cobas c 111 **system**

Data Analysis
Version 1.1

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Document information

Revision history

Manual version	Software version	Revision date	Changes
1.0	n/a	February 2008	First publication.
1.1	n/a	March 2012	Error in formula for ISE results corrected.

Edition notice

This document is for users of **cobas c** 111 instruments. Every effort has been made to ensure that all the information contained in this manual is correct at the time of printing. However, Roche Diagnostics GmbH reserves the right to make any changes necessary without notice as part of ongoing product development.

Intended use

The information contained in this document helps users of **cobas c** 111 instruments and the **cobas c** 111 Development Channel Programming Software understand the measurement principles used on **cobas c** 111 instruments, and it describes the calculations and checks executed when performing measurements.

The **cobas c** 111 instrument is a continuous random-access analyzer intended for in vitro determination of clinical chemistry and electrolyte parameters in serum, plasma, urine or whole blood (HbA1c).

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Instrument approvals

The **cobas c** 111 instrument meets the protection requirements laid down in IVD Directive 98/79/EC and the European Standard EN 591. Furthermore, our instruments are manufactured and tested according to the following international standards:

- EN/IEC 61010-1 2nd Edition
- EN/IEC 61010-2-101 1st Edition

This documentation meets the European Standard EN 591.

Regulatory compliance is demonstrated by the following labels:

CE	Complies with European Union (EU) Directive 98/79/EC.
c UL us	Issued by Underwriters Laboratories, Inc. (UL) for Canada and the US.

Where to find information

This document covers the measurement principles used with **cobas c** 111 instruments, and it describes the calculations and checks executed when performing sample, calibration, and control measurements.

👁 For information on working with **cobas c** 111 instruments, see the **cobas c** 111 Instrument Operator's Manual.

👁 For information on working with the **cobas c** 111 Development Channel Programming Software, see the **cobas c** 111 Development Channel Operator's Manual.

Conventions used in this manual

Visual cues are used to help locate and interpret information quickly. This section explains the formatting conventions used in this document.

Symbols The following symbols are used:

Symbol	Used for
👁	Cross-reference
💡	Tip

Photometric measurements

This chapter explains the calculations and checks that are applied to photometric measurements.

The following flow chart shows the individual steps that are carried out with calibration, recalibration, sample and control measurements.

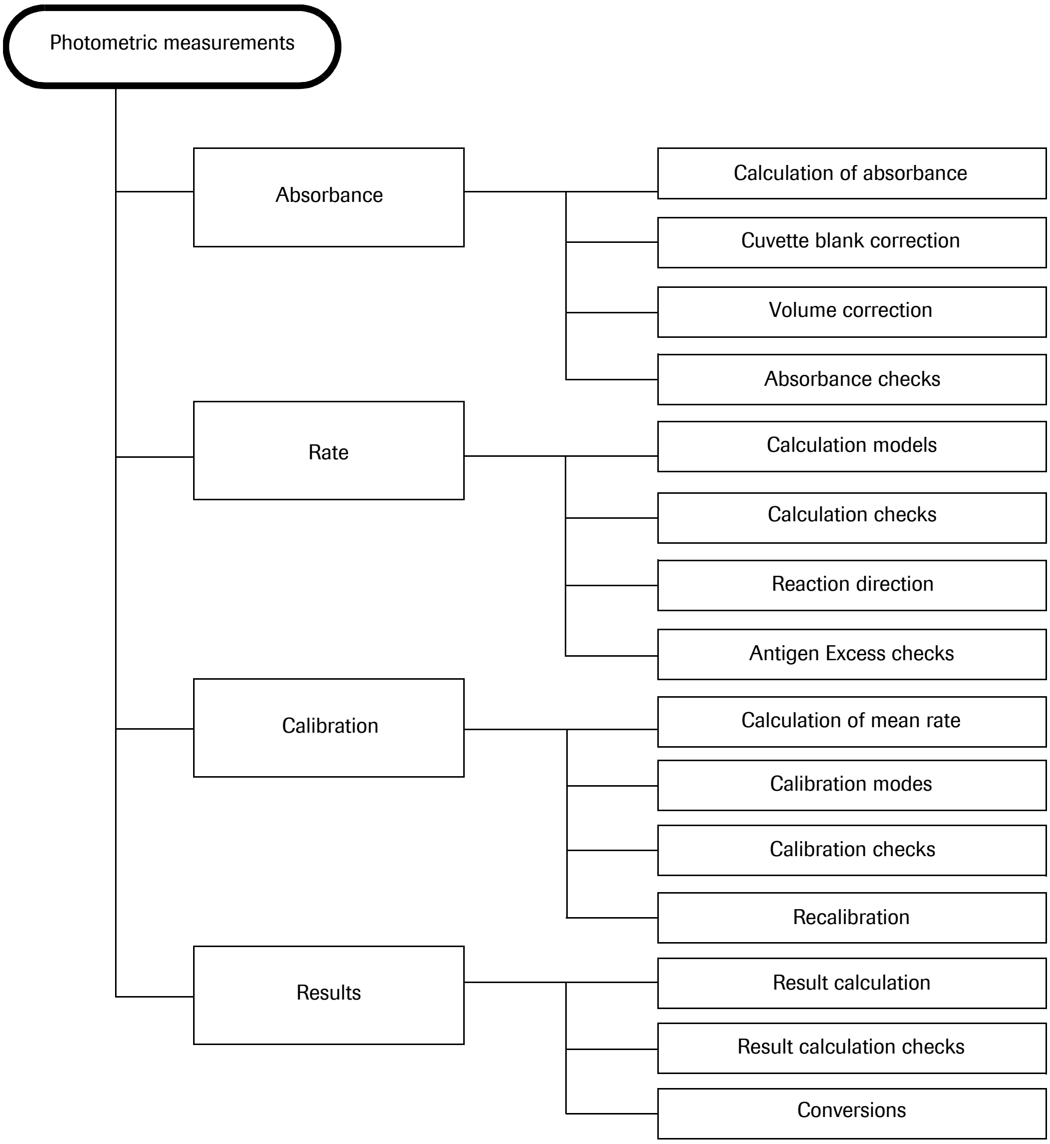


Figure 1 Chart of steps in photometric measurements

Absorbance

This section explains the absorbance calculations and the associated checks.

	Calibration	Control	Sample
Calculation of absorbance	✓	✓	✓
Cuvette blank correction			
Monochromatic measurement	✓	✓	✓
Bichromatic measurement	✓	✓	✓
Volume correction	✓	✓	✓
Absorbance checks			
High Absorbance check	✓	✓	✓
Reagent Range check	✓		
High Activity check		✓	✓
Low Activity check		✓	✓

Table 1 Calculations and checks for absorbance measurements

Calculation of absorbance

Calculation is carried out after every measurement by the absorbance photometer.

There are up to 40 absorbance measurements (cycles) consisting of 1 or 2 out of 12 available wavelengths.

The absorbance signal is corrected using the following values:

$$\text{measured absorbance} + \left(\begin{array}{c}\text{cuvette blank correction}\\ \text{(mono/bichromatic)}\end{array}\right) + \text{volume correction}$$

The values obtained from photometric measurements are converted to absorbance values. This conversion is carried out for all points measured at the requested wavelength.

$$A_{\text{raw cuv}}(\lambda, n) = -\log \frac{I_{\text{cuv}}(\lambda, n)}{I_{\text{air}}(\lambda)}$$

$A_{\text{raw cuv}}(\lambda,n)$	Raw absorbance of each point (n) at the requested wavelength λ.
$I_{\text{cuv}}(\lambda,n)$	Intensity of each point (n) at the requested wavelength λ.
$I_{\text{air}}(\lambda)$	Intensity of air (without cuvette) at the requested wavelength λ.

