cobas 6000 analyzer series

COBI-CD
Compendium of Background Information

Document information

Revision history

Manual Version	Software Version	Revision date	Changes
1.0		01.03.2006	Revision of contents with respect to new **cobas** 6000 analyzer series features
1.1	**cobas** 6000 analyzer series	June 2010	One modification in Table B-15
2.0		November 2012	Update to Template Version 4.1 General update
2.1		December 2014	Minor changes in: Technical limit check Equation for Sens.E

Edition notice

cobas 6000 analyzer series Compendium of Background Information

This document is for users of the **cobas** 6000 analyzer series.

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.

Any customer modification to the instrument will render the warranty or service agreement null and void.

Software updates are done by Roche Diagnostics Service representatives.

Intended use

This document is intended to provide background information for a better understanding of the hardware, test principles and calibration methods of the **cobas** 6000 analyzer series.

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cobas 6000 analyzer series meets the protection requirements laid down in IVD Directive 98/79/EC. Furthermore, our instruments are manufactured and tested according to the following international standards:

- IEC 61010-1: 2001
- IEC 61010-2-010: 2003
- IEC 61010-2-081: 2001
- IEC 61010-2-101: 2002
- UL 61010-1: 2nd edition
- CAN/CSA 61010-1: Second edition

The **cobas** 6000 complies with the emission and immunity requirements described in the following standard:

- IEC 61326-2-6: 2005

The Operator's manual meets the European Standard DIN EN ISO 18113-3.

Compliance is demonstrated by the following marks:

CE Complies with the IVD directive 98/79/EC.

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How to use the CD

This CD is provided as an information source for background knowledge regarding the **cobas** 6000. Some of the information on this CD is available in PDF-format and requires Adobe Reader to be installed. If you do not have this software installed, refer to the instructions for the Installation of Adobe Reader below. You may access information by selecting a topic from the table of contents on the left.

If you have any further questions, please do not hesitate to contact Roche Diagnostics Customer Service or visit us on the Web at www.roche.com/diagnostics.

Installation of Adobe Reader

We have included the files necessary to install Adobe Reader in this CD. If this software is not installed on your computer, proceed as follows:

► **To install Adobe Reader**

1. Close all running applications.
2. Change to the folder \reader on the CD-ROM.
3. Double-click on **AdbeRdr11000_en_US.exe** to start the installation routine for Adobe Reader.
4. Follow the instructions on screen.
5. It is recommended that you restart your computer after the installation process has finished.

■

Where to find information

The following documents are provided to assist in finding desired information quickly:

Operator's Manual Introduction and Part A-F: Contains information about safety, hardware modules and operating the system as well as maintenance and troubleshooting. A table of contents at the beginning and an index at the end of this book help you to find information quickly.

Online Help Contains a detailed description of the software of the **cobas** 6000. In addition to the software description, the whole Operator's Manual is embedded into the Online Help. This makes it possible to retrieve information from both Online Help and Operator's Manual using the search functions available for electronically stored documents.

Short Guide The Short Guide is a collection of operations important to routine operation presented in a practical, easy-to-use format.

COBI-CD The COBI-CD (Compendium of Background Information) provides you with background information about the technologies, test principles, their theory and calibration methods used by **cobas** 6000. It also provides a complete glossary. The information can be read and printed using Adobe Reader.

Online Help System

cobas 6000 has a context sensitive online Help feature to aid in operating **cobas** 6000. *Context sensitive* means that wherever you are located within the **cobas** 6000 software, choosing the **Help** feature displays Help text or a screenshot relating to that area of the software. The online Help offers a quick and convenient way to find information, such as explanations of screens and dialog boxes and how to perform particular processes. **cobas** 6000 offers two sources of online information, F1 Help and Direct Help

F1 Help There are two main entry points for the Online Help: a context sensitive entry via the **Help** buttons in the software or F1 on the keyboard, or the main entry via the **Help** icon in the bottom left of the screen. The context sensitive entry displays text or a screenshot relating to your current location in the software.

Direct Help **cobas** 6000 also offers Direct Help. Direct Help provides you with information about an element on the screen without having to leave the software.

Symbols and abbreviations

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

Symbols The following symbols are used:

Symbol	Used for
►	Start of procedure
■	End of procedure
•	List item
👁	Cross-reference
➔	Call-up (software reference)
	Tip
	Safety alert
	Electrical and electronic equipment marked with this symbol are covered by the European directive WEEE. The symbol denotes that the equipment must not be disposed of in the municipal waste system.

Abbreviations The following abbreviations are used:

Abbreviation	Definition
A	
ANSI	American National Standards Institute
B	
BTS	barcode transfer sheet
C	
CBT	Computer based training
cc	cubic centimeter
CLAS 2	Clinical Laboratory Automation System 2
CLIA	Clinical Laboratory Improvement Amendments
COBI-CD	compendium of background information
COI	Cutoff index
CSA	Canadian Standards Association
D	
dBA	decibel weighted against the A-frequency response curve. This curve approximates the audible range of the human ear.
DIL	diluent
E	
EC	European community
ECL	electrochemiluminescence
e.g.	*exempli gratia* – for example
EMC	electromagnetic compatibility
EN	European standard
F	
FIFO	First in first out
H	
HCFA	Health Care Financing Administration
I	
i.e.	*id est* – that is to say
IEC	International Electrical Commission
IS	Internal Standard (ISE module)
ISE	ion selective electrode
ISE Dil.	Diluent (ISE module)
ISE Ref.	Reference solution (ISE module)
IVD	In vitro Diagnostic Directive
K	
KVA	kilovolt-Ampere. Unit for expressing rating of AC electrical machinery.
L	
LDL	lower detection limit
LIS	Laboratory Information System
LLD	liquid level detection

Abbreviation	Definition
M	
MBC	matrix barcode
MSDS	material safety data sheet
N	
n/a	not applicable
NCCLS	National Committee for Clinical Laboratory Standards
P	
PC/CC	ProCell M/CleanCell M
PW	PreClean solution PreWash
Q	
QC	Quality control
R	
RCM	Reaction Calculation Mode
REF	Reference solution for ISE module
R.M.	Result message
S	
SD	standard deviation
SIP	ISE sipper syringe
SVGA	Super Video Graphics Adapter
SWA	Serum Work Area
T	
TPA	tripropylamine
U	
UL	Underwriters Laboratories Inc.
V	
VDE	Association of German Electrical Engineers (Verband Deutscher Elektrotechniker)

Measurement technology A

ISE technology

This chapter provides you with an overview of ISE technology used by the **cobas** 6000.

In this chapter *Chapter* 1

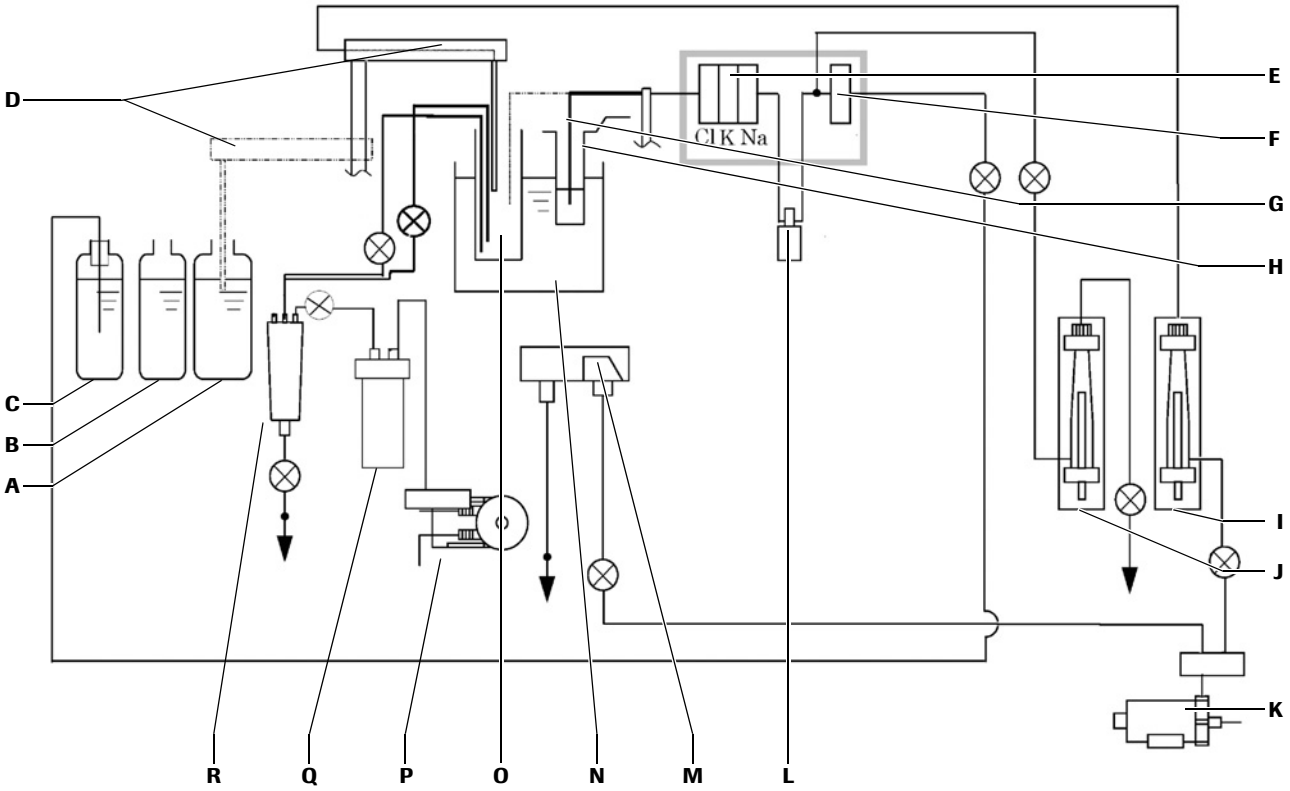
Table of contents

Components and function of the ISE unit

The figure below visualizes the liquid flow paths of the ISE unit. The following symbols are used in the figure:

Solenoid valve

To waste container



A	Internal standard (ISE IS)	**G**	Sipper nozzle	**M**	ISE rinse station
B	Diluent (ISE Dil.)	**H**	Reaction cell	**N**	Incubator bath
C	Reference solution (ISE Ref.)	**I**	ISE syringe	**O**	IS bath
D	ISE pipetter	**J**	Sipper syringe	**P**	Vacuum pump
E	Measuring electrodes	**K**	Water pump	**Q**	Vacuum tank
F	Reference electrode	**L**	Pinch valve	**R**	Vacuum reservoir

Figure A-1 ISE liquid flow path diagram

Sample probe, reaction cell, and ultrasonic mixer

The ISE unit uses the sample probe, reaction cells, and ultrasonic mixer of the **c** 501 module.

Sample probe The sample probe transports sample liquid from the sample tube to a reaction cell.

Reaction cell The reaction disk of the **c** 501 module carries the reaction cells. All reaction cells are seated in the controlled-temperature incubator bath. The incubator bath maintains the cells at the required temperature of 37°C.

Ultrasonic mixer After the sample probe has dispensed the sample into the reaction cell, the ISE pipetter adds ISE Dil.. The ultrasonic mixer of the **c** 501 module mixes the diluted sample.

ISE pipetter

The ISE pipetting system is composed of the ISE pipetter (consisting of pipetter arm and probe), the ISE syringe, and the ISE rinse station.

ISE pipetter The ISE pipetter transports ISE Dil. to the reaction cell and ISE IS to the internal standard bath.

The ISE pipetter probe is equipped with a level detector (capacitance method) which is applied to check and correct the filling volume of any bottle present in the ISE reagent compartment.

ISE syringe The ISE pipetter is connected to the ISE syringe by tubing, which controls the pipetting action.

ISE rinse station This rinse station is used for both ISE pipetter probe and ISE sipper probe.

ISE reagent compartment

The ISE reagent compartment provides five positions for reagent bottles:

- ISE IS: Two bottles
- ISE Dil.: Two bottles
- ISE Ref.: One bottle

The reagent compartment is equipped with position sensors for each reagent bottle (reflection type).

Internal standard bath

Internal standard bath (IS bath) has two chambers for heating internal standard (ISE IS) to measuring temperature (37°C). After heating, the ISE IS solution is aspirated by the sipper probe into the measuring flow path. The residual ISE IS solution is aspirated through the vacuum nozzle to empty the IS bath.

The use of two chambers allows for an optimized flow of the analysis: While the content of one chamber is ready for use, fresh ISE IS is pipetted into the other chamber where it is given time to heat up for the next measurement.

ISE sipper

The ISE sipper mechanism consists of a sipper nozzle and a sipper syringe. Between the sipper nozzle and the syringe is the ISE measuring flow path.

Sipper nozzle The sipper nozzle lowers either into ISE IS solution in the IS bath or into sample solution in a reaction cell to aspirate the respective solution into the measuring flow path.

Sipper syringe The sipper syringe provides the negative pressure for following functions:

- Aspirate sample solution or ISE IS into the measuring flow path (measurement electrodes)
- Aspirate ISE Ref. into the measuring flow path (reference electrode)
- Aspirate measured sample solution, ISE IS, and ISE Ref. from the measuring flow path into the waste container.

ISE measuring

The ISE measuring system is contained in a temperature-controlled compartment. It is composed of three ion specific electrodes and one reference electrode.

The difference between the potentials at the reference electrode and the ion-selective electrode equals the electromotive force (EMF). For every test, the EMF of both ISE IS and diluted sample solution are measured for each sort of ions (Cl^-, K^+, and Na^+). From these EMF values the results are calculated using the calibration curve.

Measurement electrodes The measurement electrodes use a special design. Membranes with ion-selective binding capacity and an open liquid junction allow the selective measurement of the ion concentrations. The electrodes are directly connected to form a flow path for the diluted sample and the ISE IS solutions.

Reference electrode The reference electrode uses the same design of the measurement electrodes. It is exclusively used as a reference for every measurement. ISE Ref. is aspirated through the electrode and a reference electrode potential is registered.

Pinch valve The pinch valve is used to control the flow of liquid that passes the electrodes.

Measurement sequence

This section describes the sequence of the ISE measurement.

Preparation First, the sample pipetter pipettes a sample into a reaction cell. Then, into this cell, ISE Dil. is pipetted by the ISE pipetter and mixing is carried out with the ultrasonic mixing unit. Next, the ISE pipetter dispenses ISE IS solution into the IS bath where it is heated to 37°C.

ISE IS measurement The ISE sipper aspirates ISE IS solution from the IS bath into the measuring flow path to perform an ISE IS measurement (single-point calibration). The residual ISE IS solution is aspirated through the vacuum nozzle to empty the IS bath.

The sipper syringe aspirates ISE Ref. from the ISE Ref. bottle to the reference electrode to perform ISE Ref. measurement.

Sample measurement The ISE sipper aspirates diluted sample from the reaction cell into the measuring flow path to perform the sample measurement.

The sipper syringe aspirates ISE Ref. from the ISE Ref. bottle to the reference electrode to perform ISE Ref. measurement.

For every ISE measurement, the analyzer measures three electromotive force values (EMF); for chloride, potassium, and sodium, where EMF denotes the difference in potential between the respective ion-selective electrode and reference electrode.

Finalization Finally, the results are calculated from the electromotive forces of ISE IS and diluted sample. The ISE system is now ready for the next analysis. If there are no more samples to be analyzed, the ISE unit performs a final ISE IS measurement and stops.

Summary This table summarizes the flow of an ISE analysis:

Step	Time	Actor	Action
		Preparation of measurement	
1	0.0 s	Sample pipetter	Pipettes sample (9.7 µl) to cell
2	12.0 s	ISE pipetter	Aspirates (348 µl) and dispenses ISE Dil. (291 µl) to cell
3	15.0 s	Ultrasonic mixing unit	Mixes sample and ISE Dil.
4		ISE pipetter	Aspirates (590 µl) and dispenses ISE IS (450 µl) to IS bath
5		IS bath	ISE IS heats to measuring temperature (37°C)
		Internal standard (ISE IS) measurement	
6	284.5 s	ISE Sipper	Aspirates ISE IS to Cl/K/Na electrodes (400 µl)
7		Sipper syringe via tubing	Aspirates ISE Ref. from the ISE Ref. bottle to reference electrode (65 µl)
8	292.0 s	Electrodes	Measure ISE IS
		Diluted sample measurement	
9	301.0 s	ISE Sipper	Aspirates sample to Cl/K/Na electrodes (250 µl)
10		Sipper syringe via tubing	Aspirates ISE Ref. from the ISE Ref. bottle to reference electrode (65 µl)
11	310.0 s	Electrodes	Measure sample
		Finalization of measurement	
12	315.0 s		Result calculation and output If there are more samples to be analyzed, go to step 1. If there are no more samples, repeat 6-8 and stop.

Table A-1 Flow of ISE analysis

c 501 – Photometric technology

This chapter provides you with an overview of the application of photometric technology used by the **cobas** 6000.

In this chapter *Chapter* **2**

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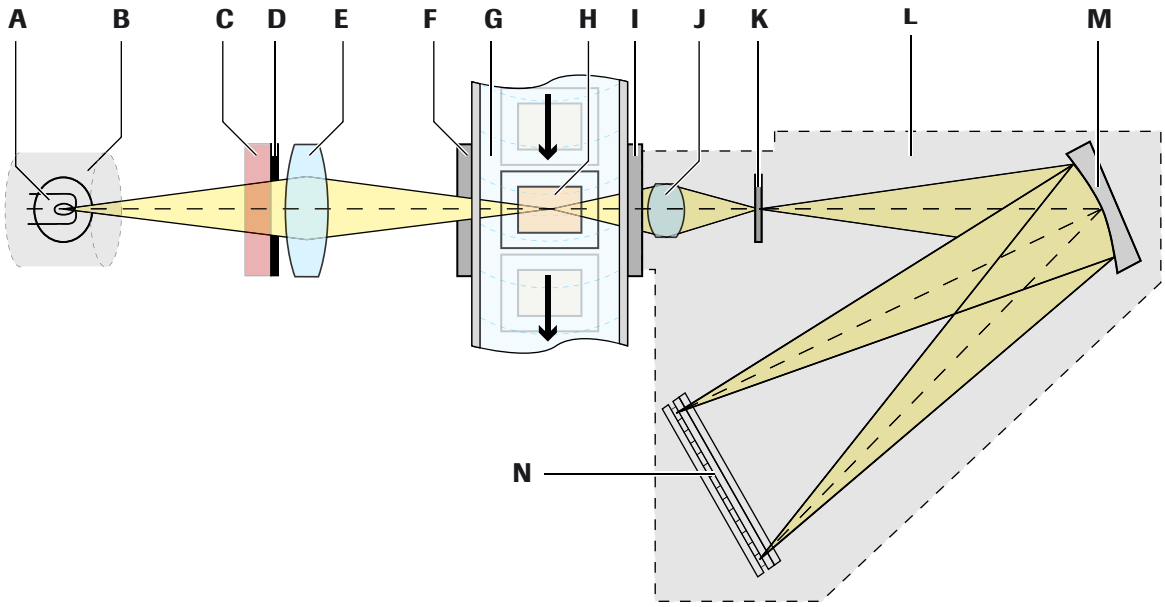
General photometer characteristics

An illustration of the light path is shown below.



A	Photometer lamp	**B**	Slit (in)	**C**	Slit
D	Water jacket	**E**	Reaction bath	**F**	Photometer
G	Infrared cut filter	**H**	Reaction cell and contents	**I**	Grating
J	Mask	**K**	Slit (out)	**L**	Detector
M	Condenser lens	**N**	Imaging lens		

Figure A-2 Photometer lightpath

When the light beam enters the photometer, it strikes a diffraction grating, which separates the light into its constituent wavelengths and reflects them onto a fixed array of 12 photodiodes. Each photodiode is permanently positioned to detect light at a different wavelength.

Absorbance readings are taken each time a reaction cell rotates past the photometer. When the reaction cell passes through the photometer lightpath, absorbance at the 12 wavelengths for each individual assay is measured.

Most Roche Diagnostics photometric tests use two wavelength readings to calculate results. Each test-specific reactant, which is measured during the reaction, absorbs light maximally at one particular wavelength. The main or primary wavelength is chosen such that it is in close proximity of the absorption maximum in order to obtain the best sensitivity. However, using the difference between readings at two wavelengths (bichromatic system) eliminates the effect of interferences sometimes found when using a single wavelength (monochromatic system) and compensates for most of the photometric noise which improves the photometric resolution.

For a test that has a reaction time of 10 minutes, the **c** 501 has a total of 70 measuring points.

Bichromatic analysis uses two wavelengths: One that is at or near the peak absorbance of the chromogen produced by the reaction, and a second wavelength at which little or no absorbance of the desired chromogen occurs.

Any absorbance (A_2) that occurs, due to interference from other substances in the sample, is measured at the secondary wavelength. This amount is then subtracted from the total absorbance (A_1) occurring at the primary wavelength to yield the net absorbance (A^C).

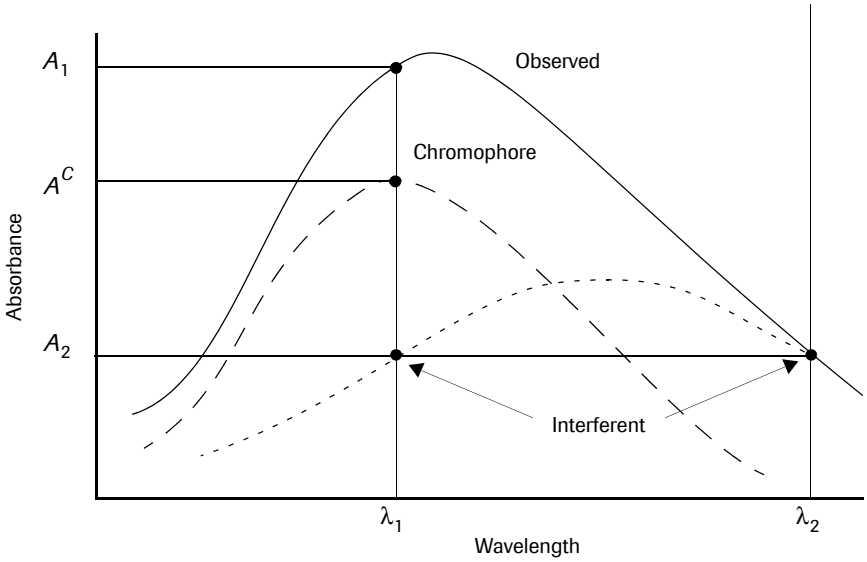


Figure A-3 Bichromatic absorbance

The optimum measuring points for each test are part of the application parameters, which are available via download.

The assay code and calibration type programmed via the application parameters determine how final results are calculated for each test.

e 601 – ECL technology

This chapter provides you with an overview of the electrochemiluminescent technology used by the **cobas** 6000. The use of the ruthenium complex and the measuring cell in which the reaction occurs are described.

In this chapter *Chapter* 3

ECL measuring principles

Electrochemiluminescent (ECL) processes are known to occur with numerous molecules including compounds of ruthenium, osmium, rhenium or other elements.

ECL is a process in which highly reactive species are generated from stable precursors at the surface of an electrode. These highly reactive species react with one another, producing light.

The development of ECL/Origen immunoassays is based on the use of a ruthenium(II)-tris(bipyridyl) $[Ru(bpy)_3]^{2+}$ complex and tripropylamine (TPA). The final chemiluminescent product is formed during the detection step.

The chemiluminescent reactions that lead to the emission of light from the ruthenium complex are initiated electrically, rather than chemically. This is achieved by applying a voltage to the immunological complexes (including the ruthenium complex) that are attached to streptavidin-coated microbeads. The advantage of electrically initiating the chemiluminescent reaction is that the entire reaction can be precisely controlled.

Use of the Ruthenium complex

ECL technology uses a ruthenium chelate as the complex for the development of light. Salts of ruthenium-tris(bipyridyl) are stable, water-soluble compounds. The bipyridyl ligands can be readily modified with reactive groups to form activated chemiluminescent compounds.

For the development of ECL immunoassays, a N-hydroxysuccinimide (NHS) ester of a modified $Ru(bpy)^3$ complex is used because it can be easily coupled with amino groups of proteins, haptens and nucleic acids. This allows the detection technology to be applied to a wide variety of analytes.

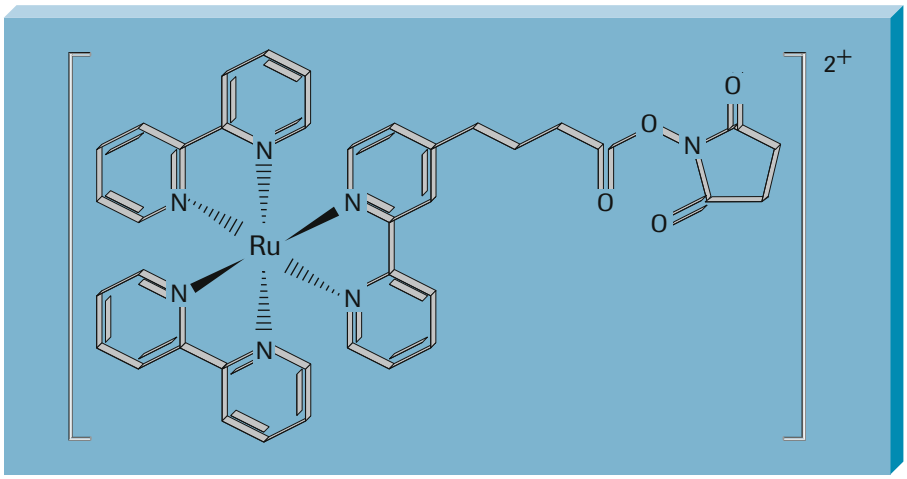


Figure A-4 The ruthenium complex

The ECL reaction at the electrode surface

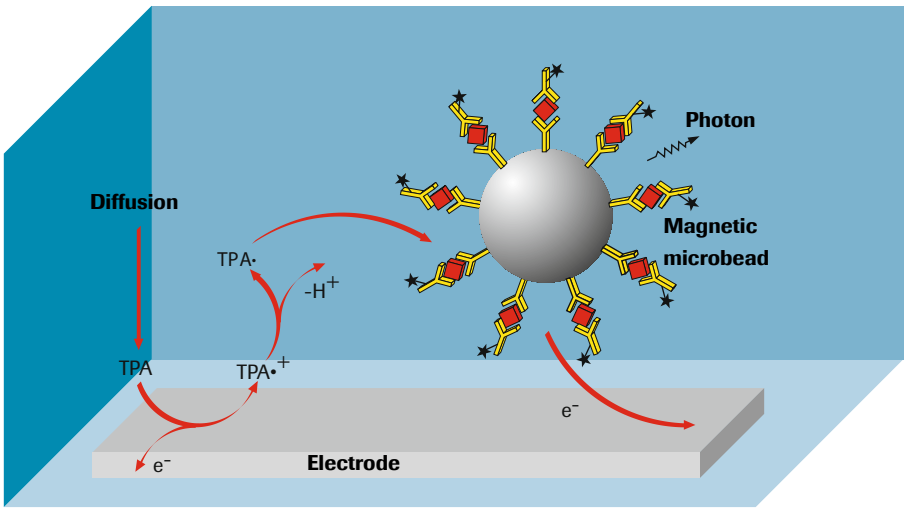


Figure A-5 Detection of a ruthenium-labeled immune complex

Two electrochemically active substances, the ruthenium complex and tripropylamine (TPA), are involved in the reactions that lead to the emission of light. Both substances remain stable as long as a voltage is not applied.

The ECL reaction of ruthenium-tris(bipyridyl)$^{2+}$ and tripropylamine occurs at the surface of a platinum electrode. The applied voltage creates an electrical field, which causes all the materials in this field to react. Tripropylamine is oxidized at the electrode, releases an electron and forms an intermediate tripropylamine radical-cation, which further reacts by releasing a proton (H^+) to form a TPA radical (TPA•).

In turn, the ruthenium complex also releases an electron at the surface of the electrode thus oxidizing to form the $Ru(bpy)_3^{3+}$ cation. This ruthenium cation is the second reaction component for the following chemiluminescent reaction with the TPA radical.

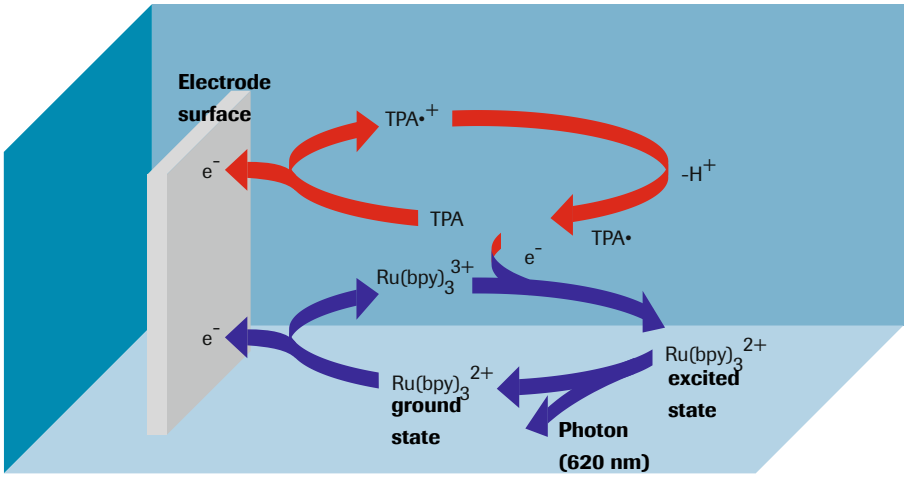


Figure A-6 The ECL reaction at the electrode surface

TPA• and $Ru(bpy)_3^{3+}$ react with one another, whereby $Ru(bpy)_3^{3+}$ is reduced to $Ru(bpy)_3^{2+}$ and at the same time forms an excited state via energy transfer. This excited state is unstable and decays with emission of a photon at 620 nm to its original state. The reaction cycle can now start again. The tripropylamine radical decays to by-products which do not affect the chemiluminescence process. TPA is used up and therefore must be present in excess. The reaction is controlled by diffusion of the TPA and the amount of ruthenium complex present. As TPA in the electrical field is depleted, the signal strength (light) is slowly reduced once the maximum is reached.

Although TPA is depleted during measurement, the ruthenium ground state complex is continually regenerated. This means that the ruthenium complex can perform many light-generating cycles during the measurement process, therefore showing an inherent amplification effect which contributes to the technology's sensitivity. Many photons can be created from one antigen-antibody complex.

ECL signal generation

The graph displays a typical ECL signal generation. Viewed from an electrical perspective, the reaction can be explained as follows: When a voltage is applied to the detection cell electrode a peak of light emission occurs over a short time interval and can be detected as the resulting ECL signal. A defined area under the curve is measured around the intensity maximum.

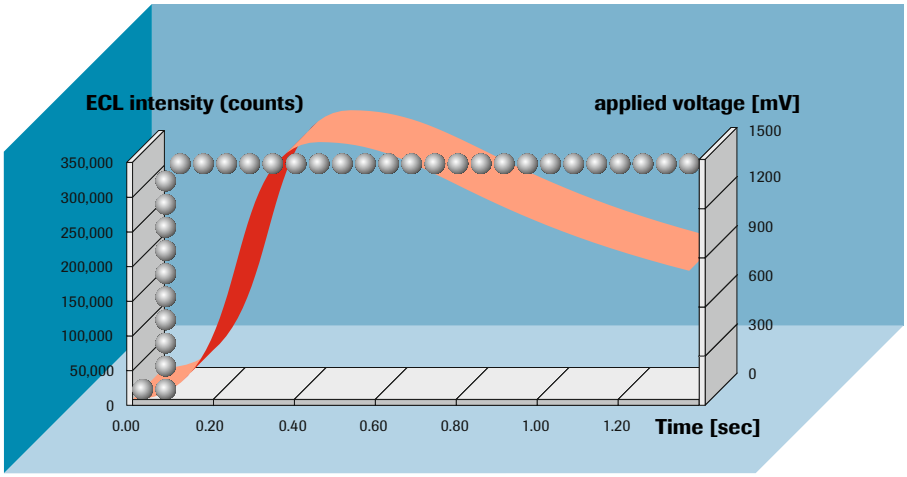


Figure A-7 ECL signal generation

The dotted line indicates the voltage at the electrode used to generate the ECL signal. The solid line is the actual light output measured by the photomultiplier detector.

