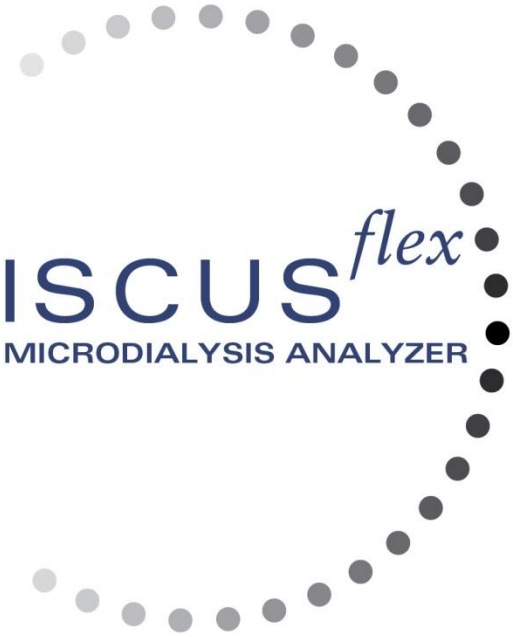
Technical Manual



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μdialysis

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Technical manual for ISCUS*flex* Microdialysis Analyzer

Printed in Sweden

All devices from M Dialysis AB are intended for use by qualified medical personnel only.

For In Vitro Diagnostic Use

Precautions:

Configuration maintenance of the computer software should only be performed by trained personnel.

No installation of non-M Dialysis software permitted.

Technical Manual (REF 8003388) ISCUS*flex* Microdialysis Analyzer

Contents

ISCUS*flex* MICRODIALYSIS ANALYZER

1. OVERVIEW

1.1. GENERAL

ISCUS*flex* Microdialysis Analyzer is a selective chemistry analyser designed for the small sample volumes obtained when sampling with microdialysis. Sample volumes used are 0.2 - 1 µL per analysis and reagent volumes 5.0 - 15 µL per analysis depending on analyte.

1.2. DESCRIPTION

The dimensions of ISCUS*flex* are 350 × 270 × 430 mm (W × D × H) and the weight of the system is 12 kg. The analyser can be placed on a table or similar capable of carrying its weight.

Bottles for rinsing and waste fluid are easily accessible behind the door on the right side of the instrument.

The transfer area for samples and reagents are located in the front of the instrument.

The movements of the microprocessor controlled sample- and reagent cannula is based on a XZ co-ordinate principle, while the sample- and reagent tray moves in the Y co-ordinate. This allows the system to locate samples, reagents and calibrator. "X" is left to right, "Z" is up.

Samples can be collected in specially designed sealed microvials as well as glass or plastic 300 µL vials that are placed in a microvial holder. The microvial holder has 16 positions for direct analysis or control samples. The sample cannula pierces the upper stopper of the vial to aspirate a sample. The sampling arm is equipped with a cannula guide that also secures the sample vial in position when sample is aspirated.

There is a reagent cassette holder for the reagent cassettes and/or separate reagent or control sample bottles. The sample/reagent cannula pierces a membrane in the cap of the reagent, calibrator and control sample bottles to aspirate reagent or calibrator.

Reagent and sample volumes are controlled by a precision glass syringe of 500 µL.

A second syringe of 1000 µL is used to rinse the cell and sample/reagent cannula with rinsing fluid. A solenoid valve is used to select between washing of the system and filling with rinsing fluid.

Absorbance measurements are made with a single-beam filter photometer, using a horizontal placement of a capillary flow cell relative to the light path. The wavelength is chosen by two different Class 1 LEDs with the wavelengths of 530 nm and 375 nm respectively.

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2. DESCRIPTION

2.1. SPECIFICATIONS AND CLASSIFICATION

See User's Manual.

2.2. SAMPLE AND REAGENT RACK

The sample and reagent rack can be tipped outwards between the analysis and the user access position. The movement is carried out by a microprocessor controlled stepper motor.

Furthermore, the racks can be tipped backwards to facilitate mixing of the reagents.

2.2.1. Sample positions

There are 16 positions for sample vials. Each of these positions has a vial sensor, which senses when a vial is placed in the hole.

2.2.2. Reagent positions

There are eight positions for reagent, calibrator and/or control sample bottles. The six leftmost positions can also hold a reagent cassette that contains vials for different reagents and a calibrator.

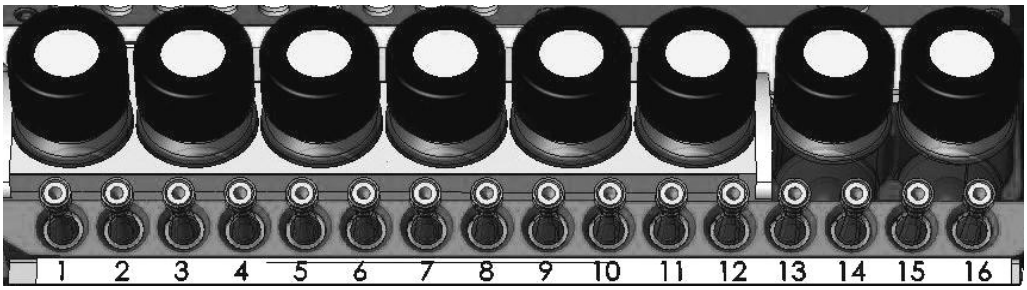

Figure 1. The sample and reagent rack

2.3. SAMPLING CANNULA AND X-Z MODULE

2.3.1. Sampling Cannula

The sampling cannula is used for aspirating both sample and reagent. The inner diameter of the main part is about 0.4 mm, but at the tip, the diameter is reduced to about 0.2 mm to allow high precision pipetting of very small volumes of sample. At the top of the cannula, there is a ferrule, which is used to connect the cannula to the tubing from the cuvette.

The sampling cannula is a crucial part of the analyser and must be handled with care in order to obtain the best analytical performance.

2.3.2. X-Z module

The X-Z module controls the movement of the sample cannula, up and down (Z-direction) and across the sample and reagent rack (X-direction).

There are optical sensors on the X-Z module, which defines the mechanical home position in the X and Z-direction. All movements in the X-direction are measured relative the X-home position.

There is also an optical sensor that detects when the sampling cannula moves relative the cannula guide. All movements in the Z-directions are measured relative the point where the cannula starts to move relative the cannula guide. I.e. when the cannula guide touches the top of e.g. a microvial, the movement of the cannula guide will be interrupted, but the cannula will continue to move a certain distance, which is measured from the point where the cannula guide stopped its movement.

2.3.3. **Wash Bin**

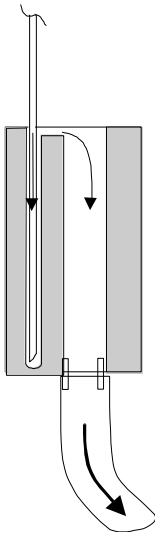

Figure 2. Wash Bin

The sample cannula is rinsed between each analysis. While the sample cannula is positioned inside the wash bin, rinsing fluid is flushed through the interior of the cannula and around the exterior to remove any remaining sample or reagent. The wash bin has a liquid level sensor that informs the system when the membrane pump has finished emptying.

2.4. FLUID SYSTEM

The transportation of fluid in the system is controlled by two syringe pumps. One pump is equipped with a 500 µL syringe, for high precision pipetting. The second pump, with a 1000 µL syringe, is used for rinsing the system between each analysis. A solenoid valve is used to select between the rinse of the system and filling the syringe.

2.4.1. **Syringe pumps**

The 500 µL syringe controls the volume of sample and reagent aspirated and the transport of the sample/reagent mixture to the cuvette. It should be filled with Rinsing Fluid at all times and no air bubble should be visible in the syringe or tubing to and from the syringe.

The high precision movement of the plunger is controlled by a microprocessor controlled stepper motor. An optosensor, that detects when the plunger is in the top position, defines the home position for the syringe pumps. When emptying the syringes, the plunger always moves to the topmost position and then moves back a distance corresponding to 3 µL nominally.

2.4.2. **Rinsing Fluid and Waste Containers**

The front bottle provides the supply of Rinsing Fluid for the syringe and wash bin and the rear bottle is used to hold the waste. Both bottles are equipped with liquid levels sensors that will warn when the rinsing fluid bottle is empty or the waste bottle is full.

See the “User’s manual” for instructions how to change rinsing fluid and empty the waste bottle.

The Rinsing Fluid contains a small amount of wetting agent (Brij®-35) and preservative dissolved in reagent grade water.

2.5. OPTICAL SYSTEM

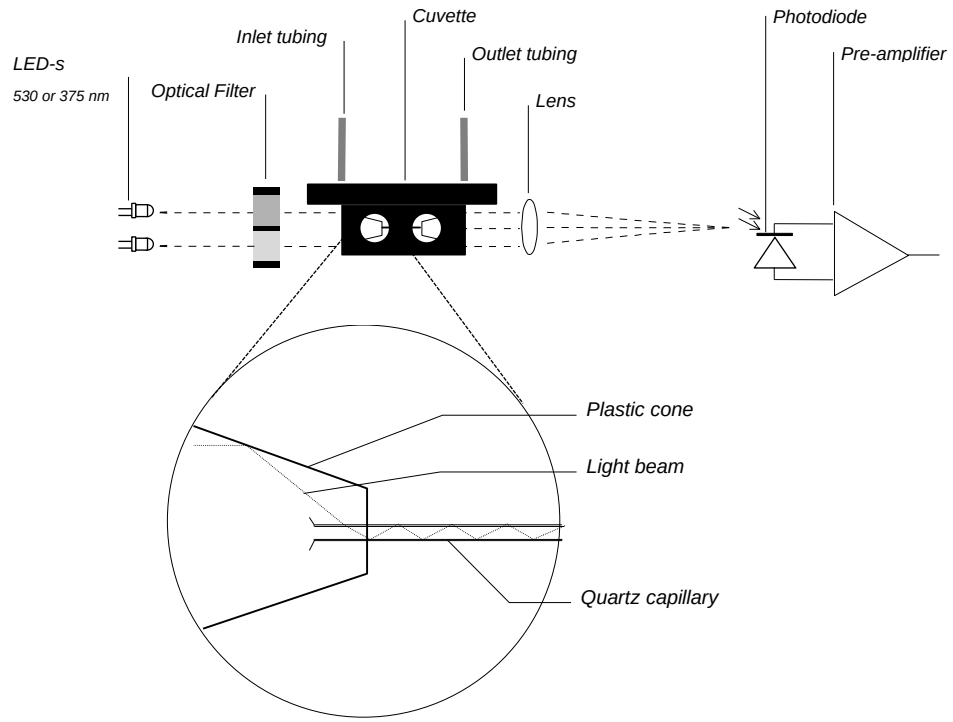

Figure 3. Principle of the optical system

The optical system of ISCUS*flex* is equipped with a single-beam filter photometer.

Two Class 1 Light Emitting Diodes (LED), with wavelengths of 530 and 375 nm receptively, are used as the light source. The active LED (530 or 375 nm) is determined by the analytical method.