Cuvette blank correction

Cuvette blank correction is carried out in every absorbance calculation.

All absorbencies are corrected using a blank, that is the absorbance of a water-filled cuvette at the corresponding wavelength.

Monochromatic measurement calculation

The blank measurement is calculated using the following formula:

$$A_{blank}(\lambda) = A_{raw\ cuv}(\lambda,1) + A_{H2O}(\lambda)$$

The absorbance is normalized using the following formula:

$$AN_{cuv}(\lambda, n) = A_{raw\ cuv}(\lambda, n) - A_{blank}(\lambda)$$

$A_{raw\ cuv}(\lambda,1)$	Raw absorbance of point 1 of an empty cuvette at the requested wavelength λ.
$A_{blank}(\lambda)$	Blank raw absorbance at wavelength λ. (Corresponds to a water-filled cuvette.)
$A_{raw\ cuv}(\lambda, n)$	Raw absorbance of each point (n) at the requested wavelength λ.
$A_{H2O}(\lambda)$	Absorbance of water at the requested wavelength. This value is calculated from the difference of a cuvette filled with water and an empty cuvette (air/water calibration system check).
$AN_{cuv}(\lambda,n)$	Normalized absorbance of each point (n) at the requested wavelength λ.

Bichromatic measurement calculation

$$AN_{cuv}(\lambda, n) = (A_{raw\ cuv}(\lambda_1, n) - (A_{blank}(\lambda_1)) - (A_{raw\ cuv}(\lambda_2, n) - A_{blank}(\lambda_2))$$

$A_{raw\ cuv}(\lambda, n)$	Raw absorbance value of all points of an empty cuvette at the requested wavelength.
$A_{blank}(\lambda)$	Absorbance value of a cuvette filled with water at the requested wavelength.
$AN_{cuv}(\lambda, n)$	Normalized absorbance values of a cuvette.
λ_1	Main wavelength.
λ_2	Reference wavelength.

Volume correction

Volume correction is carried out for every absorbance calculation.

All absorbencies are corrected using the volume ratio ($\text{initial volume}/\text{final volume}$). This is necessary since, with each addition of liquid, the reaction mixture becomes more diluted, while the path length of the light and the measuring window remain constant. Volume correction is carried out for all points.

The following equation is used for volume correction:

$$A(\lambda, n) = \frac{AN_{cuv}(\lambda,n) \cdot V_{cuv}(n)}{VF_{cuv}}$$

$A(\lambda, n)$	Volume-corrected absorbance of each point (n).
$AN_{cuv}(\lambda, n)$	Normalized absorbance of each point (n) at the wavelength λ.
$V_{cuv}(n)$	Starting volume of point n of a cuvette before the last addition is made.
VF_{cuv}	Final volume of cuvette after the last addition.

Absorbance checks

High Absorbance check

This check is carried out for every absorbance calculation.

All absorbencies used for the calculation of all measuring points of all requested wavelengths between the first and last point may not exceed 2.0 absorbance units. The first and last points refer to:

- $\mathrm{Calc_{first}}\ldots\mathrm{Calc_{last}}$ (Endpoint and Kinetic)
- $\mathrm{HighAct_{first}}\ldots\mathrm{HighAct_{last}}$ (High/Low Activity)
- $\mathrm{Ag_{first}}\ldots\mathrm{Ag_{last}}$ (Antigen Excess)

$$A_{\mathrm{raw\ cuv}}(\lambda, n) \leq 2.0$$

$A_{\mathrm{raw\ cuv}}(\lambda, n)$	Absorbance of each point (n) (between the first and last points) at requested wavelength λ.

The High Abs flag is displayed if a value between the first and last points exceeds 2.0.

Reagent Range check

This check is carried out for every absorbance calculation for the calibrator with the lowest concentration.

For the reagent blank, all absorbencies required for calculations are checked to ensure they do not exceed the preset reagent range limits.

$$RR_{\mathrm{low}} \leq A_{\mathrm{cuv}}(n) \leq RE_{\mathrm{high}}$$

$A_{\mathrm{cuv}}(n)$	Absorbance of the reagent blank of each point used in the calculations.
RR_{low}	Reagent range lower limit (test parameter).
RR_{high}	Reagent range upper limit (test parameter).

The < Reag Rng or > Reag Rng flag is displayed if the absorbance of the reagent in a cuvette is not within the specified range.

High Activity check

This check is used to identify large differences in the absorbance after the start of the reaction.

With ascending reaction curve:

$$A_{\mathrm{cuv}}(\mathrm{last}) - A_{\mathrm{cuv}}(\mathrm{first}) \leq \mathrm{HLL}$$

With descending reaction curve:

$$A_{\mathrm{cuv}}(\mathrm{first}) - A_{\mathrm{cuv}}(\mathrm{last}) \leq \mathrm{HLL}$$

$A_{\mathrm{cuv}}(\mathrm{first})$	Absorbance at the time of the high activity first point (test parameter).
$A_{\mathrm{cuv}}(\mathrm{last})$	Absorbance at the time of high activity last point (test parameter).
HLL	High activity limit (test parameter).

The High Act flag and the value of the upper test range limit are displayed in the result list if the value does not meet the conditions.

Low Activity check

This check is used to identify zero pipetting of sample and reagents.

With ascending reaction curve:

$$A_{cuv}(\text{last}) - A_{cuv}(\text{first}) \geq \text{HLL}$$

With descending reaction curve:

$$A_{cuv}(\text{first}) - A_{cuv}(\text{last}) \geq \text{HLL}$$

$A_{cuv}(\text{first})$	Absorbance at the time of the low activity first point (test parameter).
$A_{cuv}(\text{last})$	Absorbance at the time of the low activity last point (test parameter).
HLL	Low activity limit (test parameter).

The Low Act flag and the value of the lower test range limit are displayed in the result list if the value exceeds the lower test limit.

Rate

This section explains the rate calculations and the associated checks.

	Calibration	**Control**	**Sample**
Calculation model Endpoint			
Absorbance difference	✓	✓	✓
Endpoint Unstable check	✓	✓	✓
Calculation model Kinetic			
Limits of kinetic range	✓	✓	✓
Absorbance difference per minute	✓	✓	✓
Nonlinear check	✓	✓	✓
Kinetic readings check	✓	✓	✓
Reaction direction	✓	✓	✓
Antigen Excess check		✓	✓
Test range check (level rate)		✓	✓
Calculation of mean rate	✓		

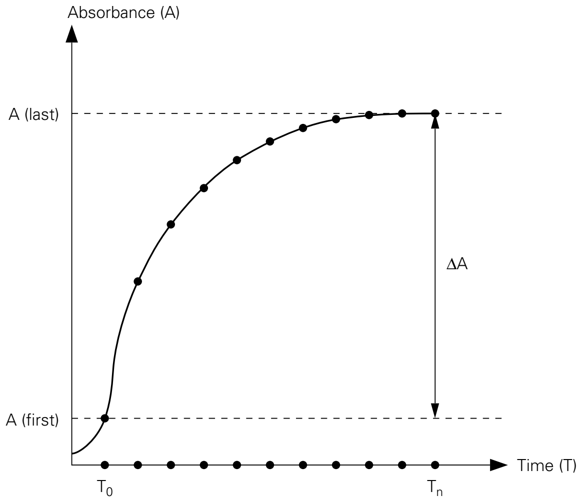
Table 2 Calculation and checks for rate determination

Calculation model Endpoint

Calculation of absorbance difference

The calculation of the absorbance difference is carried out for calibrators, controls, and samples.