ECL measuring cell

The core of the system is the ECL detection cell, which is designed as a flow-through cell. Essentially, three operating steps are performed in the measuring cell:

- Bound/free separation

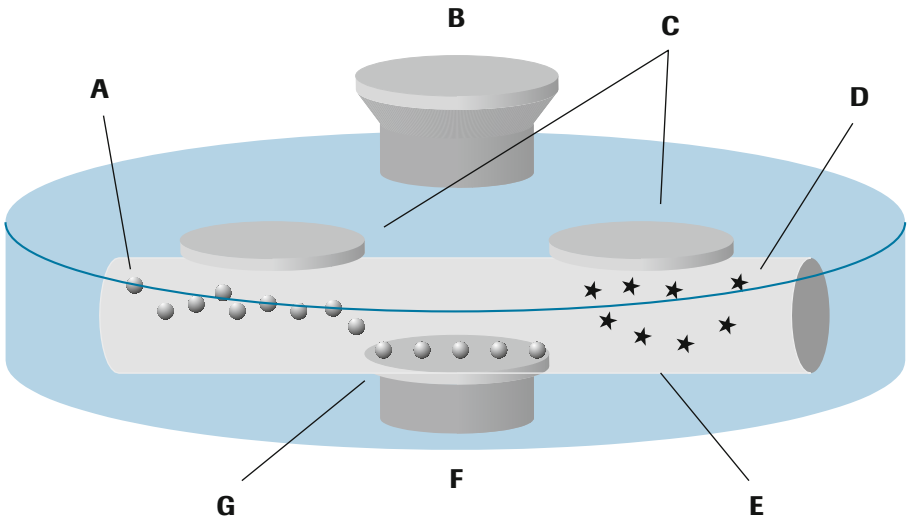
  Using a magnet, the streptavidin microbeads that are coated with antigen-antibody complexes are uniformly deposited on the working electrode. A system buffer (ProCell) is used to wash the particles on the working electrode and to flush out the excess reagent and sample materials from the measuring cell.

- ECL reaction

  The magnet is removed and a voltage is then applied to the electrode on which the microbeads, coated with antigen-antibody complexes, are deposited to initiate the ECL reaction. The light emission is measured with a photomultiplier. The system then uses the corresponding signals for the calculation of results.

- Release of microbeads and cell cleaning

  Once the measurement is completed, the paramagnetic microbeads are washed away from the electrode surface with a special cleaning solution (CleanCell). The surface of the measuring cell is regenerated by varying the potential on the electrode. The cell is then ready for another measurement.



A Magnetic microbeads with bound antigen-antibody complex
B Photomultiplier
C Counter electrode
D Unbound antibody (ruthenium-labeled)
E Flow channel
F Magnet
G Working electrode

Figure A-8 ECL measuring cell

Advantages of ECL technology

Electrochemiluminescence is a highly innovative technology that offers distinct advantages over other detection techniques.

- Extremely stable nonisotopic label allows liquid reagent convenience.
- Enhanced sensitivity in combination with short incubation times means high quality assays and fast result turnaround.
- Large measuring range of five orders of magnitude minimizes dilutions and repeats, reducing handling time and reagent costs.
- Applicable for the detection of all analytes providing a solid platform for menu expansion.

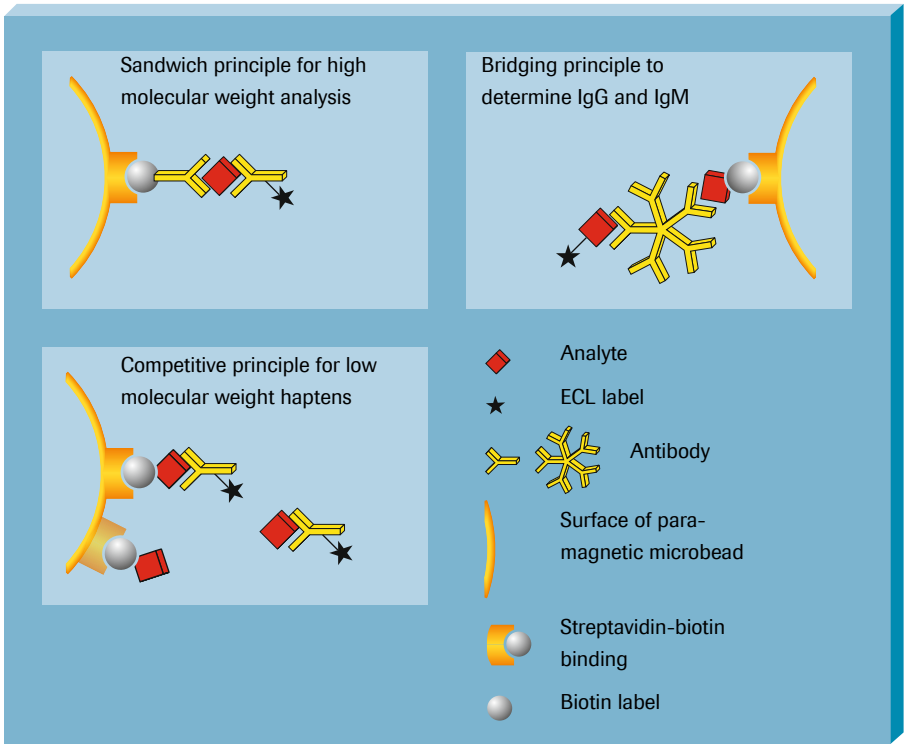


Figure A-9 ECL assay principles

Test principles B

ISE unit – Ion selective electrode principles

This chapter provides you with an overview of the ion selective electrode test principles and result calculation used by the **cobas** 6000.

In this chapter

Chapter **4**

Table of contents

Introduction

The ISE unit performs indirect measurement of electromotive force (EMF) in millivolts between ion selective electrodes and the reference electrode. Indirect measurement means that all samples are diluted at a 1:31 ratio.

The EMF values of each sample are converted to mmol/L values by a calculation algorithm that uses the EMF data together with data from a two-point calibration with two primary standards.

A one-point calibration before and after each routine sample measurement is used to offset the drift between consecutive measurements. For this one-point calibration the internal standard (IS) is used.

Calculation of unknown sample concentrations

The concentration of the sodium, potassium, and chloride in a sample is calculated from the EMF of the specific electrode by the following equation, which is derived from the Nernst Equation:

Equation B–1 $$C_s = C.\text{Value} + C_{IS} \times 10^{(E_s - E_{IS})/S}$$

C_s	Concentration of the specific ion in sample
C.Value	Compensation value
C_{IS}	Concentration of the internal standard
E_s	Electromotive force (voltage) of the unknown sample for the specific ion
E_{IS}	Electromotive force (voltage) of the internal standard for the specific ion
S	Slope

c 501 – Photometric principles

This chapter provides you with an overview of the photometric test principles and assay techniques used by the **cobas** 6000.

In this chapter *Chapter* 5

Types of photometric assays

There are two fundamental types of photometric assays on this instrument:

- Endpoint assays
- Rate assays

Measurements are taken by the photometer at specific measuring points. If measurements are taken after the reactions are completed, the intensity of the colored (or turbidity) product is an indicator of the sample component's concentration. These are called endpoint assays.

If measurements are taken as the reaction proceeds, the rate of the reaction is proportional to the sample component's concentration or activity being analyzed. These are called rate reactions. There are also modifications of these two techniques possible in this instrument, as well as a combination of the two.

Assay types and measuring points

There are four different assay types. The assay types are divided in endpoint assays and rate assays:

Fundamental assay type	Assay type	Characteristic
Endpoint assays	1 Point	Endpoint assay programmed for a single measuring point
	2 Point End	Endpoint assay with sample blank
Rate assays	Rate A	Rate assay applying least squares method on multiple measuring points
	2 Point Rate	Rate assay programmed for two measuring points

Table B–1 Assay types

For more information on endpoint assays, see:
1 Point assay on page B-24
2 Point End assay on page B-27

For more information on rate assays, see:
Rate A assay on page B-30
Rate A assay with sample blank correction on page B-33
2 Point Rate assay on page B-36

Measuring points

Independent of the programmed application parameters, the photometer measures the absorbance of a reaction mixture in fixed intervals of 7 to 15 seconds. Not all of these measurements are used for the calculation of the result. Therefore, the numbering of the photometer measuring points differs from the numbering of the measuring points used in calculations.

The figure below represents an endpoint assay programmed for two measuring points (mp_1 and mp_2) indicated by the blue vertical lines in the reaction monitor.

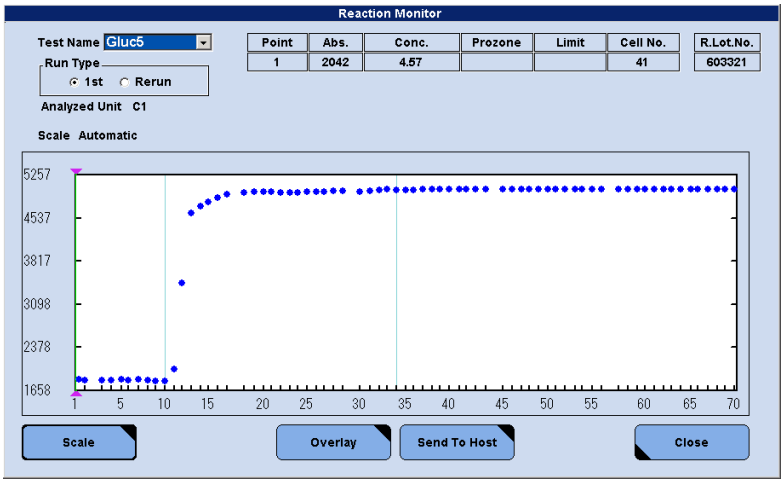


Figure B-1 Photometer measuring points

In this example, the application parameters define the 10th photometer measuring point (mp_{10}) to be mp_1 and the 34th photometer measuring point (mp_{34}) to be mp_2. In other words, mp_{10} of the instrument is set to be mp_1 of the test calculation, and mp_{34} of the instrument is set to be mp_2 of the test calculation.

Displaying assay type and measuring points

The **Analyze** tab on the **Utility** > **Application** screen displays the assay type and measuring points among other application parameters for a selected test.

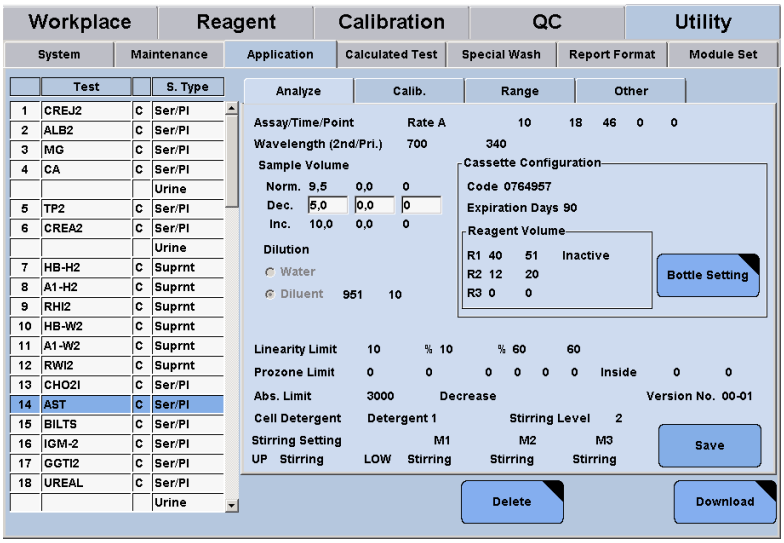


Figure B-2 Analyze tab on Utility > Application screen

► To view the assay type and measuring points for a test

1 Select **Utility** > **Application**.

2 Select the test you want to view from the test list on the left side of the screen.

3 Select the **Analyze** tab.

4 To the right of **Assay/Time/Point** there are six entries:

- The first entry displays the assay type selected.
- The second entry displays the reaction time in minutes.
- The third through sixth entries display chosen measuring points.

■

In the following sections, the entries for the **Assay/Time/Point** text boxes on **Utility** > **Application** > **Analyze** are shown as follows:

Assay/Time/Point: [Assay Type] [time] [mp_1] [mp_2] [mp_3] [mp_4]

Comprehensive assay descriptions

In the following section one example of an endpoint assay and one example of a rate assay is given, along with detailed explanations of the application parameters and result calculations.

For extended programming and calculation examples, see:
Example of a 2 Point End assay on page B-12
Example of a Rate A assay on page B-16

Example of a 2 Point End assay

A 2 Point End assay is an endpoint assay with sample blank measurement and can be programmed for two or more reagents. 2 Point means there are readings at two measuring points, mp_1 and mp_2:

- mp_1 is the sample blank which is measured before or shortly after the final reagent is added.
- mp_2 measures the absorbance of the final reaction product; it is set after addition of final reagent and after the reaction is completed.

2 Point End assay graph

A graphic representation of a 2 Point End assay using reagents dispensed at R1 and R2 timing is shown below.

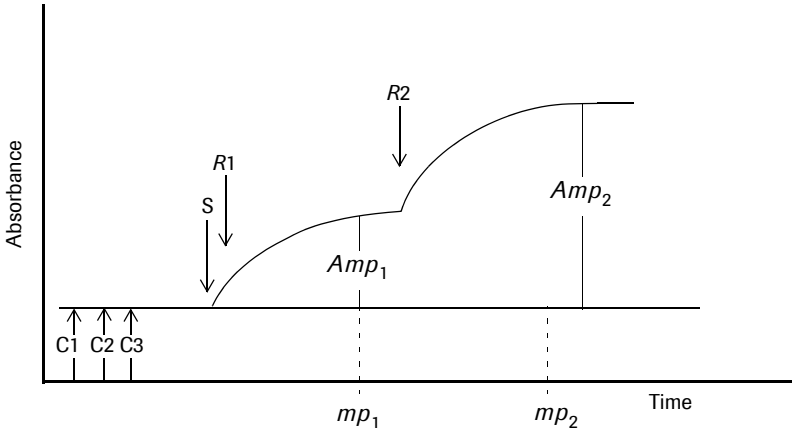


Figure B-3 2 Point End assay graph

C1, C2, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
R1	Pipetting of reagent at R1 timing
R2	Pipetting of reagent at R2 timing
mp_1	1st photometric measuring point (sample blank)
mp_2	2nd photometric measuring point (endpoint)
Amp_1 , Amp_2	Absorbances at measuring point 1 and measuring point 2

(a) See *Cell Blank Measurements report* on page B-21.

Example data

The following data from the **Utility** > **Application** screen are used for this example:

Test	Gluc5
Assay type	2 Point End
Time	5 min
Points	10.34
2nd wavelength	415 nm
Primary wavelength	340 nm

Entries on Utility > Application > Analyze

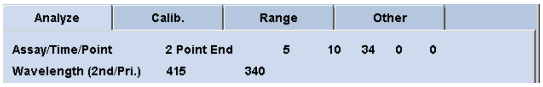


Figure B-4 Entries on **Utility** > **Application** > **Analyze**

In the later sections, the entries for the **Assay/Time/Point** text boxes on **Utility** > **Application** > **Analyze** are shown as follows:

Assay/Time/Point: [2 Point End] [5] [10] [34] [0] [0]

This means:

- The assay type is 2 Point End.
- The reaction time is 5 minutes.
- The sample blank absorbance (sample plus first reagent) is determined by the 10th photometer measurement of the respective reaction cell.
- The absorbance of the sample plus first and second reagents is determined by the 34th photometer measurement of the respective reaction cell.

Dilution factor

After the mixture of sample and R1 reagent is measured as sample blank, it is diluted by the addition of R2 reagent. Therefore, the readings cannot be subtracted, unless a correction for the dilution is taken into account. A dilution factor (d) is calculated as follows and applied to the sample + R1 absorbance:

Equation B-2

$$d = \frac{V_{samp} + V_{R1}}{V_{samp} + V_{R1} + V_{R2}}$$

$$d = \frac{2\mu L + 150\mu L}{2\mu L + 150\mu L + 50\mu L} = \frac{152}{202} = 0{,}7525$$

d	Dilution factor
V_{samp}	Sample volume
V_{R1}	R1 volume
V_{R2}	R2 volume

Reaction monitor

The two measuring points for this assay's calculation are set at the 10th and 34th photometer measurements; the first is the sample blank reading, the second is the final absorbance reading (endpoint), as indicated in the **Reaction Monitor** window below.

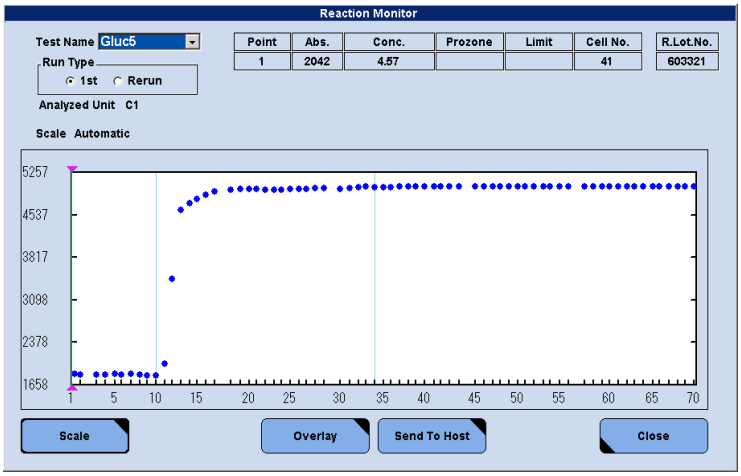


Figure B-5 **Reaction Monitor** window of a 2 Point End assay

You can move the focus from one measuring point to the next using the scroll bar below the graph. The absorbance at the measuring point that has the focus is displayed in the Abs. field above the graph. Alternatively, the absorbance values of all measuring points are listed on the **Reaction Monitor** report also:

```
                                Reaction Monitor                    19/01/04     15:03

Ser/Pl    N002050   00020-1          19/01/04       CELL 041      Gluc5      4.57
ID                                   14:30:55

      ***    (PRIMARY) - (SECONDARY)    ***
    CB1-3      1-10      11-20      21-30      31-40
     1532      2042       2160       5087       5091
     1532      1989       3551       5087       5088
     1534      1859       4603       5085       5093
               1844       4940       5085       5088
               1832       5028       5090
               1832       5070       5088
               1826       5083       5087
               1827       5088       5088
               1822       5089       5090
               1823       5091       5087
```

Figure B-6 Reaction Monitor report

The values on the **Reaction Monitor** report (as well as those in the **Abs.** field on the **Reaction Monitor** window) are absorbance × 10^4. Moreover, these values are already corrected for the water blank value, determined during the cell blank measurement.

See *Cell Blank Measurements report* on page B-21.

The real time water blank values displayed in the **CB1-3** column of the **Reaction Monitor** report serve to verify the integrity of the reaction cell immediately before sampling.

Reaction absorbance

To determine the reaction absorbance A_x, the sample blank value is corrected for dilution and then subtracted from the endpoint absorbance:

Equation B-3

$$A_x = Amp_{34} - d \cdot Amp_{10}$$

$$A_x = 0{,}5088 - 0{,}7525 \cdot 0{,}1823$$

$$A_x = 0{,}5088 - 0{,}1372 = 0{,}3716$$

The absorbance used in calculations (A_x) is 0.3716.

Calculation of concentration

The calculation of the unknown concentration of the analyte in the sample uses the following endpoint reaction formula:

Equation B-4

$$C_x = [K(A_x - A_b) + C_b] \cdot IF_A + IF_B$$

C_x	Concentration of the analyte (Gluc) in the sample
K	Calibration factor (also referred to as K factor)
A_x	Absorbance after reaction is completed (calculated above: 0.3716)
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
C_b	Concentration value for Std (1)/blank calibrator
IF_A, IF_B	Instrument constants representing a slope of 1 and an intercept of 0

K and A_b are displayed on the **Working Information** window. Select **Calibration** > **Status** > **Calibration Result** > **Working Information** to display this window.

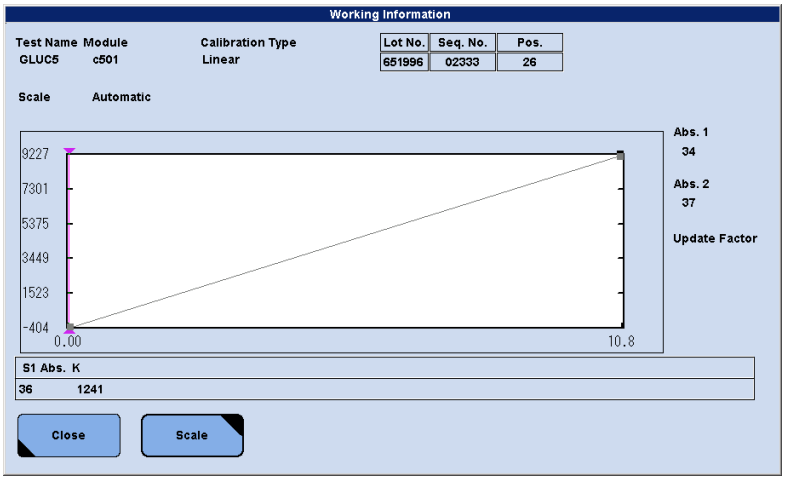


Figure B-7 **Working Information** window

When the test's concentration value for Std (1) is programmed with a decimal, the displayed K factor includes an extra digit for each number to the right of the decimal point.

A_b is the absorbance of the first standard solution, Std (1), which is a blank calibrator. This value is also displayed on the **Working Information** window in the **S1 Abs.** field.

See *Working Information window* on page B-22.

C_b, the concentration of the analyte in the first standard solution Std (1), is displayed on the **Others** tab of the **Utility** > **Application** screen. This C_b value controls the number of digits of the displayed K and the rounding of the final results. When the test's C_b value is programmed with a decimal, K includes an extra digit for each number to the right of the decimal point.

See *Others tab* on page B-23.

Example values The following values are used for this example:

K	12.41 (displayed as 1241 due to a Std (1) concentration value of 0.00)
A_x	0.3716 (calculated above)
A_b	0.0036 (displayed as 36 in the S1 Abs. field due to factor 10^4)
C_b	0.00
IF_A, IF_B	Instrument constants representing a slope of 1 and an intercept of 0

Applying these values to the above formula $C_x = [K(A_x - A_b) + C_b] \cdot IF_A + IF_B$ yields:

$C_x = 12{,}41(0{,}3716 - 0{,}0036) + 0{,}0$
$C_x = 12{,}41(0{,}3680)$
$C_x = 4{,}567$

The result is rounded to 4.57 on the report because the concentration value for Std (1), the blank calibrator, contains two zeros to the right of the decimal as displayed on **Utility** > **Application** > **Others**.

Example of a Rate A assay

For rate assays, the time course of the reaction is followed by measuring the absorbance as a function of time. That is, measurements are taken as the reaction proceeds. Rate assays use these measurements because their concentration calculations are based on the determination of the rate of change in absorbance, v:

Equation B-5 $$v_x = \left|\frac{dA_x}{dt}\right|$$

A Rate A assay is programmed for multiple measuring points. This means, there is a measuring window and every photometric measurement within this window is taken into account for the rate calculation—beginning with the reading at the first programmed measuring point ($mp_{initial}$) through the reading at the second programmed measuring point (mp_{final}).

The absorbance values are converted into the rate of change in absorbance (v) by least squares analysis. There is no need for a dilution factor because all readings are taken after the addition of the last reagent.

Rate A assay graph

A graphic representation of a Rate A assay using a reagent dispensed at R1 and R2 or R3 timing is shown below.

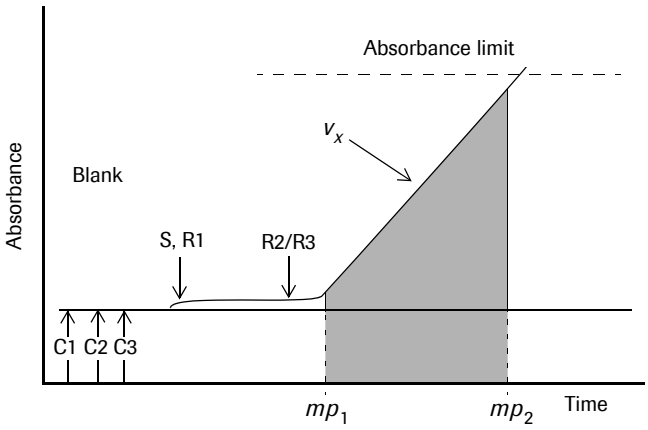


Figure B-8 Rate A assay - reagents at R1 and R2 or R3 timing

C1, C2, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
R1	Pipetting of reagent at R1 timing
R2/R3	Pipetting of reagent at R2/R3 timing
v_x	Rate of change in absorbance (slope) between mp_1 and mp_2
mp_1	First photometric measuring point
mp_2	Last photometric measuring point

(a) See *Cell Blank Measurements report* on page B-21.

Example data

The following data from **Utility** > **Application** screen are used for this example:

Test	AST
Assay	Rate A
Time	10 min
Points	18, 46
2nd wavelength	700 nm
Primary wavelength	340 nm
Conc. value for Std (1)	0.0

Entries on Utility > Application > Analyze

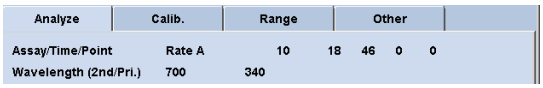


Figure B-9 Entries on Utility > Application > Analyze

In the later sections of this chapter, the entries for the **Assay/Time/Point** text boxes on **Utility** > **Application** > **Analyze** are shown as follows:

Assay/Time/Point: [Rate A] [10] [18] [46] [0] [0]

This means:

- The assay type is Rate A.
- The reaction time is 10 minutes.
- The initial absorbance reading is the 18th photometer measurement of the respective reaction cell.
- The final absorbance reading is the 46th photometer measurement of the respective reaction cell.

Reaction monitor

The rate of change in absorbance is calculated by least squares analysis of the absorbance values measured within the measuring window, as indicated in the reaction monitor below:

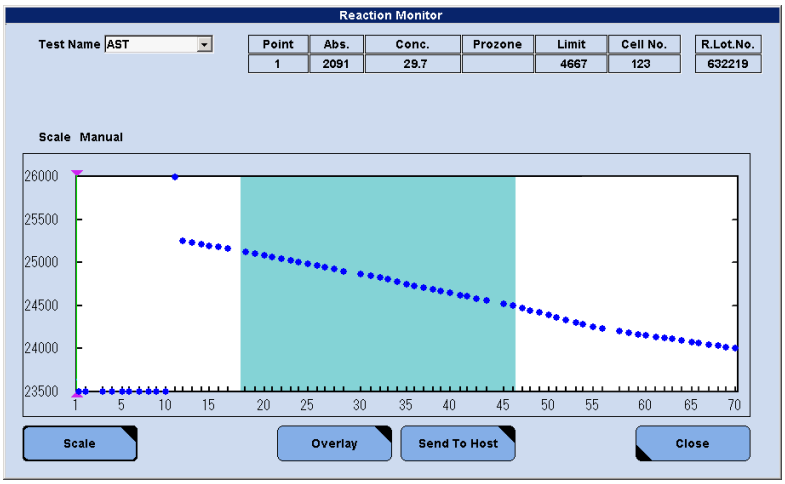


Figure B-10 **Reaction Monitor** window of a Rate A assay

The values on the reaction monitor report are reaction absorbance $\times 10^4$. Moreover, these values are already corrected for the water blank value determined during the cell blank measurement.

See *Cell Blank Measurements report* on page B-21.

The absorbance values measured between the initial and the final absorbance reading (mp_{18} through mp_{46}) represent a change over 4.04 minutes. The mathematical analysis results in a rate of change in absorbance of -0.01575 per minute.

Reaction Monitor 19/01/04 15:02

Ser/Pl N002050 00020-1 19/01/04 20 CELL 123 AST 29.7
ID 14:30:43

*** (PRIMARY)-(SECONDARY) ***

CB1-3	1-10	11-20	21-30	31-40	41-50	51-60	61-70
1535	2091	26660	25078	24830	24629	24401	24183
1535	2051	25264	25051	24814	24609	24381	24161
1534	2091	25243	25028	24796	24589	24362	24143
	2088	25236	25003	24766	24562	24340	24125
	2088	25226	24977	24746	24525	24320	24107
	2088	25193	24958	24742	24499	24298	24075
	2081	25170	24935	24703	24480	24259	24061
	2081	25132	24921	24676	24463	24238	24049
	2080	25117	24898	24660	24443	24206	24020
	2079	25094	24862	24639	24419	24194	24007

Figure B-11 Reaction Monitor report

Result calculation

The calculation of the unknown concentration of the analyte in the sample uses the following rate reaction formula:

Equation B-6 $C_x = [K(v_x - v_b) + C_b] \cdot IF_A + IF_B$

K	Calibration factor
v_x	Rate of change in absorbance (expressed in 10^4/min)
v_b	Rate of change in absorbance of the reaction with Std (1)/blank calibrator
C_b	Concentration value for Std (1)/blank calibrator
C_x	Concentration of the analyte (AST) in the sample
IF_A, IF_B	Instrument constants for a slope of 1 and an intercept of 0

K and v_b are displayed on the **Working Information** window. Select **Calibration** > **Status** > **Calibration Result** > **Working Information** to display this window.

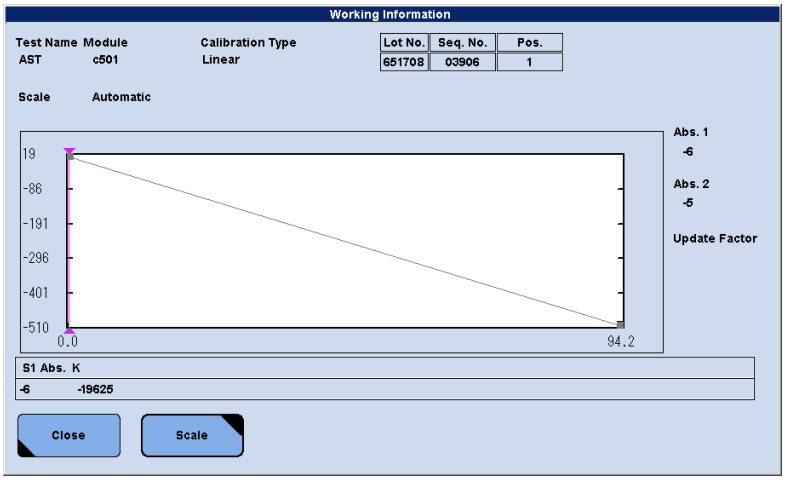


Figure B-12 Working Information window

When the test's concentration value for Std (1) is programmed with a decimal, the displayed K factor includes an extra digit for each number to the right of the decimal point. v_b is displayed in the **S1 Abs.** field of the **Working Information** window.

See *Working Information window* on page B-22.

C_b, the concentration of the analyte in the first standard solution, Std (1), is displayed on the **Others** tab of the **Utility** > **Application** screen.

See *Others tab* on page B-23.