The light beam passes through an optical filter and then through the cuvette. Passing through the optical lens, the light beam then reaches the photodiode where it is converted to an electrical signal. The electrical signal is pre-amplified in the detector unit before the signal is passed to the analogue-to-digital converter.

The measuring cuvette is designed to minimise the risk of getting air bubbles trapped. The light beam enters the cuvette through a plastic cone and is reflected, by total internal reflection, into the quartz capillary. Then, it passes through the quartz capillary, containing the sample, in a zigzag pattern also by total internal reflection between quartz and air. The effective volume of the cuvette is about 2 µL and the geometric path length 10 mm. The effective path length is shorter however, about 8 mm, since a part of the travel through the capillary occurs in the quartz wall. The temperature of the optical unit is controlled to 37 °C.

2.6. INITIALISATION

2.6.1. Finding the mechanical home position

- The transport lock of the x movement is released when the transport lock solenoid is activated on power on.

The mechanical home positions are found in the following order:

- The cannula is moved to the z home position (moved up).
- The reagent/vial holder is moved to the home position.

- The cannula is moved to the x home position (moved to the right).
- The cannula is then moved into the wash bin.

2.7. MEASURING SEQUENCE

- The measuring sequence starts with a rinse of the flow system. At this time, the light intensity through the measuring cuvette without sample is measured.
- A 1 µL air bubble is then aspirated into the cannula to separate the sample from the rinsing fluid.
- Thereafter the cannula moves to the sample vial and aspirates the sample, 0.2 to 2.5 µL depending on the analyte measured.
- Directly after aspiration of the sample, the cannula moves to the reagent bottle and aspirates the reagent, 5 to 15 µL depending on the analyte.
- After aspiration of reagent, the cannula moves up and the sample is mixed with the reagent by rapidly moving the test solution to the measuring cuvette. The test solution is stopped in the cuvette.
- After a delay, the absorbance signal is measured during 29 seconds.

After measuring, the test solution is dispensed into the wash bin followed by a rinse of the system.

2.7.1. Calibration

2.7.1.1.Reagent blank measurement

A reagent blank is first measured. In this case, the measuring cuvette is filled with the reagent and the signal is measured in exactly the same way as when measuring a sample.

The Calibrator is then measured in duplicate.

Calibration is automatically performed when new reagents are placed in the analyzer and then once every sixth hour during operation.

Calculation and checks

A third order polynomial is fitted to the data points and the quality of the blank measurement is controlled by calculating the residual square sum (*RSS*); see section 5 "DATA ANALYSIS".

The reagent blank signal is used to correct the signal for all subsequent measurements up to the next calibration event.

The reagent blank is re-analysed if the residual square sum $(RSS) > 0.8$. If the blank measurement is uncertain also on the second attempt, the last accepted blank measurement will be used.

2.7.1.2.Calibrator measurements

A third-order polynomial is fitted to the data points. The fitted polynomial from the reagent blank is then subtracted from the fitted polynomial for the Calibrator. The steepest slope (positive or negative) during the sampling period, R_{Cal}, is calculated by taking the derivative of the obtained polynomial.

The assigned value of the Calibrator divided by the average of the two calibration measurements is used as the response factor (*RF*).

$$Response\ Factor\ (RF) = \frac{2 \times Assigned\ concentration}{(R_{Cal1} + R_{Cal2})}$$

The quality of the measurement is controlled by calculating *RSS* and the *normalised RSS NormRSS* = square root of *RSS divided by* R_{Cal}.

Checks

The calibrator is reanalysed if

RSS > 0.8 and
NormRSS > 4.0.

The duplicate measurement of the calibrator is re-run if

Relative standard deviation of duplicate > 5 % or
if the response factor is outside the expected range.

2.7.2. **Sample analysis**

2.7.2.1.Sample measurements

During the measurement, the signal is sampled at rate of approximately 8 Hz during 29 seconds. The acquired data points are fitted to a third order polynomial. The signal is corrected for the reagent blank by subtracting the blank signal obtained during calibration. The derivative of the resulting polynomial is calculated and from the derivative, the maximum absorbance change during the measurement is calculated, see section 5, "DATA ANALYSIS".

The maximum absorbance change, R_{sample}, is used to calculate the concentration by multiplication with the response factor.

$$c_{glucose} = R_{sample} \times Response\ Factor$$

Checks

A sample is reanalysed if

RSS > 0.8 and
NormRSS > 4.0.

If also the second attempt fails no value for the sample will be given.

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3. ANALYTICAL METHODS

3.1. D-GLUCOSE

3.1.1. Measuring principle

Glucose is enzymatically oxidised by glucose oxidase (GOD). Peroxidase (POD) catalyses the reaction between the hydrogen peroxide formed, phenol and 4-amino-antipyrine to form the red-violet coloured quinoneimine. The rate of formation of the coloured substance is proportional to the glucose concentration.

$$\text{D-Glucose} + O_2 \rightarrow \text{gluconolactone} + H_2O_2$$

$$2\ H_2O_2 + \text{phenol} + \text{4-amino-antipyrine} \rightarrow \text{quinoneimine} + 4\ H_20$$

3.1.2. Instrumental parameters

Conditions:

Wavelength:	530 nm
Temperature:	37°C
Mode:	kinetic, steepest positive slope
Sample volume:	0.5 µL
Reagent volume:	14.5 µL
Delay time:	12 s
Sampling time:	29 s

3.1.3. Measuring range

If the calculated concentration is < 0.1 mmol/L the result is reported as "*N" and if the calculated concentration is > 25 mmol/L the result is reported as ” >N", where N is the calculated concentration.

3.1.4. Reagent

3.1.4.1.Content

	Component	Concentration in test solution
Glucose Reagent	4-amino-antipyrine	0.77 mmol/L
	Glucose oxidase	> 1.5 kU/L
	Peroxidase	> 1.5 kU/L
	Ascorbate oxidase	> 3 kU/L
Glucose Buffer	Phosphate Buffer, pH 7.0	0.1 mol/L
	Phenol	11 mmol/L
	Sodium Azide	0.4 g/L

The reagent contains sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

3.1.4.2.Stability

Reagents are stable up to expiry date when stored at +2 to +8 °C. Reconstituted reagent is stable for five days in the instrument.

3.1.5. Calibration

3.1.5.1.Calibrator

A bottle of Calibrator A. (Cat no P000057) is included in all reagent cassettes.

3.1.5.2.Checks

The calibration is rerun if

Response factor < 0.63 mol × L^{-1} / mAU × s^{-1} or
Response factor > 2.5 mol × L^{-1} / mAU × s^{-1}.

3.1.6. **Performance**

3.1.6.1.Linearity

The method is linear from 0.1 to 25 mmol/L (450 mg/L) with a deviation of < 5 % at 25 mmol/L.

The figure below shows the linearity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the linear range for ISCUS*flex* is the same.

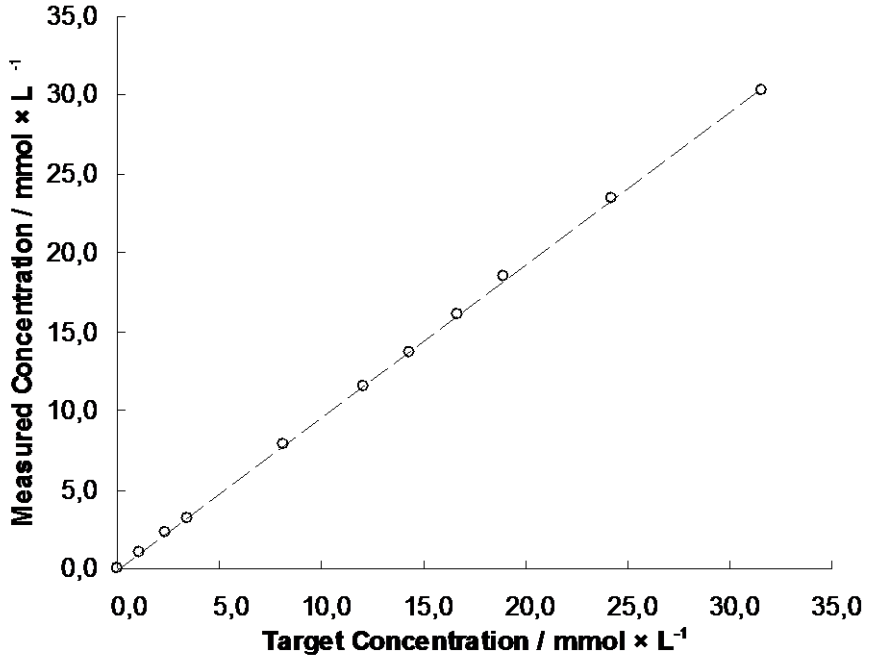

Figure 4. Linearity of the glucose method for the CMA 600 analyser obtained from samples with known concentrations of glucose.

3.1.6.2.Analytical Sensitivity

The average sensitivity of this method is 0.8 - 1 mAU × s^{-1} per mmol × L^{-1}.

3.1.6.3.Detection limit

The detection limit is 0.1 mmol/L.

3.1.6.4.Specificity

Glucose oxidase is specific for β-D-glucose. Ascorbate oxidase is added to the reagent to remove interference from ascorbic acid.

The table below shows the specificity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the specificity for ISCUS*flex* is the same.

No significant change in values was observed when the following substances were added to Ringer's solution containing 5 mmol/L glucose.

Table I

Substance (conc.)	Recovery / %
Lactate (25 mmol/L)	99
Urea (50 mmol/L)	101
Glycerol (2.5 mmol/L)	99
Ascorbate (0.5 mmol/L)	97
Uric acid (0.5 mmol/L)	98
Acetaminophen (300 mg/L)	102
Creatinine (0.15 mmol/L)	99
Acetylsalicylic acid (300 mg/L)	99
Glutathion (0.3 mmol/L)	103

As with any chemical reaction, user must be alert to the possible effect on the result due to unknown interference from medication or endogenous substances. All patient results must be evaluated considering the total clinical status of the patient.

3.1.6.5.Precision

Estimates of imprecision, obtained from replicate assays of samples with known concentrations of glucose in Ringer's solution spanning the range of linear measurements are shown in Table II.

Table II

	Within run		Between run		Total		N	
Mean / mmol × L^{-1}	SD / mmol × L^{-1}	RSD / %	SD / mmol × L^{-1}	RSD / %	SD / mmol × L^{-1}	RSD / %	Obs.	Runs
1.30	0.030	2.3%	0.033	2.6%	0.044	3.4%	100	20
5.30	0.153	2.9%	0.147	2.8%	0.210	4.0%	100	20
15.61	0.313	2.0%	0.390	2.5%	0.494	3.2%	100	20

3.1.6.6.Accuracy

The results below show the accuracy for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and method parameters, the accuracy for ISCUS*flex* is the same. Test has shown that the results from ISCUS*flex* are within ± 10% of those obtained with CMA 600.