Two points are used to determine the rate (ΔA) of the reaction. The rate is used to calculate the concentration of the sample, based on the kind of calibration mode used.



$$RC_{cuv} = A(last) - A(first)$$

RC_{cuv}	Rate in ΔA
A(last)	Absorbance at the last calculation point (last calculation point, test parameter). See the **cobas c**111 Method Sheet.
A(first)	Absorbance at the fist calculation point (first calculation point, test parameter). See the **cobas c**111 Method Sheet.

Endpoint Unstable check

This check applies only to the calculation model Endpoint. It is carried out to determine if the last five measuring points used for determinations are stable.

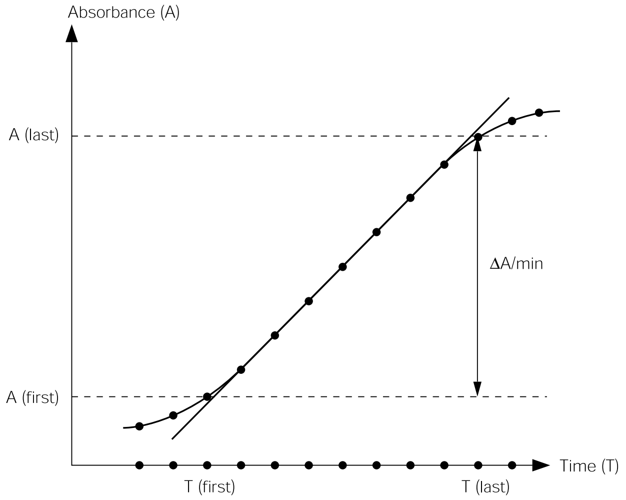
$$A(high) - A(low) \leq EP$$

A(high)	Highest absorbance obtained from the last five measuring points.
A(low)	Lowest absorbance obtained from the last five measuring points.
EP	Endpoint unstable limit in absorbance (dAbs. test parameter).

The EP Unstab flag is displayed if the absorbance difference is not within the specified range.

Calculation model Kinetic

The calculation model Kinetics is applied to calibrations, quality controls and samples. A linear regression analysis is carried out between the first and last points as specified in the **cobas c** 111 Method Sheet. The slope of regression line is the rate (ΔA per minute) of the reaction. The rate is used to calculate the concentration of the sample based on the kind of calibration mode applied on the calibration curve.



Limits of kinetic range

With the Kinetic calculation model, an inspection of the reaction is carried out to determine if the beginning and end of the kinetic range is within the defined absorbance range. If this is not the case the measurement range is reduced accordingly.

Determining the measurement range used for calculating the kinetics:

The first and last absorbance values ($A(first)$, $A(last)$) in the kinetic range are used as the first and last measuring points ($MP_{kin\ first}$, $MP_{kin\ last}$) when calculating the kinetics. All points must fulfill the following conditions:

$$RL_{low} \leq A(n) \leq RL_{high}$$

RL_{low}	Absorbance of the lower reaction limit (test parameter). Minimum value = 0.0.
$A(n)$	Absorbance of each point in the kinetic range.
RL_{high}	Absorbance of the upper reaction limit (test parameter). Maximum value = 2.0.

To avoid values that are too low when calculating the kinetics, you can perform the following checks with floating limits (test parameters):

With ascending reaction curve:

$$\text{fl. RL}_{\text{low}} = \text{RL}_{\text{low}} + (A_{\text{cuv}}(\text{sample}) - A_{\text{cuv}}(\text{cal}_{\text{ref}})) \text{ and fl. RL}_{\text{high}} = \text{RL}_{\text{high}}$$

$$\text{fl. RL}_{\text{low}} \leq A(n) \leq \text{fl. RL}_{\text{high}}$$

With descending reaction curve:

$$\text{fl. RL}_{\text{high}} = \text{RL}_{\text{high}} + (A_{\text{cuv}}(\text{sample}) - A_{\text{cuv}}(\text{cal}_{\text{ref}})) \text{ and fl. RL}_{\text{low}} = \text{RL}_{\text{low}}$$

$$\text{fl. RL}_{\text{low}} \leq A(n) \leq \text{fl. RL}_{\text{high}}$$

If the result for $(A_{\text{cuv}}(\text{sample}) - A_{\text{cuv}}(\text{cal}))$ is negative, set the value for this expression to zero. In such a case, the floating reaction limit becomes a fixed reaction limit.

$\text{fl. RL}_{\text{low}}$	Corrected Reaction Limit Low (Floating RL Low, test parameter).
$\text{fl. RL}_{\text{high}}$	Corrected Reaction Limit High (Floating RL High, test parameter).
$A_{\text{cuv}}(\text{sample})$	Sample absorbance value of a cuvette (test parameter).
$A_{\text{cuv}}(\text{cal}_{\text{RF}})$	Calibrator absorbance value of a reference calibrator cuvette (test parameter).

Calculation of the absorbance difference per minute

This calculation is performed with the Kinetics calculation model.

With the specified points (at least 2) a Passing/Bablok analysis is carried out to determine the absorbance difference per minute (Kinetic).

Nonlinear check

This check is used with the Kinetics calculation model.

The linearity of the curve is determined by analyzing the points which lie within the limits specified for the linear region. A Passing/Bablok analysis is carried out on each half of the curve to determine the straight lines. The slopes of these straight lines are determined using the differences ΔA1 and ΔA2.

If there is an even number of points within the linear range the two halves of the curve overlap at two points.

Conditions for running the test:

$$|\text{Rate}| > 0.006\,\text{abs/min}$$

Test conditions:

$$|\Delta A_1/\text{min} - \Delta A_2/\text{min}| < \text{lin}$$

ΔA_1	Absorbance change (ascending) of the first half of the curve.
ΔA_2	Absorbance change (ascending) of the second half of the curve.
lin	Linearity in Δ (ΔA/min) (linearity test parameter).

The Non Linear flag is displayed if the conditions are not fulfilled.

Kinetic Readings check

This check is used with the Kinetics calculation model.

The number of measuring points used to calculate the kinetics is checked to ensure that the defined number (within the linear region of the curve) is present.

$[MP_{kin\ last} - MP_{kin\ first} + 1] \geq KR$

$MP_{kin\ last}$	Last measuring point used for the kinetics calculation.
$MP_{kin\ first}$	First measuring point used for the kinetics calculation.
KR	Number of kinetic readings (test parameter).

The < Kin Read flag is displayed if the number of points needed for the kinetics calculation is less than the number defined, but the result is still calculated.

Reaction direction

This check is carried out for every absorbance calculation.

The rate is corrected according to the expected reaction direction. The reaction direction is preset (see **cobas c** 111 Method Sheet).

Reaction direction increasing:

$R_{cuv} = RC_{cuv} \cdot (+1)$

Reaction direction decreasing:

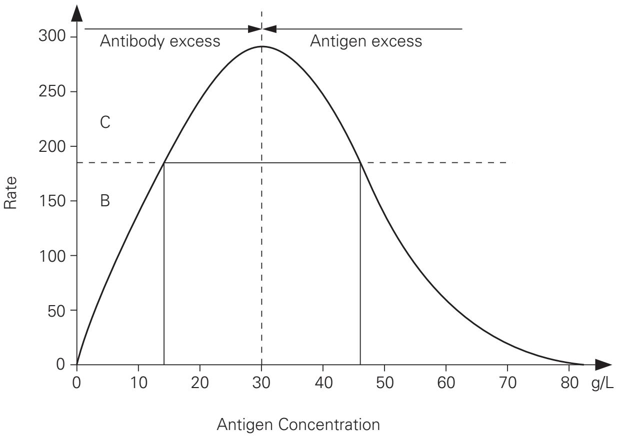
$R_{cuv} = RC_{cuv} \cdot (-1)$

R_{cuv}	Rate for calibrators, controls, or samples.
RC_{cuv}	Rate after absorbance difference calculation. (See also *Calculation model Endpoint* on page 12 and *Calculation model Kinetic* on page 13.)