Example values

The following values are used for this example:

K	-1962.5 (displayed as -19625 due to a Std (1) concentration value of 0.0)
v_x	-0.01575 (calculated by least squares method)
v_b	-0.0006 (displayed as -6 in the **S1 Abs.** field due to factor 10^4)
C_b	0.0
IF_A, IF_B	Instrument constants for a slope of 1 and an intercept of 0

Applying these values to the rate reaction formula (Equation B-6) yields:

$$C_x = \{-1962{,}5 \cdot [-0{,}01575 - (-0{,}0006)] + 0{,}0\} \cdot 1 + 0$$
$$C_x = -1962{,}5 \cdot (-0{,}01515)$$
$$C_x = 29{,}73$$

The result is rounded to 29.7 on the report because the concentration value for Std (1), the blank calibrator, contains one zero to the right of the decimal as displayed on **Utility** > **Application** > **Others**.

Reaction cell and calibration data

The following three sections are frequently referred to in other parts of this document which describe result calculations of the various types of assays:

Both the **Working Information** window and the **Others** tab of the **Utility** > **Application** screen display calibration information for individual tests and calibrators, respectively. The **Cell Blank Measurements** report contains data necessary for the calculation of absorbance values, which are the basis for all other calculations.

For more information, see:
Cell Blank Measurements report on page B-21
Working Information window on page B-22
Others tab on page B-23

Cell Blank Measurements report

Reaction absorbance in a cell is measured against the cell's water blank value (current cell blank). This cell blank report is requested as part of weekly maintenance. The values on this report are stored and compared to the real time water blank values that display on the **Reaction Monitor** report.

See *Reaction monitor* on page B-14.

If the difference between the current real time water blank values and the previous cell blank measured by the cell blank maintenance function is greater than 0.1 Abs, an alarm is issued.

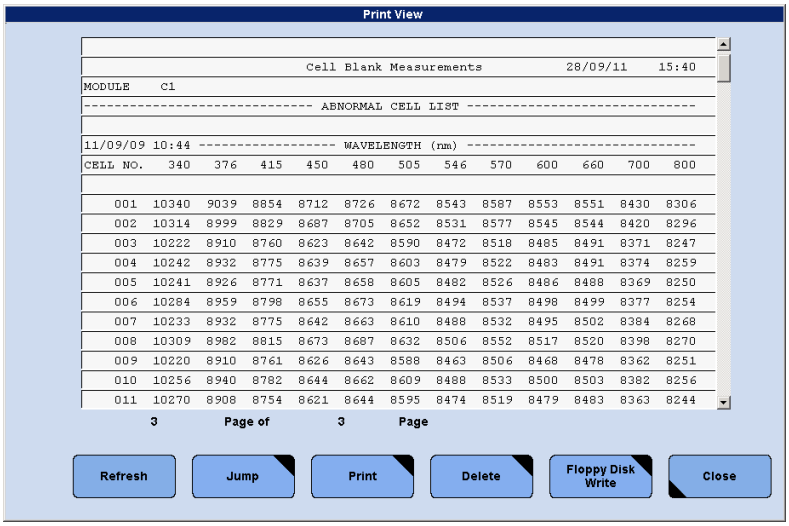


Figure B-13 Example of a **Cell Blank Measurements** report

Working Information window

➔ **Calibration** > **Status** > **Calibration Result** > **Working Information**

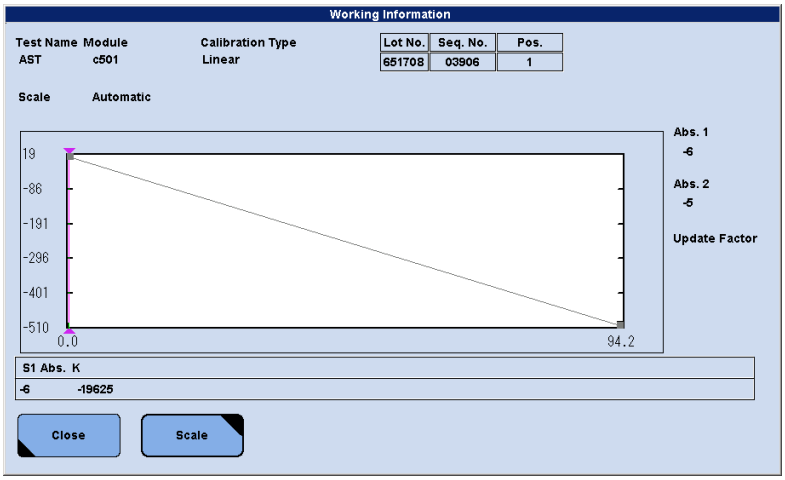


Figure B-14 **Working Information** window

S1 Abs.

The **Working Information** window displays the current calibration curve and values for the application selected under **Calibration** > **Status** > **Calibration Result**. For endpoint assays based on an RCM or Linear calibration, the value under S1 Abs. equals the blank calibrator's absorbance value × 10^4. For rate assays it is the rate of change in absorbance of the reaction with the blank calibrator.
S1 Abs. is subtracted from the reaction absorbance of all other samples including calibrators Std (2) through Std (6), controls, STAT and routine samples.

K factor

The K factor—as well as S1 Abs.—is used in the result calculation of every measured test. Given a linear calibration curve, the two main types of assays use the following formulas for result calculation:

Equation B-7 $C_x = K \cdot (A_x - A_b) + C_b$ for endpoint assays

Equation B-8 $C_x = K \cdot (v_x - v_b) + C_b$ for rate assays

K	Calibration factor
A_x	Absorbance after reaction is completed
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
C_b	Concentration value for Std (1)/blank calibrator
v_x	Rate of change in absorbance of the reaction with the sample
v_b	Rate of change in absorbance of the reaction with Std (1)/blank calibrator

On the **Working Information** window, K factors are always displayed as whole numbers. The correct decimal placement in a K factor depends on the decimal places in the concentration value for Std (1) displayed on the **Others** tab of the **Utility** > **Application** screen. If the Std (1) concentration has *n* decimal places, divide the displayed K factor by the *n*-th power of ten to obtain the correct value for result calculations.

Others tab

➔ **Utility > Application > Others**

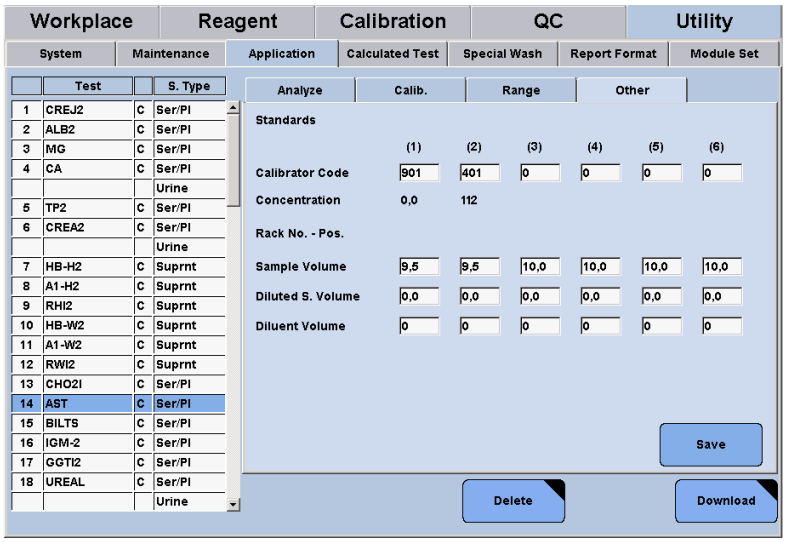


Figure B-15 Others tab on Utility > Application screen

Use this tab to display test parameters such as calibrator codes, calibrator set points, calibrator positions, and pipetting volumes.

When the test's Std (1) concentration (blank calibrator concentration) is programmed with a decimal, the displayed K factor on the **Working Information** window gets the same number of decimal places. This also determines the decimal placement in displayed results, as shown in the table below:

Std (1) concentration	K (posted)	K (calculations)	Result
0	-1219	-1219	52
0.0	-12190	-1219.0	52.3
0.00	-121904	-1219.04	52.31

Table B-2 Determination of decimal placement

Endpoint assays

In the following sections the various types of endpoint assays are explained in detail. After a brief listing of assay characteristics, a graphical representation of the absorbance in the course of the reaction is given, as well as an example of result calculation.

For details on the various types of endpoint assays, see:
1 Point assay on page B-24
2 Point End assay on page B-27

1 Point assay

Assay characteristics:

- Called 1 Point because only one measuring point is designated in the **Application** screen.
- Addition of one or more reagents is possible.
- No sample blanking required.
- The absorbance reading for this type of assay can be taken during any disk rotation after addition of the final reagent.

Entries on Utility > Application > Analyze

Assay/Time/Point: [1 Point] [time] [mp_1] [0] [0] [0]

***c** 501*

$1 \leq mp_1 \leq 70$

$1 \leq \text{time} \leq 10$

Cell blank = (C1 + C2 + C3) / 3

Reaction volume = 100-250 µL

1 Point assay graph

1 Point assay with R1 timing

A graphic representation of a 1 Point assay using a reagent dispensed at R1 timing is shown below. The figure below shows an increase in absorbance as the reaction occurs. A decrease in absorbance as the reaction occurs is also possible.

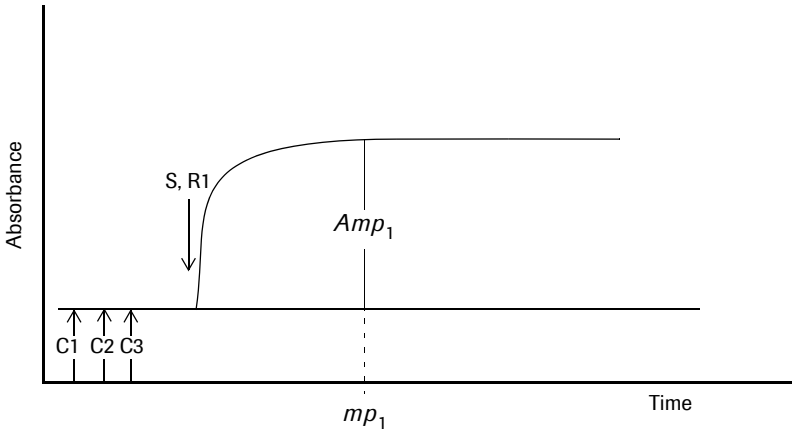


Figure B-16 1 Point End assay - reagent at R1 timing

1 Point assay with R1 and R2 or R3 timing

A graphic representation of a 1 Point assay using reagents dispensed at R1 and R2 or R3 timing is shown below.

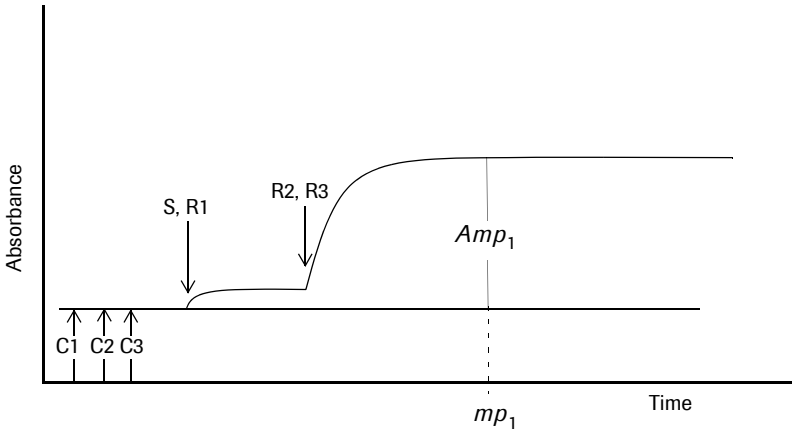


Figure B-17 1 Point End assay - reagents at R1 and R3 timing

*C*1 , *C*2 , ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
*R*1	Pipetting of reagent at R1 timing
*R*2 , *R*3	Pipetting of reagent at R2 or R3 timing
mp_1	Measuring point 1, endpoint (after reaction has reached equilibrium)
Amp_1	Absorbance at measuring point 1

(a) See *Cell Blank Measurements report* on page B-21.

Sample program and calculations

This section provides an example of an application's result calculations.

For more detailed explanations, see *Comprehensive assay descriptions* on page B-12.

Entries on Utility > Application > Analyze

The following data from **Utility** > **Application** are used for this calculation example:

Test	CHOL2
Assay/Time/Point	[1 Point] [10] [70] [0] [0] [0]

Reaction Monitor 04/11/16 09:25

Ser/Pl N002043 00076-1 04/11/16 C CELL 001 CHOL2 4.92
ID 010 09:08:37

*** (PRIMARY)-(SECONDARY) ***

CB1-3	1-10	11-20	21-30	31-40	41-50	51-60	61-70
49	1515	4689	4696	4699	4697	4691	4687
47	2615	4671	4699	4700	4697	4690	4689
46	4405	4677	4699	4701	4698	4695	4686
	4555	4682	4700	4702	4695	4692	4686
	4603	4686	4697	4701	4694	4692	4688
	4624	4688	4698	4696	4697	4694	4687
	4649	4692	4702	4702	4694	4689	4686
	4666	4692	4699	4699	4695	4689	4686
	4672	4696	4700	4698	4695	4690	4685
	4685	4693	4700	4700	4694	4690	4686

Figure B-18 **Reaction Monitor** report

Calculation of concentration

The calculation of the concentration of the analyte in the sample uses the following equation:

Equation B-9 $$C_x = [K(A_x - A_b) + C_b] \cdot IF_A + IF_B$$

Symbol	Definition	Value
A_x	Absorbance value for concentration calculation [a]	0.4686
C_x	Concentration of the analyte in the sample	
K	Calibration factor[b]	14.06
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)[b]	0.1188
C_b	Concentration value for Std (1)/blank calibrator[c]	0.00
IF_A, IF_B	Instrument constants for a slope of 1 and an intercept of 0	1.0

Table B-3 Definitions and values for quantities used in the calculation

(a) See Reaction Monitor report above.

(b) Displayed on Working Information window. For explanations, see *Working Information window* on page B-22.

(c) Displayed on Utility > Application > Others. For explanations, see *Others tab* on page B-23.

Applying these values to the above formulas (Equation B-9) yields:

$C_x = 14{,}06 \cdot (0{,}4686 - 0{,}1188) + 0{,}00 = 14{,}06 \cdot 0{,}3498$

$C_x = 4{,}918$ (4.92 on reports and the Data Review screen)

2 Point End assay

Assay Characteristics:

- Called 2 Point because there are readings at two measure points, mp_1 and mp_2, which are designated on **Utility** > **Application** > **Analyze**.
- Allows for two or more reagent additions.
- Performs sample blank measurement.
- The first absorbance reading for this type of assay can be taken during any disk rotation. Usually it is taken before or shortly after the final reagent is added.
- The second absorbance reading can be taken during any disk rotation after the final reagent is added.

Entries on Utility > Application > Analyze

Assay/Time/Point: [2 Point End] [time] [mp_1] [mp_2] [0] [0]

***c** 501*

$1 \leq mp_1 < mp_2 \leq 70$
$1 \leq \text{time} \leq 10$

Cell blank = (C1 + C2 + C3) / 3

Reaction volume = 100-250 µL (at all measuring points)

2 Point End assay graph

A graphic representation of a 2 Point End assay using reagents dispensed at R1 and R2 or R3 timing is shown below.

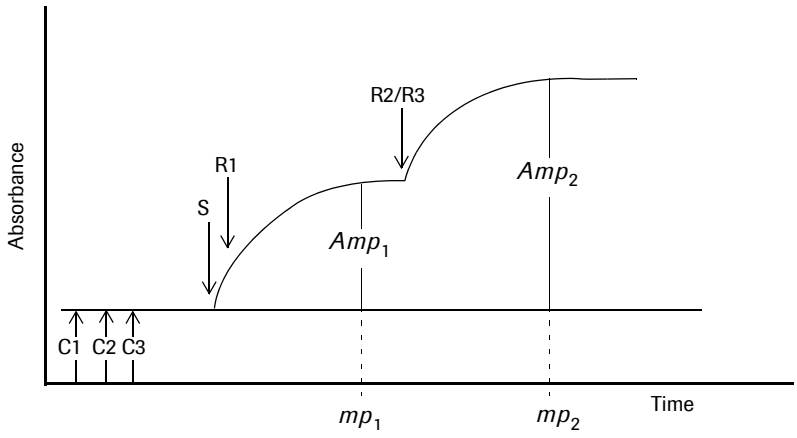


Figure B-19 2 Point End assay - reagents at R1 and R2 or R3 timing

$C1, C2, \ldots$	The reaction cell's water blank values[(a)]
S	Pipetting of sample
$R1, R2/R3$	Pipetting of reagent at R1 timing and of reagent at R2 or R3 timing
mp_1	Measuring point 1, sample blank (here before final reagent addition)
mp_2	Measuring point 2, endpoint (after reaction has reached equilibrium)
Amp_1, Amp_2	Absorbances at measuring point 1 and measuring point 2

(a) See *Cell Blank Measurements report* on page B-21.

Sample program and calculations

This section provides an example of an application's result calculations.

For more detailed explanations, see *Comprehensive assay descriptions* on page B-12.

The following data from the **Utility** > **Application** screen are used for this example:

Test	Gluc5
Assay/Time/Point	[2 Point End] [5] [10] [34] [0] [0]

The result calculation is based on a calculated value for the absorbance of the final reaction product A_x. To determine this value the sample blank reading is corrected for dilution and subtracted:

Equation B-10 $A_x = Amp_2 - d \cdot Amp_1$ with

$$d = \frac{V_{samp} + V_{R1}}{V_{samp} + V_{R1} + V_{R2}}$$

Reaction Monitor 19/01/04 15:03

Ser/Pl N002050 00020-1 19/01/04 CELL 041 Gluc5 4.57
ID 14:30:55

***	(PRIMARY) - (SECONDARY)			***
CB1-3	1-10	11-20	21-30	31-40
1532	2042	2160	5087	5091
1532	1989	3551	5087	5088
1534	1859	4603	5085	5093
	1844	4940	5085	5088
	1832	5028	5090	
	1832	5070	5088	
	1826	5083	5087	
	1827	5088	5088	
	1822	5089	5090	
	1823	5091	5087	

Figure B-20 **Reaction Monitor** report

Assuming absorbance values on the reaction monitor report are the following:

Symbol	Definition	Value
A_x	Absorbance value for concentration calculation	
Amp_2	Absorbance at measuring point 2 (34th measurement of cell)[(a)]	0.5088
Amp_1	Absorbance at measuring point 1 (10th measurement of cell)[(a)]	0.1823
d	Dilution factor	
V_{samp}	Sample volume	2 µL
V_{R1}	Volume of reagent R1	150 µL
V_{R2}	Volume of reagent R2	50 µL

Table B-4 Definitions and values for quantities used in the calculation

(a) See Reaction Monitor report above.

The absorbance at measuring point 1 is multiplied by the following to correct for dilution:

$$d = \frac{(2\mu L + 150\mu L)}{(2\mu L + 150\mu L + 50\mu L)} = \frac{152}{202} = 0{,}7525$$

Therefore:

$$A_x = 0{,}5088 - 0{,}7525 \cdot 0{,}1823$$
$$A_x = 0{,}5088 - 0{,}1372 = 0{,}3716$$

Calculation of concentration

The calculation of the concentration of the analyte in the sample uses the following equation:

Equation B-11 $C_x = [K(A_x - A_b) + C_b] \cdot IF_A + IF_B$

Symbol	Definition	Value
C_x	Concentration of the analyte in the sample	
K	Calibration factor[(a)]	12.41
A_x	Absorbance value calculated above	0.3716
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)[(a)]	0.0036
C_b	Concentration value for Std (1)/blank calibrator[(b)]	0.00
IF_A , IF_B	Instrument constants for a slope of 1 and an intercept of 0	1.0

Table B-5 Definitions and values for quantities used in the calculation

(a) Displayed on **Working Information** window. For explanations, see *Working Information window* on page B-22.

(b) Displayed on **Utility** > **Application** > **Others**. For explanations, see *Others tab* on page B-23.

Applying these values to the above formula (Equation B-11) yields:

$$C_x = 12{,}41(0{,}3716 - 0{,}0036) + 0{,}00$$
$$C_x = 12{,}41 \cdot 0{,}3680$$
$$C_x = 4{,}567$$ (4.57 on reports and the **Data Review** screen)

Rate assays

The following sections explain in detail the various types of rate assays. After a brief listing of assay characteristics, a graphical representation of the absorbance in the course of the reaction is given, as well as an example of result calculation.

For details on the various types of rate assays, see:
Rate A assay on page B-30
Rate A assay with sample blank correction on page B-33
2 Point Rate assay on page B-36

Rate A assay

Assay Characteristics:

- One or more reagent additions are possible.
- Rate of change in absorbance is calculated by least squares method.
- Substrate depletion is monitored for linearity.

Entries on Utility > Application > Analyze

Assay/Time/Point: [Rate A] [time] [mp_1] [mp_2] [0] [0]

***c** 501*

$1 \leq mp_1 < mp_2 \leq 70$; $mp_1 + 2 < mp_2$; $1 \leq \text{time} \leq 10$

Cell blank = (C1 + C2 + C3) / 3

Reaction volume = 100-250 µL

Rate A assay graph

A graphic representation of a Rate A assay using a reagent dispensed at R1 is shown below.

Rate A assay with R1 timing

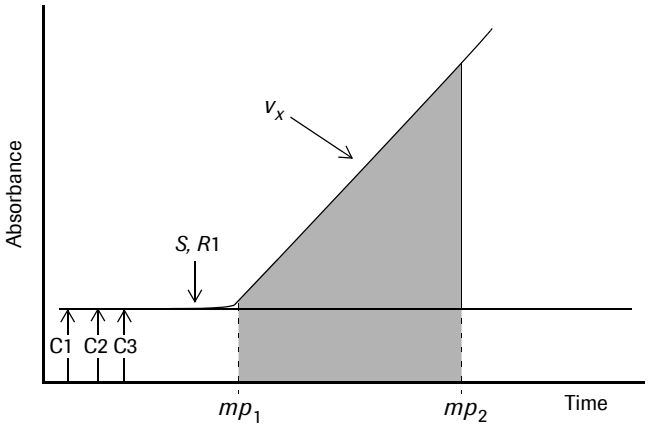


Figure B-21 Rate A assay - reagent at R1 timing

Rate A assay with R1 and R2 or R3 timing

A graphic representation of a Rate A assay using reagents dispensed at R1 and R2 or R3 timing is shown below.

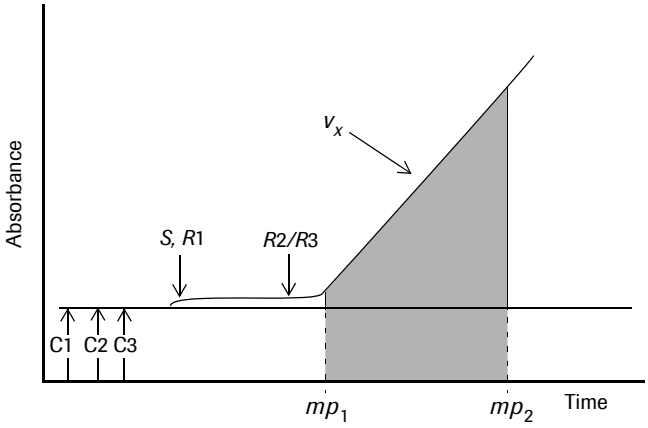


Figure B-22 Rate A assay - reagents at R1 and R2 or R3 timing

$C1$, $C2$, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
$R1$	Pipetting of reagent at R1 timing
$R2$, $R3$	Pipetting of reagent at R2 or R3 timing
v_x	Rate of change in absorbance (slope) between mp_1 and mp_2
mp_1	Measuring point 1 (initial measuring point)
mp_2	Measuring point 2 (final measuring point)

(a) See *Cell Blank Measurements report* on page B-21.

Sample program and calculations

This section provides an example of an application's result calculations.

For more detailed explanations, see *Comprehensive assay descriptions* on page B-12.

The following data from the **Utility** > **Application** screen are used for this example:

Test	AST
Assay/Time/Point	[Rate A] [10] [18] [46] [0] [0]

```
                                  Reaction Monitor                    19/01/04      15:02

Ser/Pl      N002050     00020-1      19/01/04        20 CELL 123          AST      29.7
ID                                   14:30:43

   ***      (PRIMARY)-(SECONDARY)      ***
  CB1-3        1-10        11-20       21-30       31-40       41-50       51-60       61-70
     1535      2091        26660       25078       24830       24629       24401       24183
     1535      2051        25264       25051       24814       24609       24381       24161
     1534      2091        25243       25028       24796       24589       24362       24143
               2088        25236       25003       24766       24562       24340       24125
               2088        25226       24977       24746       24525       24320       24107
               2088        25193       24958       24742       24499       24298       24075
               2081        25170       24935       24703       24480       24259       24061
               2081        25132       24921       24676       24463       24238       24049
               2080        25117       24898       24660       24443       24206       24020
               2079        25094       24862       24639       24419       24194       24007
```

Figure B-23 **Reaction Monitor** report

Calculation of concentration

The calculation of the unknown concentration of the analyte in the sample uses the following equation:

Equation B-12 $C_x = [K(v_x - v_b) + C_b] \cdot IF_A + IF_B$ with

$v_x = v(mp_2,mp_1)$

Symbol	Definition	Value
v_x	Rate of change in absorbance of the reaction with the sample	
$v(mp_2,mp_1)$	Rate of change in absorbance between mp_1 (18th measurement of cell) and mp_2 (46th measurement)[(a)]	
C_x	Concentration of the analyte in the sample	
K	Calibration factor[(b)]	-1962.5
v_x	Rate of change in absorbance of the reaction with the sample	-0.01575
v_b	Rate of change in absorbance of the reaction with Std (1)/blank calibrator[(b)]	-0.0006
C_b	Concentration value for Std (1)/blank calibrator[(c)]	0.0
IF_A, IF_B	Instrument constants representing a slope of 1 and an intercept of 0	1.0

Table B-6 Definitions and values for quantities used in the calculation

(a) See Reaction Monitor report above.

(b) Displayed on Working Information window. For explanations, see *Working Information window* on page B-22.

(c) Displayed on Utility > Application > Others. For explanations, see *Others tab* on page B-23.

Applying these values to the above formula (Equation B-12) yields:

$C_x = -1962{,}5 \cdot (-0{,}01575 + 0{,}0006) + 0{,}0$

$C_x = -1962{,}5 \cdot (-0{,}01515)$

$C_x = 29{,}73$ (29.7 on reports and the Data Review screen)

Rate A assay with sample blank correction

Assay Characteristics:

- Assay with sample blank measurement.
- One or more reagent additions are possible.
- Rate of change in absorbance is calculated by least squares method.
- Substrate depletion is monitored for linearity.

Entries on Utility > Application > Analyze

Assay/Time/Point: [Rate A] [time] [mp_1] [mp_2] [mp_3] [mp_4]

***c** 501*

$1 \leq mp_3 < mp_4 < mp_1 < mp_2 \leq 70$
$(mp_3 + 2) < mp_4$; $(mp_1 + 2) < mp_2$
$1 \leq$ time ≤ 10
Cell blank = (C1 + C2 + C3) / 3
Reaction volume = 100-250 µL (at all measure points)

Rate A assay with sample blank graph

A graphic representation of a Rate A assay using a reagent dispensed at R1 and R3 timing is shown below.

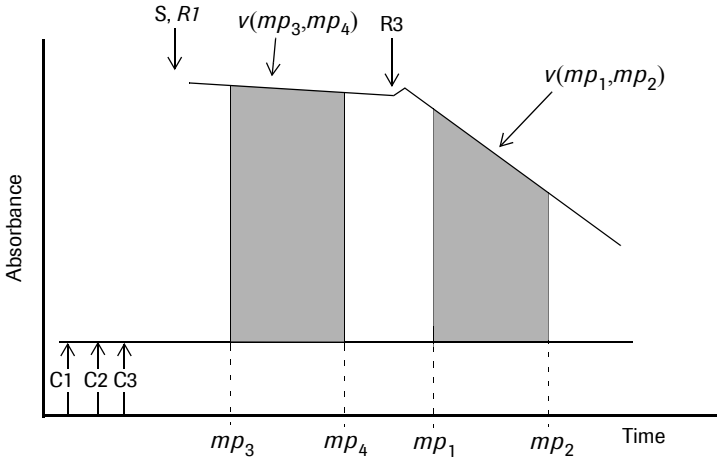


Figure B-24 Rate A assay with sample blank

$C1$, $C2$, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
$R1$, $R3$	Pipetting of reagent at R1 timing and of reagent at R3 timing
mp_1 , mp_2	Measuring point 1 and 2 (initial and final measuring points for rate reaction)
mp_3 , mp_4	Measuring point 3 and 4 (initial and final measuring points for sample blank)
$v(mp_1,mp_2)$	Rate of change in absorbance (slope) between mp_1 and mp_2
$v(mp_3,mp_4)$	Rate of change in absorbance (slope) between mp_3 and mp_4

(a) See *Cell Blank Measurements report* on page B-21.

Sample program and calculations

The following data from the **Utility** > **Application** screen are used for this example:

Test	CREAJ
Assay/Time/Points	[Rate A] [10] [42] [52] [24] [34]

Calculation of the rate of change in absorbance uses the following equation:

Equation B-13 $v_x = v(mp_2,mp_1) - d \cdot v(mp_4,mp_3)$ with

$$d = \frac{V_{samp} + V_{R1}}{V_{samp} + V_{R1} + V_{R2}}$$

Reaction Monitor 16/01/04 17:16

S21 S0815 00020-1 16/01/04 20 CELL 013 CREAJ 381
ID 14:00:28

***	(PRIMARY)-(SECONDARY)		***				
CB1-3	1-10	11-20	21-30	31-40	41-50	51-60	61-70
108	1309	1174	1148	1126	1871	2432	2849
106	1277	1169	1147	1121	1921	2474	2882
106	1202	1165	1142	1122	1986	2521	2909
	1196	1166	1141	1119	2044	2554	2942
	1188	1162	1140	1385	2147	2597	2971
	1184	1161	1136	1499	2201	2640	2996
	1178	1159	1136	1593	2254	2711	3027
	1174	1154	1133	1674	2296	2749	3055
	1173	1154	1133	1732	2345	2786	3078
	1172	1150	1125	1804	2394	2816	3107

Figure B-25 **Reaction Monitor** report

Symbol	Definition	Value
v_x	Rate of change in absorbance of the reaction with the sample	
$v(mp_3,mp_4)$	Rate of change in absorbance between mp_3 and mp_4	– 0.0015
$v(mp_1,mp_2)$	Rate of change in absorbance between mp_1 and mp_2	0.0371
d	Dilution factor	
V_{samp}	Sample volume	10 µL
V_{R1}	Reagent 1 volume	104 µL
V_{R2}	Reagent 2 volume	33 µL

Table B-7 Definitions and values for quantities used in the calculation

The rate of change in absorbance between 24th and 34th measurement of the reaction cell (sample blank) is multiplied by the following to correct for dilution:

$$d = \frac{10\mu L + 104\mu L}{10\mu L + 104\mu L + 33\mu L} = \frac{114}{147} = 0{,}7755$$

Therefore:

$$v_x = 0{,}0371 - 0{,}7755 \cdot (-0{,}0015) = 0{,}0383$$

Calculation of concentration

The calculation of the unknown concentration of the analyte in the sample uses the following equation:

Equation B–14 $C_x = [K(v_x - v_b) + C_b] \cdot IF_A + IF_B$

Symbol	Definition	Value
C_x	Concentration of the analyte in the sample	
K	Calibration factor[(a)]	9896
v_x	Rate of change in absorbance of the reaction with the sample (calculated above)	0.0383
v_b	Rate of change in absorbance of the reaction with Std (1)/blank calibrator[(a)]	– 0.0002
C_b	Concentration value for Std (1)/blank calibrator[(b)]	0
IF_A , IF_B	Instrument constants representing a slope of 1 and an intercept of 0	1.0

Table B–8 Definitions and values for quantities used in the calculation

(a) Displayed on **Working Information** window. For explanations, see *Working Information window* on page B-22.