Analytical recovery of glucose added to microdialysis samples (at 2 to 20 mmol/L) gave a mean recovery of 103 % (range 88.2 - 112.6 %).

Comparison of 40 pooled and spiked microdialysis samples using Cobas Mira S with Roche Unimate GLUC PAP reagent gave the following correlation. (y = CMA 600 , x = COBAS):

$Y = 0.979\ x - 0.01$ mmol/L $N = 40$

$R^2 = 0.994$ $S_{xy} = 0.46$ mmol/L

The results are presented in figure 5.

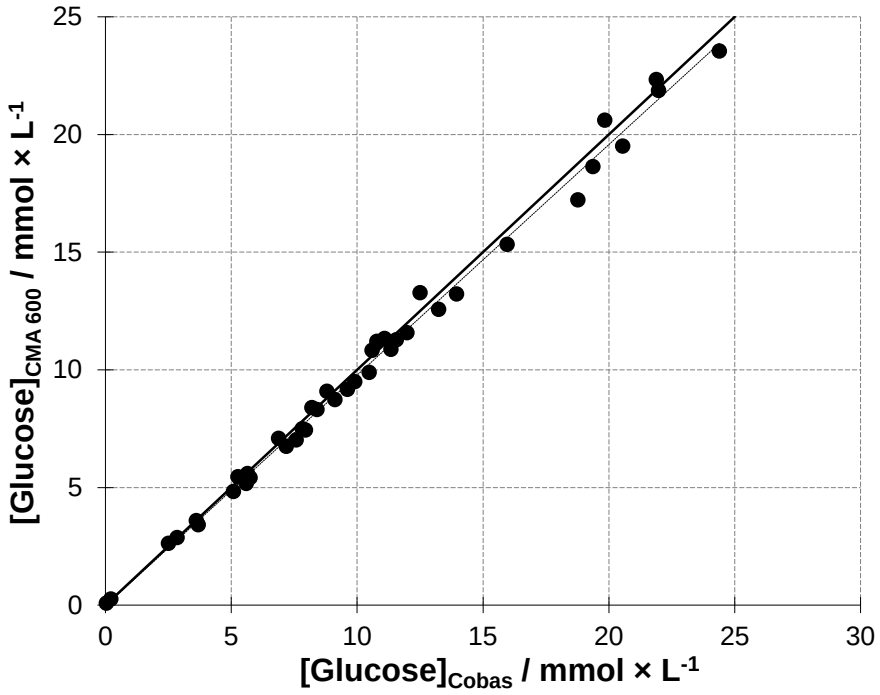

Figure 5. *Correlation between CMA600 and Cobas Mira S (Roche Unimate GLUC PAP). $Glucose_{CMA600}$ =0.979 × $Glucose_{Cobas}$ - 0.01 mmol/L; R^2 = 0.994; SE = 0.46 mmol/L, N = 40.*

3.2. L-LACTATE

3.2.1. Measuring principle

Lactate is enzymatically oxidised by lactate oxidase. Peroxidase (POD) catalyses the reaction between the hydrogen peroxide formed, 4-amino-antipyrine and 4-chlorophenol to form a red-violet coloured quinoneimine. The rate of formation of the coloured substance is proportional to the lactate concentration.

$$\text{L-Lactate} + O_2 \rightarrow \text{pyruvate} + H_2O_2$$

$$H_2O_2 + \text{4-chloro-phenol} + \text{4-amino-antipyrine} \rightarrow \text{quinoneimine} + 2\ H_2O + HCl$$

3.2.2. Instrument parameters

Conditions:

Wavelength:	530 nm
Temperature:	37°C
Mode:	kinetic, steepest positive slope
Sample volume:	0.2 µL
Reagent volume:	14.8 µL
Delay time:	17 s
Sampling time:	29 s

3.2.3. Measuring range

If the calculated concentration is < 0.1 mmol/L the result is reported as "*N" and if the calculated concentration is > 12 mmol/L the result is reported as ” >N", where N is the calculated concentration.

3.2.4. Reagent

3.2.4.1.Content

	Component	Concentration in test solution
Lactate Reagent	4-amino-antipyrine	0.4 mmol/L
	Lactate oxidase	> 0.5 kU/L
	Peroxidase	> 0.5 kU/L
	Ascorbate oxidase	> 10 kU/L
Lactate Buffer	PIPES Buffer, pH 6.8	0.1 mol/L
	4-Chloro-phenol	5.4 mmol/L
	Sodium Oxalate	7.5 mmol/L
	EDTA-disodium salt	3 mmol/L
	Sodium Azide	0.4 g/L

The reagent contains sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

3.2.4.2.Stability

Reagents are stable up to expiry date when stored at +2 to +8 °C. Reconstituted reagent is stable for five days in the instrument.

3.2.5. Calibration

3.2.5.1.Calibrator

A bottle of Calibrator A. (Cat no P000057) is included in all reagent cassettes.

3.2.5.2.Checks

The calibration is rerun if

Response factor < 1.56 mmol × L^{-1} / mAU × s^{-1} or
Response factor > 6.25 mmol × L^{-1} / mAU × s^{-1}.

3.2.6. Performance

3.2.6.1.Linearity

The method is linear from 0.1 to 12 mmol/L (108 mg/dL lactic acid) with a deviation of < 5 % at 12 mmol/L.

The figure below shows the linearity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the linear range for ISCUS*flex* is the same.

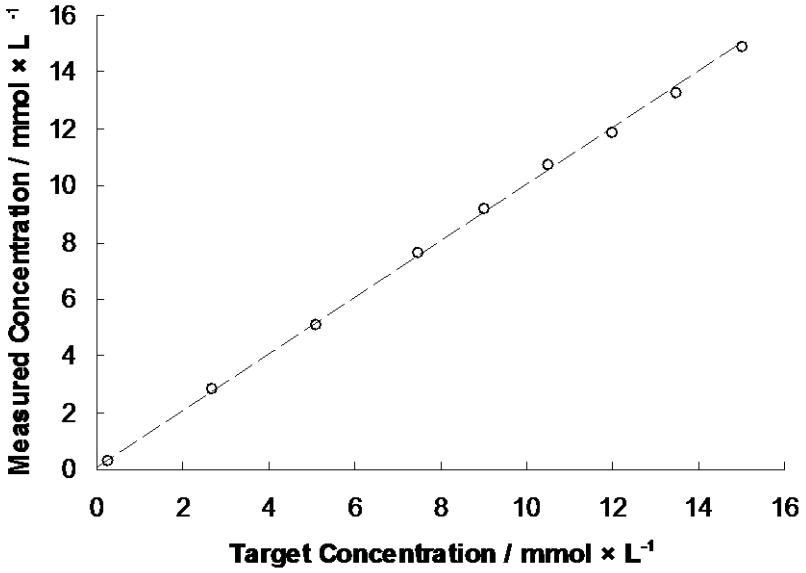

Figure 6. Linearity of the lactate method for the CMA 600 analyser obtained from samples with known concentrations of lactate in Ringer's solution.

3.2.6.2.Analytical Sensitivity

The average sensitivity of this method is 0.28 – 0.36 mAU × s^{-1} per mmol × L^{-1}.

3.2.6.3.Detection limit

The detection limit is 0.1 mmol/L.

3.2.6.4.Specificity

Lactate oxidase is specific for L-lactate. Ascorbate oxidase is added to the reagent to remove interference from ascorbic acid.

The table below shows the specificity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the specificity for ISCUS*flex* is the same.

No significant change in values was observed when the following substances were added to Ringer's solution containing 1.2 mmol/L lactate.

Table III

Substance (conc.)	Recovery / %
Glucose (25 mmol/L)	97
Glycerol (2.5 mmol/L)	98
Ascorbate (0.5 mmol/L)	97
Uric acid (0.5 mmol/L)	103
Acetaminophen (300 mg/L)	104
Creatinine (0.15 mmol/L)	102
Acetylsalicylic acid (300 mg/L)	100
Glutathion (0.3 mmol/L)	101

As with any chemical reaction, user must be alert to the possible effect on the result due to unknown interference from medication or endogenous substances. All patient results must be evaluated considering the total clinical status of the patient.

3.2.6.5.Precision

Estimates of imprecision, obtained from replicate assays of samples with known concentrations of lactate in Ringer's solution spanning the range of linear measurements are shown in Table IV.

Table IV

	Within run		Between run		Total		N	
Mean / mmol × L^{-1}	SD / mmol × L^{-1}	RSD / %	SD / mmol × L^{-1}	RSD / %	SD / mmol × L^{-1}	RSD / %	Obs.	Runs
0.79	0.021	2.7%	0.031	4.0%	0.037	4.7%	100	20
3.16	0.082	2.6%	0.068	2.1%	0.106	3.4%	100	20
9.13	0.148	1.6%	0.201	2.2%	0.246	2.7%	100	20

3.2.6.6.Accuracy

The results below show the accuracy for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and method parameters, the accuracy for ISCUS*flex* is the same. Test has shown that the results from ISCUS*flex* are within ± 10% of those obtained with CMA 600.

Analytical recovery of lactate added to microdialysis samples (at 0.44 to 7.5 mmol/L) gave a mean recovery of 96 % (range 74 - 114 %).

Comparison of 39 pooled and spiked microdialysis samples using Cobas Mira S with RANDOX Lactate PAP (LC 2389) reagent gave the following correlation. (y = CMA 600, x = COBAS):

$Y = 0.945\ x + 0.22$ mmol/L $N = 39$

$R^2 = 0.988$ $S_{xy} = 0.27$ mmol/L

The results are presented in figure 7.