Antigen Excess check

This check is carried out with controls and samples. It can be used to determine an antigen excess. The binding of an antigen to a specific antibody produces cross-linked complexes which scatter light.

In the presence of excess antibody, the absorbance increases with increasing antigen concentration. With antigen excess, the absorbance will decrease with increasing antigen concentration so that the same ΔA refers to two different concentrations. The point at which the signal begins to decrease is the beginning of the antigen excess region of the Heidelberger Curve. The Antigen (AG) Rate should be determined with the Antigen Excess check first.



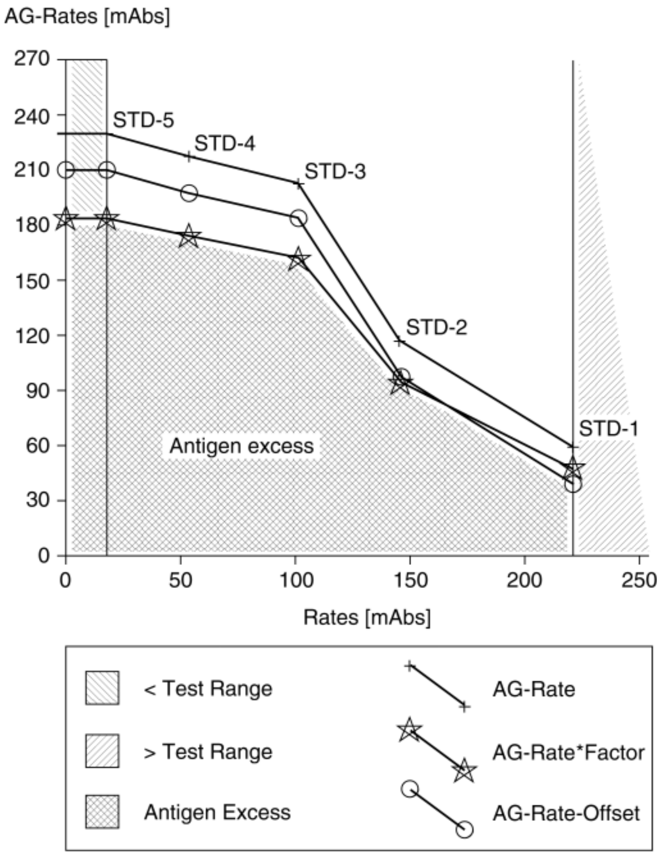
Determination of antigen (AG) rates

The test parameters AG First, AG Last and the Endpoint calculation model are required for determining antigen rates.

The AG rate of the standards is determined by the mean of the replicate AG rates.

Carrying out the antigen excess check

The following figure shows a Rate/AG-Rate curve for the standards. The graph was created using linear interpolation.



The rates of the samples are determined using the corresponding antigen rates of the standards from the Rate/AG-Rate curve.

For rates outside the range between STD-5 and STD-1, the following applies:

- If they are below the lowest standard the antigen rate is assigned to the lowest standard.
- If they are above the highest standard the antigen excess check is not carried out.

$$A_{cuv} \geq \frac{AR_{std} \cdot F_{AG}[\%]}{100[\%]} \text{ and } AR_{cuv} \geq AR_{std} - \text{offset}$$

AR_{std}	Antigen rate of the standard.
F_{AG}	Test-specific factor.
AR_{cuv}	Antigen rate of a sample.
offset	Defined rate (for **cobas c** 111 approximately 0.004 Abs.).

The AG Excess flag is displayed if the checks are not fulfilled.

Test Range check (level rate)

This check determines whether the rate values from the nonlinear calibration modes are within the monotonic curve area.

$$R_{WP\ low} \leq R_{samp\text{-}n} \leq R_{WP\ high}$$

$R_{samp\text{-}n}$	Rate of controls or samples.
$R_{WP\ low}$	Rate of the lowest deflection point, determined from Monotonic Check (test parameter).
$R_{WP\ high}$	Rate of the highest deflection point, determined from Monotonic Check (test parameter).

If the rate is not in the monotonic curve area, no valid end results are obtained.

The > Test Rng flag is displayed if the upper test range limit is exceeded.

The < Test Rng flag is displayed if the value falls below the lower test range limit.

Calculation of mean rate

This check is carried out for calibrators only.

The test parameter Replicate may be set to Single or Duplicate. If set to Duplicate, the calibrators (1…6) are analyzed twice. The individual rates of the replicates are averaged to provide a mean rate for the calibrators.

The mean is determined from the replicate results.

$$RM_{samp\text{-}n} = \frac{[R_{samp\text{-}n}(1) + \ldots + R_{samp\text{-}n}(r)]}{r}$$

$RM_{samp\text{-}n}$	Mean rate of calibrators.
$RM_{samp\text{-}n}(r)$	Rate of calibrators (equivalent to R_{cuv}).
r	Number of replicates.

Quantitative calibration

This section explains the calibration modes and the associated checks. The following table gives an overview:

	Calibration	Control	Sample
Calibration modes			
Linear Interpolation	✓		
Linear Regression	✓		
Logit/Log 4	✓		
Logit/Log 5	✓		
Exponential 5	✓		
Calibration checks			
Curve Direction check	✓		
Curve Non Monotonic check	✓		
Calculation Error check	✓		
Curve Out of Range check	✓		

Table 3 Calibration modes and checks

The calibration yields parameters which enable the calibration curve to be calculated.

The calibration curve can be used to convert the rate of the sample into the concentration of the analyte being measured.

For determining the calibration curve, the following mathematical modes are used:

Mode	Number of calibrations	Curve parameters
Linear Interpolation	3…6	none
Linear Regression	0…6	R_0, F
Logit/Log 4	4…6	R_0, K_c, A, B
Logit/Log 5	5…6	R_0, K_c, A, B, C
Exponential 5	5…6	R_0, K_c, A, B, C

Table 4 Calibration modes and their parameters

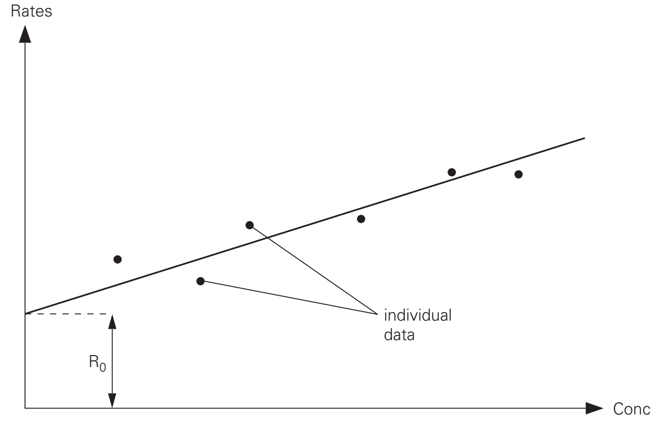
For the Linear Regression mode, if the number of calibrators = 0, the preset curve parameters R_0 and F are used. If the number of calibrators = 1, the preset curve parameters R_0 or F are used.

Calibration mode Linear Interpolation

Linear interpolation analysis is carried out on the rates of up to six calibrators to plot a calibration curve.

Calibration mode Linear Regression

Linear regression analysis is carried out on the rates of all calibrators to find the line of "best fit". If only one calibrator has been set, either the slope of the linear regression is fixed and the offset of the curve is determined from this calibrator, or the rate of one calibrator is fixed and the slope determined.