(b) Displayed on **Utility** > **Application** > **Others**. For explanations, see *Others tab* on page B-23.

Applying these values to the above formula (Equation B-14) yields:

$C_x = 9896 \cdot [0{,}0383 - (-0{,}0002)] + 0$

$C_x = 9896 \cdot 0{,}0385$

$C_x = 381$ (381 on reports and the **Data Review** screen)

2 Point Rate assay

Assay Characteristics:

- Rate assay measures rate of change in absorbance.
- Called 2 Point because there are 2 measuring points (or duplicate readings at mp_1 and mp_2).
- The first absorbance reading for this type of assay can be taken during any disk rotation after the final reagent is added.
- This reaction is monitored for substrate depletion, but not for linearity.

Entries on Utility > Application > Analyze

Assay/Time/Point: [2 Point Rate] [time] [mp_1] [mp_2] [0] [0]

***c** 501*

$1 \leq mp_1 < mp_2 \leq 70$

$1 \leq \text{time} \leq 10$

Cell Blank = (C1 + C2 + C3) / 3

Reaction volume = 100-250 µL (at all measuring points)

2 Point Rate assay graph – R1 and R2 or R3 timing

A graphic representation of a 2 Point Rate assay using reagents dispensed at R1 and R2 or R3 timing is shown below.

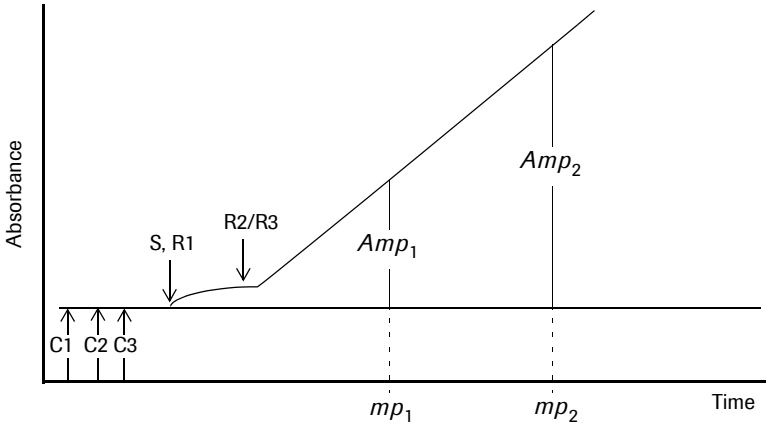


Figure B-26 2 Point Rate assay - reagents at R1 and R2 or R3 timing

$C1$, $C2$, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
$R1$	Pipetting of reagent at R1 timing
$R2$, $R3$	Pipetting of reagent at R2 or R3 timing
mp_1 , mp_2	Measuring point 1 and 2
Amp_1 , Amp_2	Absorbances at measuring point 1 and measuring point 2

(a) See *Cell Blank Measurements report* on page B-21.

Sample program and calculations

The following data from the **Utility** > **Application** screen are used for this example:

Test	CREAJ
Assay/Time/Point	[2 Point Rate] [10] [18] [29] [0] [0]

Reaction Monitor 04/11/16 13:33

Ser/Pl N 00076-1 04/11/16 C CELL 001 CREAJ 486.7
ID 010 12:55:01

***	(PRIMARY)-(SECONDARY)		***				
CB1-3	1-10	11-20	21-30	31-40	41-50	51-60	61-70
28	1370	1383	1923	2329	2604	2826	3005
27	1314	1407	1967	2365	2623	2847	3021
28	1227	1475	2011	2389	2646	2867	3029
	1218	1539	2045	2420	2672	2880	3042
	1213	1585	2088	2451	2714	2898	3059
	1207	1643	2128	2472	2734	2917	3070
	1204	1695	2156	2503	2757	2945	3080
	1201	1790	2193	2532	2772	2963	3095
	1200	1840	2232	2551	2793	2978	3103
	1198	1888	2298	2576	2815	2986	3116

Figure B-27 **Reaction Monitor** report

The result calculation is based on a calculated value for the rate of change in absorbance of the reaction mixture v_x. To determine this value, readings are subtracted and divided by the time between measuring points 1 and 2:

Equation B-15 $v_x = (Amp_2 - Amp_1)/t$

Symbol	Definition	Value
v_x	Rate of change in absorbance	
Amp_2	Absorbance at measuring point 2 (a)	0.2232
Amp_1	Absorbance at measuring point 1	0.1790
t	Time between mp_1 and mp_2	1.4917 min

Table B-9 Definitions and values for quantities used in the calculation

(a) See Reaction monitor above.

Applying these values to the above formula (Equation B-15) yields:

$v_x = (0{,}2232 - 0{,}1790)/1{,}4917 = 0{,}029631$

Calculation of concentration

The calculation of the unknown concentration of the analyte in the sample uses the following equation:

Equation B-16 $C_x = [K(v_x - v_b) + C_b] \cdot IF_A + IF_B$

Symbol	Definition	Value
C_x	Concentration of the analyte in the sample	
K	Calibration factor[(a)]	16479.6
v_x	Rate of change in absorbance of the reaction with the sample	0.029631
v_b	Rate of change in absorbance of the reaction with Std (1)/blank calibrator[(a)]	0.0001
C_b	Concentration value for Std (1)/blank calibrator[(b)]	0.0
IF_A , IF_B	Instrument constants for a slope of 1 and intercept of 0	1.0

Table B-10 Definitions and values for quantities used in the calculation

(a) Displayed on **Working Information window**. For explanations, see *Working Information window* on page B-22.

(b) Displayed on **Utility** > **Application** > **Others**. For explanations, see *Others tab* on page B-23.

Therefore:

$C_x = 16479{,}6 \cdot (0{,}029631 - 0{,}0001) + 0{,}0$
$C_x = 16479{,}6 \cdot 0{,}029531$
$C_x = 486{,}659$ (486.7 on reports and the **Data Review** screen)

Prozone check

There are two prozone check methods available:

- Antigen readdition method
- Reaction rate method

Both of these methods can be applied to any type of assay.

For more information, see:
Antigen readdition method on page B-39
Reaction rate method on page B-44

Antigen readdition method

Prozone checks applying the antigen readdition method compare the absorbance before and after a final reagent addition at R2 or R3 timing, as indicated below:

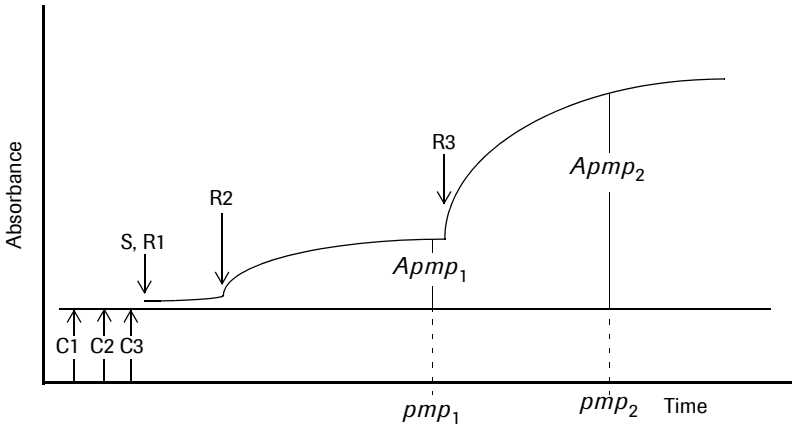


Figure B-28 Prozone check - antigen readdition method

$C1$, $C2$, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
$R1$, $R2$, $R3$	Pipetting of reagent at R1, R2, and R3 timing
pmp_1 , pmp_2	Prozone measuring points 1 and 2
$Apmp_1$,$Apmp_2$	Absorbance at pmp_1 and pmp_2

(a) See *Cell Blank Measurements report* on page B-21.

Settings and calculation

Program a prozone check on the **Analyze** tab of the **Utility** > **Application** screen according to the following description:

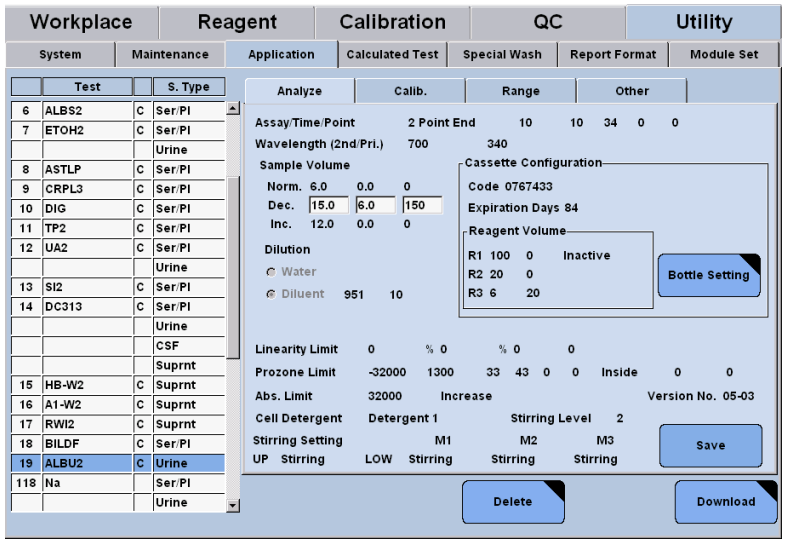


Figure B-29 Application parameters of an application with prozone check (antigen readdition method)

To the right of the **Prozone Limit** field there are nine boxes:

[lower limit] [upper limit] [pmp_1] [pmp_2] [0] [0] [comp.] [0] [0]

- The first two boxes indicate the lower and upper prozone limits (in Abs × 10^4).
- The next four boxes are for the prozone measuring points (*pmp*):
  - 3rd entry: First prozone measuring point (pmp_1)
  - 4th entry: Second prozone measuring point (pmp_2)
  - 5th entry: Set to zero for the antigen readdition method
  - 6th entry: Set to zero for the antigen readdition method

  Appropriate values are: $2 \leq pmp_1 < pmp_2 \leq 70$. If all entries are set to zero, prozone check is not performed.
- The seventh box (**Inside/Outside**) indicates in which case a data alarm (>*Proz*) is issued: If the entry is set to Inside, an alarm is issued in case the obtained check value lies *inside* the defined range between the lower and upper prozone limits (first two boxes).
  Vice versa, if the entry is set to **Outside**, an alarm is issued in case the obtained check value lies *outside* the defined range.
- The eighth and ninth boxes are not used (set to zero) for this method.

Prozone check value calculation

The calculation of the prozone check value uses the following equation:

Equation B-17

$$PC = Apmp_2 - d \cdot Apmp_1 \text{ with}$$

$$d = \frac{V_{samp} + V_{R1}}{V_{samp} + V_{R1} + V_{R2}}$$

PC	Prozone check value
$Apmp_2$	Absorbance at prozone measuring point 2
$Apmp_1$	Absorbance at prozone measuring point 1
d	Dilution factor
V_{samp}	Sample volume
V_{R1}	R1 volume
V_{R2}	R2 volume

Reaction graph in the Reaction Monitor window

This section shows the reaction graph after addition of extra antigen on the **Reaction Monitor** window (**Workplace** > **Data Review** > **Reaction Monitor**).

Increasing absorbance

Reaction graph with absorbance increase after addition of extra antigen.

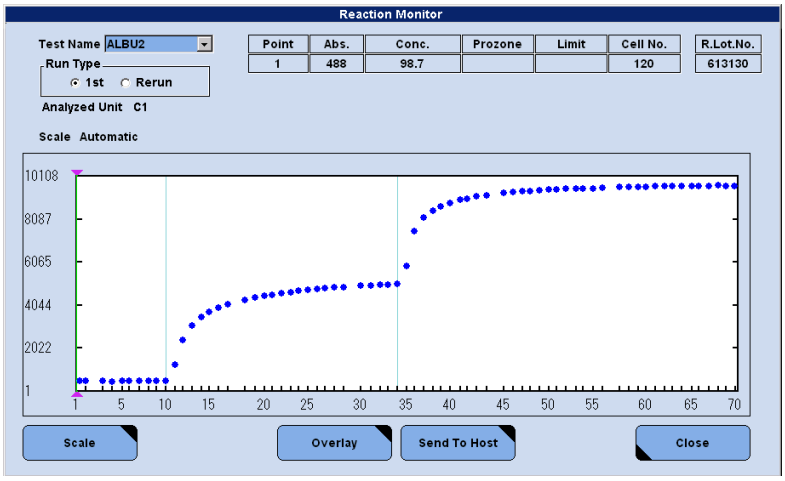
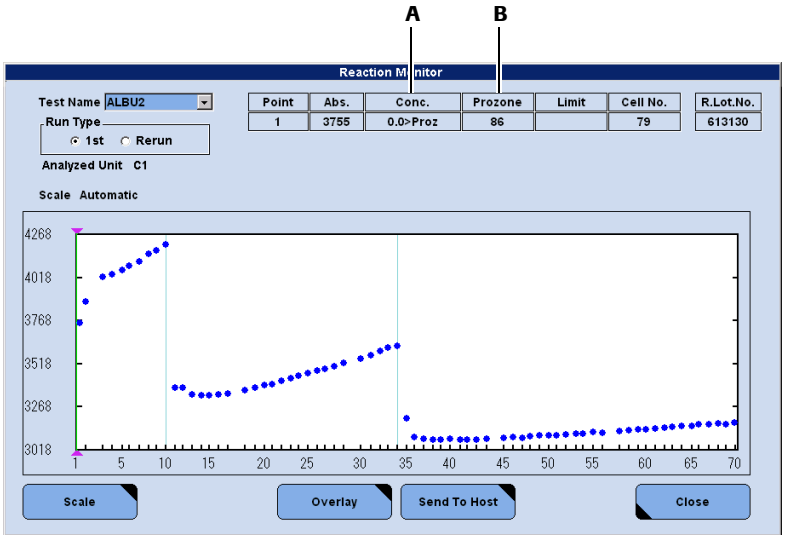


Figure B-30 **Reaction Monitor**: Increasing absorbance

Decreasing absorbance Reaction graph with absorbance decrease after addition of extra antigen.



A *>Proz* flag **B** Prozone check value

Figure B-31 **Reaction Monitor**: Decreasing absorbance

Comprehensive calculation example

This section provides an example of a 2 Point End assay with prozone check (antigen readdition method) with the calculation of the prozone check value.

The following data from the **Utility** > **Application** screen are used for this example:

Test	ALBU2
Assay/Time/Point	[2 Point End] [10] [10] [34] [0] [0]
Prozone Limit	[-32000] [1300] [33] [43] [0] [0] [Inside] [0] [0]

See Figure B-29 on page B-40

The following **Reaction Monitor** report is used for this example:

```
                          Reaction Monitor                        13/04/10  15:30

Urine     N000023   00048-3   13/04/10  C         CELL 079    ALBU2   0.0 >Proz      86
ID 0002                       09:04:44

***  (PRIMARY) - (SECONDARY)  ***
    CB1-3      1-10      11-20      21-30      31-40      41-50      51-60      61-70
      783      3755       3377       3396       3568       3075       3104       3141
      777      3880       3379       3419       3593       3076       3106       3146
      780      4019       3338       3432       3611       3079       3111       3153
               4034       3336       3448       3623       3082       3111       3156
               4060       3336       3461       3201       3088       3121       3157
               4083       3338       3479       3091       3093       3118       3166
               4112       3346       3488       3084       3088       3128       3167
               4155       3364       3504       3077       3098       3131       3170
               4174       3379       3520       3079       3103       3137       3168
               4211       3394       3548       3081       3101       3137       3177
```

Figure B-32 **Reaction Monitor** report

Prozone check value calculation

The calculation of the prozone check value uses the following equation:

Equation B-18

$$PC = Apmp_2 - d \cdot Apmp_1 \text{ with}$$

$$d_{R3} = \frac{V_{samp} + V_{R1} + V_{R2}}{V_{samp} + V_{R1} + V_{R2} + V_{R3}}$$

Symbol	Definition	Value
PC	Prozone check value	
$Apmp_2$	Absorbance at prozone measuring point 2	3079[a]
$Apmp_1$	Absorbance at prozone measuring point 1	3611[a]
d_{R3}	Dilution factor (correcting for R3 addition)	
V_{samp}	Sample volume	6.0 µL[b]
V_{R1}	R1 volume (reagent 1 + system water)	100 µL + 0 µL[c]
V_{R2}	R2 volume (reagent 2 + system water)	20 µL + 0 µL[c]
V_{R3}	R3 volume (reagent 3 + system water)	6 µL + 20 µL[c]

Table B-11 Definitions and values for quantities used in the calculation

(a) Displayed on the Reaction Monitor report, see Figure B-32 on page B-42.

(b) Displayed on **Utility** > **Application** > **Analyze** > **Sample Volume**, see Figure B-29 on page B-40.

(c) Displayed on **Utility** > **Application** > **Analyze** > **Cassette Configuration** > **Reagent Volume**, see Figure B-29 on page B-40.

Applying these values to the above formulas (Equation B-18) yields:

$$d_{R3} = \frac{(6\mu L + 100\mu L + 20\mu L)}{(6\mu L + 100\mu L + 20\mu L + 6\mu L + 20\mu L)} = \frac{126}{152} = 0{,}8289$$

Therefore:

$$PC = 3079 - (0{,}8289 \cdot 3611) = 86$$

The calculated prozone check value is compared to the lower and upper prozone limits on **Utility** > **Application** > **Analyze**. In the above calculated example the prozone check value is 86. This value lies inside the defined prozone limits (-32000 to 1300), and the seventh box is also set to **Inside**. Thus, a data alarm (>*Proz*) is issued: The test result is flagged on the **Reaction Monitor** window, on the **Data Review** screen, and the prozone data alarm is printed on the patient report.

Reaction rate method

Prozone checks applying the reaction rate method compare the rate of change in absorbance at two different times after final reagent addition, as indicated below:

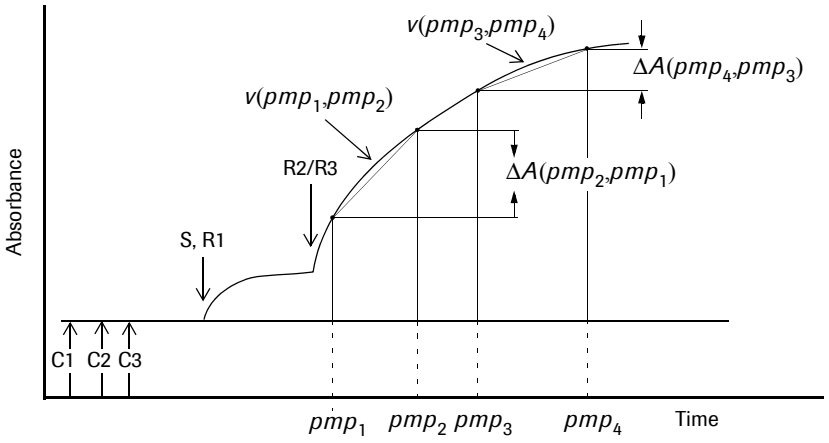


Figure B-33 Prozone check - reaction rate method

$C1$, $C2$, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
$R1$	Pipetting of reagent at R1 timing
$R2$, $R3$	Pipetting of reagent at R2 or R3 timing
pmp_n	Prozone measuring point n, with n = 1, 2, 3, and 4
$v(pmp_n,pmp_m)$	Rate of change in absorbance between pmp_n and pmp_m
$\Delta A(pmp_n,pmp_m)$	Absorbance difference between pmp_n and pmp_m

(a) See *Cell Blank Measurements report* on page B-21.

Settings and calculation

Program a prozone check on the **Analyze** tab of the **Utility** > **Application** screen according to the following description:

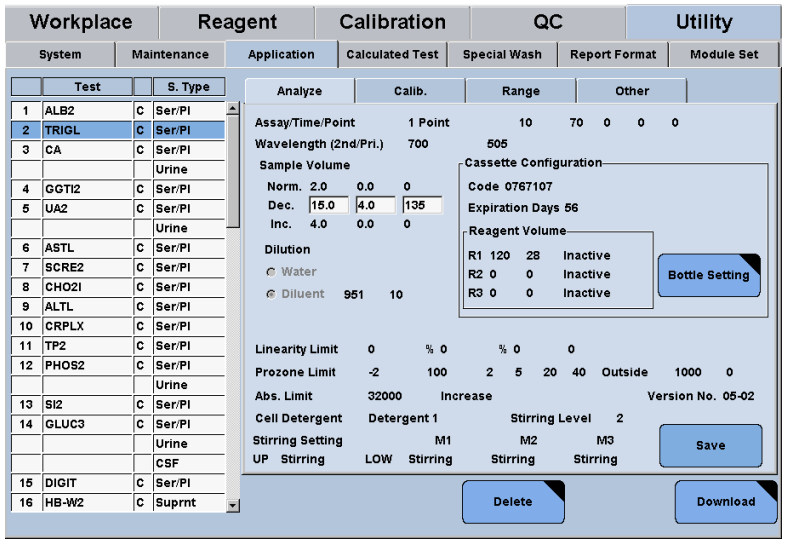


Figure B-34 Application parameters of an application with prozone check (reaction rate method)

To the right of the **Prozone Limit** field there are nine boxes:

[lower limit] [upper limit] [pmp_1] [pmp_2] [pmp_3] [pmp_4] [comp.] [0] [0]

- The first two boxes indicate the lower and upper prozone limits (in Abs × 10^4).
- The next four boxes are for the prozone measuring points (*pmp*):
  - 3rd entry: First prozone measuring point (pmp_1)
  - 4th entry: Second prozone measuring point (pmp_2)
  - 5th entry: Third prozone measuring point (pmp_3)
  - 6th entry: Fourth prozone measuring point (pmp_4)

  Appropriate values are: $1 \leq pmp_1 < pmp_2 \leq 70$ and $1 \leq pmp_3 < pmp_4 \leq 70$. If all entries are set to zero, prozone check is not performed.
- The seventh box (**Inside/Outside**) indicates in which case a data alarm (>*Kin*) is issued: If the entry is set to **Inside**, an alarm is issued in case the obtained check value lies inside the defined range between the lower and upper prozone limits (first two boxes).
  Vice versa if the entry is set to **Outside**, an alarm is issued in case the obtained check value lies outside the defined range.

- The eighth and ninth boxes define additional conditions for the reaction rate method. These allow you to neglect the prozone check in case the reaction rates get too low.

  The entry in the eighth box defines the limit (in Abs × 10^4) for the difference in absorbance between pmp_1 and pmp_2. If the measured difference between these points falls below the limit, the prozone check is neglected.—In other words:

  If $|Apmp_2 - Apmp_1| < F \times 10^{-4}$, reaction rate prozone check is not performed, where F is defined in the eighth box.

  Likewise, the ninth box defines the limit between pmp_3 and pmp_4. If the measured difference falls below the limit, the prozone check is neglected.—In other words:

  If $|Apmp_4 - Apmp_3| < G \times 10^{-4}$, reaction rate prozone check is not performed, where G is defined in the last box of the Prozone Limit line.

Prozone check value calculation

The calculation of the prozone check value uses the following equation:

Equation B-19

$$PC = [v(pmp_3,pmp_4)/v(pmp_1,pmp_2)] \times 100 \text{ with}$$

$$v(pmp_3,pmp_4) = (Apmp_4 - Apmp_3)/(pmp_4 - pmp_3)$$

$$v(pmp_1,pmp_2) = (Apmp_2 - Apmp_1)/(pmp_2 - pmp_1)$$

PC	Prozone check value
pmp_n	Prozone measuring point n (with n = 1, 2, 3, or 4)
$v(pmp_n,pmp_m)$	Rate of change in absorbance between pmp_n and pmp_m
$Apmp_n - Apmp_m$	Absorbance difference between pmp_n and pmp_m
$pmp_n - pmp_m$	Time difference between pmp_n and pmp_m

The calculated prozone check value (PC) is displayed on the **Reaction Monitor** window and compared to the range between the lower and upper prozone limits, which is defined in the first two boxes in the **Prozone Check** line. Under the following conditions a prozone data alarm is issued:

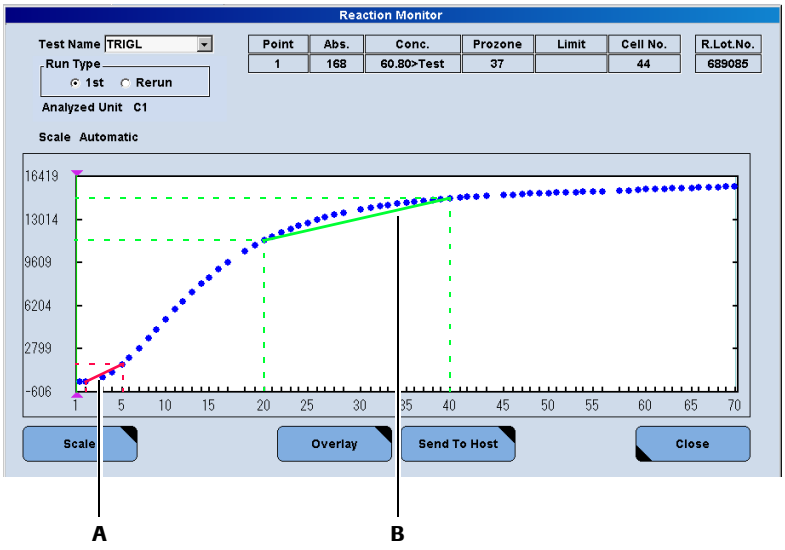
- A prozone data alarm is issued if PC lies inside the prozone interval and **Inside** is displayed in the seventh text box in the **Prozone Check** line.
- Likewise, a prozone data alarm is issued if PC lies outside the prozone interval and **Outside** is displayed in the seventh text box.

In case of an alarm, the test result is flagged with >*Kin* on the **Reaction Monitor** window, on the **Data Review** screen, and the prozone data alarm is printed on the patient report.

Reaction graph in the Reaction Monitor window

This section shows the reaction graph which is producing no alarm or a >*Kin* alarm. The reaction graph is on the **Reaction Monitor** window (**Workplace** > **Data Review** > **Reaction Monitor**).

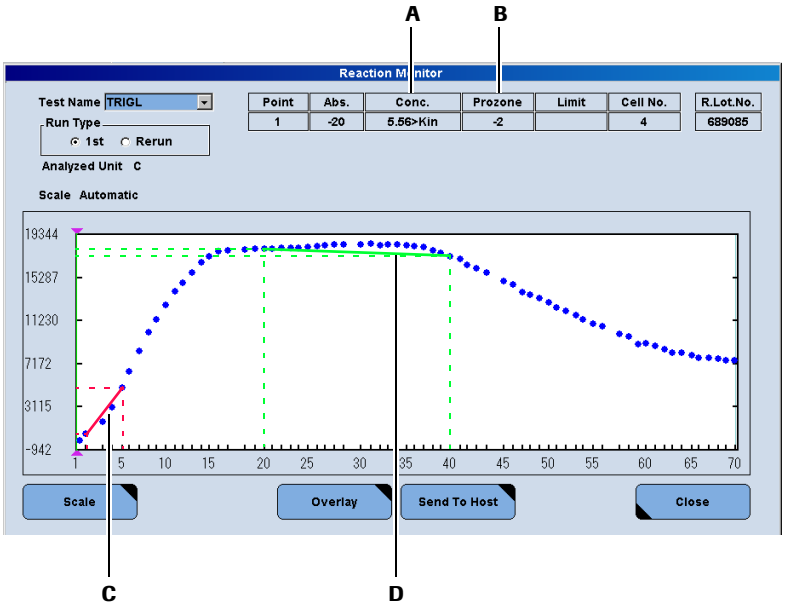
Normal reaction curve Reaction graph with a normal reaction curve. No alarm occurs.



A $v(pmp_1 pmp_2)$ **B** $v(pmp_3 pmp_4)$

Figure B-35 **Reaction Monitor**: Normal reaction curve

Abnormal reaction curve Reaction graph with an abnormal reaction curve. A >*Kin* alarm occurs.



A *>Kin* flag **C** $v(pmp_1 pmp_2)$

B Prozone check value **D** $v(pmp_3 pmp_4)$

Figure B-36 **Reaction Monitor**: Abnormal reaction curve

Comprehensive calculation example

This section provides an example of a 1 Point End assay with prozone check (reaction rate method) with the calculation of the prozone check value.

The following data from the **Utility** > **Application** screen are used for this example:

Test	TRIGL
Assay/Time/Point	[1 Point End] [10] [70] [0] [0] [0]
Prozone Limit	[-2] [100] [2] [5] [20] [40] [Outside] [1000] [0]

See Figure B-34 on page B-45

The following **Reaction Monitor** report is used for this example:

```
                          Reaction Monitor                      13/04/10  15:30

Ser/Pl    N000052   00028-2   13/04/10  C         CELL 004    TRIGL   5.56 >Kin
ID 005                        09:04:44

***  (PRIMARY) - (SECONDARY)  ***
    CB1-3      1-10     11-20     21-30     31-40     41-50     51-60     61-70
     -111       -20     13936     17999     18422     16982     12450      8838
     -110       577     14779     18025     18266     16454     12138      8504
     -110      1686     15763     18066     18366     16149     11743      8230
               3067     16659     18093     18375     15748     11292      8201
               4933     17276     18143     18336     14897     10964      7994
               6465     17734     18212     18193     14590     10646      7687
               8398     17832     18280     18113     13859      9954      7693
              10151     17923     18363     17825     13651      9737      7601
              11323     17961     18403     17570     13366      9032      7455
              12728     17992     18378     17285     12894      9042      7490
```

Figure B-37 **Reaction Monitor** report

Prozone check value calculation

The calculation of the prozone check value uses the following equation:

Equation B-20

$$PC = [v(pmp_3,pmp_4)/v(pmp_1,pmp_2)] \times 100 \text{ with}$$

$$v(pmp_3,pmp_4) = (Apmp_4 - Apmp_3)/(pmp_4 - pmp_3)$$

$$v(pmp_1,pmp_2) = (Apmp_2 - Apmp_1)/(pmp_2 - pmp_1)$$

Symbol	Definition	Value
PC	Prozone check value	
$Apmp_1$	Absorbance at prozone measuring point 1	577[(a)]
$Apmp_2$	Absorbance at prozone measuring point 2	4933[(a)]
$Apmp_3$	Absorbance at prozone measuring point 3	17992[(a)]
$Apmp_4$	Absorbance at prozone measuring point 4	17285[(a)]
pmp_1	Prozone measuring point 1	2[(b)]
pmp_2	Prozone measuring point 2	5[(b)]
pmp_3	Prozone measuring point 3	20[(b)]
pmp_4	Prozone measuring point 4	40[(b)]

Table B-12 Definitions and values for quantities used in the calculation

(a) Displayed on the Reaction Monitor report, see Figure B-37 on page B-48.

(b) Displayed on **Utility** > **Application** > **Analyze** > **Prozone Limit**, see Figure B-34 on page B-45.

Applying these values to the above formulas (Equation B-20) yields:

$$v(pmp_1,pmp_2) = (4933 - 577)/(5 - 2) = 1452$$

$$v(pmp_3,pmp_4) = (17285 - 17992)/(40 - 20) = -35,4$$

Therefore:

$$PC = \frac{-35,4}{1452} \times 100 = -2,4\ \%$$

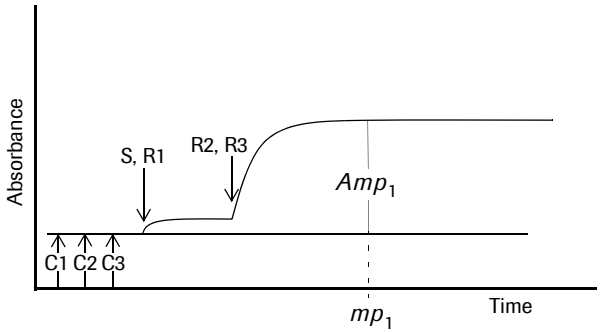
The calculated prozone check value is compared to the lower and upper prozone limits on **Utility** > **Application** > **Analyze**. In the above calculated example the prozone check value is -2.4%. This value lies outside the defined prozone limits, and the seventh box is also set to **Outside**. Thus, a data alarm (>*Kin*) is issued: The test result is flagged on the **Reaction Monitor** window, on the **Data Review** screen, and the prozone data alarm is printed on the patient report.

