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Construction of control charts for RM, Dw sample types

Initial analyses

Initially the results obtained by a laboratory can be compared with intervals constructed from the control limits coming with the reference material and given in the INSTRUCTIONS. Values within the intervals are initially acceptable. If a specific value falls outside the limits it can be due to mere chance. Otherwise it can be a consequence of the use of another method than the reference method. If all laboratories use the reference methods, approximately 5% of the results in an analysis fall outside $\pm 2s_0$ and 0.3% outside $\pm 3s_0$ by chance alone. For many laboratories the probability of getting results outside the limits is less than that, but for a specific laboratory it can be greater due to the fact that the laboratory's mean value differs greatly from the one given.

If results occur more frequently than is stated above the cause should be sought. By immediately analysing new vials, it can be decided whether the deviations were caused by chance.

Preliminary control chart with own limits

The control limits coming with the reference material include *variations between laboratories* (including variation between analysts) and should therefore be substituted by the laboratory's own limits for the different analyses as soon as possible. The intervals from these limits should be narrower than the intervals from the limits coming with the material.

It can be appropriate to initially perform analyses more frequently than ordinarily planned to rapidly (normally within 3 months) obtain own limits for the construction of control charts.

For the construction of control charts with only a laboratory's own variation, the results (but not clearly erroneous) of the laboratory should be used square-root transformed. **See the example later in this document.**

Square-root transformation of colony counts

Square-root transformation renders the results more normally distributed and with a more uniform variance. After transformation, the laboratory calculates its own mean value (mv), mv±2s and mv±3s, where s is its own standard deviation. Thereafter the mean and the limits are back transformed to the cfu scale by taking the square. No rounding off of the values should be done until the values are back transformed.

Construction of the chart

A preliminary control chart can be constructed when the laboratory has obtained 5–10 results for an analysis. The laboratory calculates the mean value and limits by using square-root values and then back transformation. The obtained limit values are upper and lower warning limits (2s) and action limits (3s), respectively.

In a diagram with colony count on the vertical axis and the analyses along the horizontal axis, horizontal lines can mark the mean value and the different warning and action limits. The mean value will not divide the intervals obtained from the two pairs of limit values in equal parts, because the calculations were done with square-root transformed values.

Redrawing of control chart

At least when 20 results are obtained from the same batch of RM for one analysis the mean value and limits should be recalculated in the same way as for the preliminary chart. The limits and mean value can then be recalculated whenever there is a reason. The limits must be based on relevant consecutive analyses from *one batch of RM*. The series of results should be long enough to properly reflect the current capacity of the laboratory.

If the laboratory during a fairly long period of time is always within *its* 2s limits for an analysis it should do recalculations with current results to obtain a narrower interval. If it is outside the 3s limits more than expected without a relevant reason it should also do recalculations to obtain a broader interval.

A new batch of RM

When a new batch of RM must be introduced, because the previous one has been used up or is too old, some modifications usually need to be made to the control intervals. The new batch can have a somewhat different mean value than the old one. However, the assumption is made that the variations (standard deviations) for the various analyses are the same as before.

Since the mean values in the specific laboratory usually differ somewhat from those coming with the RM they must first be adjusted. For every analysis the *ratio* between the old value of the laboratory and the one accompanying the old RM (both square-root transformed) can be used as a correction factor. A relevant mean value for the new batch is obtained by multiplication of the correction factor and the mean value (square-root transformed) accompanying the new RM. By use of their own known standard deviations, limits for the new RM can be calculated as described above.

When approximately 10 analyses have been done with the new RM, (preliminary) mean values and limits can be calculated from these instead, to reflect the new material only. Adjustments are then made when necessary.

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Example of calculations and control chart

Example of obtained results

Ten consecutive results of *total coliform bacteria*: 56, 47, 69, 61, 71, 63, 80, 66, 59 and 68 colonies per 5 ml.

Control chart for initial analyses

As an example are here given the mean value 66 and the limits 39, 47, 88 and 100 cfu per 5 ml for total coliform bacteria. These values are marked on one axis in a diagram. From these markings lines are drawn parallel with the other axis. These lines constitute the limits for the two original pairs of intervals. The line for the mean value divides each of the two intervals in two somewhat unequally sized parts. Immediately after they are obtained the colony counts can be put into the diagram after each other in a temporal series and compared with the interval limits. See Figure 1.