Calculation of curve parameter R_0 (intercept):

$$R_0 = \frac{\{\Sigma[CT_{std\text{-}n}^{\,2}] \cdot \Sigma[RM_{std\text{-}n}]\} - \{\Sigma[CT_{std\text{-}n} \cdot RM_{std\text{-}n}] \cdot \Sigma[CT_{std\text{-}n}]\}}{\{\Sigma[CT_{std\text{-}n}^{\,2}] \cdot N\} - \{\Sigma[CT_{std\text{-}n}]\}^2}$$

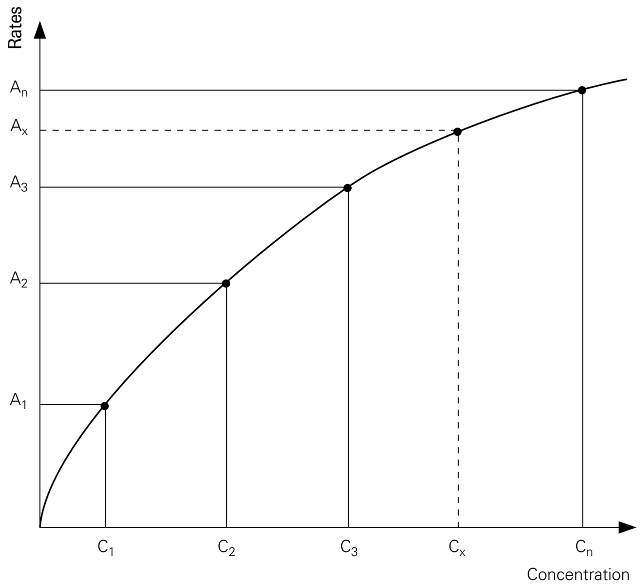
Calculation of curve parameter F (factor):

$$F = \frac{\{\Sigma[CT_{std\text{-}n}]\}^2 - \{\Sigma[CT_{std\text{-}n}^{\,2}] \cdot n\}}{\{\Sigma[CT_{std\text{-}n}] \cdot \Sigma[RM_{std\text{-}n}]\} - \{\Sigma[CT_{std\text{-}n} \cdot RM_{std\text{-}n}] \cdot n\}}$$

R_0, F	Curve parameters.
$CT_{std\text{-}n}$	Calibrator values.
$RM_{std\text{-}n}$	Mean calibrator rate.
n	Number of calibrators.

Calibration mode Logit/Log 4

The Logit/Log 4 mode is used to calculate nonlinear calibration curves. Between four and six calibrators can be entered.



The curve parameters are calculated with a maximum of 150 iterations, using the following equation:

$$RM_{\text{std-n}} = R_0 + \frac{K_c}{1 + \exp[-(A + B \cdot \ln CT_{\text{std-n}})]}$$

$RM_{\text{std-n}}$	Mean calibrator rate.
$CT_{\text{std-n}}$	Calibrator values.
R_0, K_c, A, B	Curve parameters.

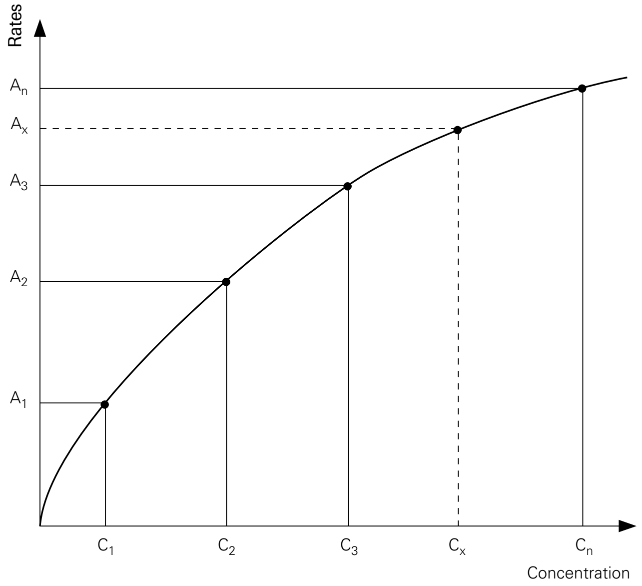
Calibration mode Logit/Log 5

The Logit/Log 5 mode is used to calculate nonlinear calibration curves. Five or six calibrators can be entered.

The curve parameters are calculated with a maximum of 150 iterations, using the following equation:

$$RM_{\text{std n}} = R_0 + \frac{K_c}{1 + \exp[-(A + B \cdot \ln CT_{\text{std n}} + C \cdot CT_{\text{std n}})]}$$

$RM_{\text{std n}}$	Mean calibrator rate.
$CT_{\text{std n}}$	Calibrator values.
R_0, K_c, A, B	Curve parameters.



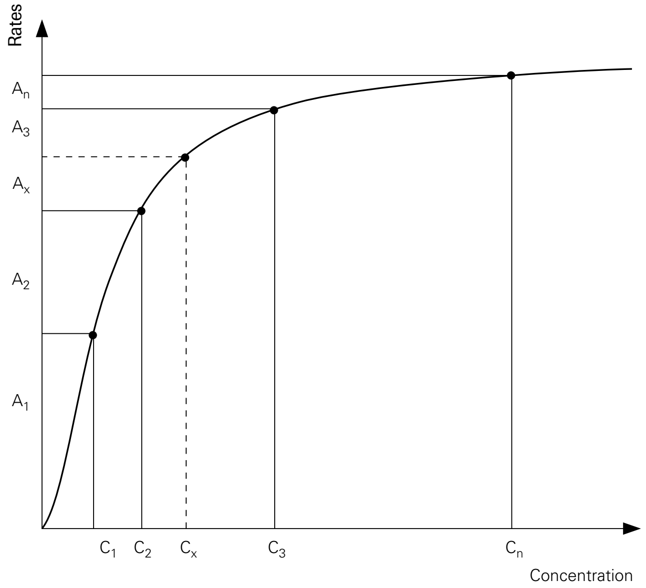
Calibration mode Exponential 5

The Exponential 5 mode is used to calculate nonlinear calibration curves. Five or six calibrators can be entered.

The curve parameters are calculated with a maximum of 150 iterations, using the following equation:

$$RM_{\text{std n}} = R_0 + K \cdot \exp[A \cdot \ln CT_{\text{std n}} + B \cdot (\ln CT_{\text{std n}})^2 + C \cdot (\ln CT_{\text{std n}})^3$$

$RM_{\text{std n}}$	Mean calibrator rate.
$CT_{\text{std n}}$	Calibrator values.
R_0, K_c, A, B, C	Curve parameters.



Calibration checks

Curve Direction check

This check determines if the direction of the calibration curve is correct. It is used to generate the Curv Dir flag if calibrators are placed in the wrong order on the instrument.

With ascending calibration curve:

$$RM_{\text{std highest}} - RM_{\text{std lowest}} > 0$$

With descending calibration curve:

$$RM_{\text{std lowest}} - RM_{\text{std highest}} > 0$$

$RM_{\text{std highest}}$	Mean rate value of the standard with the highest analyte concentration or activity.
$RM_{\text{std lowest}}$	Mean rate value of the standard with the lowest analyte concentration or activity.

The Curv Dir flag is displayed if the calibration curve direction is not correct.

Curve Non-Monotonic check

This check determines if the calibration curve is monotonic within the test range.

The Non Mono flag is displayed if the curve is not monotonic within the defined test range. This flag is also displayed if the calculation model Linear Interpolation is used and the test range is defined outside the defined standard target value.