Summary of assay techniques

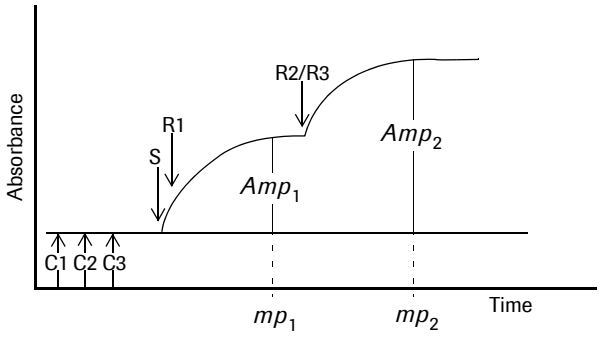
Assay type	Measuring points	Calculation of unknown
1 Point	$1 \le mp_1 \le 70$	$C_x = [K(A_x - A_b) + C_b] \cdot IF_A + IF_B$
2 Point End	$1 \le mp_1 < mp_2 \le 70$	$C_x = [K(A_x - A_b) + C_b] \cdot IF_A + IF_B$
2 Point Rate	$1 \le mp_1 < mp_2 \le 70$	$C_x = [K(v_x - v_b) + C_b] \cdot IF_A + IF_B$
Rate A with sample blank	$1 \le mp_3 < mp_4 < mp_1 < mp_2 \le 70$, $mp_3 + 2 < mp_4$, $mp_1 + 2 < mp_2$	$C_x = [K(v(mp_3,mp_4) - v_b) + C_b] \cdot IF_A + IF_B$
Rate A	$1 \le mp_1 < mp_2 \le 70$, $mp_1 + 2 < mp_2$	$C_x = [K(v_x - v_b) + C_b] \cdot IF_A + IF_B$ with $v_x = v(mp_2,mp_1) - d \cdot v(mp_4,mp_3)$

Table B-13 Summary of assay techniques

Endpoint assay example graphs

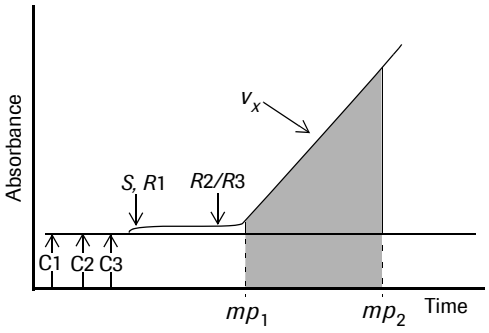


1 Point assay

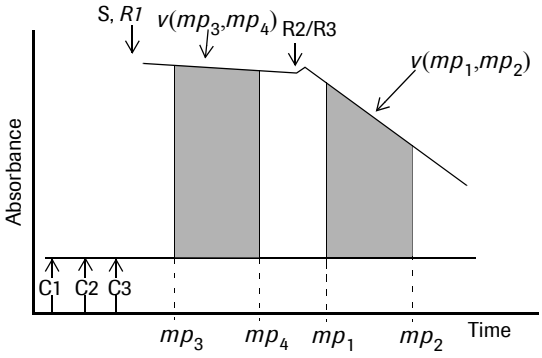


2 Point End assay

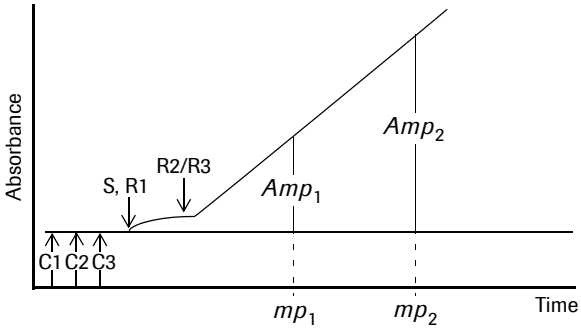
Rate assay example graphs



Rate A assay



Rate A with sample blank correction



2 Point Rate assay

Table B-14 Reaction time courses for individual assay types

c 501 – Serum index principles

This chapter provides you with an overview of the serum index test principles used by the **cobas** 6000.

In this chapter *Chapter* 6

Table of contents

Introduction

A number of diseases result in increased amounts of chromogens such as bilirubin or hemoglobin, or lipemic particles, which increase the turbidity. These chromogens interfere with many photometric assays. However, this interference can be quantified by means of serum index measurements.

Serum indices are calculations of absorbance measurements that provide a semi-quantitative representation of levels of icterus, hemolysis, or lipemia (turbidity) present in patient samples.

Definition of serum indices

The icterus index, I, is reported in icterus units that are linear, up to 60 mg/dL, and semi-quantitative. For example, an icterus index of 20 is equivalent to a known bilirubin concentration of approximately 20 mg/dL.

The hemolysis index, H, is reported in hemolysis units that are linear, up to 1000 mg/dL, and semi-quantitative. For example, a hemolysis index of 500 is equivalent to a known hemoglobin concentration of approximately 500 mg/dL.

The lipemia index, L, is reported in lipemia units corresponding to Intralipid® (Kabi-Pharmacia, Inc.), an artificial lipid material. These units are linear, up to 2000, and semi-quantitative. Hence, the L index provides an estimate of sample turbidity, not its concentration of triglycerides.

Measurement of serum indices

The analyzer takes an aliquot of the patient sample, dilutes it with 0.9% NaCl, and then measures the absorbances at three pairs of wavelengths:

- For measurement of lipemia (L), wavelengths 700/660 nm are used because this range is free from influence by hemolysis and icterus (see Figure B-38 below).
- Hemolysis (H) is measured at 600/570 nm and correction is made for absorption due to lipemia.
- Icterus (I) is measured at 505/480 nm and correction is made for absorption due to lipemia and hemolysis.

Shown below are example absorption spectra of turbid serum, hemolytic solution, and bilirubin solution.

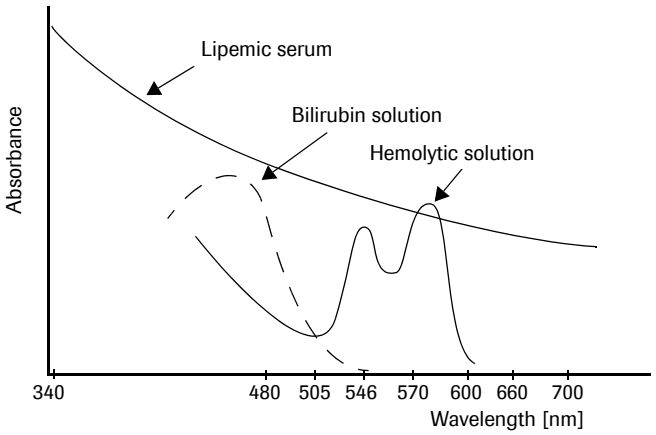


Figure B-38 Example absorption spectra of a turbid (lipemic) serum, a hemolytic solution, and a bilirubin solution

Calculation of serum indices

To obtain the serum indices L, H, and I from the sample's absorbances, the system uses the following formulas:

Equation B-21 $$L = \frac{1}{C} \cdot (Abs_1)$$

Equation B-22 $$H = \frac{1}{A} \cdot (Abs_2 - B \cdot Abs_1)$$

Equation B-23 $$I = \frac{1}{D} \cdot (Abs_3 - E \cdot Abs_2 - F \cdot Abs_1)$$

L, H, I	Serum indices for lipemia, hemolysis, icterus
C, A, D	Factors for conversion of absorbance values ($\times 10^4$) to serum indices
Abs_1	Bichromatic absorbance readings at 700 and 660 nm for lipemia
Abs_2	Bichromatic absorbance readings at 600 and 570 nm for hemolysis
Abs_3	Bichromatic absorbance readings at 505 and 480 nm for icterus
B	Corrects hemoglobin measurement Abs_2 for lipemia
E, F	Correct bilirubin measurement Abs_3 for hemoglobin and for lipemia

C, A, and D are sample dilution-dependent and unit-dependent scaling factors to provide semi-quantitative interference levels.
B, E and F are correcting factors which correct overlapping interference spectra. They are independent of sample dilution since they are based on ratios of absorbances.

Serum indices can be programmed in either conventional or SI units. Make sure that the correct scaling factors are set for the units you chose. The units should be the same as those used in test results.

➔ For more information refer to the instructions for use of the serum index reagent.

Evaluating serum indices

The results should fall in the following ranges, corresponding to an approximate amount of the chromogen indicated:

Serum index		Conventional units	SI units	
Lipemia index	L	0-1000 mg/dL		Turbidity
Hemolysis index	H	0-1000 mg/dL	0-620 µmol/L	Hemoglobin
Icterus index	I	0-60 mg/dL	0-1000 µmol/L	Total bilirubin

Table B-15

Once the serum indices are determined, refer to the Limitations section of the instructions for use of the application to assess the results. This indicates the index up to which potential interference is within the Roche Diagnostics specification or when the hemolysed, icteric, or lipemic sample may not be used with the respective application.

Serum index data alarms

Upper limits for serum indices can be defined individually for each test. Limit values are loaded with the application and displayed in the serum index boxes (**L**, **H**, and **I**) on the **Range** tab of the **Utility** > **Application** screen. If a measured serum index value is greater than the corresponding value in the **L**, **H**, or **I** box, an alarm is issued. When serum index limits are set to 0, the serum index check will be neglected.

The following examples show how different data alarms are issued when test-specific limit values for serum indices are exceeded in a patient sample.

Examples The application GLUC2 has an **I** index limit of 60.

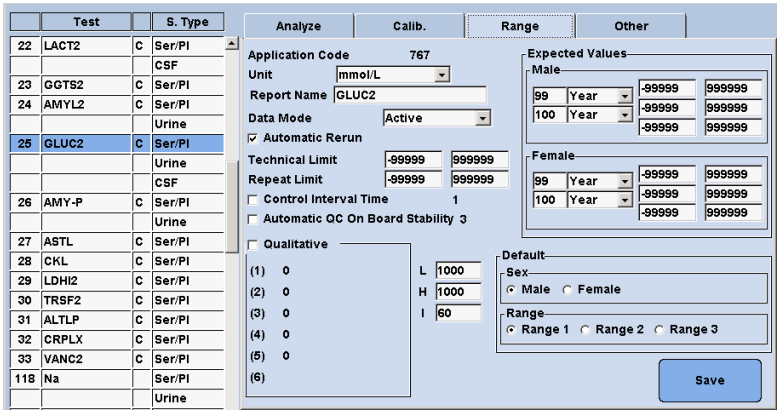


Figure B-39 Utility > Application > Range: GLUC2

If the obtained **I** index is greater than 60, a *>I.I* alarm is issued.

Another application, ALB2, is programmed with serum index limits for **L**, **H**, and **I** indices. If one obtained serum index value exceeds the **L** limit, a *>I.L* alarm is issued. If for example **L** and **I** are exceed, a *> I.LI* alarm is issued.

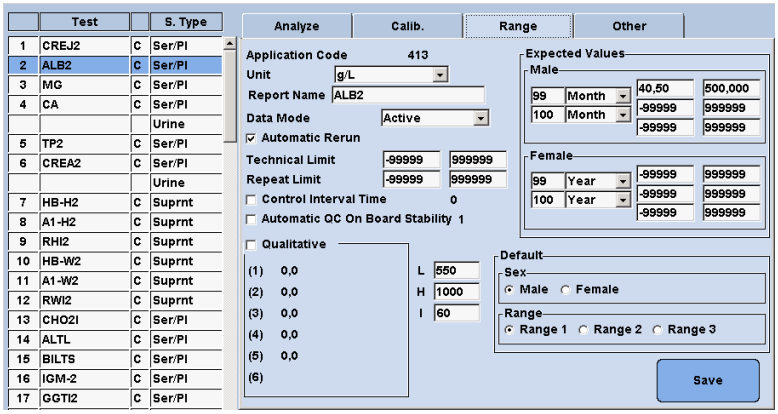


Figure B-40 Utility > Application > Range: ALB2

The measurement of the sample yielded an **H** index of 557, an **I** index of 89, and a **L** index of 631. The results are displayed on the **Data Review** screen.

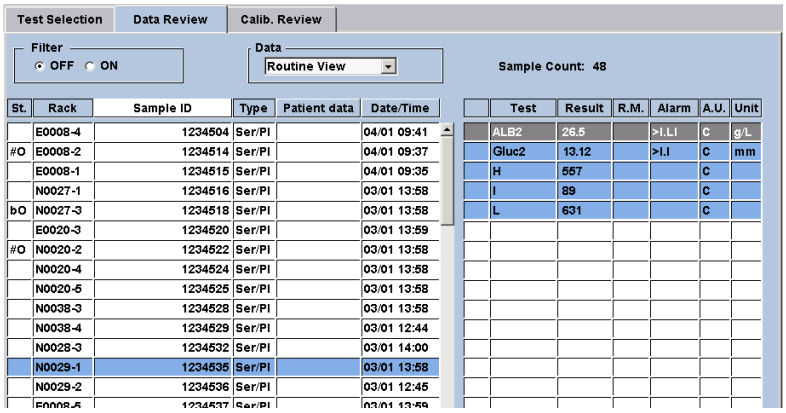


Figure B-41 Workplace > Data Review: GLUC2 and ALB2

For the GLUC2 the limit of 60 for the **I** index is exceeded. Therefore a *>I.I* data alarm is attached to the result.

For ALB2, more than one limit value is exceeded—namely **L** and **I**. Therefore a *>I.LI* data alarm is attached to the result.

e 601 – Immunology principles

This chapter provides you with an overview of the immunology test principles used by the **cobas** 6000.

In this chapter *Chapter* 7

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e 601 test principles

3 test principles are available on the **e** 601: Competitive principle for extremely small analytes, sandwich principle (1 or 2 steps) for larger analytes, and bridging principle to detect antibodies in the sample.

For detailed descriptions of these principles, see:
Competitive principle on page B-61
Sandwich principle on page B-63
Bridging principle on page B-65

Competitive principle

This principle is applied to analytes of low molecular weight, such as FT3.

- In the first step, sample and a specific anti-T3 antibody labeled with a ruthenium complex are combined in an assay cup.
- After the first incubation, biotinylated T3 and streptavidin-coated paramagnetic microbeads are added. The still free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex is bound to the microbead via interaction of biotin and streptavidin.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell. The immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by ProCell.
- In the ECL reaction, the conjugate is a ruthenium-based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is indirectly proportional to the amount of antigen in the patient sample.

Evaluation and calculation of concentration of the antigen are carried out by means of a calibration curve that was established using standards of known antigen concentration.

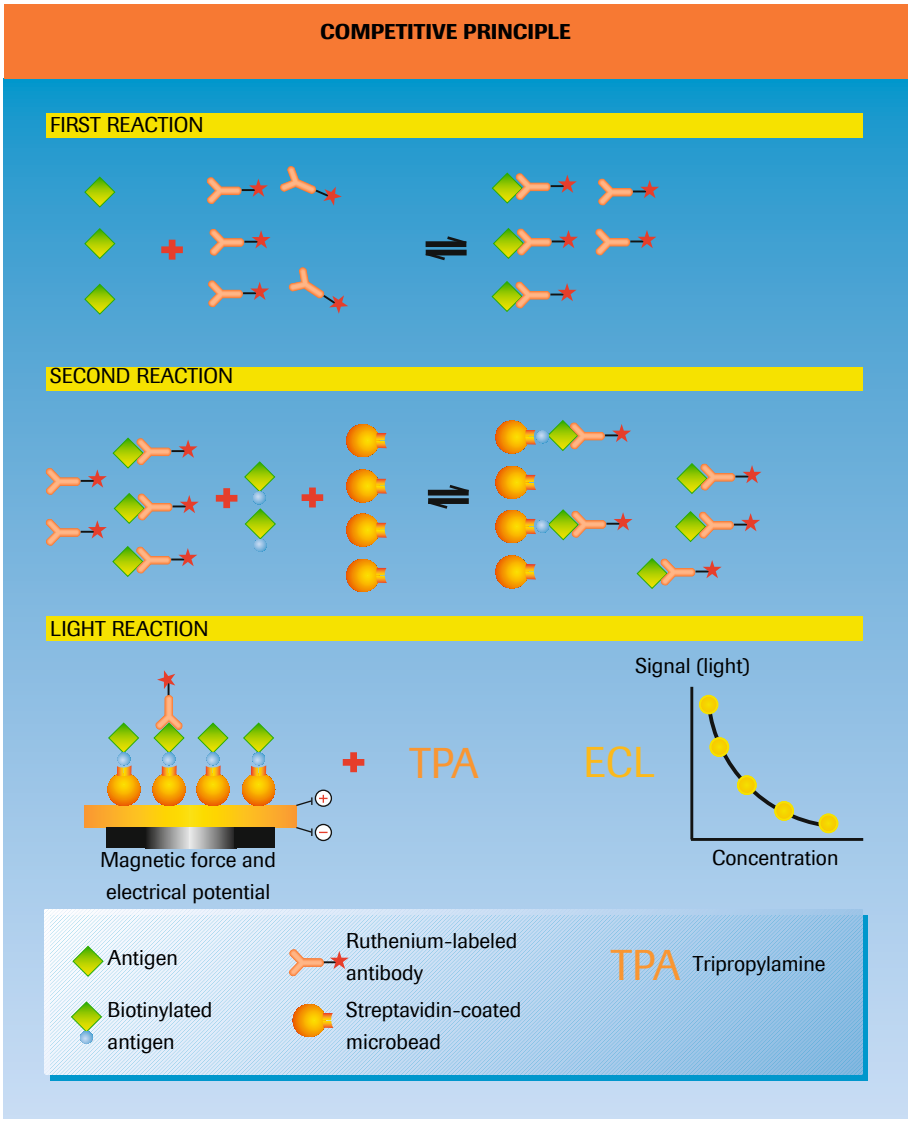

Figure B-42 Competitive principle

Sandwich principle

The sandwich principle is applied to higher molecular weight analytes, such as thyroid-stimulating hormone (TSH).

- In the first step, patient sample is combined with a reagent containing biotinylated TSH antibody and a ruthenium-labeled TSH-specific antibody in an assay cup. During a 9-minute incubation step, antibodies capture the TSH present in the sample.
- In the second step, streptavidin-coated paramagnetic microbeads are added. During a second 9-minute incubation, the biotinylated antibody attaches to the streptavidin-coated surface of the microbeads.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by ProCell.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of TSH in the sample.

Evaluation and calculation of concentration of the antigen or analyte are carried out by means of a calibration curve that was established using standards of known antigen concentration.

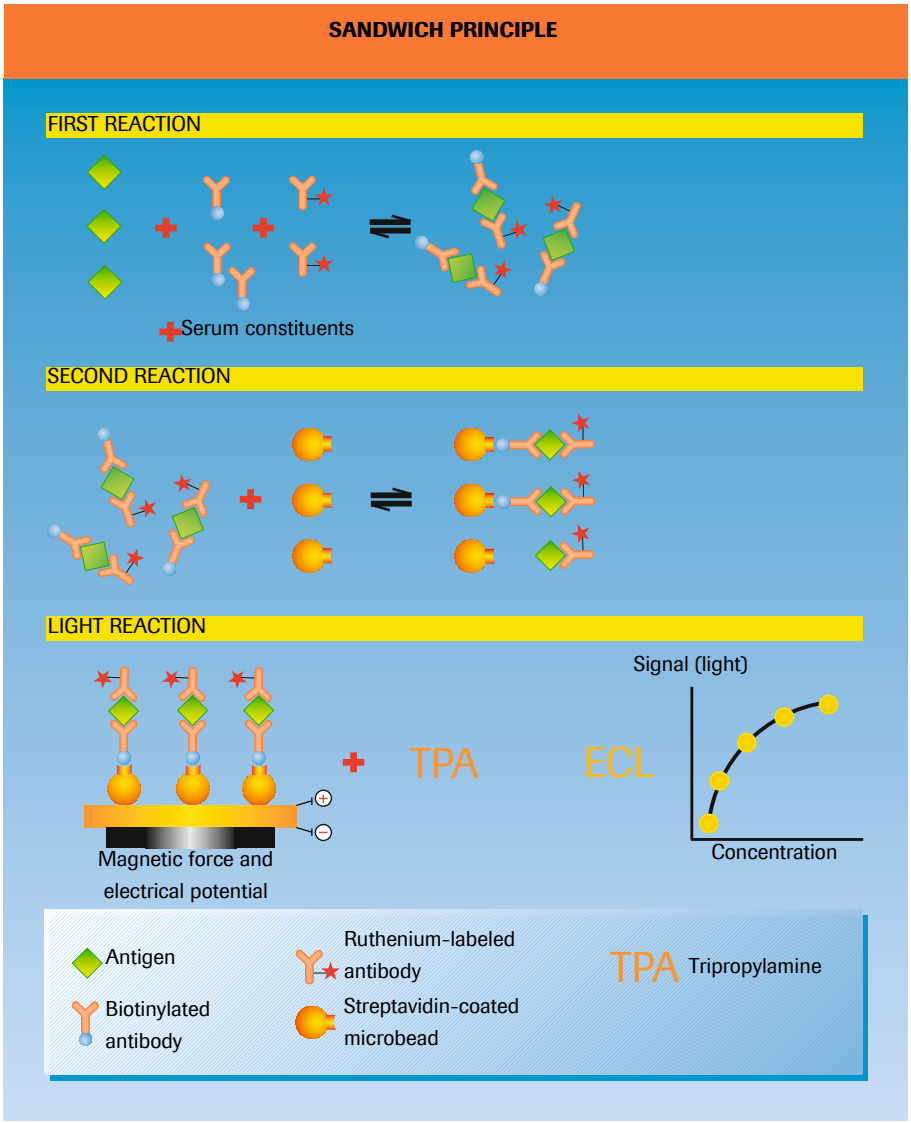

Figure B-43 Sandwich principle

Bridging principle

The bridging principle is similar to the sandwich principle, except that the assay is designed to detect antibodies, not antigens, (e.g., IgG, IgM and IgA). This is accomplished by including biotinylated and ruthenium-labeled antigens in the reagents for which the targeted antibody has affinity.

- In the first step, serum antibodies bind with the biotinylated and ruthenium-labeled antigens to form an immune complex.
- The immune complex then reacts with streptavidin-coated microbeads via the biotinylated antigen.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by ProCell.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of analyte in the sample.

Evaluation and calculation of the concentration of the antibody are carried out by means of a calibration curve that was established using standards of known antibody concentrations.

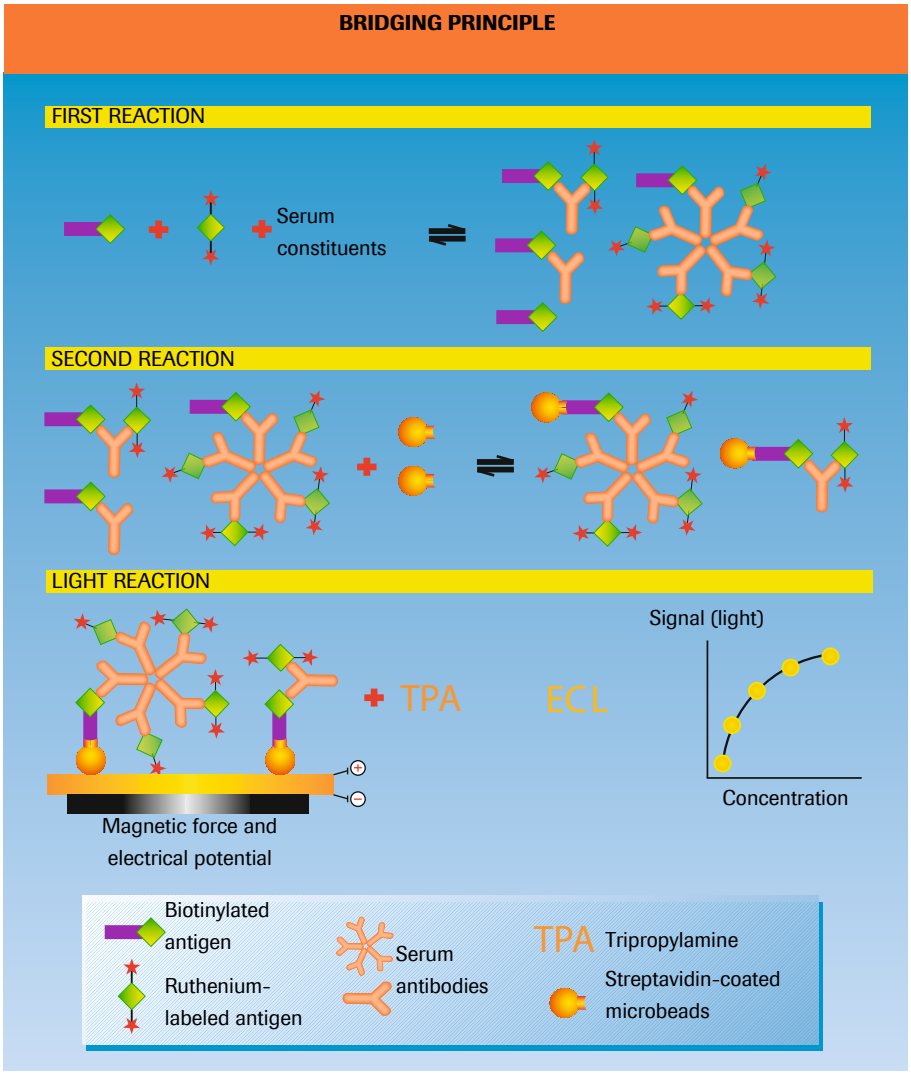

Figure B-44 Bridging principle

Calibration C

ISE unit – Ion selective electrode calibration

This chapter provides you with an overview of the calibration of ion-selective electrode tests used by the **cobas** 6000.

In this chapter *Chapter* 8

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ISE calibration

A full calibration of Na^+, K^+, and Cl^- in the ISE unit requires the aqueous standards ISE Standard 1 (low), ISE Standard 2 (high) and ISE Standard 3 (high). Additionally, an internal standard (IS) is measured. A full calibration is required at least every 24 hours

For each calibration, the standard solutions are aspirated into the electrode cartridges, and—after equilibration occurs at the electrode membrane—the electromotive force (EMF, voltage) is measured.

The slope of the calibration will be calculated based upon these readings and the assigned value of the standards.

Calibration checks

If any of the data alarms listed below occurs during a calibration, an *Std.E* alarm is issued. The calibration curve of the affected test is not updated. Choose **Alarm** (global button) to verify which data alarm has occurred.

Calibration data alarm	
ADC.E	ADC abnormal
Calc.?	Calculation not possible
ISE.E	ISE voltage level error
ISE.N	ISE noise error
<Mix	Mixing power low level
Mix.E	Ultrasonic mixing error
Samp.S	Sample short

Table C-1 Data alarms giving rise to an Std.E alarm when occurring in calibration

Calibration error

The compensated limit is the difference, in per cent, between the current calibration and the previous one. If the compensated limit exceeds the maximum compensated limit, a *Cal.E* alarm is issued. The maximum compensated limit is defined in the **Compensated Limit** text box on the **Utility** > **Application** > **Calib** screen.

The compensated limit is calculated as follows:

Equation C-1

$$CI = \frac{U_{Pre} - U_{Cur}}{(U_{Pre} + U_{Cur})/2} \times 100$$

Cl	Compensated limit
U_{Pre}	Previous value (slope or S3 concentration)
U_{Cur}	Current value (slope or S3 concentration)

Slope calculation

The slope is calculated in millivolts (mV) from the aqueous high and low standards. The slope is calculated according to the following formula:

Equation C-2 $$S = \frac{E_H - E_L}{\log\left(\frac{C_H}{C_L}\right)}$$

S	Slope
E_H	EMF (voltage) of high standard
E_L	EMF (voltage) of low standard
C_H	Concentration of high standard
C_L	Concentration of low standard

Due to factors such as the condition of the electrodes, the measured slope may deviate from this ideal slope. Therefore, the slope obtained should fall within the following ranges:

Na^+	50 to 68 mV
K^+	50 to 68 mV
Cl^-	-68 to -40 mV

Internal standard calculation

In any ISE measurement system a number of junctions between wires, membranes, and reagents exist. The internal standard compensates for system-related variations.

After the slope is established during a calibration, the internal standard concentration is calculated. The concentration of Na^+, K^+, and Cl^- in the internal standard is calculated from the electromotive force (EMF, voltage) of each electrode measured during calibration according to the formula below.

Equation C-3 $$C_{IS} = C_L \times 10^{(E_{IS} - E_L)/S}$$

C_{IS}	Concentration of the specific ion in the internal standard
C_L	Input concentration of the low standard
E_{IS}	EMF (voltage) of the internal standard for the specific ion
E_L	EMF (voltage) of the low standard for the specific ion
S	Slope

The calculated value of the internal standard, as well as the voltage, is shown on the Calibration report.

One-point calibration

An internal standard, labeled as ISE Internal Standard (IS), is measured during calibration as well as before and after each routine sample. These measurements are used to correct for system-related drifts (junction potential differences, differences in electrode conditions, and the like).

Compensation value calculation

The concentration of ions in the ISE Std 3 is calculated according to the following formula:

Equation C-4 $$S_3Conc = C_{IS} \times 10^{(E_C - E_{IS})/S}$$

S_3Conc	Concentration of ions in the ISE Std 3
C_{IS}	Concentration of the internal standard, determined during calibration
E_C	EMF (voltage) of the ISE Std 3 for the specific ion
E_{IS}	EMF (voltage) of the internal standard for the specific ion
S	Slope

The formula for finding the compensation value (C. Value):

C. Value = assigned value (ISE Std 3) - calculated value (ISE Std 3)

This compensation value is automatically updated after each successful calibration.

During calibration the compensation value is compared to the compensation value of the previous calibration. If the percent difference is greater than the **Compensated Limit** on **Utility** > **Application** > **Calib.**, a *Cal.E* alarm is issued.

The ISE unit is in an optimal condition when the compensation values (C. value) are stable and negligibly low.

Reference electrode

A 1M KCl solution is measured concurrently with each sample analysis. The reference electrode is used for this purpose. The voltage of the reference electrode serves as a reference point for all measurements. That is, all reported voltages are readings from which the voltage of the reference electrode has been subtracted.

Reference electrode

c 501 – Photometric calibration

This chapter provides you with an overview of the calibration types used by the **cobas** 6000 for photometric assays. The K factor, calibration updates and calculating results are also discussed.

In this chapter *Chapter* 9

Calibration checks

For each photometric application, the following checks that automatically verify the reliability of calibrations are available. If a check value lies outside the configured check limits, an alarm is issued. This section briefly explains the calibration checks and the associated alarms.