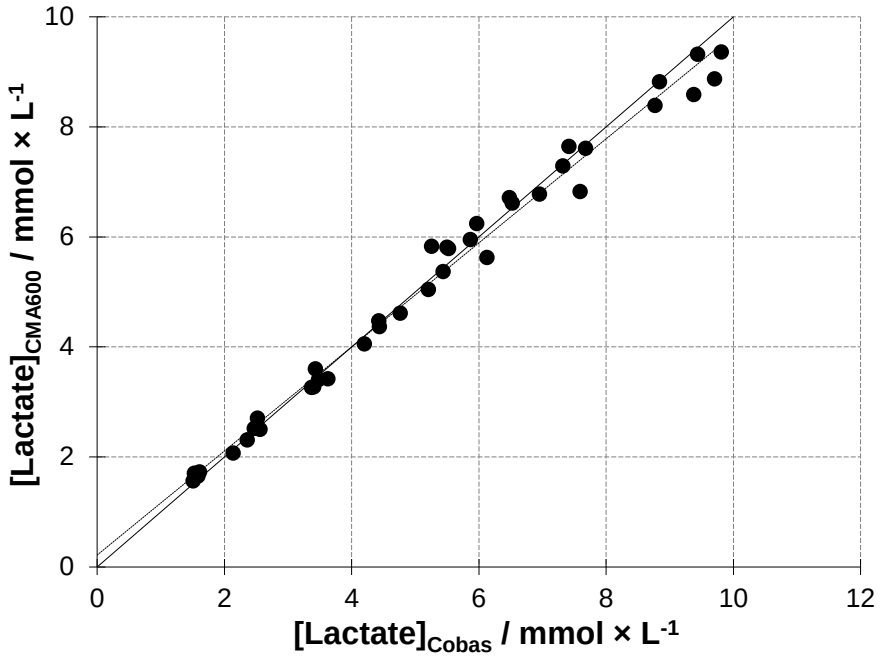

Figure 7. *Correlation between CMA600 and Cobas Mira S (RANDOX Lactate PAP). $Lactate_{CMA600}$ =0.945 × $Lactate_{Cobas}$ + 0.22 mmol/L; R^2 = 0.988; SE = 0.27 mmol/L, N = 39.*

3.3. GLYCEROL

3.3.1. Measuring principle

Glycerol is phosphorylated by adenosine triphosphate (ATP) and glycerol kinase (GK) to glycerol-3-phosphate, which is subsequently oxidised in the presence of glycerol-3-phosphate oxidase (GPO). Peroxidase catalyses the reaction between the hydrogen peroxide formed, 3,5-dichloro-2-hydroxybenzene sulphonic acid (DCHBS) and 4-amino-antipyrine to form the red-violet coloured quinoneimine. (N-(4-antipyryl)-3-3-chloro5-sulphonate-p-benzo-quiononeimine, ACBS). The rate of formation of the coloured substance is proportional to the glycerol concentration.

$$\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \rightarrow \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{DCHBS} + \text{4-amino-antipyrine} \rightarrow \text{ACBS} + 2\ H_20 + \text{HCl}$$

3.3.2. Instrument parameters

Conditions:

Wavelength:	530 nm
Temperature:	37°C
Mode:	kinetic, steepest positive slope
Sample volume:	0.5 µL
Reagent volume:	14.5 µL
Delay time:	17 s
Sampling time:	29 s

3.3.3. Measuring range

If the calculated concentration is < 10 µmol/L the result is reported as "*N" and if the calculated concentration is > 1500 µmol/L the result is reported as ” >N", where N is the calculated concentration.

3.3.4. Reagent

3.3.4.1.Content

	Component	Concentration in test solution
Glycerol Reagent	4-amino-antipyrine	0.4 mmol/L
	ATP	1.0 mmol/L
	Glycerol kinase	> 0.4 kU/L
	Glycerol-3-phosphate oxidase	> 1.5 kU/L
	Peroxidase	> 1 kU/L
	Ascorbate oxidase	> 7 kU/L
Glycerol Buffer	PIPES Buffer, pH 7.6	0.04 mol/L
	DCHBS	1.5 mmol/L
	Magnesium ions	17.5 mmol/L
	Sodium Azide	0.2 g/L

The reagent contains sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

3.3.4.2.Stability

Reagents are stable up to expiry date when stored at +2 to +8 °C. Reconstituted reagent is stable for five days in the instrument.

3.3.5. **Calibration**

3.3.5.1.Calibrator

A bottle of Calibrator A. (Cat no P000057) is included in all reagent cassettes.

3.3.5.2.Checks

The calibration is rerun if

Response factor < 0.08 mmol × L^{-1} / mAU × s^{-1} or
Response factor > 0.31 mmol × L^{-1} / mAU × s^{-1}.

3.3.6. **Performance**

3.3.6.1.Linearity

The method is linear from 10 to 1500 µmol/L (14 mg/dL) with a deviation of < 5 % at 1500 µmol/L.

The figure below shows the linearity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the linear range for ISCUS*flex* is the same.

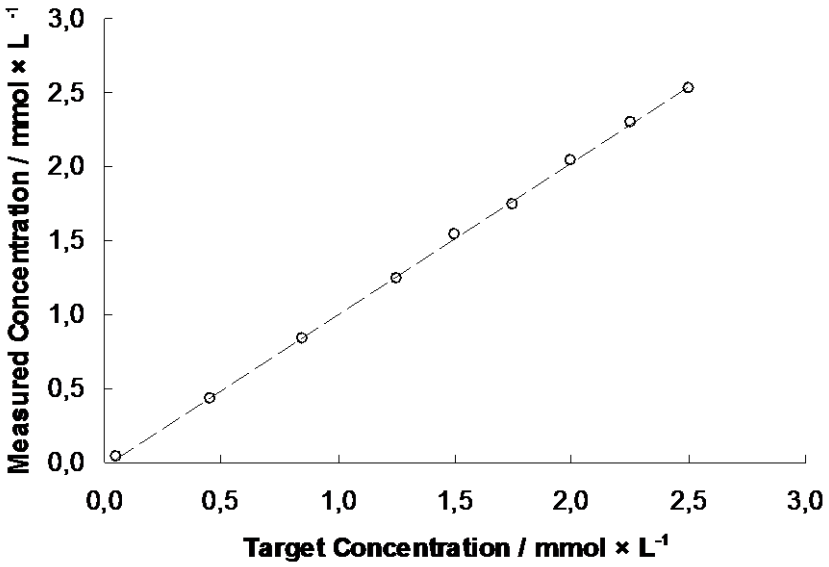

Figure 8. Linearity of the glycerol method for the CMA 600 analyser obtained from samples with known concentrations of glycerol.

3.3.6.2.Analytical Sensitivity

The average sensitivity of this method is 6.7 - 8.4 mAU × s^{-1} per mmol × L^{-1}.

3.3.6.3.Detection limit

The detection limit is 10 µmol/L.

3.3.6.4.Specificity

In this method, glycerol is converted to glycerol-3-phosphate. Any endogenous glycerol-3-phosphate will be detected as glycerol. The concentration of glycerol-3-phosphate is usually low compared to the level of glycerol.

Ascorbate oxidase is added to the reagent to remove interference from ascorbic acid.

The table below shows the specificity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the specificity for ISCUS*flex* is the same.

No significant changes in values were observed when the following substances were added to Ringer's solution containing 150 µmol/L glycerol.

Table V

Substance (conc.)	Recovery / %
Glucose (25 mmol/L)	100
Lactate (25 mmol/L)	102
Urea (50 mmol/L)	101
Ascorbate (0.5 mmol/L)	95
Uric acid (0.5 mmol/L)	93
Acetaminophen (300 mg/L)	98
Creatinine (0.15 mmol/L	99
Acetylsalicylic acid (600 mg/L)	97
Glutathion (0.3 mmol/L)	105

As with any chemical reaction, user must be alert to the possible effect on the result due to unknown interference from medication or endogenous substances. All patient results must be evaluated considering the total clinical status of the patient.

3.3.6.5.Precision

Estimates of imprecision, obtained from replicate assays of samples with known concentrations of glycerol in Ringer's solution spanning the range of linear measurements are shown in Table.

Table VI

	Within run		Between run		Total		N	
Mean / µmol × L^{-1}	SD / µmol × L^{-1}	RSD / %	SD / µmol × L^{-1}	RSD / %	SD / µmol × L^{-1}	RSD / %	Obs	Runs
67.5	2.2	3.2%	3.7	5.4%	4.2	6.2%	100	20
260	6.7	2.6%	8.4	3.2%	10.6	4.1%	100	20
810	18.9	2.3%	17.5	2.2%	25.5	3.2%	100	20

3.3.6.6.Accuracy

The results below show the accuracy for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and method parameters, the accuracy for ISCUS*flex* is the same. Test has shown that the results from ISCUS*flex* are within ± 10% of those obtained with CMA 600.

Analytical recovery of glycerol added to microdialysis samples (at 125 to 650 µmol/L) gave a mean recovery of 104 % (range 91 - 114 %).

Comparison of 39 pooled and spiked microdialysis samples using Cobas Mira S with RANDOX Glycerol PAP (GY 105) reagent gave the following correlation. (y = CMA 600, x = COBAS):

Y = 1.08 x - 13 µmol/L *N = 33*
R^2 = 0.974 *S_{xy} = 37 µmol/L*

The results are presented in figure 9.

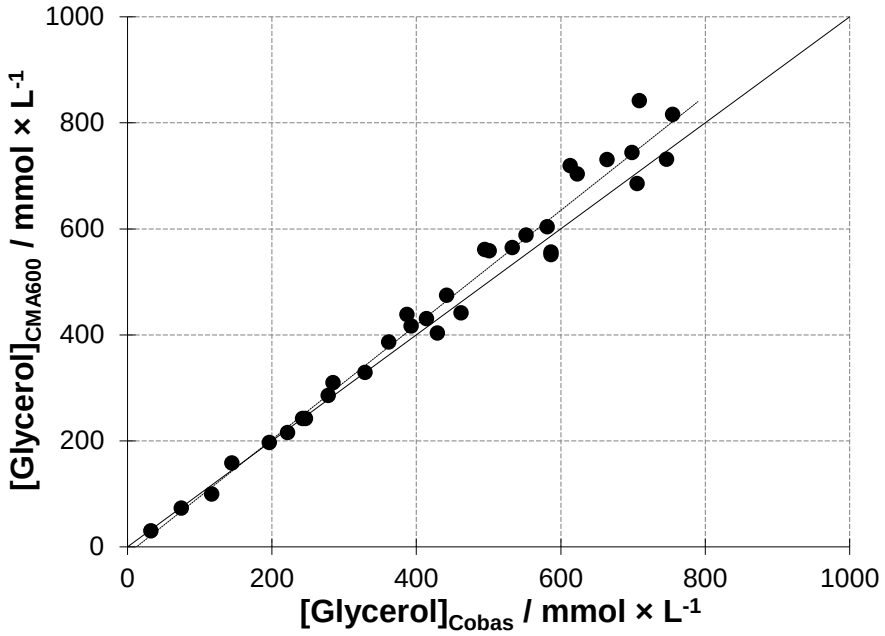

Figure 9. *Correlation between CMA600 and Cobas Mira S (RANDOX Glycerol PAP). $Glycerol_{CMA600}$ =1.08 × $Glycerol_{Cobas}$ - 13 µmol/L; R^2 = 0.974; SE = 37 µmol/L, N = 33.*

3.4. PYRUVATE

3.4.1. Measuring principle

Pyruvate is enzymatically oxidised by pyruvate oxidase (PyrOx) in presence of inorganic phosphate (P_i). Peroxidase (POD) catalyse the reaction between the hydrogen peroxide formed, TOOS and 4-amino-antipyrine to form the red-violet coloured quinonediimine. The rate of formation of the coloured substance is proportional to the pyruvate concentration.

$$\text{Pyruvate} + P_i + O_2 \rightarrow \text{acetylphosphate} + CO_2 + H_2O_2$$

$$2\ H_2O_2 + \text{TOOS} + \text{4-amino-antipyrine} \rightarrow \text{quinonediimine} + 4\ H_20$$

3.4.2. Instrumental parameters

Conditions:

Wavelength:	530 nm
Temperature:	37°C
Mode:	kinetic, steepest positive slope
Sample volume:	0.5 µL
Reagent volume:	14.5 µL
Delay time:	17 s
Sampling time:	29 s

3.4.3. Measuring range

If the calculated concentration is < 10 µmol/L the result is reported as "*N" and if the calculated concentration is > 1.5 mmol/L the result is reported as ” >N", where N is the calculated concentration.

