermore, these parameters cover all three technical units of the XN-Series, XN-L Series and XR-Series analysers, the FCM detector, the RBC / platelet detector and the HGB detector. An overview of how the major parameters monitor all technical units is displayed in figure 2.

MCHC
MCHC is considered as the most important parameter for laboratories as it is an excellent indicator of accurate results and naturally varies only within a very tight biological range. This is because MCHC is the quotient of HGB and HCT. These two parameters have a small margin of possible values. Therefore, unusual result for MCHC is seldom caused by clinical reasons. The more likely reason is analyser malfunction. Another advantage is that two different technical detectors, the RBC/PLT channel and haemoglobin channel, are used to calculate MCHC values and are therefore monitored simultaneously. Therefore, great significance is placed on this parameter when assessing the results of XbarM control.
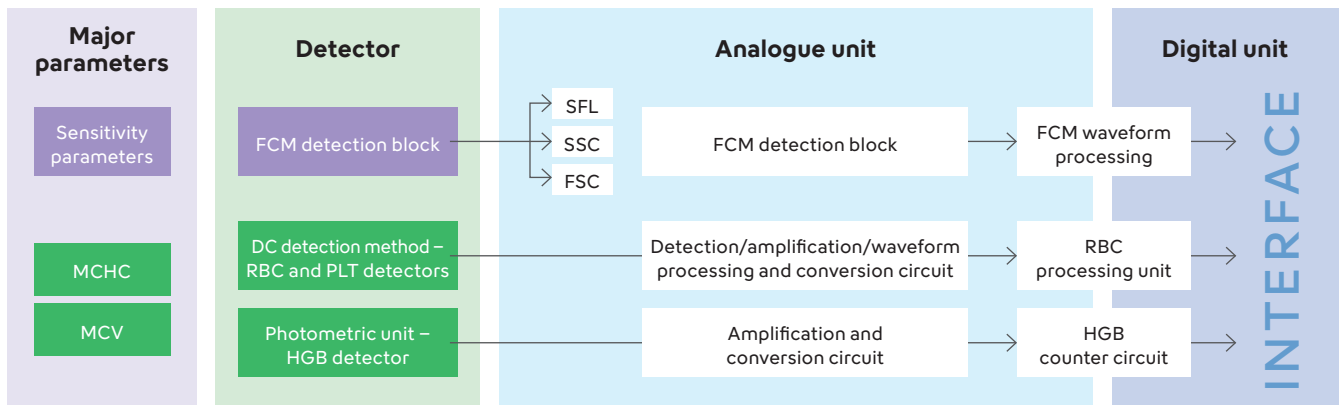
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Fig. 2 Overview of the XbarM major parameters covering all technical units of the analyser.
SFL: side fluorescence light, SSC: side scattered light, FSC: forward scattered light

MCV
MCV is considered a relatively stable parameter which tends to have a low variability in healthy individuals because red blood cells have a relatively uniform size. Since MCV is a direct measurement of the size of red blood cells, it is less influenced by external factors and only slightly impacted by demographics, like gender and age. This makes MCV a valuable parameter for monitoring the analyser´s performance.

The sensitivity parameters
Sensitivity parameters are also major parameters because they reflect the current analyser adjustments, also called 'sensitivity', in different measurement channels and are independent of concentration values. These sensitivity parameters correspond to the channels' scattergrams, which illustrate the respective cell populations as clusters and their relative position in the associated system of coordinates. For example, WDF-X and WDF-Y mark the position of the neutrophils cluster in the WDF scattergram, as displayed in figure 3. Importantly, the strict monitoring

of these parameters by XbarM is of great significance, since these sensitivity parameters have very narrow and biological-specific variation, cover all main technical units used for flow cytometry in the analyser and are always directly or indirectly influenced by calibration or sensitivity adjustment.

Supporting parameters
The second group, 'supporting parameters', contains the common count parameters of the red blood cells and platelets, haemoglobin and haematocrit, as well as some statistical parameters (MCH) and the white blood cell count. These parameters are, by nature, subject to a higher degree of biological variation and are dependent on the patient population. This group of parameters is therefore less significant for the XbarM control, as it is not possible to identify changes precisely, and is thus less sensitive. For this reason, stabilised QC materials are more applicable as a control for these parameters and therefore they are considered as 'supporting parameters' for XbarM.