Calibration checks	Associated data alarms
SD limit check	SD.E
Duplicate limit check	Dup.E
Sensitivity limit check	Sens.E
S1 Abs. limit check	S1A.E
Std. check	Std.E

Table C-2 Calibration checks and associated data alarms

The limits of the calibration checks are configured under **Utility** > **Application** > **Calib.**

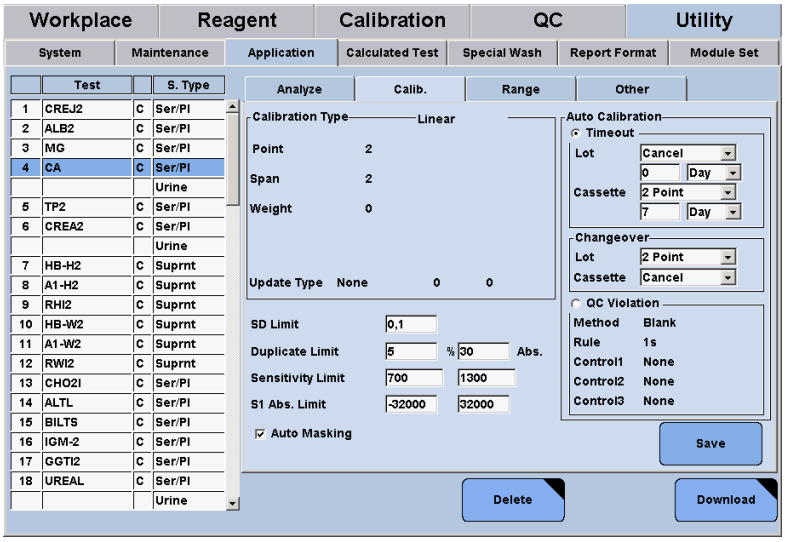


Figure C-1 **Calib.** tab of the **Utility** > **Application** screen

SD limit

When performing calibrations for tests with non-linear and non-spline calibration curves the instrument performs the following checks:
For each calibrator, an absorbance value is calculated from the given concentration and the current calibration curve. This calculated absorbance is compared to the measured absorbance. If the difference of the two exceeds the SD limit value, an *SD.E* alarm is issued. The SD limit value is defined in the **SD Limit** box (in Abs × 10^4). An SD limit value of 999.9 denotes the check will be omitted.

In case an *SD.E* alarm occurs, measurement is still possible and the calibration curve is updated. However, trace the cause of the alarm before you proceed to sample measurement. The SD value is printed out together with the result of calibration.

Duplicate limit

All photometric calibrators are run in duplicate. The duplicate check calculates the % error and the absolute absorbance error (difference) between these duplicate measurements. The obtained check values are compared to the % error limit and the absorbance error limit. The % error limit is defined in the first **Duplicate Limit** box. The absorbance error limit is defined in the second **Duplicate Limit** box (**Abs.**). The corresponding check values $DE_{\%}$ and $DE_{\text{Abs.}}$ are calculated as follows:

$$DE_{\%} = \frac{|Abs2 - Abs1|}{(Abs2 + Abs1)/2} \cdot 100 \text{ and } DE_{\text{Abs.}} = |Abs2 - Abs1|,$$

where $Abs1$ and $Abs2$ denote the two absorbance readings, taken for each calibrator (duplicate readings).

If both the % error and the absorbance error are out of range, a *Dup.E* alarm is issued indicating a failed calibration. The calibration curve of the affected test is not updated.

For more details see also *Duplicate limit check (Dup.E)* on page D-11.

Sensitivity limit

Sensitivity, here, refers to the ratio of an absorbance difference to a concentration difference. It is calculated from the measured absorbances and given concentration values of the blank calibrator (S_1) and the span calibrator (S_N):

$(Abs(S_N) - Abs(S_1))/(Conc(S_N) - Conc(S_1))$, where

The sensitivity obtained in a calibration must lie within certain limits: The lower limit is defined in the first **Sensitivity Limit** box. The upper limit is defined in the second **Sensitivity Limit** box. If the obtained sensitivity is not within these limits, a *Sens.E* alarm is issued indicating a failed calibration. The calibration curve of the affected test is not updated.

S1 Abs. limit

This check sets an upper and lower absorbance limit for the blank calibrator, Std (1). If the absorbance for Std (1) falls outside these limits, the system issues a *S1A.E* alarm indicating an erroneous calibration. The calibration curve of the affected test is not updated. An **S1 Abs. Limit** minimum of -32000 and maximum of 32000 denotes the check will be omitted.

Std. check

If any of the data alarms listed below occurs in a calibration, an *Std.E* alarm is issued. The calibration curve of the affected test is not updated. Choose **Alarm** (global button) to verify which data alarm has occurred.

Data alarm	Data flag
ABS over	>Abs
ADC abnormal	ADC.E
Calculation not possible	Calc.?
Cell blank abnormal	>Cuvet
Duplicate error (c 501)	Dup.E
Linearity abnormal	>Lin
Mixing power low level	<Mix
Ultrasonic mixing error	Mix.E
Prozone error 1, Prozone error 2 / Kinetic unstable	>Proz, >Kin[(a)]
Reaction limit over (substrate depletion)	>React
Reagent short	Reag.S
S1ABS abnormal	S1A.E
Sample short	Samp.S

Table C-3 Data alarms giving rise to an *Std.E* alarm when occurring during calibration

(a) Not for Std.1

Updated and non-updated calibration data

The table below shows the data output when data alarm is issued during a calibration. If the working curve is not updated, take necessary measures and perform recalibration. Recalibration may also be required depending on the cause of an alarm even if the working curve is updated.

Data alarm	Working curve	Saving on hard disk	Display on Alarm screen
SD.E	Updated	Yes	Provided
Dup.E	Not updated	No	Not provided
Sens.E	Not updated	No	Provided
S1A.E	Not updated	No	Not provided
Std.E	Not updated	No	Provided

Table C-4 Data output in case of data alarm during calibration

Calibration overview

Calibration types

The term calibration refers to the determination of a valid relation between the measured signal [absorbance or (for rate assays) a rate of change in absorbance] and the concentration of the analyte of interest. The graphical representation of such a signal/concentration relation is the calibration curve also referred to as working curve.

The system uses different types of mathematical models to describe this relation. These math models are referred to as calibration types.

Calibration methods

Up to six calibrators—abbreviated Std (1), Std (2)... Std (6)—can be used for a full calibration. However, not all of these need to be used in every update of a calibration. Select one of four different calibration methods to define which calibrators are to be used.

Calibration update types

For calibration methods where only one calibrator is remeasured (**Blank** and **Span**), there are three possibilities how the calibration curve is corrected. This choice is made by setting the calibration update type.

For more information, see:
Calibration types on page C-15
Calibration methods on page C-16
Calibration update types on page C-20

Calibration types

Linear calibrations are used for tests when the absorbance readings plotted against calibrator concentrations lie on a straight line. If a linear calibration is based on two calibrator measurements, it is termed linear two-point calibration. If it is based on more than two calibrators, it is termed linear multipoint calibration.

Nonlinear calibrations are used for tests whose absorbances at different concentrations form a nonlinear but reproducible plot. At least three and a maximum of six calibrators are required for calibration. Available calibration types are RCM, RCM2T1, and RCM2 T2.

In addition, there are two calibration types whose calibration curves are piecewise defined interpolation functions: Spline and Line Graph. The following table provides an overview of all available calibration types.

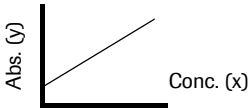
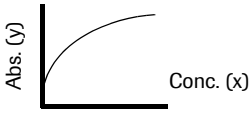
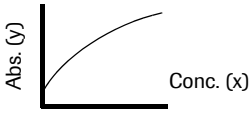
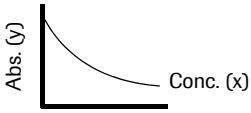
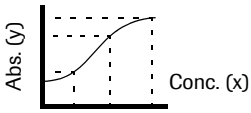
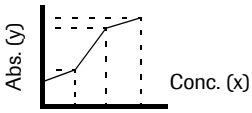
Calibration type	Math model		Cross-reference
Linear	$y = a + b \cdot x$		*Linear calibration* on page C-24
RCM	$y = \frac{a-d}{1+\left(\frac{x}{b}\right)^c} + d$		*RCM calibration* on page C-28
RCM2T1	$y = a + b \cdot \frac{\sinh z}{1+z^2}$ with $z = c \cdot x + d$		See *RCM2T1 calibration* on page C-30
RCM2T2	$y = a + r \cdot (1 + s \cdot x)^{-2}$		*RCM2T2 calibration* on page C-32
Spline	Piecewise polynomials of higher degree for the interpolation between calibrator data points		*Spline calibration* on page C-34
Line Graph	Polygon of linear interpolations with slopes of $(A_N - A_{N-1})/(C_N - C_{N-1})$		*Line Graph calibration* on page C-36

Table C-5 Overview of calibration types

K factor

A K factor is used in the calculation of sample results. Any test requiring more than just a blank during calibration will have its K factor calculated via the measured absorbances of the blank calibrator Std (1) and the other calibrator(s).

For more details, see *K factor calculation* on page C-21.

A fixed K factor is used for some tests and is derived at the time of system installation. The respective tests have only their blank (baseline) values updated during calibration.

Calibration methods

A calibration determines the relation between a measured signal and the concentration of an analyte. This relation, however, is dependent on various conditions (including lot variations, age of reagents, instrument parameters) and therefore needs to be updated regularly.

A calibration update can be described either as an adjustment of parameters of the calibration curve or as an adjustment of the measured signal (signal correction) to compensate for changed conditions. Both of these descriptions are mathematically equivalent.

Calibrations can be updated manually or automatically. A calibration update does not necessarily include all calibrators used in the full calibration of a test. According to the number of calibrators used, calibrations updates are termed one-point or two-point. In case of one-point calibration update, the signal correction is a simple proportional adjustment: $s' = r \cdot s$, where s and s' denote the signals obtained with the original and the current system, respectively. In case of a two-point calibration update, the signal correction is linear: $s' = p \cdot s + q$.

On **cobas** 6000 systems, there are four methods available to update calibrations: **Blank** and **Span** (which are both one-point calibrations), **2 Point**, and **Full**. These calibration methods are listed below along with corresponding calibrators and calibration types.

Calibration method	Calibrator(s) needed		Applicable calibration type
Blank	Std (1) calibrator	For **c** 501 tests water is used as blank calibrator.	Linear, RCM, RCM2T1, RCM2T1, Spline, Line Graph
Span	Std (N) with N > 1	For this method you must use a calibrator other than Std (1).	Linear, RCM, RCM2T1, RCM2T1
2 Point	Std (1) calibrator and one additional Std (N), N > 1		Linear, RCM, RCM2T1, RCM2T1
Full	Std (1), Std (2), Std (3)… Std (N)	All calibrators specified for the application[a]	RCM, RCM2T1, RCM2T1, Spline, Line Graph

Table C-6 Calibration methods

(a) Displayed on Utility > Application > Others.

In the following sections, we explain these calibration methods and show how the calibration curve parameters are updated. The following parameters are used throughout:

Definition of parameters

S1Abs	Calibration curve parameter displayed in the **S1 Abs.** column of the **Calibration Result** window[a] and on **Working Information** window[b]
K	Calibration curve parameter displayed in the **K** column
A, B	Calibration curve parameters displayed in the columns **A** and **B**
'	A diacritical mark (') denotes an updated parameter. For example, B' is the new B parameter of the calibration curve after the calibration update.

(a) To display this window, choose **Calibration** > **Status** > **Calibration Result**.

(b) To display this window, choose **Calibration** > **Status** > **Calibration Result** > **Working Information**.

Blank calibration

A Blank calibration is a one-point calibration. Tests are calibrated with the Std (1) calibrator only, and the signal correction is a simple proportional adjustment. The calculation method for various applicable calibration types are listed below.

Calibration type	S1 Abs.	K	A	B
Linear[(a)]	$\mathrm{S1Abs}' = s_b$	$K' = \frac{\mathrm{S1Abs}}{s_b} \cdot K$		
RCM	$\mathrm{S1Abs}' = s_b$	Previous value	Previous value	$B' = \frac{s_b}{\mathrm{S1Abs}} \cdot B$
RCM2T1	$\mathrm{S1Abs}' = \frac{s_b}{\widehat{s}_b} \cdot \mathrm{S1Abs}$	$K' = \frac{s_b}{\widehat{s}_b} \cdot K$	Previous value	Previous value
RCM2T2	$\mathrm{S1Abs}' = \frac{s_b}{\mathrm{S1Abs} + K} \cdot \mathrm{S1Abs}$	$K' = \frac{s_b}{\mathrm{S1Abs} + K} \cdot K$	Previous value	

Table C-7 Applicable calibration types for Blank calibration updates

(a) For linear calibrations the fixed factor is not updated.

s_b Mean signal of Std (1) calibrator from current update measurements (absorbance or rate of change in absorbance)

$\widehat{s}_b$ Signal value calculated from the (non-updated) RCM2T1 calibration curve for Std (1) calibrator $\widehat{s}_b = \mathrm{S1Abs} + K \cdot \sinh B / (1 + B^2)$

Span calibration

A **Span** calibration is a one-point calibration. Tests are calibrated with only one calibrator, and this has to be a standard solution other than Std (1). The signal correction is a simple proportional adjustment.

The calibrator that corresponds to the **Span** point (entered on **Utility** > **Application** > **Calib.**) is measured and the previously measured calibration curve is corrected for each applicable calibration type as listed below.

Calibration type	S1 Abs.	K	A	B
Linear	$\mathrm{S1Abs}' = \frac{s_N}{\widehat{s}_N} \cdot \mathrm{S1Abs}$	$K' = \frac{\widehat{s}_N}{s_N} \cdot K$		
RCM	$\mathrm{S1Abs}' = \frac{s_N}{\widehat{s}_N} \cdot \mathrm{S1Abs}$	Previous value	Previous value	$B' = \frac{s_N}{\widehat{s}_N} \cdot B$
RCM2T1	$\mathrm{S1Abs}' = \frac{s_N}{\widehat{s}_N} \cdot \mathrm{S1Abs}$	$K' = \frac{s_N}{\widehat{s}_N} \cdot K$	Previous value	Previous value
RCM2T2	$\mathrm{S1Abs}' = \frac{s_N}{\mathrm{S1Abs} + K} \cdot \mathrm{S1Abs}$	$K' = \frac{s_N}{\mathrm{S1Abs} + K} \cdot K$	Previous value	

Table C-8 Applicable calibration types for **Span** calibration updates

s_N	Mean signal of Std (N) from current update measurements (absorbance or rate of change in absorbance)
$\widehat{s}_N$	Signal value calculated from the (non-updated) calibration curve for the given concentration value of Std (N)

The calculated signal value $\widehat{s}_N$ is obtained simply by insertion of the given concentration value x of Std (N) into the function of the calibration curve. For a Linear calibration, for example, $\widehat{s}_N = \mathrm{S1Abs} + (1/K) \cdot x$. Likewise for an RCM calibration,

Equation C-5

$$\widehat{s}_N = \frac{\mathrm{S1Abs} - B}{1 + \left(\frac{x}{K}\right)^A} + B.$$

2 Point calibration

Tests are calibrated using Std (1) calibrator and one calibrator Std (N) with N > 1. For this calibration update, the signal correction is linear: $s' = q + p \cdot s$.

The number of the second calibrator Std (N) is displayed in the **Span** box on **Utility** > **Application** > **Calib.** The calculation method depends on the calibration type as listed below.

Calibration type	S1 Abs.	K	A	B
Linear	$\mathrm{S1Abs}' = s_b$	$K' = \frac{1}{p} \cdot K$		
RCM	$\mathrm{S1Abs}' = s_b + (s_b - p \cdot \mathrm{S1Abs})\left(\frac{x}{K}\right)^A$	Previous value	Previous value	$B' = p \cdot B$
RCM2T1	$\mathrm{S1Abs}' = s_b - p \cdot K \frac{\sinh B}{1 + B}$	$K' = p \cdot K$	Previous value	Previous value
RCM2T2	$\mathrm{S1Abs}' = s_b - p \cdot K$	$K' = p \cdot K$	Previous value	

Table C-9 Applicable calibration types for **2 Point** calibration updates

p	Calibration update parameter $p = (s_N - s_b)/(\hat{s}_N - \hat{s}_b)$
s_b	The currently measured signal (absorbance or rate of change in absorbance) for Std (1) calibrator
s_N	The currently measured signal (absorbance or rate of change in absorbance) for the calibrator Std (N)
$\hat{s}_b$	Signal value calculated from the (non-updated) calibration curve for Std (1) calibrator
$\hat{s}_N$	Signal value calculated from the (non-updated) calibration curve for the given concentration value of Std (N)

Full calibration

Tests are calibrated using all calibrators specified on **Utility** > **Application** > **Others**. After this calibration, all parameters of the calibration curve are updated. The parameters of a test's calibration curve are displayed on the **Calibration Result** window (choose **Calibration** > **Status** > **Calibration Result**). Parameters of linear calibration curves are updated by linear regression, and nonlinear calibration curves are updated using a nonlinear regression algorithm.

Applicable calibration types are Linear multipoint (with more than two calibrators), RCM, RCM2T1, RCM2T2, Spline, and Line Graph.

Calibration update types

If you are updating a calibration using either a **Blank** update or a **Span** update you may select how the calibration is updated from **Utility** > **Application** > **Calib.** using the **Update Type** box.

The following calibration types can be updated by either the **Blank** or **Span** method and may use this update feature: RCM, RCM2T1, RCM2T2, Spline, and Line Graph. The calibrator used is either Std (1), for a **Blank** calibration, or it is defined in the **Span** box on **Utility** > **Application** > **Calib.** > **Calibration Type** area.

There are three update types available: **None**, **Difference**, and **Ratio**.

None If **None** is chosen as the entry in the **Update Type** box, then neither **Difference** nor **Ratio** calibration update values are applied. The calibration update occurs as described for either a blank or span calibration, depending on the calibration type chosen.

Difference The absorbance difference to the previous calibration is measured for the one defined calibrator only. This difference is then added to each of the test's standard absorbance values. This moves the calibration curve up or down, maintaining its original slope.

Ratio The test's absorbance value is measured with the one defined calibrator only. The ratio of this value to the previous yields an adjustment factor. Each of the test's standard absorbance values is then multiplied by this factor. This adjusts the slope of the calibration curve, maintaining its original y-intercept.

K factor calculation

This section shows how K factors are calculated from absorbance and concentration values for tests that are based on linear two-point calibration curves. Two examples are given: One for an endpoint assay and one for a rate assay.

After a successful calibration, an updated S1 Abs. value is shown on both the **Working Information** window and the first column of the **S1** on the **Calibration Monitor** report.

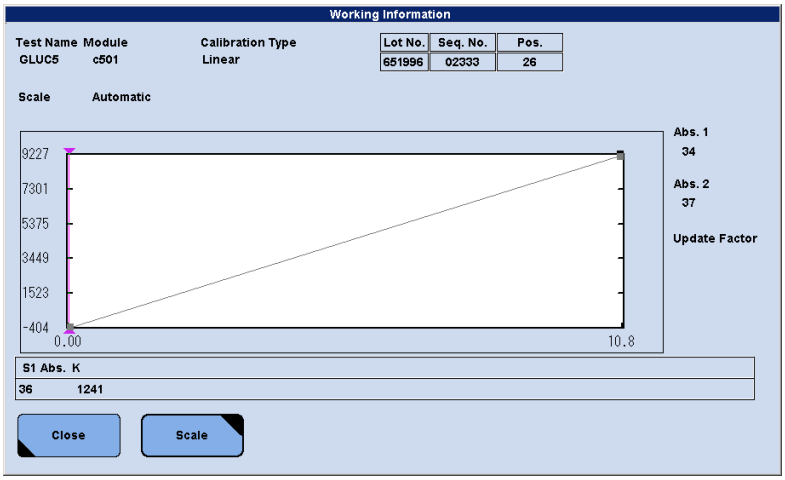


Figure C-2 **Working Information** window

The absorbance value (or rate of change in absorbance) of the second calibrator is printed in the first column under **S2** on the **Calibration Monitor** report.

These new values are used to calculate the K factor. When displayed on the **Working Information** window, the K factor is automatically rounded and multiplied by the correct power of 10, according to the decimal placement of the Std (1) concentration.

Endpoint assay example

The formula for endpoint assays is:

Equation C-6 $K = (C_N - C_b)/(A_N - A_b)$

C_b	Concentration value for Std (1)/blank calibrator
C_N	Concentration value for the second calibrator Std (N), N > 1
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
A_N	Absorbance of the second calibrator Std (N), N > 1

A glucose test is calibrated with water as Std (1) and a second calibrator with a concentration of 10.8. The mean of the measured absorbances is 0.0036 for Std (1) and 0.8739 for the second calibrator. The K factor is calculated as follows:

Equation C-7 $K = (10{,}8 - 0{,}00)/(0{,}8739 - 0{,}0036)$

$K = 10{,}8/0{,}8703 = 12{,}41$

This K factor can now be used to calculate results from test absorbances.

For more information on calculation of endpoint assay results, see:
Example of a 2 Point End assay on page B-12
Calculation of concentration on page B-15

Rate assay example

The formula for rate assays is:

Equation C-8 $K = (C_N - C_b)/(v_N - v_b)$

C_b	Concentration value for Std (1)/blank calibrator
C_N	Concentration value for the second calibrator Std (N), N > 1
v_b	Rate of change in absorbance of the reaction with Std (1)/blank calibrator
v_N	Rate of change in absorbance of the reaction with the calibrator Std (N), N > 1

An AST (aspartate aminotransferase) test is calibrated with water as Std (1) and a second calibrator with a concentration of 94.2 U/L. The mean of the measured rates of change in absorbance are $v_b = -0{,}0006$ for Std (1) and $v_x = -0{,}01575$ for the second calibrator. The K factor is calculated as follows:

Equation C-9 $K = (94{,}2 - 0{,}0)/[-0{,}0486 - (-0{,}0006)]$

$K = 94{,}2/(-0{,}0480) = -1962{,}5$

This K factor can now be used to calculate results from test absorbance rates.

For more information on calculation of rate assay results, see:
Example of a Rate A assay on page B-16
Result calculation on page B-19

Introduction to weighting

It is possible to apply a weighting function during the curve fitting process that favors those calibrator points with a lower absorbance (or rate of change in absorbance). This may result in a more accurate curve fit in that particular concentration range.

Calculation without weighting

When weighting is not used (entry of 0 in the **Weight** field on **Utility** > **Application** > **Calib.**), the curve fit is optimized by varying the parameters of the calibration function to minimize the sum of the residuals. The residuals are the squares of the differences between the actual absorbances for each calibrator and the absorbance calculated from the calibration function. In other words:

Equation C-10 $$\sum_{i=1}^{n} [A_i - f(C_i)]^2 \rightarrow \min$$

A_i	Actual absorbance (or rate of change in absorbance) of calibrator i
$f(C_i)$	Absorbance (or rate of change of absorbance) of calibrator i calculated by the calibration function from its concentration (C_i)
$i = 1 \ldots n$	Numbers of calibrators used

Calculation with weighting

When weighting is used (entry of 1 or 2 in the **Weight** field on **Utility** > **Application** > **Calib.**), each of the residuals is multiplied by a weight factor during the curve fitting process thus:

Equation C-11 $$\sum_{i=1}^{n} \{w_i [A_i - f(C_i)]\}^2 \rightarrow \min$$

w_i	Weight factor for calibrator point i
All other symbols	As described above

Weighting factors

The weighting factor is inversely related to the absorbance of the calibrator, so that those with a smaller absorbance will have a larger weighting factor.

- If an entry of 1 is made in the **Weight** field, then the weighting factor for calibrator point i is: $w_i = 1/[g(A_i)]$, where $g(A_i)$ is a function of the absorbance (or rate of change in absorbance) of calibrator i.
- If an entry of 2 is made in the **Weight** field, then the weighting factor for calibrator point i is: $w_i = 1/[g(A_i)]^2$.

Linear calibration

Water is commonly used as a zero or blank calibrator. For Linear 2 Point calibration, the absorbance of water and a second calibrator is measured. These two points are used to establish a linear plot, and its slope is used in the calculation of subsequent control and patient results.

Parameters on **Utility** > **Application** > **Calib.**:

Calib Type	Linear
Point	2
Weight	0, 1, 2
Span	2 to 6 (for 2 Point use 2)

Linear two-point calibration graph

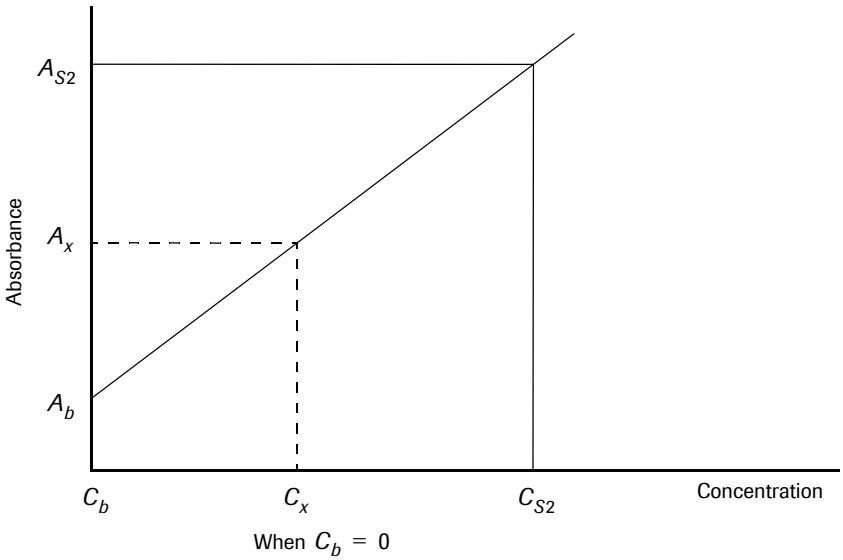


Figure C-3 Linear 2 Point calibration graph - $C_b = 0$

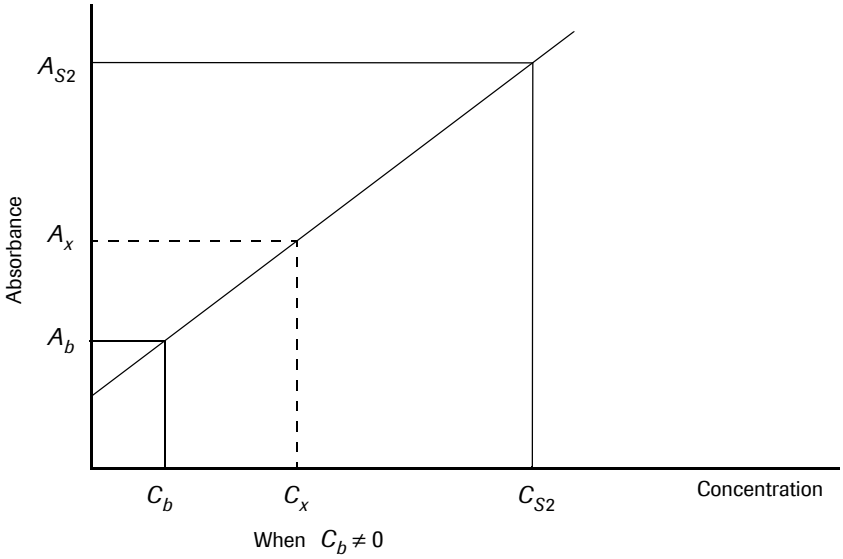


Figure C-4 Linear 2 Point calibration graph - $C_b \neq 0$

A_x	Sample absorbance value
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
A_{S2}	Absorbance of Std (2)
C_b	Concentration value for Std (1)/blank calibrator
C_x	Concentration of the analyte in the sample
C_{S2}	Concentration value for Std (2)

Linear two-point calculation

The math model for a Linear calibration is the equation for a straight line $y = a + b \cdot x$, where a is the y-intercept and b is the slope. For our purpose, we interpret this equation's variables in as follows:

$x = C$	Concentration of the analyte
$y = A$	Absorbance (or rate of change in absorbance for rate assays)
a	Absorbance when the concentration of the analyte is 0
b	Ratio of the change in absorbance to the change in concentration

Slope The slope of a straight line can be derived either by the formula $b = (\Delta y)/(\Delta x)$ (when two points are used) or by the least squares method (when multiple points are used). For the first case, comparison with Figure C-4 shows that $\Delta y = A_{S2} - A_b$ and $\Delta x = C_{S2} - C_b$.
The formula for the slope can then be solved to $b = (A_{S2} - A_b)/(C_{S2} - C_b)$. This equation shows that b is equal to the reciprocal K factor defined earlier.
Therefore, $b = 1/K$.

y-intercept Comparison with Figure C-4 shows that the y-intercept $a = A_b - (b \cdot C_b)$, where A_b is the absorbance and C_b the concentration value for Std (1)/blank calibrator.

With slope and y-intercept thus determined, it is now possible to solve the equation $y = a + b \cdot x$ to x, to calculate the analyte concentration in a patient sample C_x:

Equation C-12 $y = a + b \cdot x$ yields

$$x = \frac{1}{b}(y - a), \text{ where}$$

$$a = A_b - (b \cdot C_b) \qquad b = 1/K \qquad x = C_x \qquad y = A_x$$

By substitution of a, b, x, and y the following equation is obtained:

Equation C-13 $C_x = K[A_x - (A_b - b \cdot C_b)]$ which is equivalent to

$$C_x = [K(A_x - A_b) + C_b]$$

Two additional constants are applied to this formula to correct the result for systematic bias deriving from the instrument. The final formula for calculation of the concentration is

Equation C-14 $C_x = [K(A_x - A_b) + C_b] \cdot IF_A + IF_B$

C_x	Concentration of the analyte in the sample
K	K factor
A_x	Sample absorbance value
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
C_b	Concentration value for Std (1)/blank calibrator
IF_A, IF_B	Instrument constants representing a slope of 1 and an intercept of 0

Assay types

Linear 2 Point calibration can be used with the following assay types:

- 1 Point assay
- 2 Point End assay
- 2 Point Rate assay
- Rate A assay

RCM calibration

The RCM calibration applies a working curve in which the absorbance increases or decreases in a nonlinear manner as the concentration increases.

Parameters on **Utility** > **Application** > **Calib.**:

Calib Type	RCM
Point	2 to 6
Weight	0,1,2
Span	2 to 6

RCM calibration graph

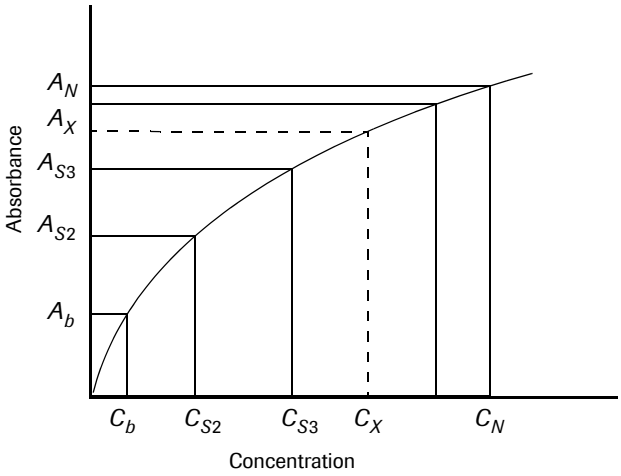


Figure C-5 Nonlinear RCM calibration graph

A_x	Sample absorbance value
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
A_{S2} , A_{S3} , ...	Absorbance value for Std (2) to Std (6)
A_N	Absorbance of Std (N)
C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C_{S2} , C_{S3} , ...	Concentration value for Std (2) to Std (6)
C_N	Concentration value for Std (N)

RCM calculation

The math model for the RCM calibration curve approximation is shown below:

Equation C-15 $$A = \frac{a-d}{1+\left(\frac{C}{b}\right)^{c}} + d$$

A	Absorbance (or rate of change in absorbance for rate assays)
C	Concentration of the analyte
a	Parameter representing the absorbance at zero concentration (A_b).
b	Parameter representing the concentration where the absorbance or absorbance rate is 1/2 of the span between A_{inf} and A_b.
c	Parameter describing the curvature of the calibration curve.
d	Parameter representing the predicted absorbance or absorbance rate for infinite concentration (A_{inf}).

The above calibration curve parameters correspond to the values on the **Working Information** window as follows (to display this window, select **Calibration** > **Status** > **Calibration Result** > **Working Information**):

S1 Abs. column displays parameter a.
K column displays parameter b.
A column displays parameter c.
B column displays parameter d.