3.4.4. Reagent

3.4.4.1.Content

	Component	Concentration in test solution
Pyruvate Reagent	4-amino-antipyrine	0.3 mmol/L
	Pyruvate oxidase	> 0.25 kU/L
	Peroxidase	> 0.8 kU/L
	Tiamine pyrophosphate	0.2 mmol/L
	FAD	10 µmol/L
	Ascorbate oxidase	> 10 kU/L
Pyruvate Buffer	Citrate Buffer, pH 6.1	0.1 mol/L
	Potassium dihydrogenphosphate	10 mmol/L
	$MgCl_2$	10 mmol/L
	TOOS	1.5 mmol/L
	Sodium Azide	0.3 g/L

The reagent contains sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

3.4.4.2.Stability

Reagents are stable up to expiry date when stored at +2 to +8 °C. Reconstituted reagent is stable for five days in the instrument.

3.4.5. Calibration

3.4.5.1.Calibrator

A bottle of Calibrator A. (Cat no P000057) is included in all reagent cassettes.

3.4.5.2.Checks

The calibration is rerun if

Response factor < 0.08 mmol × L^{-1} / mAU × s^{-1} or
Response factor > 0.33 mmol × L^{-1} / mAU × s^{-1}.

3.4.6. **Performance**

3.4.6.1.Linearity

The method is linear from 10 to 1500 µmol/L with a deviation of < 5 % at 1500 mmol/L.

The figure below shows the linearity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the linear range for ISCUS*flex* is the same.

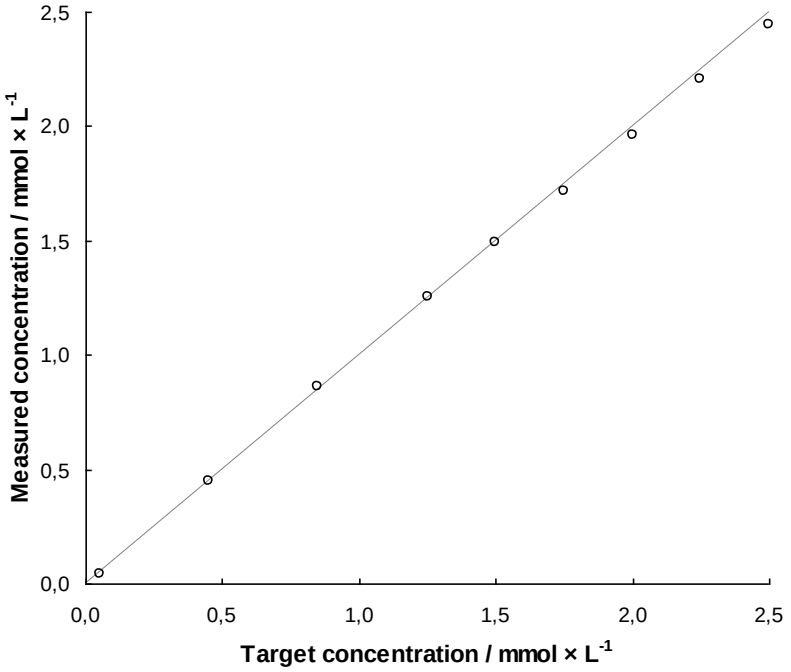

Figure 10. Linearity of the pyruvate method for the CMA 600 analyser obtained from samples with known concentrations of pyruvate.

3.4.6.2.Analytical Sensitivity

The average sensitivity of this method is 4 – 5.6 mAU × s^{-1} per mmol × L^{-1}.

3.4.6.3.Detection limit

The detection limit is 10 µmol/L.

3.4.6.4.Specificity

Pyruvate oxidase is specific for pyruvate. Ascorbate oxidase is added to the reagent to remove interference from ascorbic acid.

The table below shows the specificity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the specificity for ISCUS*flex* is the same.

No significant change in values was obtained when the following substances were added to Ringer's solution containing 250 µmol/L pyruvate.

Table VII

Substance (conc.)	Recovery / %
Glucose (55 mmol/L)	96.8
Lactate (25 mmol/L)	99.6
Urea (50 mmol/L)	99.4
Glycerol (2.5 mmol/L)	100.0
Ascorbate (0.2 mmol/L)	98.1
Uric acid (0.5 mmol/L)	100.1
Acetaminophen (300 mg/L)	97.5
Creatinine (1 mmol/L)	98.2
Acetylsalicylic acid (300 mg/L)	97.8
3-hydroxypyruvat (0.1 mmol/L)	104.0
2-oxobutyrate(0,1 mmol/L)	101.6
Acetoacetate(0,1 mmol/L)	100.8
2-oxoglutarate(0,1 mmol/L)	98.2
Glutathion (0.3 mmol/L)	95.1

As with any chemical reaction, user must be alert to the possible effect on the result due to unknown interference from medication or endogenous substances. All patient results must be evaluated considering the total clinical status of the patient.

3.4.6.5.Precision

Estimates of imprecision, obtained from replicate assays of samples with known concentrations of pyruvate in Ringer's solution spanning the range of linear measurements are shown in the following table.

Table VIII

	Within run		Between run		Total		N	
Mean / µmol × L^{-1}	SD / µmol × L^{-1}	RSD / %	SD / µmol × L^{-1}	RSD / %	SD / µmol × L^{-1}	RSD / %	Obs.	Runs
53.2	2.9	5.4%	5.1	9.6%	5.8	10.9%	100	20
229	6.0	2.6%	7.2	3.2%	9.3	4.0%	100	20
685	24.5	3.6%	23.4	3.4%	33.6	4.9%	100	20

3.4.6.6.Accuracy

The results below show the accuracy for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and method parameters, the accuracy for ISCUS*flex* is the same. Test has shown that the results from ISCUS*flex* are within ± 10% of those obtained with CMA 600.

Analysis of Sigma Metabolite Control (S 3005, Lot 027H6027) ultrafiltrated through a Microcon-10 ultrafiltration device (Amicon, Inc.) gave a mean value of 171 µmol/L (RSD= 2.3%, n=3), assigned value 180 µmol/L (range 150 - 200 µmol/L).

Analytical recovery of pyruvate added to ultrafiltrated Sigma Metabolite Control (at 125 and 250 µmol/L) gave a mean recovery of 106 % (range 104.7 - 108.1 %).

Comparison of 44 samples using HPLC gave the following correlation. (y = CMA 600, x = HPLC):

Y = 0.972 x - 5.5 µmol/L *N = 44*
R^2 = 0.921 *S_{xy} = 16.1 µmol/L*

The results are presented in figure 5.

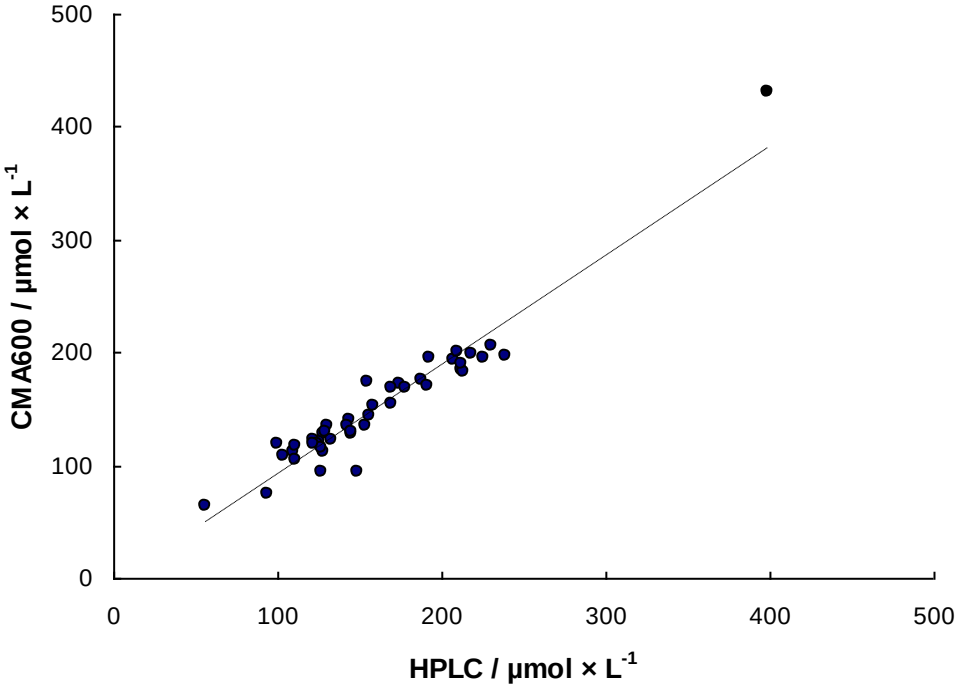

Figure 11. Correlation between CMA600 and HPLC.
$Pyruvate_{CMA600}$ =0.972 × $Pyruvate_{HPLC}$ - 5.5 µmol/L; R^2 = 0.921; SE =16.1 µmol/L, N = 44.

3.5. GLUTAMATE

3.5.1. Measuring principle

Glutamate is enzymatically oxidised by glutamate oxidase (GltOx). Peroxidase (POD) catalyse the reaction between the hydrogen peroxide formed, TOOS and 4-amino-antipyrine to form the red-violet coloured quinonediimine. The rate of formation of the coloured substance is proportional to the glutamate concentration.

$$\text{Glutamate} + O_2 \rightarrow \text{2-oxoglutarate} + H_2O_2$$

$$2\ H_2O_2 + \text{TOOS} + \text{4-amino-antipyrine} \rightarrow \text{quinonediimine} + 4\ H_20$$

3.5.2. Instrumental parameters

Conditions:

Wavelength:	530 nm
Temperature:	37°C
Mode:	kinetic, steepest positive slope
Sample volume:	1.5 µL
Reagent volume:	7.5 µL
Delay time:	17 s
Sampling time:	29 s

3.5.3. Measuring range

If the calculated concentration is < 1 µmol/L the result is reported as "*N" and if the calculated concentration is > 150 µmol/L the result is reported as ” >N", where N is the calculated concentration.

3.5.4. Reagent

3.5.4.1.Content

	Component	Concentration in test solution
Glutamate Reagent	4-amino-antipyrine	0.3 mmol/L
	Glutamate oxidase	> 0.25 kU/L
	Peroxidase	> 0.8 kU/L
	Ascorbate oxidase	> 17 kU/L
Glutamate Buffer	PIPES Buffer, pH 6.8	0.1 mol/L
	TOOS	1.5 mmol/L
	Sodium Azide	0.3 g/L

The reagent contains sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

3.5.4.2.Stability

Reagents are stable up to expiry date when stored at +2 to +8 °C. Reconstituted reagent is stable for five days in the instrument.

3.5.5. Calibration

3.5.5.1.Calibrator

A bottle of Calibrator A. (Cat no P000057) is included in all reagent cassettes.