Other parameters
The last group of 'other parameters' contains parameters, mainly white blood cell differential parameters and distribution dependent parameters, which are highly variable depending on the patient population, season, wards, or the kind of samples generally measured on the analyser. The variation of the 'other parameters' is so high that these parameters are not significant for the XbarM control program.

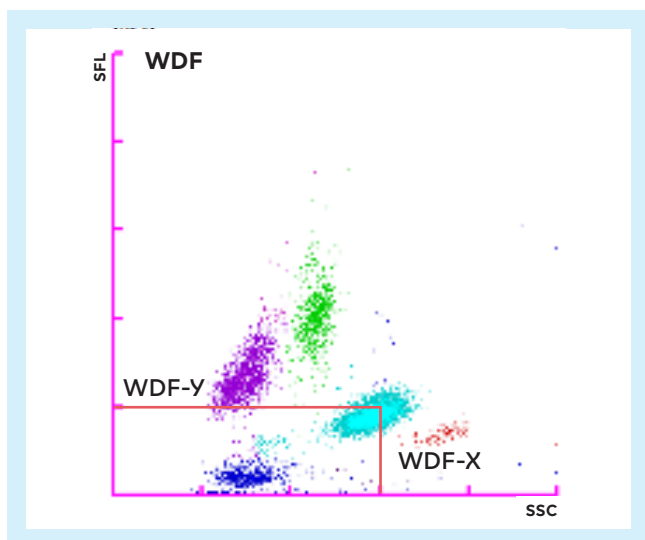In Table 1, all parameters are listed and divided into their respective parameter groups.

Fig. 3 Sensitivity parameters WDF-Y and WDF-X in the WDF scattergram on XN-Series.

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Table 1 XbarM parameter classification for XN-Series, XN-L Series and XR-Series.

Major parameters	Supporting parameters	Other parameters
WNR-X*	RBC	RDW-SD
WNR-Y*	HGB	RDW-CV
WNR-Z*	HCT	MicroR
WDF-X	MCH	MacroR
WDF-Y	PLT	PDW
WDF-Z	WBC	PCT
RET-RBC-X	NEUT-RI*	MPV
RET-RBC-Y	NEUT-GI*	P-LCR
RET-RBC-Z	RET	NRBC*
RET-RBC-WX*	RBC-O	NEUT
RET-RBC-WY*	PLT-O	LYMPH
MCHC	PLT-O	MONO
MCV	PLT-F-X	MONO
DLT-RBC	PLT-F-Y	EO
DLT-PLT-O	PLT-F-Z	BASO
	WPC-X	IG
	WPC-Y	IRF
	WPC-Z	LFR
		MFR
		HFR
		RET-H*e*
		RBC-H*e*
		HYPO-H*e*
		HYPER-H*e*
		PLT-F
		IPF

* Only available for XN-Series and XR-Series.

How is the XbarM control program set up?

A necessary precondition for proper analyser monitoring with XbarM is a well-adjusted and well-calibrated analyser. Sysmex supports adjusting the XbarM monitoring according to the laboratory analyser needs.

1. Selection of batch size
As previously described, new averages for each parameter are incorporated into the XbarM file when enough individual results have been obtained to form a batch. The selected batch size, therefore, influences the sensitivity of the XbarM control. If, for example, a batch is composed of 200 samples, some laboratories might only generate a maximum of one point a day in the XbarM control program. A trend or a change would therefore only be noticed after several days, which is far too late. Conversely, a batch size of 5 samples would be too small, as a large laboratory would generate approximately 100 or more points every day with a high coefficient of variation due to the diminished smoothing

algorithm. This would make it difficult to detect long-term drifts, as too many values would be produced every day and as a result, the previous values would virtually be eliminated from the control program. These extreme examples demonstrate that the batch size for the XbarM control must be adapted to the sample throughput of the individual laboratory.