The formula for sample concentration calculation is shown below:

Equation C-16 $$C_x = (C + C_b) \cdot IF_A + IF_B \text{ with}$$

$$C = b \cdot \left(\frac{a - A_x}{A_x - d}\right)^{1/c}$$

C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C	Concentration value before instrument constants adjustment
IF_A, IF_B	Instrument constants representing a slope of 1 and an intercept of 0
A_x	Sample absorbance value
a, b, c, d	Calibration curve parameters as in Equation C-15

Assay types

Nonlinear RCM calibration can be used with the following assay types:

- 1 Point assay
- 2 Point End assay
- 2 Point Rate assay
- Rate A assay

RCM2T1 calibration

The RCM2T1 calibration applies a working curve in which the absorbance increases in a nonlinear manner as the concentration increases.

Parameters on **Utility** > **Application** > **Calib.**:

Calib Type	RCM2T1
Point	2 to 6
Weight	0,1,2
Span	2 to 6

RCM2T1 calibration graph

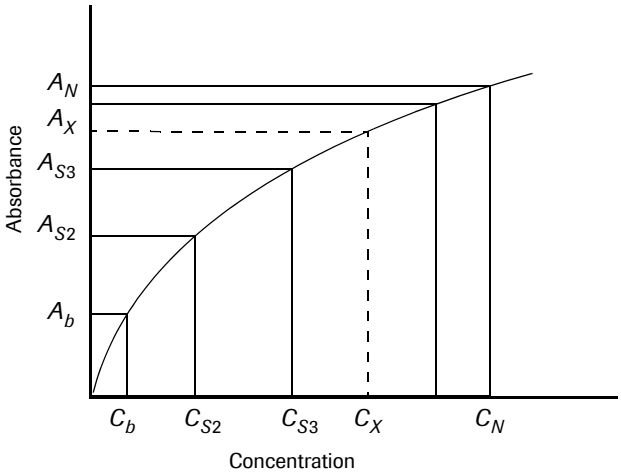


Figure C-6 Nonlinear RCM2T1 calibration graph

A_x	Sample absorbance value
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
A_{S2} , A_{S3} , ...	Absorbance value for Std (2) to Std (6)
A_N	Absorbance of Std (N)
C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C_{S2} , C_{S3} , ...	Concentration value for Std (2) to Std (6)
C_N	Concentration value for Std (N)

RCM2T1 calculation

The math model for the RCM2T1 calibration curve approximation is shown below:

Equation C-17 $A = a + b \cdot \frac{\sinh z}{1 + z^2}$ with $z = c \cdot C + d$

A	Absorbance (or rate of change in absorbance for rate assays)
C	Concentration of the analyte
a , b , c , d	Calibration curve parameters determined by nonlinear regression algorithm

The above calibration curve parameters correspond to the values on the **Working Information** window as follows (to display this window, select **Calibration** > **Status** > **Calibration Result** > **Working Information**):

S1 Abs. column displays parameter a .
K column displays parameter b .
A column displays parameter c .
B column displays parameter d .

The model function for RCM2T1 (Equation C-17) cannot be inverted analytically. However, the iteration series $z_{n+1} = arc\sinh[y \cdot (1 + z_n^2)]$ can be used to solve the equation $y = \sinh z / (1 + z^2)$. Thus, the formula for the sample concentration is as follows:

Equation C-18 $C_x = (C + C_b) \cdot IF_A + IF_B$ where C is calculated by iteration.

C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C	Concentration value before instrument constants adjustment
IF_A , IF_B	Instrument constants representing a slope of 1 and an intercept of 0

Assay types

Nonlinear RCM2T1 calibration can be used with the following assay types:

- 1 Point assay
- 2 Point End assay
- 2 Point Rate assay
- Rate A assay

RCM2T2 calibration

The RCM2T2 calibration applies a working curve in which the absorbance decreases in a nonlinear manner as the concentration increases.

Parameters on **Utility** > **Application** > **Calib.**:

Calib Type	RCM2T2
Point	2 to 6
Weight	0,1,2
Span	2 to 6

RCM2T2 calibration graph

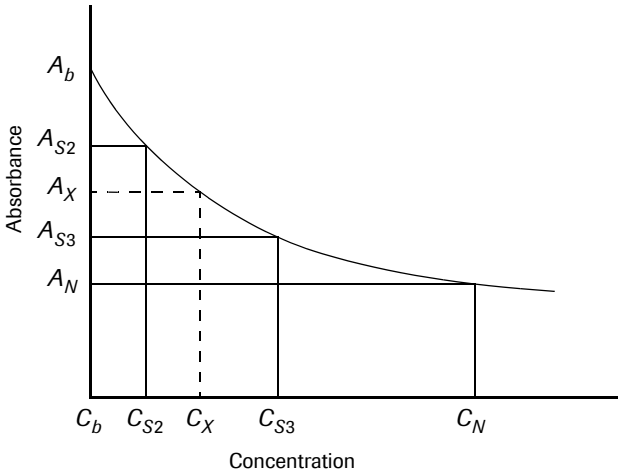


Figure C-7 Nonlinear RCM2T2 calibration graph

A_x	Sample absorbance value
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
A_{S2} , A_{S3} , ...	Absorbance value for Std (2) to Std (6)
A_N	Absorbance of Std (N)
C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C_{S2} , C_{S3} , ...	Concentration value for Std (2) to Std (6)
C_N	Concentration value for Std (N)

RCM2T2 calculation

The math model for the RCM2T2 calibration curve approximation is shown below:

Equation C-19 $A = a + r \cdot (1 + s \cdot C)^{-2}$

A	Absorbance (or rate of change in absorbance for rate assays)
C	Concentration of the analyte
a, r, s	Calibration curve parameters determined by nonlinear regression algorithm

The above calibration curve parameters correspond to the values on the **Working Information** window as follows (to display this window, select **Calibration** > **Status** > **Calibration Result** > **Working Information**):

S1 Abs. column displays parameter a.
K column displays parameter r.
A column displays parameter s.

The formula for sample concentration calculation is shown below:

Equation C-20 $C_x = (C + C_b) \cdot IF_A + IF_B$ where

$$C = \frac{1}{s} \cdot \left(\sqrt{\frac{r}{A_x - a}} - 1 \right)$$

C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C	Concentration value before instrument constants adjustment
IF_A, IF_B	Instrument constants representing a slope of 1 and an intercept of 0
A_x	Sample absorbance value
a, r, s	Calibration curve parameters as in Equation C-19

Assay types

Nonlinear RCM2T2 calibration can be used with the following assay types:

- 1 Point assay
- 2 Point End assay
- 2 Point Rate assay
- Rate A assay

Spline calibration

When this calibration type is applied, the ranges between the data points of the measured calibrators are approximated by third degree polynomials so that a smooth calibration curve is obtained.

Parameters on **Utility** > **Application** > **Calib.**:

Calib Type	Spline
Point	3 to 6
Weight	0,1,2
Span	2 to 6

Spline calibration graph

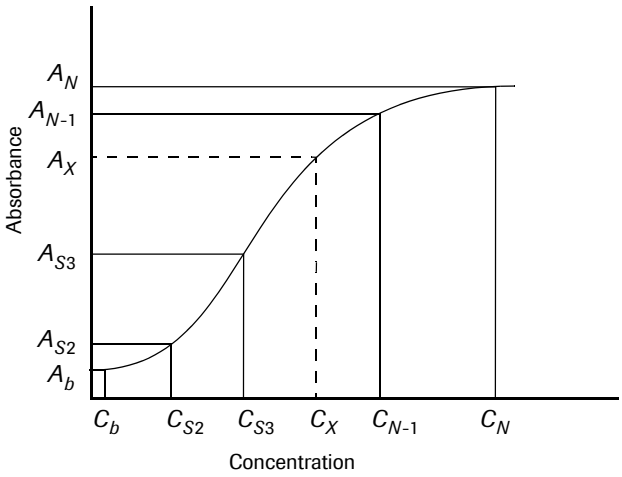


Figure C-8 Nonlinear spline calibration graph

A_x	Sample absorbance value
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
A_{S2} , A_{S3} , ...A_N	Absorbance of Std (2), Std (3), ...Std (N)
C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C_{S2} , C_{S3} , ... C_N	Concentration value for Std (2), Std (3), ...Std (N)

Spline calculation

In the math model of a spline calibration, data points of calibrators are taken as supporting points for the determination of interpolating functions. The simplest interpolating functions are used in linear interpolation, where adjacent data points are connected by a straight line. This method is used for Line Graph calibrations.

See *Line Graph calibration* on page C-36.

In contrast to the angular polygon of a Line Graph calibration, a smooth curve will result when using piecewise polynomials of a higher degree for the interpolation. The routine applied for Spline calibrations determines a smooth cubic spline approximation using third degree polynomials.

Assay types

Nonlinear spline calibration can be used with the following assay types:

- 1 Point assay
- 2 Point End assay
- 2 Point Rate assay
- Rate A assay

Line Graph calibration

When this calibration type is applied, the ranges between the data points of the measured calibrators are approximated by linear interpolation. An angular polygon is obtained as calibration curve.

Parameters on **Utility** > **Application** > **Calib.**

Calib Type	Line Graph
Point	3 to 6
Weight	0,1,2
Span	2 to 6

Line Graph calibration graph

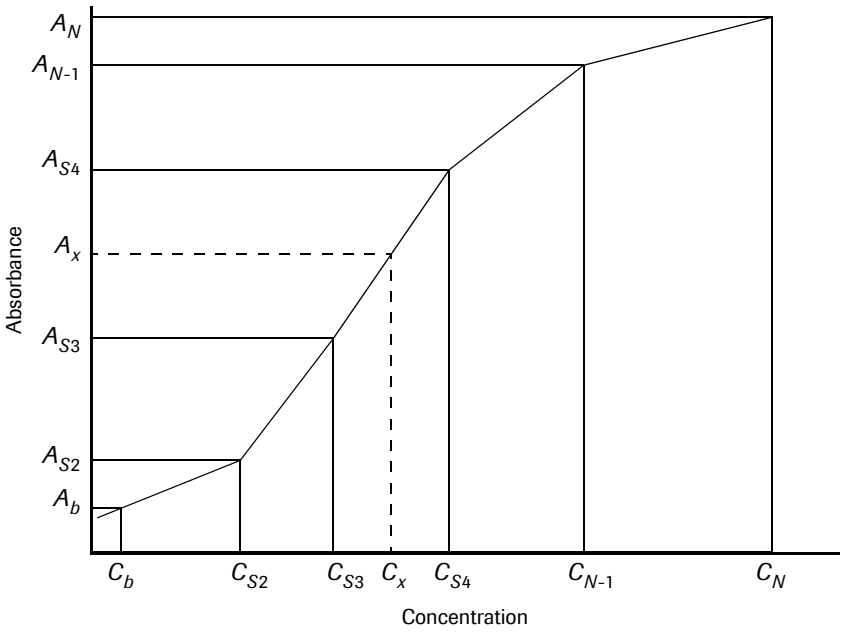


Figure C-9 Nonlinear line calibration graph

A_x	Sample absorbance value
A_b	Absorbance of Std (1)/blank calibrator (S1 Abs.)
A_{S2} , A_{S3} , ...A_N	Absorbance of Std (2), Std (3), ...Std (N)
C_x	Concentration of the analyte in the sample
C_b	Concentration value for Std (1)/blank calibrator
C_{S2} , C_{S3} , ...C_N	Concentration value for Std (2), Std (3), ...Std (N)

Line Graph calculation

The math model for Line Graph calibration curve approximation is shown below:

Equation C-21 $$K_{N-1} = \frac{C_N - C_{N-1}}{A_N - A_{N-1}}$$

K_{N-1}	Calibration factor for the interval between C_{N-1} and C_N, or A_{N-1} and A_N, respectively
A_N	Absorbance of Std (N)
A_{N-1}	Absorbance of Std (N-1)
C_N	Concentration value for Std (N)
C_{N-1}	Concentration value for Std (N-1)

The formula for sample concentration is shown below:

Equation C-22 $$C_x = [K_{N-1}(A_x - A_{N-1}) + C_{N-1}] \cdot IF_A + IF_B \text{ for } A_x \in [A_{N-1}, A_N]$$

C_x	Concentration of the analyte in the sample
A_x	Sample absorbance value
IF_A, IF_B	Instrument constants representing a slope of 1 and an intercept of 0
All other symbols	See legend above.

For a calibration based upon N standard solutions—Std (1) to Std (N)—there are N - 1 calibration curve intervals. The sample absorbance value (or rate of change in absorbance for rate assays) A_x determines which of the calibration curve intervals and which of the calibration factors is relevant for the calculation of C_x. If A_x lies between A_{N-1} and A_N the relevant calibration factor is K_{N-1}.

Assay types

Nonlinear Line Graph calibration can be used with the following assay types:

- 1 Point assay
- 2 Point End assay
- 2 Point Rate assay
- Rate A

e 601 - Immunology calibration

This chapter provides you with an overview of the calibration methods used by the **cobas** 6000 for immunology assays. Master, lot and reagent calibration are described, as well as calibration validation and stability.

In this chapter

Chapter **10**

Table of contents

Introduction

Calibration is required to determine the concentration of an unknown substance as accurately as possible independent of reagent lot, reagent conditions, and analyzer conditions. For this, a master calibration curve is generated at Roche Diagnostics during production of the reagent. At the customer site, the analyzer generates an update of the master curve by measuring 2 calibrators under routine laboratory conditions.

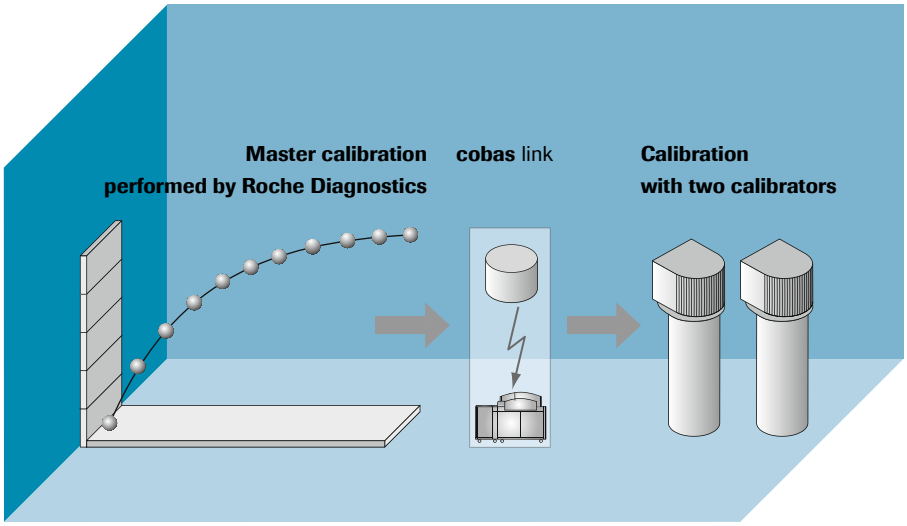


Figure C-10 Calibration procedure

The calibration curve produced from the master calibration and the measured calibration is specific to each reagent lot and in some cases, to an individual reagent pack. The result of a calibration is validated automatically by the analyzer and can be further validated by the operator.

Lot-specific calibrator data for each application are available by data transfer from **cobas** link.

Master calibration

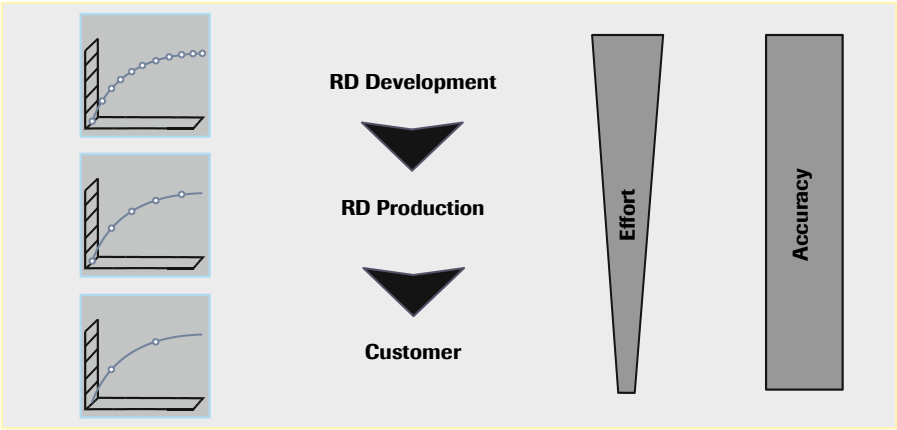


Figure C-11 Calibration concept

A reference standardization curve utilizing master test kit reagents and certified reference standard material [e.g., World Health Organization (WHO) reference material] is measured at Roche Diagnostics. This curve uses 10 to 12 points (n = 10 to 12). The reference standard curve is the basis for the production of master calibrators. Reference standardization can also be performed using a reference method (for example, GC-MS) or versus a commercially available method (for example, from the main competitor).

A lot-specific master calibration curve (n = 5 or 6) is measured at Roche Diagnostics using lot-specific test kit reagents and master calibrators. The shape of the lot-specific master curve is characterized by a four-parameter Rodbard function. The lot-specific Rodbard parameters characterizing this curve are encoded in the **cobas e** pack barcode.

The data characterizing this curve (including CalSet assigned values) are transferred from **cobas** link.

At the customer site the calibration curve is adapted by measuring two calibrator levels.

Lot calibration

A lot calibration (**L-Calib**) is a calibration performed with a fresh reagent pack that has been on the analyzer no longer than 24 hours. All quality criteria must be fulfilled. The lot calibration is valid for all other reagent packs of the same lot.

Reagent pack calibration

A reagent pack calibration (**R-Calib**) is performed with a reagent that has been on the analyzer more than 24 hours.

See *Calibration checks* on page C-45.

A reagent pack calibration is valid for one specific reagent pack only. The reagent pack calibration is compared to the most recent stored **L-Calib** for validation.

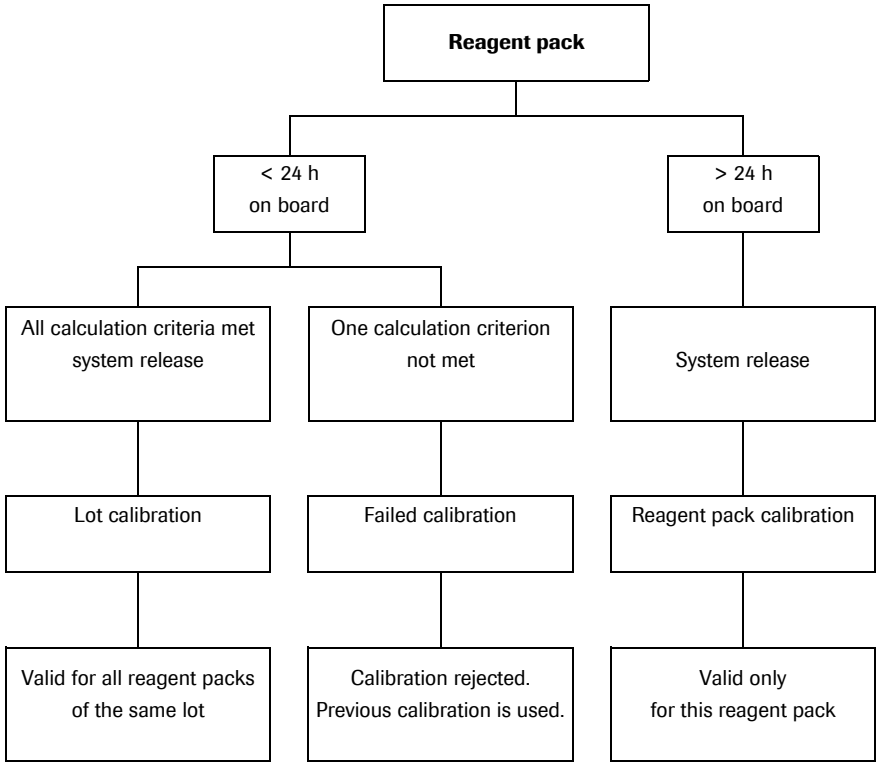


Figure C-12 Reagent pack calibration flow chart

Calibration stability

Calibration stability falls into the following two categories:

- Lot calibration stability—which relates to the long term stability of the instrument conditions and over the shelf life of reagents
- Reagent pack calibration stability—which relates to the age of the reagent when stored on board

Calibration frequency

The calibration stability depends on the following requirements:

- Test specific requirements
- National requirements

Please note calibrations have to be carried out according test specific requirements mentioned in the test specific instructions for use.

Obligatory calibration

Obligatory calibration is listed below:

- New reagent lot: calibration

Recommended calibration

Typical recommended calibrations are listed below:

- Using the same reagent lot: calibration every 31 days
- Using the same reagent pack: calibration every 7 days
- As required, for example, if quality control findings are outside the specified limits or according national requirements.

Calibration checks

Calibrations are automatically checked for the following:

Quantitative assays	Qualitative assays
Missing values	Missing values
Monotony of curve	Slope
Calibration factor	Minimum signal
Minimum signal	Maximum signal
Minimum difference	Minimum acceptable difference
Deviation of duplication	Deviation of duplication

Table C-10 Quality criteria for quantitative and qualitative assays

Missing values

Duplicate determinations of two calibrators are used to adjust the master calibration curve stored on the reagent pack bar code. Currently, all Elecsys reagents use only two calibrators.

If no value is missing, the calibration succeeded. If one value is missing, the calibration fails.

Monotony of curve (quantitative assays only)

All measured calibrator values must fall in ascending (sandwich or bridging principle) or descending (competition principle) order. If the monotony is not given, the calibration fails.

Slope (qualitative assays only)

It is checked whether or not the calibration curve behaves monotonously. That is, all measured calibrator values must fall in ascending (sandwich or bridging principle) or descending (competition principle) order. If this is not the case, or the slope is less than or greater than the slope encoded in the reagent bar code, the calibration fails.

Calibration factor (quantitative assays only)

Each new lot calibration (**L-Calib**) uses a calibration factor of 1. For all subsequent reagent pack calibrations (**R-Calib**), a new calibration factor is calculated. The calibration factor is the quotient of the slopes of the currently performed calibration and the last valid lot calibration.

Status	Calibration factor range
Succeeded	Within 0.8-1.2
Failed	< 0.8, or > 1.2

Table C-11 Criteria for calibration factor validation

The calibration factor criterion is only used in validating reagent pack calibrations.

Minimum signal

The measured signal of the calibrator replicate must be above the minimum value. Values are lot dependent and are encoded in the reagent barcode.

If one of either calibrator replicates falls below the recommended minimum signal level, the calibration fails.

Min/max signal (qualitative assays only)

The measured signal of the calibrator should fall between the designated minimum and maximum signal. Minimum and maximum signals are test dependent and are encoded in the reagent bar code.

If one of either calibrator replicates falls out of the specified min/max signal range, the calibration fails.

Minimum difference (quantitative assays only)

Defined as the difference in percent of the values between calibrator 1 and 2. This difference must amount to at least 30% for the calibration to be accepted.

Minimum acceptable difference (qualitative assays only)

The difference between the negative and positive calibrator signal values must be greater than the encoded value limit. This limit is test dependent and is encoded in the reagent bar code.

Deviation of duplicate measurement (Dupl.)

The deviation of duplicate measurements is a check of the signal values for each replicate of a calibrator. If the difference between the duplicate measurements is above a certain limit, the calibration fails.

System errors (Sys.E)

If a hardware error occurs during a calibrator measurement, the calibration has failed.

Cutoff (qualitative assays only)

Qualitative assays are calibrated by a scaling factor, or the cutoff value. The actual cutoff value is calculated by means of the cutoff formula on the basis of at least one reactive or non-reactive calibrator. Each sample receives a scaled result value, the cutoff value, which allows for the classification of samples being reactive or non-reactive.

Borderline (qualitative assays only)

For some assays it is possible that in a range around a Cutoff Index = 1, no determination regarding reactive or non-reactive results can be made. This range is called the borderline or borderline area.

Calibration of quantitative assays

The following is a description of the applied calculation methods for heterogeneous immunology results. To calculate quantitative tests the system uses the following three calibration functions to convert measured signals into concentration values:

- Rodbard function
- Linear calibration function
- Linear-reciprocal calibration function

The calibration function used by the system is encoded in the 2D bar code on the appropriate reagent pack. The calculations are performed automatically by the analyzer, including the correction for samples diluted by the analyzer.

Rodbard function

The conversion of the measured signal into a concentration using the Rodbard function is as follows:

Equation C-23

$$y = \frac{a - d}{1 + \left(\frac{x}{b}\right)^{c}} + d$$

x	Sample concentration
a , b , c , d	Rodbard function parameters
y	Signal

Parameters b and c define the shape of the curve and parameters a and d define the position of the curve.

Under the controlled conditions of automation on the analyzer, the shape of the calibration curve is very stable and, therefore, it is possible to calibrate this nonlinear function with only two calibrators and the information of the shape parameters b and c . The curve position parameters a and d are calculated with each calibration.

The following inverse formula is used to determine the sample concentration.

Equation C-24

$$x = b \cdot \left(\frac{a - y}{y - d}\right)^{1/c}$$

y	Signal
a , b , c , d	Rodbard function parameters
x	Sample concentration

Linear reciprocal calibration function

The conversion of the measured signal into a concentration is as follows:

Equation C-25 $$\frac{1}{y} = b \cdot x + a$$

y	Signal
x	Concentration
a , b	Calibration curve parameters (y-intercept and slope)

Calibrations using a linear reciprocal calibration curve are always performed using two calibrators.

The following inverse formula is used to determine the sample concentration.

Equation C-26 $$x = \frac{1 - a \cdot y}{b \cdot y}$$

x	Sample concentration
a , b	Calibration curve parameters
y	Signal

Calibration of qualitative assays

In order to assess patient samples as reactive, non-reactive, or border area, a cutoff value is calculated.

Two standards, Std 1 and Std 2, are used for calibration. These calibrators produce effective signals from which the cutoff value is calculated.

Based on the test there are different equations to calculate the cutoff value. The test-specific equation as well as the corresponding test-specific factors a, b, and c can be found in the respective product information of the test. This information is also encoded on the reagent barcode.

The cutoff formula is lot independent.

Result calculation for qualitative assays

In order to calculate the result of a qualitative assay (cutoff test), the system compares the effective signal of the measurement with the cutoff value of the calibration. For that purpose, a cutoff index is calculated as follows:

Equation C-27

$$COI = \frac{S_{eff} - (c \times S1_{eff})}{Cutoff}$$

COI	Cutoff index
S_{eff}	Effective signal of sample measurement
c	Factor for reduction of a constant blank value signal
$S1_{eff}$	Effective signal of standard 1
$Cutoff$	Cutoff value of the calibrator

The analyzer uses all decimal places in the cutoff index to judge a result as reactive or non-reactive.

The result message (**R.M.**) is assigned before the analyzer rounds and displays the cutoff index to 2 decimal places.

The test result is evaluated depending on the test principle (sandwich or competitive).

Sandwich assays

Samples with a cutoff index ≥ 1.00 are considered reactive; those with a cutoff index < 1.00 are considered non-reactive. For some assays a border area is introduced.

Rounding cutoff index

For qualitative sandwich assay, if the actual cutoff index is 1.001 to 1.004 before rounding, the cutoff index displayed after rounding is 1.00, and the result message **reac** (reactive) is added.

On the other hand, if the actual cutoff index is 0.9995 to 0.9999 before rounding, the cutoff index displayed is still 1.00 after rounding, but in this case the result message **n-re** (non-reactive) is added.

Competitive assays Samples with a cutoff index > 1.00 are considered non-reactive; those with a cutoff index ≤ 1.00 are considered reactive.

Rounding cutoff index

For qualitative competitive assay, if the actual cutoff index is 1.001 to 1.004 before rounding, the cutoff index displayed after rounding is 1.00, and the result message **n-re** (non-reactive) is added.

On the other hand, if the actual cutoff index is 0.9995 to 0.9999 before rounding, the cutoff index displayed is still 1.00 after rounding, but in this case the result message **reac** (reactive) is added.

Border area Some assays have a border area, i.e. that for a defined cutoff index range e.g. 0.900-1.00 or 1.00-1.10 no clear decision can be taken and the attached result message is b for border area.

Calculating data alarms D

c 501 - Calculating data alarms

This chapter provides you with an overview of how some important data alarms are calculated by the **cobas** 6000.

In this chapter *Chapter* 11

Table of contents

Introduction

Several methods are used by the system to ensure that final results are valid. Data alarms appear on the results printout to indicate possible data errors. Some of these also activate the audible alarm and initiate the display of the alarm indicator on the global **Alarm** button.

Prozone limit check (>Proz, >Kin)

Some homogenous immunoassays use the principle of antigen/antibody complex formation (agglutination or precipitation) as a measurement technique. The turbidity caused by this specific agglutination or precipitation can be measured by photometric means.

The antigen/antibody complex formation is predictable as long as an excess of reagent (antibody) exists. However, in patient samples with very high levels of antigen, the reaction may begin to reverse (deagglutination) because of the effect of the excess antigen. This is called a prozone effect and without checking for this phenomenon, abnormally high samples may give incorrect or even false normal results.

There are two prozone check methods available: Antigen readdition method and reaction rate method. Both of these methods can be applied to any type of assay.

Antigen readdition (>Proz)

The system may perform a check for the prozone effect by adding a dilution of the antigen as an additional reagent (R2 or R3). If the reaction continues in the same direction (increasing or decreasing absorbance) as in the initial reaction, then an excess of reagent (antibody) still exists—prozone effect is not occurring. If the reaction proceeds in the opposite direction, after additional antigen is added, then prozone effect is occurring and the result is invalid. The corresponding data alarm is printed on the patient report.

The antigen readdition method is applied when two prozone measuring points are defined on **Utility** > **Application** > **Analyze** ([pmp_1] [pmp_2] [0] [0]).

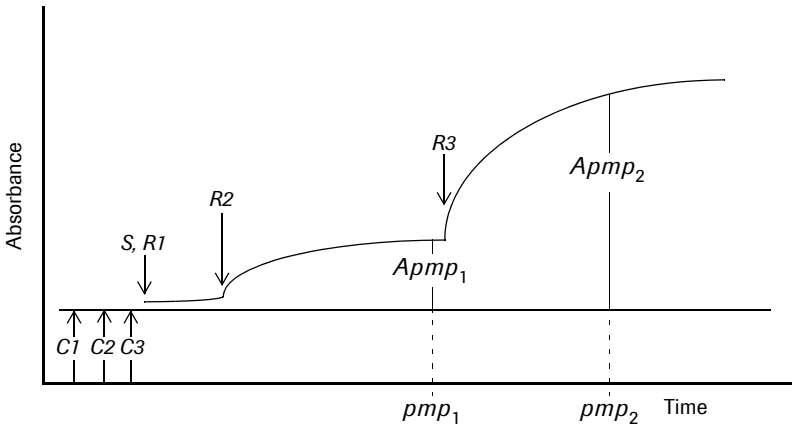


Figure D-1 Prozone check - antigen readdition method

$C1$, $C2$, ...	The reaction cell's water blank values[(a)]
S	Pipetting of sample
$R1$, $R2$, $R3$	Pipetting of reagent at R1, R2, and R3 timing
pmp_1 , pmp_2	Prozone measuring points 1 and 2
$Apmp_1$,$Apmp_2$	Absorbance at pmp_1 and pmp_2

(a) See Chapter 5 *c 501 - Photometric principles*: *Cell Blank Measurements report* on page B-21.

Reaction rate method (>Kin)

The reaction rate method verifies the existence of an excess of reagent (antibody) not by repeated addition of antigen but by observation of the reaction rate in the course of the reaction. This method is applied when four prozone measuring points are defined on **Utility** > **Application** > **Analyze**
([pmp_1] [pmp_2] [pmp_3] [pmp_4]).