Preliminary control chart with own limits

A. Calculation of square-root transformed results

1   *The square root*s of the ten values above are calculated. They are approx.: 7.483, 6.856, 8.307, 7.810, 8.426, 7.937, 8.944, 8.124, 7.681, and 8.246. These values are used in calculations without appreciable rounding off.

2   *Mean value (mv)* calculated from the ten values is approx. 7.9814.

3   *Standard deviation (s)* calculated from the ten values is approx. 0.5732.

B. Calculation of limits and construction of the diagram

4   mv-3s, mv-2s, mv+2s and mv+3s are calculated ($\approx$ 6.262, 6.835, 9.128, 9.701).

5   These four values as well as mv are squared and after rounding off equal 39, 47, 83, 94 and 64. They constitute *warning limits* (inner limits) and *action limits* (outer limits) as well as the *mean value*.

6   From these five squared values the preliminary control chart is drawn in the same way as the initial one, and new colony counts are put in. The diagram can be redrawn or form a continuation of the initial one after the use of the initial limits has ceased, as in Figure 1.

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Figure 1 *Example of a horizontal control chart (time series on the X-axis)*

Total coliform bacteria
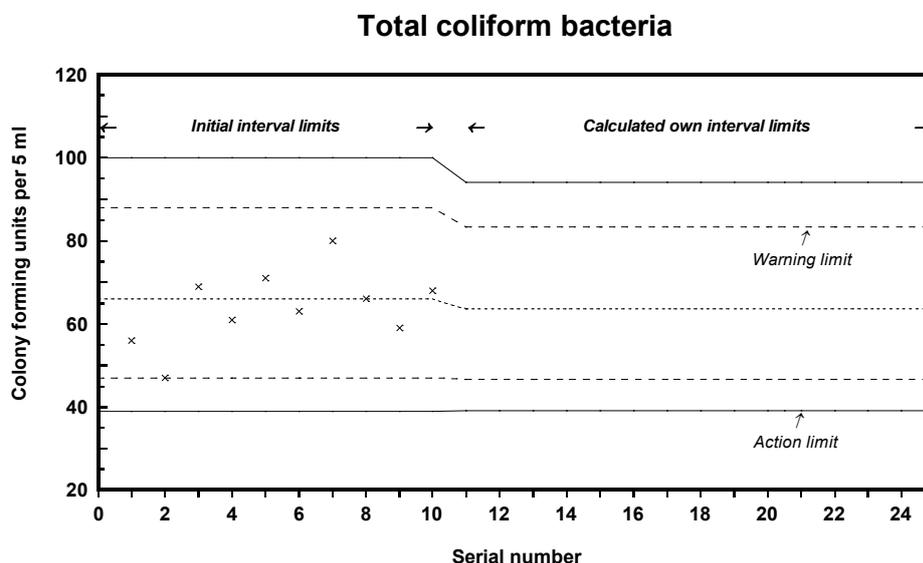
Redrawing of control chart (done when appropriate)

New calculations as in A and B above are made when more results or only a portion of the latest values should form the basis of the diagram. The diagram is then adjusted with the new limits and the new mean value.

New batch of RM

1   The most recently used square-root transformed values (called mv_{old} and s_{old}) from the previous batch are taken for a specific analysis.

2   The square-root transformed initial mean value ($\sqrt{cfu}$ in Appendix A) for both the old and the new batches (here called $\sqrt{cfu}_{old}$ and $\sqrt{cfu}_{new}$), respectively, are taken for the analysis of consideration.

3   The ratio $mv_{old} / \sqrt{cfu}_{old}$ is calculated and multiplied by $\sqrt{cfu}_{new}$. In this way the adjusted initial mean value mv_{new} is obtained for the new batch.

4   New values $mv\pm2s$ and $mv\pm3s$ are calculated as in section B above by the use of mv_{new} and s_{old}.

5   New interval limits are obtained after taking the square of these values and then the control chart is adjusted or redrawn.

6   When as many values as necessary have been obtained (approx. 10) for calculation of a new relevant value of s for the new batch, the diagram is redrawn by use of the calculations as if no diagram has ever been constructed previously (as described in sections A and B above).