It is optimal if the selected setting results are between 5 and a maximum of 10 points to be added to the XbarM file per shift. However, the batch size should generally not be set to a value much greater than 50, as this would reduce the sensitivity considerably. When selecting the batch size, it should be considered that there are varying numbers of analysis for complete blood counts (CBC), differential blood counts (DIFF), reticulocytes (RET), fluorescence-based platelet count (PLT-F) and white blood count from the white blood cell precursor channel (WPC). As a recommendation, the batch size should not be set to below 10, even if the number of requests for some tests can be comparably low. Such value would cause the system to be too sensitive and would ultimately restrict the XbarM function excessively, as trends would be hidden within the variance of the patient population. For example, at a sample throughput of 590 CBC, 220 DIFF and 40 RET orders per day meeting the inclusion criteria, the batch size for CBC should be set to 50, the DIFF batch size should be between 20 and 40 and the batch size for RET should not be below 10. Note: The ratio of samples fulfilling the XbarM inclusion criteria to all ordered samples is laboratory-specific and should also be taken into account when fine-tuning the batch size.

To display batch size settings, select [IPU Setting] in the analyser menu followed by [QC Setting] on the bottom left-hand side of the selection menu.

2. Settings for ranges and mean values
A percentage range and a mean can be entered individually for each parameter. The data points are displayed graphically around the mean up to the selected limits, similar to the XN Check QC file. The calculated values are shown as red crosses if they exceed or fall below the set limits. These ranges determine when the XbarM error message is triggered. Therefore, the narrower the ranges, the more quickly they will be exceeded, thus triggering the XbarM error message. As explained previously in the section about the importance of individual parameter groups, the ranges should be selected depending on whether a parameter is subject to high or low biological variation and how useful an XbarM error message caused by these particular parameters would be.

Users with a new system are advised to use the XbarM mean and limit values provided by Sysmex Europe SE. Calculation of mean values for individual laboratories after installation of a new analyser is only necessary for major parameters and, if needed, for supporting parameters. After measuring for a minimum period of four weeks and a minimum of 50 XbarM generated data points, limits and means of the major parameters can be changed.

Your local Sysmex representative will be happy to help you to select the most suitable values for your laboratory.

What are the advantages of the XbarM control program?

XbarM has no limitation regarding commutability as it is using patient blood samples instead of stabilised materials. Stabilised quality control materials are crucial due to direct comparability to known values (assay target values) but they only represent a snapshot of the analyser's condition when the procedure is carried out. In contrast, the XbarM control function is a long-term and continuous control process, which runs nearly real-time over the entire working day and can reveal any drifts in the results as well as between two QC measurements. Therefore, the XbarM control program monitors the functionality of all the reagents, e.g. before and after reagent replacement, and the analyser itself, e.g. before and after calibration or sensitivity adjustment, in an optimal manner. Sensitivity parameters in stabilised materials change during the lifetime of a QC material lot and hence cannot be used in monitoring the analyser. In contrast to stabilised material, the sensitivity parameters of healthy fresh blood show a stable behaviour and allow a closed meshed monitoring of the flow cytometry measurements technology including the laser unit and reagents.

A particular advantage of the XbarM control program is that it does not require any extra input from the operator because, once activated and set up correctly, it runs automatically in the background. If pre-determined criteria are violated, an 'XbarM error' is generated so that the results from the last batch of samples can be withheld or locked if necessary, until the measurements can be verified by determining the cause of this violation, thus, providing maximum safety with minimum effort.

Finally, it is important to note that no additional costs are incurred because routine blood sample results are used in this control system.

What are the strengths and weaknesses of the XbarM control program?

Based on the use of fresh blood, the XbarM function is particularly well suited to monitor any processes related to the reagents used, the stability of analyser performance, or the sensitivity of the electronics. This includes, for example, reagent replacement, use of an expired reagent, insufficient lysis due to defective measurement chambers, or incorrect laser adjustment. These analyser-related system errors are detected optimally by the XbarM control system and are generally easy to identify and correct. On the other hand, the opposite applies to sample errors and user errors. Sample errors generally occur at irregular intervals and these sporadic errors cannot be identified by the XbarM control system on account of its mathematical approach. Sample errors can only be identified by carefully examining the individual result. If an operator suspects that a sample error has occurred, the sample material should be checked by the operator and it may be necessary to perform several measurements to rule out a possible system error. In contrast, user errors are typically caused by the operator and should, in principle, be detectable by the XbarM control program if operators are not frequently changed. If the errors are random, it is more difficult to detect them with the XbarM control system. User errors can generally be detected by quality control procedures with control blood samples, since it is necessary for the samples to be handled in a correct manner during these procedures. These errors can be eliminated by providing further training.

What to do if an XbarM error alert occurs?