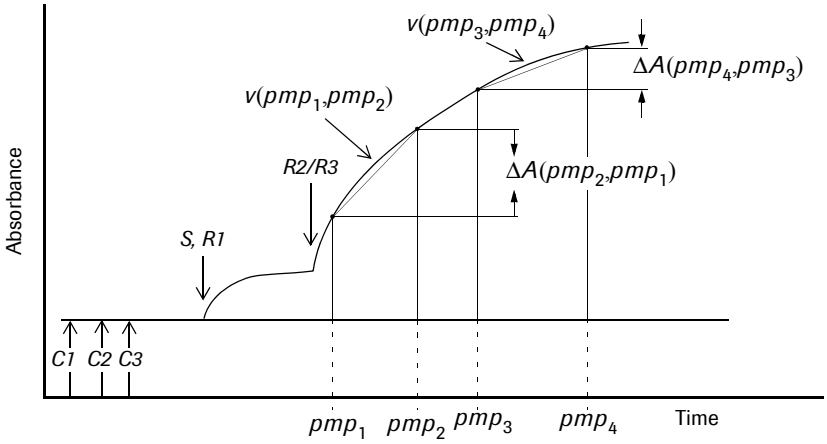


Figure D-2 Prozone check - reaction rate method

$v(pmp_1,pmp_2)$ Rate of change in absorbance between pmp_1 and pmp_2.

All other symbols See Figure D-1 above.

For more information, see Chapter 5 *c 501 - Photometric principles*.

Linearity limit check (>Lin)

To verify the linearity of a rate reaction (rate of change in absorbance), the percentage of nonlinearity is calculated. This values must be less than the **Linearity Limit**, defined on the **Utility** > **Application** > **Analyze**. If the calculated value is above the limit, a *>Lin* data alarm is issued. If any absorbance reading taken during the programmed interval exceeds the Abs. Limit parameter, that absorbance reading is excluded from the least squares rate calculation.

Depending on the number of measuring points of an application, the system calculates the nonlinearity in one of the following ways:

- If there are less than 5 measuring points, linearity check is not performed.
- If there are 6 to 16 measuring points, two times five points are used in the calculation.
- If there are 17 or more measuring points, two times eleven points are used in the calculation.

6-16 measuring points

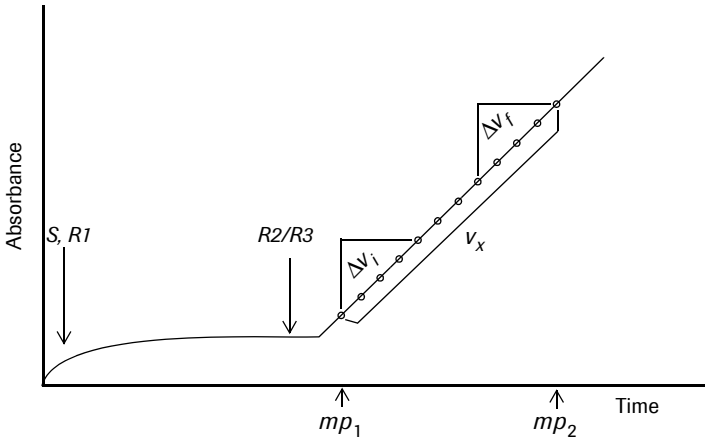


Figure D-3 Linearity Verification - >Lin

S	Pipetting of sample
R1, R2/R3	Pipetting of reagent at R1 timing, and at R2 or R3 timing
mp_1	First photometric measuring point
mp_2	Last photometric measuring point
v_x	Rate of change in absorbance calculated for all measuring points between mp_1 and mp_2 by least squares analysis
v_i	Rate of change in absorbance calculated for the initial five measuring points
v_f	Rate of change in absorbance calculated for the final five measuring points

The percentage of nonlinearity is the difference between the slope of the initial part of the curve and the slope of the final part of the curve scaled to the overall slope. An alarm is issued, if $[(v_i - v_f)/(v_x)] \cdot 100 > LL_1$, where LL_1 is the value of the first box in the **Linearity Limit** line on **Utility** > **Application** > **Analyze**.

17 or more measuring points

In principle, the percentage of nonlinearity for 17 or more measuring points is calculated in the same way as for 6 to 16 measuring points. The only difference is that v_i and v_f are calculated on the basis of the initial and final eleven measuring points, respectively.

An alarm is issued, if $[(v_i - v_f)/(v_x)] \cdot 100 > LL_2$, where LL_2 is the value of the second box in the **Linearity Limit** line on **Utility** > **Application** > **Analyze**.

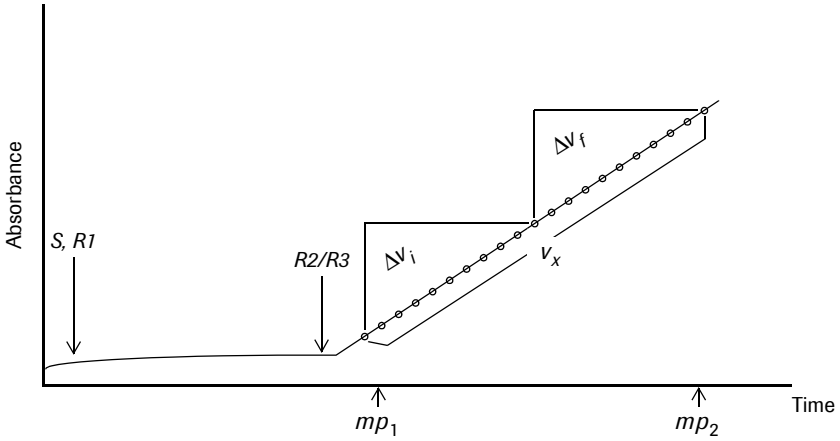


Figure D-4 Linearity Verification - >Lin

S	Pipetting of sample
R1, R2/R3	Pipetting of reagent at R1 timing, and at R2 or R3 timing
mp_1	First photometric measuring point
mp_2	Last photometric measuring point
v_x	Rate of change in absorbance calculated for all measuring points between mp_1 and mp_2 by least squares analysis
v_i	Rate of change in absorbance calculated for the initial eleven measuring points
v_f	Rate of change in absorbance calculated for the final eleven measuring points

Additional conditions for the linearity check

To the right of the **Linearity Limit** field on the **Utility** > **Application** > **Analyze** there are four boxes:

Linearity Limit [limit 6-16 mp]% [limit ≥ 17 mp]% [condition 1] [condition 2]

- The first two boxes indicate the linearity limits (in Abs × 10^4/min) for 6 to 16 and 17 or more measuring points, respectively.
- The third and fourth boxes define additional conditions for the linearity check.

  The entry in the third box defines a minimum rate of change in absorbance (in Abs × 10^4/min) for v_x. If the measured rate falls below this minimum, the linearity check is neglected.—That is, the third box defines the variable T for the following condition:

  ◦ If $|v_x| < T \times 10^{-4}$, linearity check is not performed.

  The entry in the fourth box defines a minimum difference between v_i and v_f (in Abs × 10^4/min). If the measured difference $|v_i - v_f|$ falls below this minimum, the linearity check is neglected.—That is, the fourth box defines the variable D for the following condition:

  ◦ If $|v_i - v_f| < D \times 10^{-4}$, linearity check is not performed.

The linearity limit check is applied **before** calculation of a calculated test or a compensated test, or **before** application of an instrument factor, in order to prevent further calculation with invalid results.

Sensitivity limit check (Sens.E)

An upper and lower sensitivity limit is designated on **Utility** > **Application** > **Calib.** for each photometric application. These values relate to the minimum and maximum absorbance changes which must be satisfied between the blank and span calibrators during calibration. The sensitivity observed during calibration is calculated as follows:

Equation D-1

$$\frac{Abs(S_N) - Abs(S_1)}{Conc(S_N) - Conc(S_1)}$$

where S_N is the Span calibrator and S_1 is the blank. If the sensitivity observed is not within the sensitivity limits, a *Sens.E* alarm is issued indicating a failed calibration. All calibrations that affect the factor setting for the test will be error checked against the sensitivity limit calculated from the Span Calibrator.

Duplicate limit check (Dup.E)

A duplicate limit for calibrator acceptability is designated on **Utility** > **Application** > **Calib**. The entry in the first **Duplicate Limit** text box defines the % error limit. The entry in the second box defines the absorbance error limit. The corresponding check values are calculated as follows:

$$DE_{\%} = \frac{|Abs2 - Abs1|}{(Abs2 + Abs1)/2} \cdot 100 \; ; \; DE_{\text{Abs.}} = |Abs2 - Abs1|$$

$DE_{\%}$	Relative duplicate error: Calculated value for the % error of a calibrator's absorbance readings (duplicate)
$DE_{\text{Abs.}}$	Absolute duplicate error
$Abs1$, $Abs2$	Two absorbance readings, taken for each calibrator (duplicate readings)

All photometric calibrators are run in duplicate. If both the % error and the absorbance error are out of range, a *Dup.E* alarm is issued indicating a failed calibration.

The following flowchart describes how a decision is made to flag a calibration for violating the duplication limit.

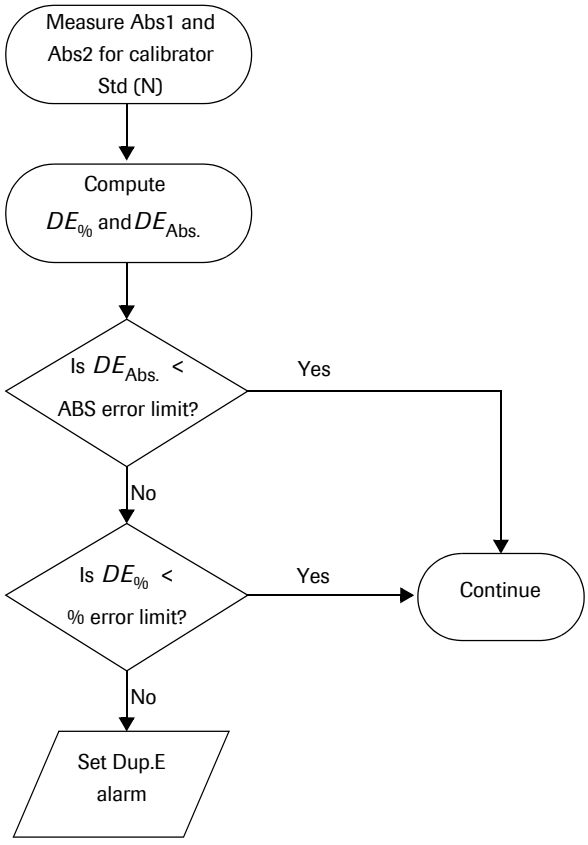


Figure D-5 Dup.E limit flowchart

Technical limit check (>Test, <Test)

If a result does not fall into the concentration range specified by the upper and lower **Technical Limits** on **Utility** > **Application** > **Range**, a data alarm >*Test* or <*Test* is issued. >*Test* indicates results that exceed the upper limit. <*Test* indicates results below the lower limit.

In order to prevent further calculation with invalid results, the technical limit check is applied **before**:

1. Calculation of a compensated test (there is no technical limit check for calculated tests).
2. Application of an instrument factor.
3. Rounding.
4. The dilution factor from automatic sample dilution is considered.

Repeat limit check (>Rept, <Rept)

The repeat limit is checked with the final concentration (printed concentration) as well as the normal value (expected value) check.

The relationship between parameters on the **Utility** > **Application** > **Range** screen and check values of the technical limit, repeat limit and normal values is as follows.

Check Item Range Screen	Application Screen	Check Value	Check Range
Technical Limit	[t1] - [t2]	Conc.1	[t1 × γ] - [t2 × γ]
Repeat Limit	[a1] - [a2]	Conc.2	[a1] - [a2]
Normal Value	[n1] - [n2]	Conc.2	[n1] - [n2]

Table D-1 Relationship between parameters and check values

Conc.1	Original concentration (measured) C_1
Conc.2	Final output concentration $C2 = (1/\gamma) \cdot C1$
γ	Concentration conversion coefficient as calculated in the Technical Limit Checking section[(a)]
t1, t2	lower technical limit and upper technical limit
a1, a2	lower repeat limit and upper repeat limit
n1, n2	lower expected value and upper expected value

(a) See *Technical limit check (>Test, <Test)* on page D-12.

When the result (Conc.1) is less than the lower technical limit (t1), the result is flagged with a <*Test* data alarm. When the result (Conc.1) is greater than the upper technical limit (t2), the result is flagged with a >*Test* data alarm.

When the result (Conc.2) is less than the lower repeat limit (a1), the result is flagged with a <*Rept* data alarm. When the result (Conc.2) is greater than the upper repeat limit (a2), the result is flagged with a >*Rept* data alarm.

When the result (Conc.2) is less than the lower expected value (n1), the result is flagged with L on the **Data Monitor** report. When the result (Conc.2) is greater than the upper expected value (n2), the result is flagged with an H on the **Data Monitor** report.

The repeat limit check is applied **after** calculation of a calculated test or a compensated test, or **after** application of an instrument factor.

Abs. limit check (>React)

In rate assays, correct data cannot be obtained if the concentration or activity value is beyond the quantitative range. For this reason, a check is performed with reference to a set upper or lower absorbance limit. For rate assays with ascending absorbances, the limit is an upper limit; for assays with descending absorbances, the limit is a lower limit. The reaction limit value is displayed on **Utility** > **Application** > **Analyze**.

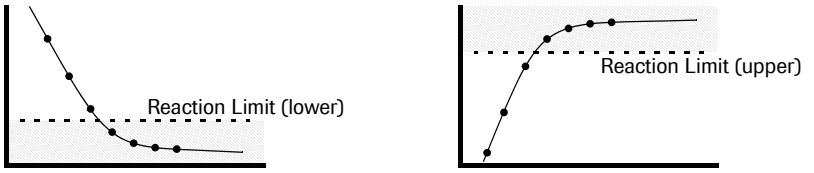


Figure D-6 Reaction limits for ascending and descending rate assays

A data alarm (*>React*) is issued if 3 or less measuring points remain within the set absorbance limit. The alarm is not issued if there are 4 or more measuring points within the absorbance limit.

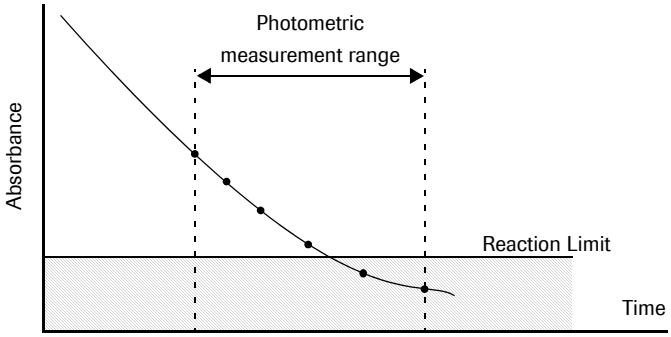
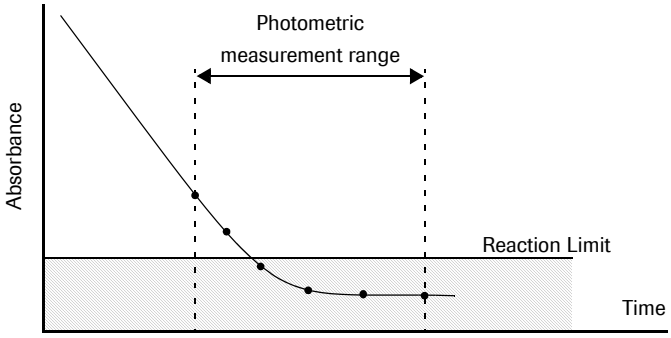
Data alarm	Number of points within Reaction Limit	Photometric points used for calculation	Reaction process
None	4	3 points within reaction limit	
>React	3 or less	3 or less Points within reaction limit	

Table D-2 Relationship between reaction limit check and photometric points

Automatic correction of Reaction Limit absorbance

The reaction limit check is performed with reference to the absorbance at the main wavelength. The system automatically corrects the given reaction limit value by adding absorbance due to sample turbidity, etc.:

Reaction limit absorbance after correction =
input reaction limit absorbance + (L_1 - L_b)

L_1: Main wavelength absorbance of sample at photometric point 1

L_b: Main wavelength absorbance of reagent blank at measuring point 1

When L1 - Lb ≤ 0, the reaction limit absorbance is not corrected.

The absorbance limit check is applied **before** calculation of a calculated test or a compensated test, or **before** application of an instrument factor.

Abs. limit check (>React)

Quality control E

Applying QC rules

This chapter provides you with an overview of the application of quality control rules used by the **cobas** 6000. The multi-rule Shewhart-type method using the Westgard algorithm is described as well as possible alarms generated.

In this chapter *Chapter* 12

Introduction

If selected in the software, the system can utilize the **Realtime QC** to evaluate QC by a multi-rule Shewhart-type method using the Westgard algorithm. For each test, this algorithm applies a set of rules selected on **QC** > **Individual** > **Realtime QC** > **Rules**.

Any combination of rules may be specified. A pair of controls for each test being processed is compared against a known standard deviation (SD) and mean. If one or both of the controls fail a rule, the system continues applying the testing criteria for all selected rules. When at least one rule violation is found, the appropriate data alarm for that rule is issued, and both the graph on the screen and the QC results on **Workplace** > **Data Review** are flagged. The QC alarm for the last rule violated is issued. The following is an explanation of each QC rule, using a display example where appropriate.

All data alarms are listed in the Data alarms chapter of the Operator's Manual.

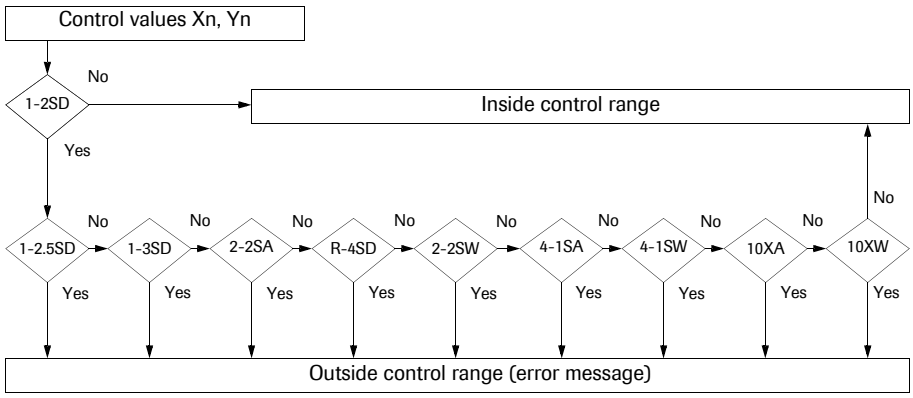


Figure E-1 Application of Westgard rules

Rule 1: 1-2SD

1-2SD represents the control rule where one control result exceeds limits defined as the mean ± 2SD. Each control sample pair X and Y is compared against its respective expected mean and standard deviation. If both X and Y are within the mean ± 2SD, the QC results are accepted. No alarm is issued and no additional rules are checked for this control pair. If either X or Y results are outside the mean ± 2SD, the test fails, but no alarm is issued. The next selected rule is then applied and tested.

Rule 2: 1-2.5SD (Q2.5SD alarm)

1-2.5SD symbolizes the control rule violated when one control result exceeds the limit defined as the mean ± 2.5SD.

If tighter QC restrictions are desired, this rule may be selected in place of Rule 2: 1-3SD. If both the 1-3SD and 1-2.5SD rules are selected in the **QC** > **Individual** > **Realtime QC** > **Select Rules** window and a set of controls fails both rules, the *Q3SD* alarm is issued. The deviation of a single sample is compared against 2.5 SD for each control. If either control X or Y is outside the mean ± 2.5 SD, the rule is violated. A *Q2.5SD* data alarm is issued for an indeterminate QC error, and a " ▲" displays on the Yoden plot

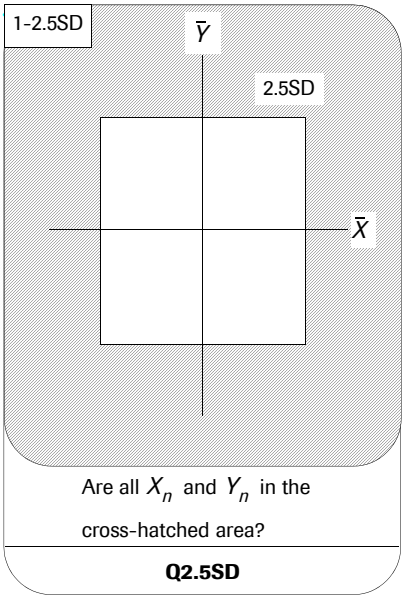


Figure E-2 Q2.5SD alarm situation

Rule 3: 1-3SD (Q3SD alarm)

1-3SD is the control rule violated when a single control X or Y result exceeds the limit defined as the mean ± 3SD. Each control sample X and Y is compared against its respective expected mean and standard deviation. If both X and Y are within the mean ± 3SD, the QC results are accepted.

If either X or Y results are outside the mean ± 3SD, a *Q3SD* alarm is issued indicating an indeterminate QC error has occurred. A "▲" displays on the screen in the appropriate part.

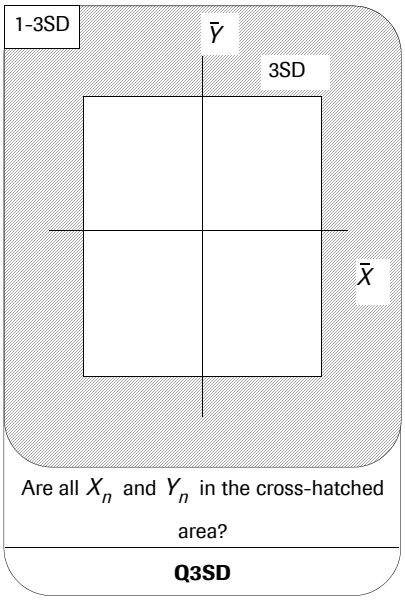


Figure E-3 Q3SD alarm situation

Rule 4: 2-2SA (S2-2Sa alarm)

The results of one assay on each control are evaluated (a total of two control results are tested).

The results of the most recent control pair of X and Y are compared against standard deviations. If both X and Y deviate outside ± 2SD and both are either above or below the mean, the rule is violated. A *S2-2Sa* data alarm is issued indicating a systematic QC error has occurred. A "●" displays on the Yoden plot.

The *S2-2Sa* alarm is issued when the two results (both X and Y in this case) are outside the ± 2SD limit, across control materials. This is a systematic violation.

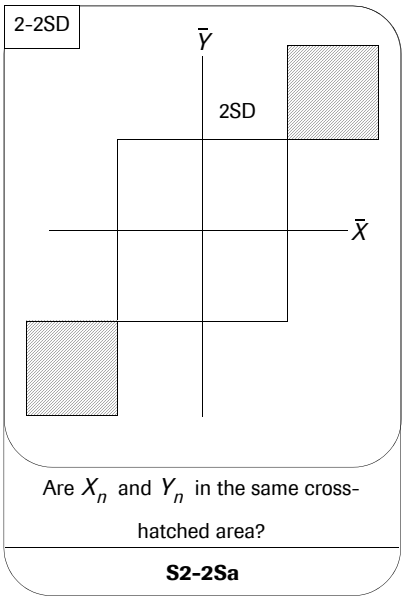


Figure E-4 S2-2Sa alarm situation

Rule 5: R-4SD (R4SD alarm)

R-4SD is the control rule in which there is a range or a difference between the control materials that exceeds 4SD, as would be the case if the X control exceeded the -2SD limit and the Y control exceeded the +2SD limit.

The run size specified when R-4SD is selected on the **QC** > **Individual** > **Realtime QC** > **Select Rules** window determines the number of consecutive control X and Y samples tested. The maximum deviations of X minus the minimum deviations of Y, and the maximum deviations of Y minus the minimum deviations of X are computed. If either of these differences is greater than 4SD, the rule is violated. A *R4SD* data alarm is issued for a random QC error, and a "■" displays on the Yoden plot.

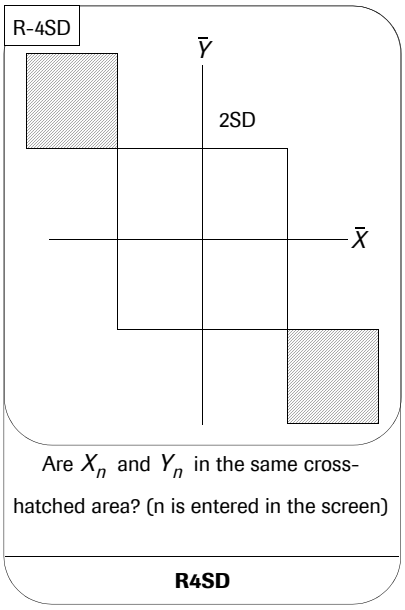


Figure E-5 R4SD alarm situation

Rule 6: 2-2SW (S2-2Sw alarm)

The results of the two most recent assays of each control are evaluated. A total of four control results are tested. If either or both X and Y results deviate outside ± 2SD, the rule is violated. A *S2-2Sw* data alarm is issued indicating a systematic QC error has occurred. A "●" displays on the Yoden plot.

The *S2-2Sw* alarm is issued when two consecutive control results are outside of the 2SD limit, within a control material. This is a systematic violation.

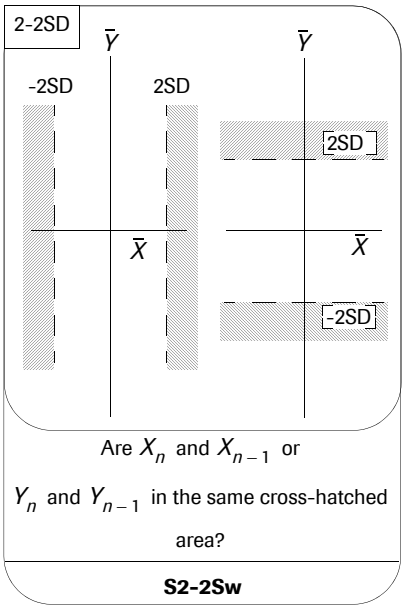


Figure E-6 S2-2Sw alarm situation

Rule 7: 4–1SA (S4–1Sa alarm)

4-1SA is the control rule violated when four consecutive control results exceed the same limit, either mean + 1SD or mean - 1SD. The *S4-1Sa* alarm is issued when three control results are outside the ± 1SD limit and one control result is outside the ± 2SD limit across control materials. This is a systematic alarm.

The results of two consecutive assays of each control are evaluated (total four samples tested). If insufficient data are available, the test is not performed. If all X and Y results exceed ± 1SD, and either the current X or Y value exceeds ± 2SD, and all are on the same side of the mean, then the rule is violated. A *S4-1Sa* data alarm is issued for a systematic QC alarm, and a "●" displays on the Yoden plot.

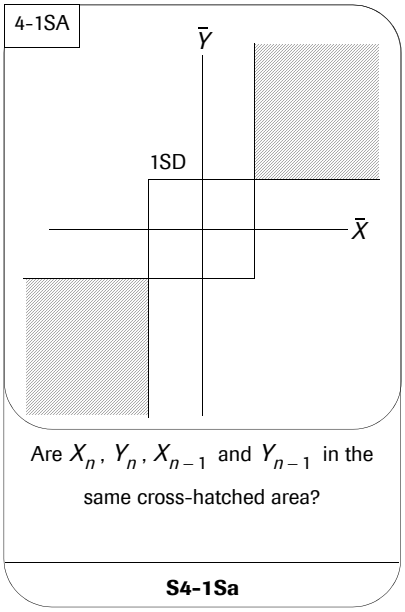


Figure E-7 S4–1Sa alarm situation

Rule 8: 4–1SW (S4–1Sw alarm)

The results of four consecutive assays of each control are evaluated (total of eight samples tested). If fewer than four samples are available, the test is not performed. If all X or Y results exceed one standard deviation, and fall on the same side of the mean, and the current X or Y value exceeds ± 2SD then the rule is violated. A *S4-1Sw* data alarm is issued for a systematic QC alarm, and a "●" displays on the Yoden plot.

The *S4-1Sw* alarm is issued when three consecutive control results are outside the ± 1SD limit and one control result is outside the ± 2SD limit within a control material. This is a systematic violation.

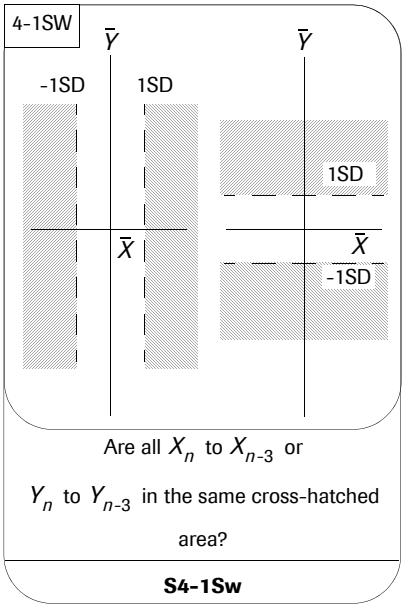


Figure E-8 S4-1Sw alarm situation

Rule 9: 10XA (S10Xa alarm)

10X is the control rule where there are 10 consecutive control observations (5 pairs) on the same side of the mean. The *S10Xa* alarm is issued when nine consecutive control results are on the same side of the mean and one control is outside the ± 2SD limit, across control materials. This is a systematic violation.

The results of five consecutive assays of each control are evaluated (total 10 samples tested). If fewer than five samples are available for each control, the test is not performed. The signs of all sample deviations for both controls are compared with zero. If all are nonzero and have the same sign, and one of the current X and Y samples exceeds 2SD, then the rule is violated. A *S10Xa* data processing alarm is issued for a systematic QC alarm, and a "●" displays on the Yoden plot.

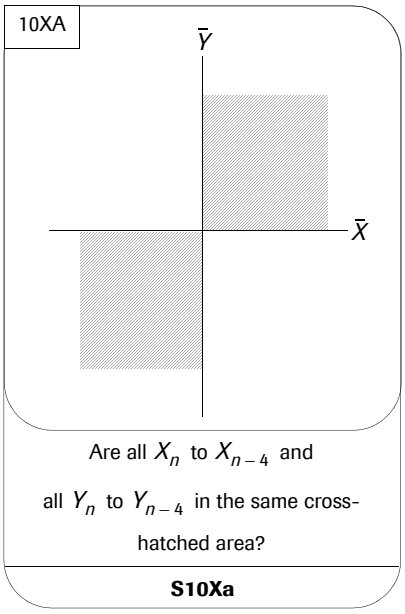


Figure E-9 S10Xa alarm situation

Rule 10: 10XW (S10Xw alarm)

The results of 10 consecutive assays of each control are evaluated (total 20 samples tested). If fewer than 10 samples are available for each control, the test is not performed. All sample deviations are compared with zero. If all are nonzero and have the same sign, and the current sample (X or Y) exceeds ± 2SD, the rule is violated. A *S10Xw* data alarm is issued for a systematic QC alarm, and a "●" displays on the Yoden plot.

The *S10Xw* alarm is issued when nine consecutive control results are on the same side of the mean and one control result is outside the ± 2SD limit within a control material. This is a systematic violation.

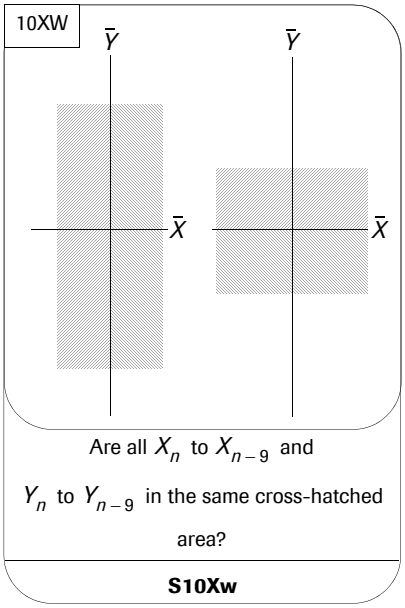


Figure E-10 S10Xw alarm situation

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