3.5.5.2.Checks

The calibration is rerun if

Response factor < 0.025 mmol × L^{-1} / mAU × s^{-1} or
Response factor > 0.125 mmol × L^{-1} / mAU × s^{-1}.

3.5.6. **Performance**

3.5.6.1.Linearity

The method is linear from 1 to 150 µmol/L with a deviation of < 5 % at 150 µmol/L.

The figure below shows the linearity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the linear range for ISCUS*flex* is the same.

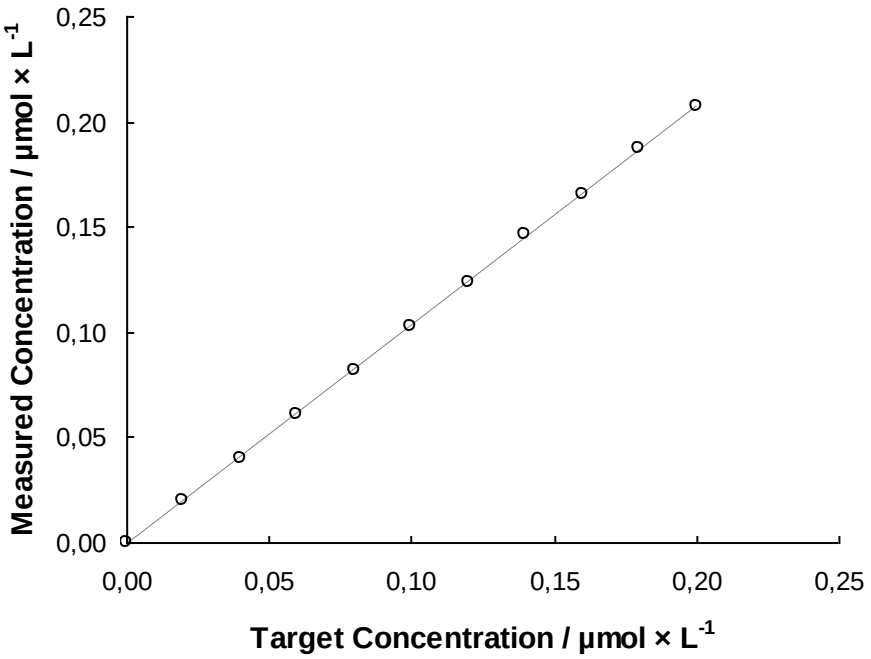

Figure 12. Linearity of the glutamate method for the CMA 600 analyser obtained from samples with known concentrations of glutamate.

3.5.6.2.Analytical Sensitivity

The average sensitivity of this method is 18 - 20 mAU × s^{-1} per mmol × L^{-1}.

3.5.6.3.Detection limit

The detection limit is about 1 µmol/L.

3.5.6.4.Specificity

Glutamate oxidase is specific for glutamate. Ascorbate oxidase is added to the reagent to remove interference from ascorbic acid.

The table below shows the specificity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the specificity for ISCUS*flex* is the same.

No significant change in values was observed when the following substances were added to Ringer's solution containing 25 µmol/L glutamate except for glutathion at 50 µmol/L, which shows a negative interference.

Table IX

Substance (conc.)	Recovery / %
Pyruvate (150 µmol/L)	104
Ascorbate (0.5 mmol/L)	96
Acetaminophen (300 mg/L)	94
Creatinine (1 mmol/L)	94
Acetylsalicylic acid (300 mg/L)	94
Glutamine (1 mmol/L)	100
Aspartate (0.1 mmol/L)	95
Histidin(1 mmol/L)	95
Glutathion (0.05 mmol/L)	60

As with any chemical reaction, user must be alert to the possible effect on the result due to unknown interference from medication or endogenous substances. All patient results must be evaluated considering the total clinical status of the patient.

3.5.6.5.Precision

Estimates of imprecision, obtained from replicate assays of samples with known concentrations of glutamate in Ringer's solution spanning the range of linear measurements are shown in the following table.

Table X

	Within run		Between run		Total		N	
Mean / µmol × L^{-1}	SD / µmol × L^{-1}	RSD / %	SD / µmol × L^{-1}	RSD / %	SD / µmol × L^{-1}	RSD / %	Obs.	Runs
9.7	0.34	3.5%	0.27	2.7%	0.43	4.4%	100	20
40.0	0.48	1.2%	1.09	2.7%	1.17	2.9%	100	20
115.5	1.1	1.0%	3.3	2.9%	3.4	3.0%	100	20

3.5.6.6.Accuracy

The results below show the accuracy for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and method parameters, the accuracy for ISCUS*flex* is the same. Test has shown that the results from ISCUS*flex* are within ± 10% of those obtained with CMA 600.

Analytical recovery of glutamate added to ultrafiltrated Sigma Metabolite Control (at 12.5 and 25 µmol/L) gave a mean recovery of 112 % (range 106.4 - 117.6 %).

Comparison of 44 samples using HPLC gave the following correlation. (y = CMA 600, x = HPLC):

$Y = 1.08\ x - 0.65\ \mu mol/L$ $N = 46$

$R^2 = 0.875$ $S_{xy} = 3.2\ \mu mol/L$

The results are presented in figure 5.

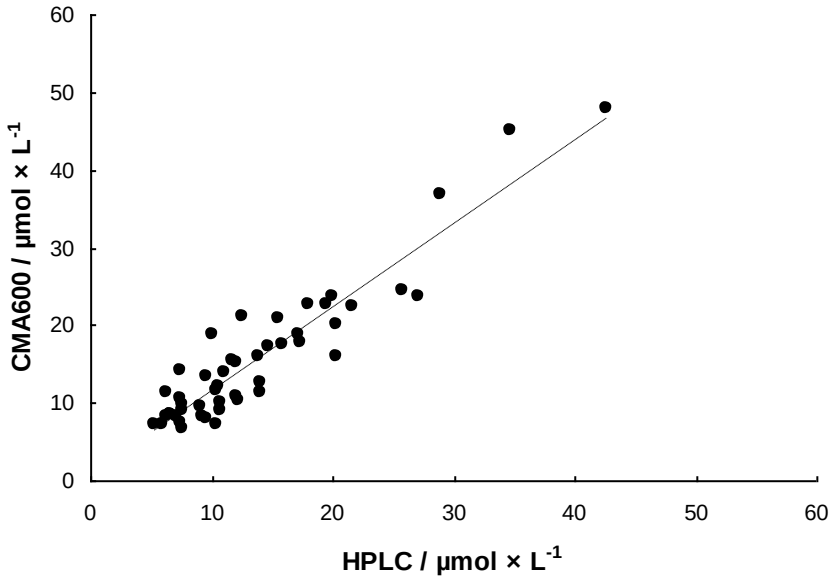

Figure 13. Correlation between CMA600 and HPLC. $Glutamate_{CMA600} = 1.08 \times Glutamate_{HPLC} + 0.65$ µmol/L; $R^2 = 0.875$; SE =3.23 µmol/L, N = 46.

3.6. UREA

3.6.1. Measuring principle

Urea is hydrolysed in the presence of urease to ammonium ions and carbon dioxide. The ammonium ions formed react with 2-oxoglutarate in the presence of glutamate dehydrogenase (GlDH) and NADH to form glutamate and NAD^+. The rate of utilisation of NADH is measured photometrically at 365 nm and is proportional to the urea concentration.

$$\text{Urea} + H_2O \rightarrow 2\ NH_3 + CO_2$$

$$\text{2-oxoglutarate} + NH_4^+ + \text{NADH} \rightarrow \text{L-glutamate} + NAD^+ + H_2O$$

3.6.2. Instrument parameters

Conditions:

Wavelength:	375 nm
Temperature:	37°C
Mode:	kinetic, steepest negative slope
Sample volume:	0.5 µL
Reagent volume:	14.5 µL
Delay time:	22 s
Sampling time:	29 s

3.6.3. Measuring range

If the calculated concentration is < 0.5 mmol/L the result is reported as "< D.L" and if the calculated concentration is > 25 mmol/L the result is reported as ” >N", where N is the calculated concentration.

3.6.4. Reagent

3.6.4.1.Content

1. Reagent: 5 bottles of lyophilisate for 6 mL
2. Buffer: 5 bottles of 6 mL

Reagent sufficient for 5 × 350 determinations

	Component	Concentration in test solution
Urea Reagent	Adenosine-5-diphosphate	3 mmol/L
	NADH	0.2 mmol/L
	2-oxoglutarate	15 mmol/L
	Urease	> 15 kU/L
	Glutamate dehydrogenase	> 1 kU/L
Urea Buffer	Tris Buffer, pH 8.0	0.1 mol/L
	Sodium Azide	0.2 g/L

The reagent contains sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

3.6.4.2.Stability

Reagents are stable up to expiry date when stored at +2 to +8 °C. Reconstituted reagent is stable for three days in the instrument. Note: The linear range is narrowed upon ageing of the reagent.

3.6.5. Calibration.

3.6.5.1.Calibrator

Use Calibrator A, Cat no P000057; a bottle is included in each reagent set.

3.6.5.2.Checks

The calibration is rerun if

Response factor < 4.43 mmol × L^{-1} / mAU × s^{-1} or
Response factor > 26.6 mmol × L^{-1} / mAU × s^{-1}.

3.6.6. **Performance.**

3.6.6.1.Linearity

The method is linear from 0.5 to 25 mmol/L (150 mg/dL) with a deviation of < 5 % at 25 mmol/L with a freshly prepared reagent. After three days, the upper limit of the linear range has decreased to about 17 mmol/L (100 mg/dL).

The figure below shows the linearity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and method parameters, the linear range for ISCUS*flex* is the same.

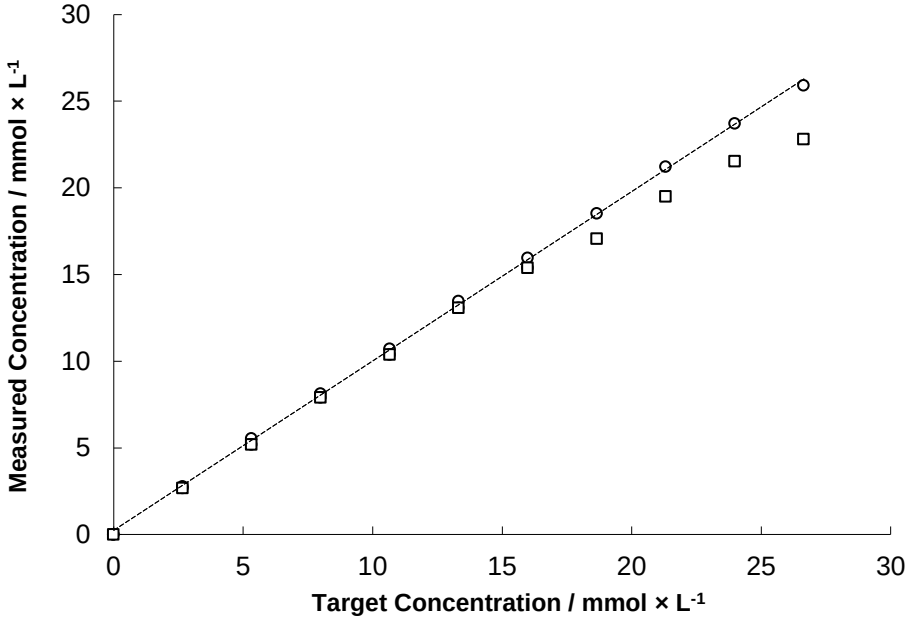

Figure 14. Linearity of urea method with a freshly prepared reagent (o) and a three day old reagent (□). Obtained from samples with known concentrations of urea.