Before any serious analyser faults occur, the XbarM control program reacts to the problem and the analyser produces an XbarM error signal. The error message should immediately prompt the operator to carry out specific checks and in particular:

1. **Review the XbarM file:**
   - Which parameter(s) is/are affected? (Major, Supporting, Other)
   - What are the standard deviation and coefficient of variation in the XbarM control display?
   - How large is the percentage deviation from the average?
   - Was there a calibration, adjustment or spare part replacement beforehand?

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2. **Check settings in the XbarM file:**
    - Are the ranges and averages appropriate?
    - How long has the trend been observed and is there a plausible explanation based on the type of samples measured during this period (particular patient population / ward / operator)?

3. **Check reagents:**
    - Are all the reagents correctly connected? Was an expired reagent inadvertently used?
    - How is the precision (repeatability – carry out repeated measurements) of the system?
    - How accurate is the system (view QC charts for control blood samples)?
    - Is there a difference between two systems (back-up and routine system; perform comparative measurements if possible)?

When you are sure that the analyser is at fault, the problem can often be isolated or resolved by speaking to your local Sysmex representative. If the operator has already checked the points listed above, they can provide comprehensive details on the course of events and speed up the error resolution process. Once the situation has been clarified and, if necessary, the error has been resolved, the routine measurement results can be released. In the worst-case scenario, the latest measurements have to be repeated once again. The operator can therefore release these results in the certainty that no false measurements have left the laboratory.

What kind of XbarM behaviour can occur?

There are multiple examples of how XbarM indicates analyser or setting errors, but changes in the workflow or patient population can also lead to changes in the XbarM behaviour. In the following examples, five potential behaviours in XbarM charts are displayed, with possible root causes for the error and actions that should be taken.

1. Bias to XbarM mean

A bias in XbarM is only relevant when using customised XbarM values, as displayed in figure 4. Deviation with non-customised values is to be expected due to analyser-specific behaviour. After customisation, the most likely reasons for the displayed behaviour are setting errors. For the major parameters, the settings for the mean should be checked and auto-calculation should be performed again if necessary. For non-customised supporting or other parameters, no actions are necessary.
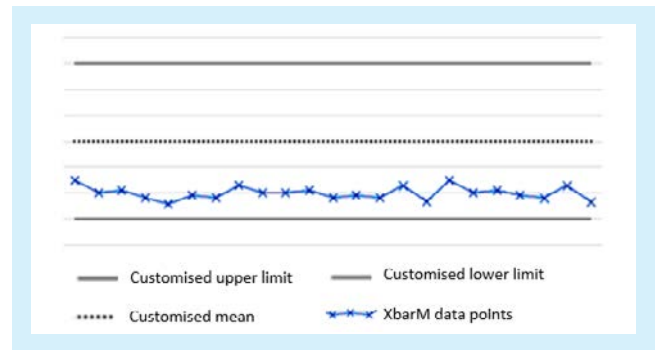

Fig. 4 XbarM troubleshooting – Bias to mean.

2. Step in XbarM mean

The second possible error is a change of the XbarM mean, as displayed in figure 5. If the step occurs right after calibration or sensitivity adjustment, this step is acceptable as long as the limits provided by Sysmex are not violated. The mean can then be adjusted via the auto-calculation function (for major parameters). If there was no event beforehand, the technical status should be checked, such as calibration, sensitivity adjustment, reagent changes, QC and maintenance at this time point. If no explanation can be found, a systematic error is possible, which should be investigated immediately.
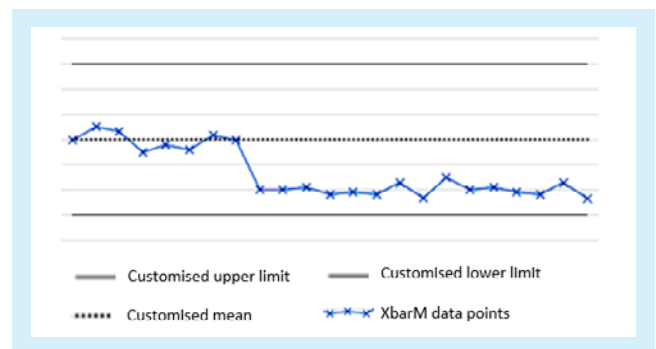

Fig. 5 XbarM troubleshooting – Change of XbarM mean.