Calculation Error check

The Calc Error flag is displayed if the calibration curve cannot be calculated.

Curve out of Range check

With this check the calibration curve is inspected to make sure it is within preset limits. This check varies according to the calibration mode.

- With Linear Interpolation, the check is not carried out.
- With Logit/Log4, Logit/Log5, Exponential 5, the check is carried out for each standard.

Theoretical rate for each expected standard concentration:

$$RT_{std\text{-}n} = f(CT_{std\text{-}n})$$

Theoretical minimum rate for each expected standard concentration:

$$RL_{std\text{-}n} = f_{low}(CT_{std\text{-}n})$$

Theoretical maximum rate for each expected standard concentration:

$$RH_{std\text{-}n} = f_{high}(CT_{std\text{-}n})$$

$$RL_{std\text{-}n} \leq RT_{std\text{-}n} \leq RH_{std\text{-}n}$$

$RT_{std\text{-}n}$	Theoretical rate of the standard.
$CT_{std\text{-}n}$	Expected standard concentration.
$RL_{std\text{-}n}$	Theoretical minimum rate of the standard.
f_{high}, f_{low}	Test-specific curve parameters (dependent on the calibration mode).
$RH_{std\text{-}n}$	Theoretical maximum rate of the standard.

Linear Regression

This check establishes whether the calibration parameters R_0 and F are within the accepted range.

$$R_{0\ low} \leq R_0 \leq R_{0\ high}$$

$$F_{low} \leq F \leq F_{high}$$

R_0, F	Parameters in the calculated calibration.
$R_{0\ low}$, F_{low}	Lower parameter limit.
$R_{0\ high}$, F_{high}	Upper parameter limit.

The Out of Rng flag is displayed if the check criteria are not met.

Results

This section explains the calibration modes and checks used to determine the results.

	Calibration	Control	Sample
Quantitative result calculation			
Linear Interpolation	✓	✓	✓
Linear Regression	✓	✓	✓
Logit/Log 4	✓	✓	✓
Logit/Log 5	✓	✓	✓
Exponential 5	✓	✓	✓
Corrections			
Volume correction	✓	✓	✓
Compensation correction	✓	✓	✓
Correlation correction	✓	✓	✓
Calculation of replicate mean	✓		
Result calculation checks			
Replicate Deviation check	✓		
Stand. Curve Deviation check	✓		
Test Range check (level concentration)		✓	✓
Reference Range check			✓
Laboratory conversion	✓	✓	✓

Table 5 Result calculation models and checks

Quantitative result calculation

The curve parameters of the various calibration modes and the rates of the calibrations are used to calculate the results.

Linear Interpolation calibration mode

The standards are used to determine the result. If the rate is out of the range of the standard rates, it is not possible to calculate the result. The result is then flagged with Calc Error.

The standard concentrations right and left of the sample concentration in the calibration curve are used to calculate the sample result using linear interpolation.

Linear Regression calibration mode

This mode is used with controls and samples.

Results are determined according to the following equation:

$$CC_{samp\text{-}n} = (RR_{samp\text{-}n} - R_0) \cdot F$$

$CC_{samp\text{-}n}$	Sample or control result in standard units.
$RR_{samp\text{-}n}$	Rate of sample or control.
R_0, F	Curve parameters.

Logit/Log 4 calibration mode

This mode is used with controls and samples.

The results are determined according to the following equation:

$$CC_{samp\text{-}n} = \exp\left\{\frac{A + \ln\left[\frac{K_c}{RR_{samp\text{-}n} - R_0} - 1\right]}{-B}\right\}$$

$CC_{samp\text{-}n}$	Sample or control result in standard units.
$RR_{samp\text{-}n}$	Rate of sample or control.
R_0, K_c, A, B	Curve parameters.

If the standard curve is not defined for a particular rate, the Calc Range flag is displayed.

Logit/Log 5 calibration mode

This mode is used with controls and samples.

The results are determined according to the following equation:

$$f(x) = A \cdot x + B \cdot x + C \cdot \exp(x) - \ln\frac{RR_{samp\text{-}n} - R_0}{K_c - [RR_{samp\text{-}n} - R_0]}$$

$f(x)$	Sample or control result in standard units.
$RR_{samp\text{-}n}$	Rate of sample or control.
R_0, K_c, A, B, C	Curve parameters.

Exponential 5 calibration mode

Results are determined according to the following equation:

$$f(x) = A \cdot x + B \cdot x^2 + C \cdot x^3 - \ln\frac{RR_{samp\text{-}n} - R_0}{K_c}$$

$f(x)$	Sample or control result in standard units.
$RR_{samp\text{-}n}$	Rate of sample or control.
R_0, K_c, A, B, C	Curve parameters.

Corrections

Volume correction after dilution

Volume correction after dilution is carried out with quantitative analysis for calibrations, controls and samples.

The calculation of the results may require correction (for example due to dilution) or additional calculations. Various checks will then be carried out.

If a control or patient sample has been diluted the corresponding correction factor is applied.

For calibrations, controls and samples in general the results are determined according to the following equation:

$$CV_{\text{samp-n}} = CC_{\text{samp-n}} \cdot F_{\text{pred}}$$

For diluted samples:

$$CV_{\text{samp-n}} = CC_{\text{samp-n}} \cdot F_{\text{pred}} \cdot F_{\text{postd}}$$

$CV_{\text{samp-n}}$	Results with dilution correction of controls or samples in standard units.
$CC_{\text{samp-n}}$	Results after calculation of controls or samples in standard units.
F_{postd}	Sample postdilution factor.
F_{pred}	Sample predilution factor.

The ∨ flag is displayed if F_{postd} is used.

Compensation correction

Compensation correction is carried out after every result calculation. In this way, results can be adapted to other methods.

With the compensation offset, additive corrections on the result can be made. These corrections have to be verified experimentally.

$$C_{\text{samp-n}} = CV_{\text{samp-n}} + O_{\text{comp}}$$

$C_{\text{samp-n}}$	Results of controls or samples.
$CV_{\text{samp-n}}$	Results (including dilution correction) of controls or samples.
O_{comp}	Compensation offset.

Correlation correction

Carried out after every result calculation. In this way, results of different instruments in the same lab can be correlated.

The correlation factor and offset are the slope and y-intercept, respectively, of the straight line obtained. These values must be determined experimentally.

$$C_{\text{samp-n}} = (CV_{\text{samp-n}} \cdot F_{\text{corr}}) + O_{\text{corr}}$$

$C_{\text{samp-n}}$	Correlated result of controls or samples in standard units.
$CV_{\text{samp-n}}$	Results (including dilution correction) of controls, or samples.
F_{corr}	Lab correlation factor (test parameter).
O_{corr}	Lab correlation offset (test parameter).

Calculation of replicate mean

This calculation is carried out for calibrators according to the following equation:

$$CM_{samp\text{-}n} = \frac{1}{r} \cdot [C_{samp\text{-}n}(1) + \ldots + C_{samp\text{-}n}(r)]$$

$CM_{samp\text{-}n}$	Mean replicate concentration.
$C_{samp\text{-}n}(r)$	Replicate concentration of calibrators.
r	Number of replicates.

The calculation of concentration of replicate r and the mean determined by linear interpolation are used for the Replicate Deviation check.

$$C_{lin\text{-}int\text{-}n}(r) = f_{lin\text{-}int\text{-}n}[R_{std\text{-}n}(r)]$$

$$CM_{lin\text{-}int\text{-}n} = \frac{1}{r} \cdot [C_{lin\text{-}int\text{-}n}(1) + \ldots + C_{lin\text{-}int\text{-}n}(r)]$$

$C_{lin\text{-}int\text{-}n}(r)$	Replicate concentration of calibrators.
$f_{lin\text{-}int\text{-}n}$	Linear interpolation function.
$R_{std\text{-}n}(r)$	Rate of replicate r.
$CM_{lin\text{-}int\text{-}n}$	Mean replicate concentration.
r	Number of replicates.