3.6.6.2.Analytical Sensitivity

The average sensitivity of this method is 0.05 - 0.09 mAU × s^{-1} per mmol × L^{-1}.

3.6.6.3.Detection limit

The detection limit is 0.5 mmol/L.

3.6.6.4.Specificity

In the method, urea is converted to ammonium ions. Endogenous ammonia will be detected as urea. This interference is in practice insignificant, since the levels of ammonia in microdialysis samples are very low compared to the concentration of urea, << 0.5 mmol/L.

The table below shows the specificity for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and the method parameters, the specificity for ISCUS*flex* is the same.

No significant change in values was observed when the following substances were added to Ringer's solution containing 5.2 mmol/L urea.

Table XI

Substance (conc.)	Recovery / %
Glucose (25 mmol/L)	102
Lactate (25 mmol/L)	102
Glycerol (2.5 mmol/L)	101
Uric acid (0.5 mmol/L)	100
Acetaminophen (300 mg/L)	101
Creatinine (0.15 mmol/L)	99
Acetylsalicylic acid (600 mg/L)	102
Glutathion (0.3 mmol/L)	105

As with any chemical reaction, user must be alert to the possible effect on the result due to unknown interference from medication or endogenous substances. All patient results must be evaluated considering the total clinical status of the patient.

3.6.6.5.Precision

Estimates of imprecision, obtained from replicate assays of samples with known concentrations of urea in Ringer's solution spanning the range of linear measurements are shown in following table.

Table XII

	Within run		Between run		Total		N	
Mean / mmol × L^{-1}	SD / mmol × L^{-1}	RSD / %	SD / mmol × L^{-1}	RSD / %	SD / mmol × L^{-1}	RSD / %	Obs	Runs
1.5	0.119	7.9%	0.079	5.2%	0.142	9.4%	100	20
5.8	0.131	2.2%	0.126	2.2%	0.180	3.1%	100	20
17.6	0.194	1.1%	0.289	1.6%	0.343	2.0%	100	20

3.6.6.6.Accuracy

The results below show the accuracy for the CMA 600 analyser. Since ISCUS*flex* uses the same reagent, optical unit and method parameters, the accuracy for ISCUS*flex* is the same. Test has shown that the results from ISCUS*flex* are within ± 10% of those obtained with CMA 600.

Analytical recovery of urea added to microdialysis samples (at 1.25 to 16 mmol/L) gave a mean recovery of 104 % (range 88 - 117 %).

Comparison of 32 pooled and spiked microdialysis samples using Cobas Mira S with Roche Unimate UREA reagent gave the following correlation. (y = CMA 600, x = COBAS):

$y = 1.04\ x + 0.44$ mmol/L $N = 32$

$r^2 = 0.991$ $S_{xy} = 0.45$ mmol/L

The results are presented in figure 15.

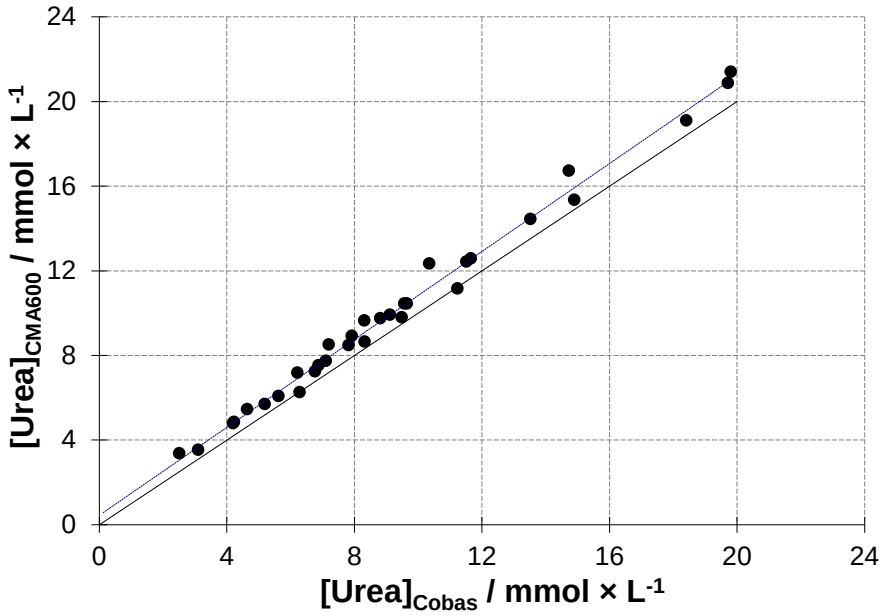

Figure 15. Correlation between CMA600 and Cobas Mira S (Roche Unimate UREA).
$Urea_{CMA600}$ =1.04 × $Urea_{Cobas}$ +0.44 mmol/L; R^2 = 0.991; SE = 0.45 mmol/L, N = 32.

3.7. L/P RATIO

The L/P-ratio is calculated on current Lactate and Pyruvate data. For an L/P-ratio point to be calculated both a Lactate and a Pyruvate result must exist for a given time point. If one of Lactate and Pyruvate is missing for a given time point no L/P-ratio is calculated for that time point.

The L/P-ratio is always calculated using the mmol value for both analytes regardless of the selected presentation unit, thus no scaling errors exist.

Furthermore, the L/P-ratio is always calculated as a concentration ratio, which differs slightly from the mass ratio as Lactate and Pyruvate have different molecular weights. The difference between concentration and mass L/P-ratios is 2.2 %.

3.8. L/G RATIO

The L/G-ratio is calculated on current Glucose and Lactate data. For a L/G -ratio point to be calculated both a Glucose and a Lactate result must exist for a given time point. If one of Glucose and Lactate is missing for a given time point no L/G -ratio is calculated for that time point.

The L/G -ratio is always calculated using the mmol value for both analytes regardless of the selected presentation unit, thus no scaling errors exist.

Furthermore, the L/G -ratio is always calculated as a concentration ratio. The mass ratio is 50% of the concentration ratio as Glucose has a molecular weight, which is twice as big as the molecular weight for Lactate.

3.9. CALIBRATOR

3.9.1. Calibrator A

3.9.1.1. Intended use

The Calibrator A is intended for calibration of the Glucose, Lactate, Glycerol, Pyruvate, Glutamate and Urea.

3.9.1.2. Content and stability

Analyte	Assigned concentration
D-Glucose	5.55 mmol/L
L-Lactate	2.5 mmol/L
Urea	13.3 mmol/L
Glycerol	475 µmol/L
Pyruvate	250 µmol/L
L-Glutamate	25 µmol/L

The calibrator is prepared in saturated benzoic acid. The assigned values for glucose, lactate, glycerol and urea are within ± 1 %; the values for pyruvate and glutamate are within ± 3 %.

Unopened Calibrator is stable up to expiry date when stored at +2 to +8 °C. The Calibrator is stable for five days in the instrument.

3.10. CONTROL SAMPLES

Good laboratory practice requires that quality control samples should be included in every run to check assay performance.

3.10.1. Intended Use

The Control Samples are intended to be used as assayed quality control samples for the M Dialysis Analyzers.

3.10.2. Contents and stability

Each package contains

5 vials Control Sample Low of 5 mL
5 vials Control Sample Elevated of 5 mL

Unopened Control Samples are stable up to expiry date when stored at +2 to +8 °C. Opened vials are stable for two weeks at +2 to +8 °C or five days on board in the instrument.

3.10.3. Usage

The use of quality control samples is often regulated by local quality assurance programs.

Control samples are usually analysed after changing the reagents, after calibration and in connection with analysis of patient samples. By analysing the control samples, performance of the analytical system, including everything from Analyzer, Reagents, Calibrator and calibration can be followed.

See the User Manual how to use the Control Samples with the ISCUS*flex* Microdialysis Analyzer.

3.11. RINSING FLUID

The rinsing fluid is used to rinse the fluid system of the analyser between each determination.

3.11.1. Content and Stability

One bottle contains 500 mL of rinsing fluid, which is sufficient for about 1600 determinations.

The Rinsing Fluid contains a small amount of wetting agent (Brij®-35) and preservative dissolved in reagent grade water.

The rinsing fluid is stable up to expiry date when stored at +2 to +30° C. It is recommended to replace the rinsing fluid when changing reagents in the analyser.

4. OPERATION

See User's manual.

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5. DATA ANALYSIS

5.1. CALCULATION OF ABSORBANCE

Since ISCUS*flex* utilises a single beam photometer, in order to get absorbance values, the light through the cuvette without any sample, P_0, has to be known. With ISCUS*flex*, this is done by measuring the "water level", i.e. the signal from the photo detector when the cuvette is filled with Rinsing Fluid. This measurement precedes each analysis. Normally the water level is between 700 and 2500 mV, depending on the filter used. The absorbance during the analysis is then calculated as,

$$Absorbance = Log \frac{P_0}{P},$$

where P is the measured signal from the photo detector during the analysis.

5.2. KINETIC CALCULATIONS

The analyser uses kinetic determinations in order to get the sample results as soon as possible. Instead of waiting for the enzymatic reaction to reach completion, that may take several minutes, the instrument reads the absorbance with a sampling rate of approximately 8 Hz during the first 29 seconds of the reaction and uses the maximal reaction rate during the measurement for quantification.

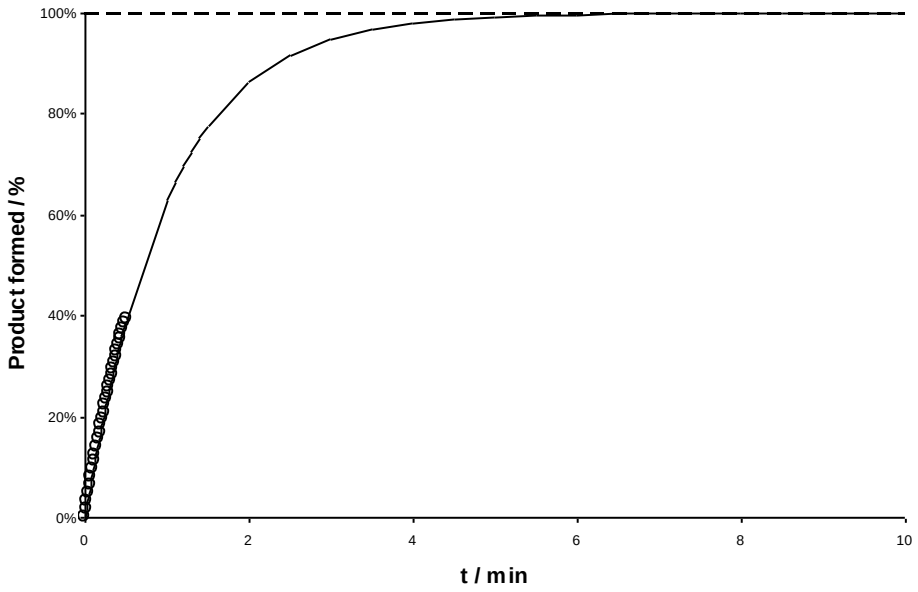

Figure 16. Typical reaction curve for a first order reaction. In this example, it takes about 7 minutes to reach the endpoint. However, by doing a kinetic measurement the result can be obtained within 30 seconds.