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3. Single outlier

Single outliers are mostly related to the patient population, as displayed in figure 6. If the XbarM chart shows a normal behaviour afterwards, the outlier can be ignored, and no further investigation is needed. If the trend continues, corrective actions must be taken, as described in the following example about 'trend'.
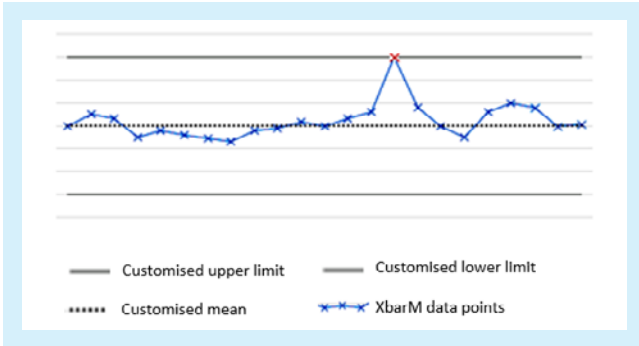

Fig. 6 XbarM troubleshooting – Single outlier.

4. Increase in the coefficient of variation

An increase in CV or a CV higher than expected most likely represents a setting or operational change, as displayed in figure 7. An analyser problem is also possible. Most likely reasons for this behaviour can be a change of batch size during the routine, a batch size that was set too small, or the CV that is too high from the beginning. Another explanation is a change in the workflow. In any case, the operational change has to be identified and either adapted or the XbarM settings adjusted accordingly. In case these changes do not solve the problem, your local Sysmex representative will be happy to assist you in identifying and resolving the problem.
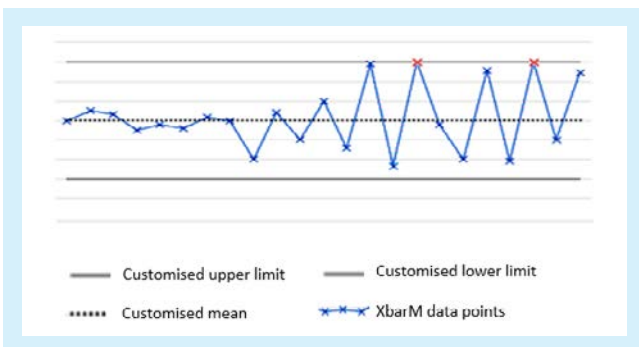

Fig. 7 XbarM troubleshooting – CV increase.

5. XbarM trend

A trend in XbarM represents a systematic error that needs to be investigated immediately, as displayed in figure 8. This was first observed at the start of the COVID-19 pandemic due to changes in the patient population. During this period, almost only sick people went for blood analysis which highly impacted the XbarM behaviour. If the reason for the trend is plausible, the mean needs to be adapted. If the systematic error cannot be identified, your local Sysmex representative will be happy to assist you in identifying and resolving the problem.
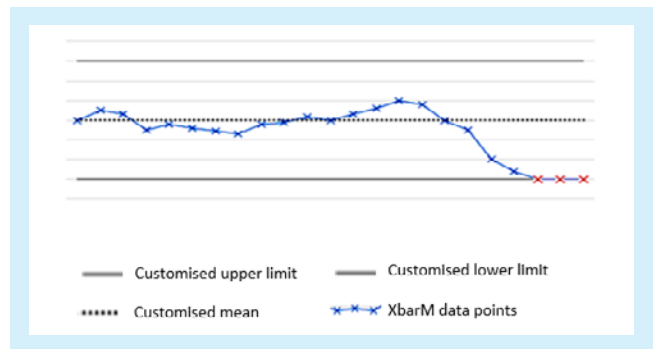

Fig. 7 XbarM troubleshooting – CV increase.

Conclusion

The Sysmex quality control concept is subject to constant surveillance and improvements. As a crucial part of this concept, the XbarM control program contributes to improved monitoring of the analyser at an early stage with no additional costs or effort during daily laboratory routines.

Therefore, XbarM, like all other QC tools, needs to be well-adjusted to contribute with the other QC tools to a highly sensitive but also quality-specific concept. Correct handling of the analyser and its control materials by the operator is crucial for reliable measurement results. If correct handling and set-up of the system is given, XbarM results in a sensitive tool for analyser surveillance.

Reference

[1] **Bull B *et al.* (1974):** A study of various estimators for the derivation of quality control procedures from patient erythrocyte indices. Am J Clin Pathol 61: 473–81.