Result calculation checks

Replicate Deviation check

This check is carried out for calibrators only. It examines the scattering of replicates of calibrators.

$$RD_n = |CM_{lin\text{-}n}| \cdot F + O$$

The following equation is applied to each replicate:

$$[CM_{lin\text{-}n} - RD_n] \le C_{lin\ int\text{-}n}(r) \le [CM_{lin\text{-}n} + RD_n]$$

$C_{lin\ int\text{-}n}(r)$	Replicate results of calibrators. Linear interpolation is applied for non-linear calibration modes. Linear regression is applied for linear calibration modes. A differentiation based on the underlaying calibration mode is not required.
$CM_{lin\text{-}n}$	Replicate mean.
r	Number of replicates.
RD_n	Scattering range.
F, O	Test parameters for replicate deviation (Factor, Offset).

The > Repl Dev flag is displayed if a result exceeds the preset deviation.

Standard Curve Deviation check

This check is carried out with quantitative analysis for the main calibration. It is used to inspect the deviation of each calibrator from the curve.

The scattering range within the monotonic range is determined for each calibrator, using the following formula:

$$SD = |CT_{std\text{-}n}| \cdot F + O$$

The following formula applies for each replicate:

$$[CT_{std\text{-}n} - SD] \le CM_{std\text{-}n}(r) \le [CT_{std\text{-}n} + SD]$$

$CT_{std\text{-}n}$	Nominal calibrator concentration.
$CM_{std\text{-}n}$	Calibrator mean.
r	Number of replicates.
F, O	Test parameters for standard deviation (Factor, Offset).

The >Std Dev flag is displayed if the replicates are not within the defined range.

Test Range (level concentration) check

This check is used with quantitative analysis. This check determines wether the concentration is within the measuring range (test range).

$TR_{low} \leq CM_{samp\text{-}n} \leq TR_{high}$

$CM_{samp\text{-}n}$	Control or sample concentration.
TR_{low}	Lower limit of the test range (test parameter).
TR_{high}	Upper limit of the test range (test parameter).

The following flags are displayed if the result is not in the test range:

- < Test Rng is displayed if the result is below the test range lower limit.
- > Test Rng is displayed if the result exceeds the test range upper limit.

Reference Range check

This check is carried out for samples with quantitative analysis. It compares the sample result with the preset reference ranges.

Each sample concentration is determined using the following equation:

$RR_{low} \leq C_{samp\text{-}n} \leq RR_{high}$

RR_{low}	Lower limit of reference range (test parameter).
$C_{samp\text{-}n}$	Sample results in laboratory units.
RR_{high}	Upper limit of reference range (test parameter).

The following flags are displayed if the result is not in the reference range:

- <RR is displayed if the result is below the reference range.
- >RR is displayed if the result is above the reference range.

Laboratory conversion

Results can be adjusted by using a laboratory conversion factor.

Laboratory units

The conversion is carried out after every result calculation.

The sample units can be converted into any desired unit by means of a conversion factor.

Conversion to another unit is carried out for replicates, control and sample results, and the means of calibrators.

For replicates of calibrators:

$$CL_{samp\text{-}n}(r) = C_{samp\text{-}n} \cdot F_{unit}$$

For control, sample or mean of calibrators:

$$CML_{samp\text{-}n}(r) = CM_{samp\text{-}n} \cdot F_{unit}$$

$CL_{samp\text{-}n}(r)$	Replicate results of calibrators in laboratory units.
$C_{samp\text{-}n}$	Results in standard units of controls and samples or replicates of calibrators.
$CML_{samp\text{-}n}$	Control and sample results or mean of calibrators in laboratory units. Only calibrators can be run in duplicates and a mean must then be calculated. For QC and sample results no mean calculation is required, since no duplicates or triplicates are run.
$CM_{samp\text{-}n}$	Control and sample results or mean of calibrators in standard units.
F_{unit}	Factor in laboratory units (test parameter).
r	Number of replicates.

Quality control

The **cobas c** 111 instrument allows the performance of real-time quality control, which consists of the actual control measurements and the preset quality control rules.

👁 For information on configuring and performing QC on the **cobas c** 111instrument, see the **cobas c** 111 Instrument Operator's Manual.

Quality control methods

Quality Control is used to detect the following types of errors:

- Random errors are usually method-dependent and are caused by the scattering of measurements.
- Systematic errors are usually instrument-dependent and are caused by, for example, inaccurate pipetting.

Ideally, quality control methods identify random and systematic errors which are indications of the precision and accuracy of the analytical system. Quality control materials used on **cobas c** 111instrument can only be defined as precision.

Precision

The common quantitative criteria for imprecision are the standard deviation and the coefficient of variation.

$$s = \sqrt{\frac{\Sigma(x_i - x_m)^2}{(n-1)}}$$

$$CV[\%] = \frac{s}{x_m} \cdot 100$$

s	Standard deviation.
x_i	Value of sample i.
x_m	Arithmetic mean value of all samples.
n	Number of samples.
CV	Coefficient of variation.

Quality control checks

For the assessment of the precision the reproducibility of a measurement is investigated.

The following precision checks (rules) are used to assess the system performance. Violation of a rule is indicated by a flag.

Rule/Flag	**Comment**
R 1(2.5s)	This rule checks whether the precision is within the predefined limits. A flag is generated if one value is outside ±2.5s.
R 1(3s)	This rule checks for random errors. A flag is generated if one value is outside ±3s.
R 2(2s)	This rule checks for systematic errors. A flag is generated if two values are outside ±2s.

Table 6 Quality control rules and flags

ISE measurements

This section explains the calculations and checks used in electrolyte measurements.

The diagram below shows the individual steps carried out in an ISE calibration or an ISE sample or control measurement:

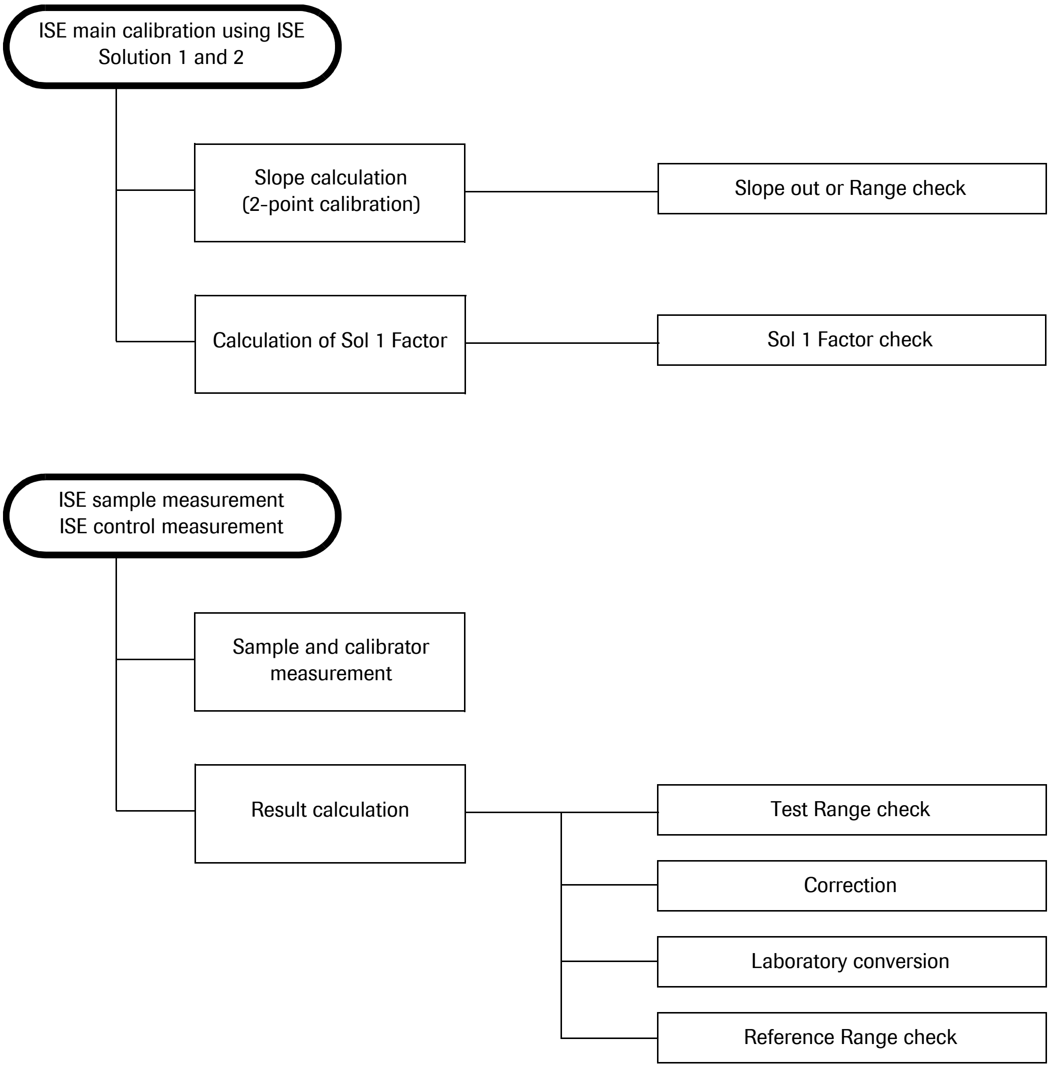


Figure 2 Charts for steps in ISE measurements

Test principle

An Ion-Selective Electrode (ISE) makes use of the unique properties of certain membrane materials to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The electrode has a selective membrane in contact with both the test solution and an internal filling solution. The internal filling solution contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions will closely associate with the membrane on each side. The membrane EMF is determined by difference in concentration of the test ion in the test solution and the internal filling solution. The EMF for a specific ion in solution develops according to the Nernst equation.

Nernst equation

Electrolyte activity is measured by determining the potential difference between the measurement and reference electrodes. The relationship between the potential difference and the ionic activity is given by the Nernst equation.

If the ionic strength of the calibrating solution chosen is equal to that of the sample, the electrode system can be calibrated with acceptable accuracy in terms of the ionic concentration instead of the activity.

The potential measured is directly related to the concentration.

$$E = E_0 + S \cdot \log a_i$$

E	Potential difference between the measurement and reference electrodes.
E_0	Standard potential of the electrode assembly. (Depends on the construction of the electrode.)
S	Electrode slope (Nernst Factor).
a_i	Ionic activity of the electrolyte being measured.

Calculation of Sol 1 factor

$$C_{\text{sol1 calc}} = \frac{C_{\text{sol1}} \cdot 10^{\frac{E_{\text{sol1-1}} - E_{\text{ISEcal-1}}}{S}} + C_{\text{sol1}} \cdot 10^{\frac{E_{\text{sol1-2}} - E_{\text{ISEcal-2}}}{S}}}{2}$$

$$\text{Sol1Factor} = \frac{C_{\text{sol1 targ}}}{C_{\text{sol1 calc}}}$$

S	Slope in mV/dec
C_{sol1}	Known concentration of ISE Solution 1.
E_{sol1}	Electrode potential of ISE Solution 1 in mV.
$E_{\text{ISE cal}}$	Electrode potential of the ISE calibrator in mV.
$C_{\text{sol1 targ}}$	Known concentration of ISE Solution 1.
$C_{\text{sol1 calc}}$	Calculated concentration of ISE Solution 1.
Sol1Factor	ISE Solution 1 factor.

Calibration

To calibrate the electrodes the slope of the measurement system must be calculated.

Slope calculation (2-point calibration)

This calculation is performed for sodium, potassium or chloride. The slope of the electrodes is determined by measuring two different standard solutions. It is calculated with the following equation (based on the Nernst equation):

$$S = \frac{E_{sol\ 1} - E_{sol\ 2}}{\log \frac{C_{sol\ 1}}{C_{sol\ 2}}}$$

S	Slope in mV/dec.
$E_{sol\ 1}$	Electrode potential of ISE Solution 1 in mV.
$E_{sol\ 2}$	Electrode potential of ISE Solution 2 in mV.
$C_{sol\ 1}$	Known concentration of ISE Solution 1.
$C_{sol\ 2}$	Known concentration of ISE Solution 2.

Slope out of Range check

This check is used in a calibration to determine if the slope lies within the defined range.

The slope must fulfill the following conditions:

$S_{low} < S < S_{high}$

S_{low}	Lower limit of slope range in mV/dec.
S	Slope in mV/dec.
S_{high}	Upper limit of slope range in mV/dec.

The Out of Rng flag is displayed if the check criteria are not fulfilled.

Sol 1 Factor check

To check if a new ISE Solution 1 factor is correct, the rate values for the second ISE Solution 1 measurement and its subsequent calibrator measurement of the main calibration are used. With these rate values, together with the slope and the Sol 1 Factor, the concentration of each ion (sodium, potassium, and chloride) in the ISE Solution 1 is calculated.

$C_{low} < C_{calc} < C_{high}$

C_{low}	Concentration calculated for ISE Solution 1 using the Sol 1 Factor determined during the main calibration.
C_{calc}	Lower limit of the recalculated ISE Solution 1 concentration.
C_{high}	Upper limit of the recalculated ISE Solution 1 concentration.

The limits for all ISE modes are:

Ion	C_{low}	C_{high}
Sodium	148.8 mmol/L	151.2 mmol/L
Potassium	4.94 mmol/L	5.06 mmol/L
Chloride	113.9 mmol/L	116.1 mmol/L

Table 7 Limits of recalculated ISE solutions

The Sol 1 F Dev flag is displayed if the check criteria are not met.

In addition, the Sol 1 factor must fulfill the following condition:

$0.85 \leq \text{Sol 1 factor} \leq 1.15$

The Sol 1 F Dev flag is displayed if the check criterion is not fulfilled.

Results

Each ISE measurement consists of the measurement of the sample and the measurement of the corresponding ISE calibrator. The electrode potential results from the respective sample or calibrator measurement.

Calculation of concentration

The sodium, potassium, and chloride concentration is determined using the following equation:

$$C_{\text{samp QC}} = C_{\text{sol 1}} \cdot 10\left[\frac{E_{\text{samp QC}} - E_{\text{ISE cal indir/urine}}}{S}\right] \cdot F$$

$C_{\text{samp QC}}$	Ionic concentration of sample or control.
$C_{\text{sol 1}}$	Nominal concentration of ISE Solution 1.
$E_{\text{samp QC}}$	Electrode potential of sample or control in mV.
$E_{\text{ISE cal indir/urine}}$	Electrode potential of ISE calibrator in mV.
F	ISE Solution 1 factor.
S	Slope in mV/dec.

Calculation checks

Test range	👁 See *Test Range (level concentration) check* on page 30.
Correlation correction	👁 See *Correlation correction* on page 38.
Laboratory conversion	👁 See *Laboratory conversion* on page 38.
Reference range	👁 See *Reference Range check* on page 30.