5.2.1. **Polynomial regression**

Theoretically, the absorbance signal obtained should follow an exponential function, as in the example shown above, if the reaction follows first order kinetics with respect to the analyte. In practice, this is not always the case. Especially at the beginning of the reaction, a short lag period is often seen. To handle this situation, a third-order polynomial,

$$signal = A_0 + A_1 \times t + A_2 \times t^2 + A_3 \times t^3,$$

is fitted to the acquired data points.

5.2.2. **Blank measurement**

When the small air bubble, aspirated before the sample and reagent, passes the detector, the absorbance signal rises sharply for a short moment. Then it takes some seconds for the detector to recover. This effect will show up as a slowly decaying absorbance signal when there is no analyte present in the sample.

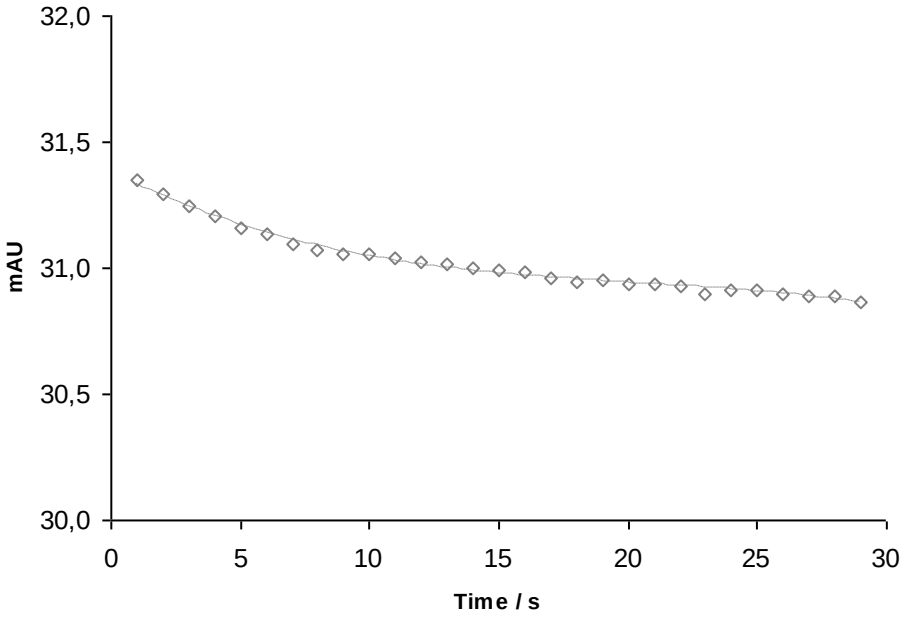

Figure 17. Raw data from blank measurement.

When analyte is present in the sample, the signal obtained will be superimposed on this slightly decaying signal. At very low analyte concentrations, this may lead to an incorrect estimate of the reaction rate. Therefore, the signal for a reagent blank is measured at every calibration event and used for correction of this effect. The blank data points are fitted to a third order polynomial in the same way as those for the samples,

$$blank = B_0 + B_1 \times t + B_2 \times t^2 + B_3 \times t^3.$$

The reaction rate, i.e. the slope of the absorbance versus time curve, is not dependent on the initial absorbance of the reagent. Therefore, it is not necessary to measure a reagent blank for each sample as is often done when using end-point determinations. It suffices to measure the blank signal at each calibration event, that is, once every sixth hour.

5.2.3. **Calculating the slope**

To obtain the slope of the measured signal is simple once the signal has been fitted to the polynomial. Firstly, the polynomial obtained for the blank is subtracted from the polynomial for the sample,

$$corrected\ signal = (A_0 - B_0) + (A_1 - B_1) \times t + (A_2 - B_2) \times t^2 + (A_3 - B_3) \times t^3.$$

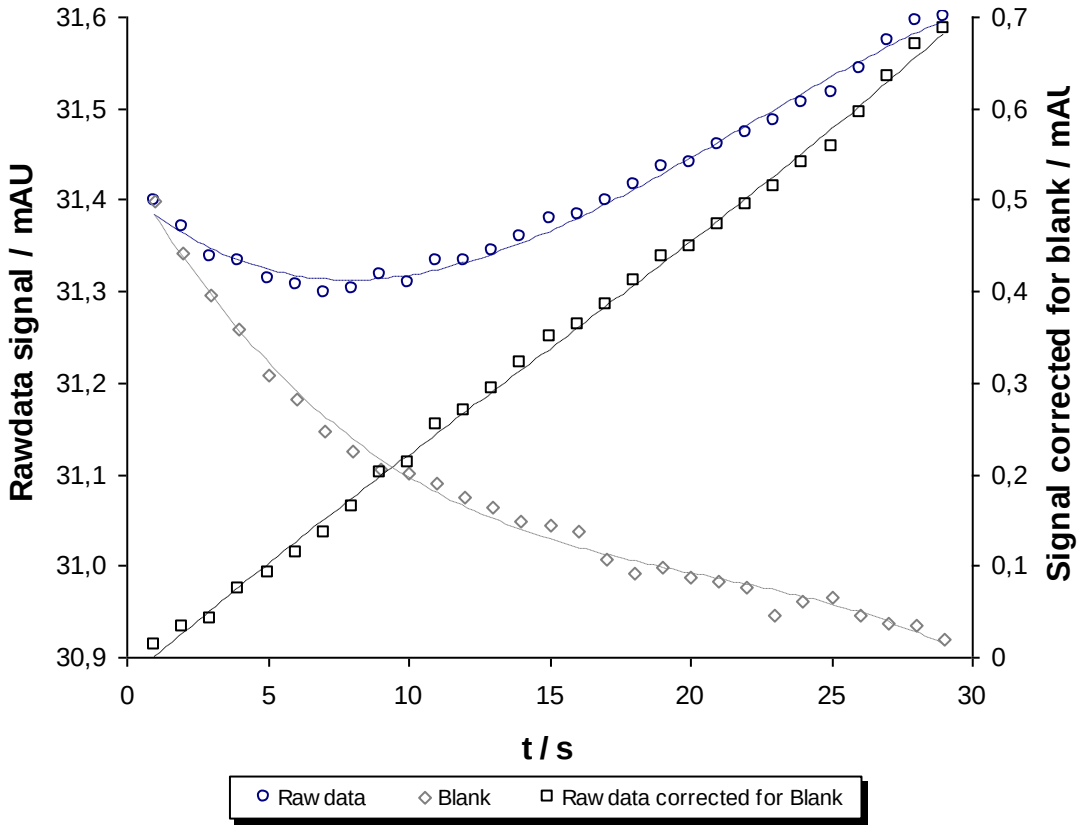

Figure 18. *Correction of raw data for blank signal. These signals were obtained for a very low concentration of glucose.*

Then, the slope is easily calculated by taking the derivative of the new polynomial.

$$slope\ (t) = (A_1 - B_1) + 2 \times (A_2 - B_2) \times t + 3 \times (A_3 - B_3) \times t^2 =$$
$$= C_1 + 2 \times C_2 \times t + 3 \times C_3 \times t^2$$

To find the maximal reaction rate, i.e. maximum if the absorbance is supposed to increase or minimum if the absorbance is decreasing, we first look for the extreme value of the polynomial by finding where the second derivative is zero,

$$2 \times C_2 + 6 \times C_3 \times t = 0 \Rightarrow t_{extreme} = - C_2 / 3 C_3$$

if $t_{extreme}$ is between 0 and 29, the extreme value is found during the measurement and the value for

$$slope\ (t_{extreme}) = slope\ (- C_2 / 3 C_3)$$

is compared with the value for the slope at the start or end to find the maximal reaction rate, i.e. optimal slope, during the measurement. If $t_{extreme}$ is less than zero or greater than 29 the optimal slope will be found either at the start or at the end of the measurement.

$$slope_{opt} = max(slope_{start}, slope_{end}, slope_{extreme}), \text{ if } t_{extreme} = 0 \ldots 29 \text{ else}$$

$$slope_{opt} = max(slope_{start}, slope_{end})$$

The optimal slope during the measurement is used in further calculations.

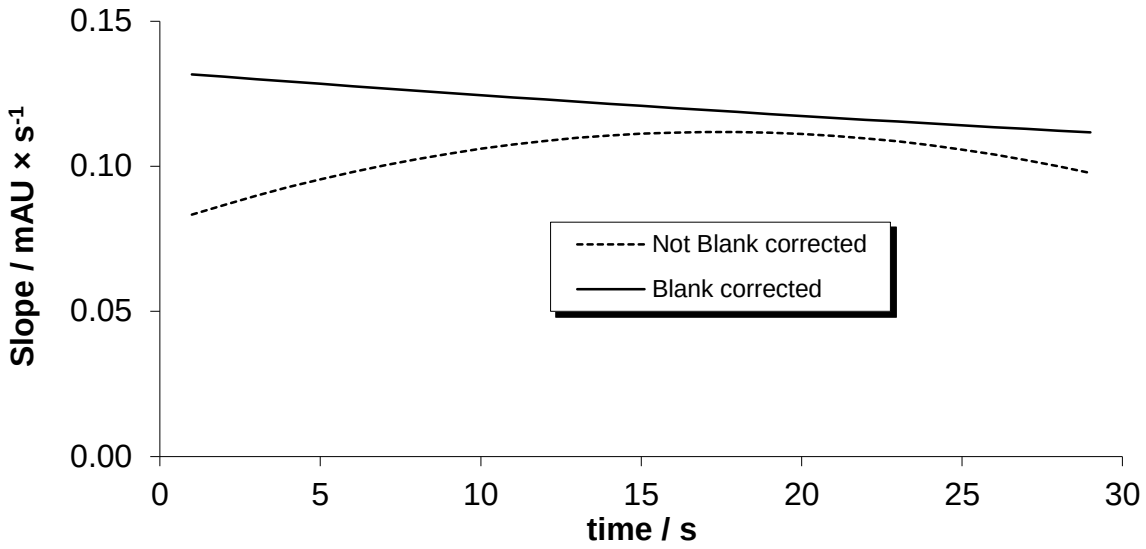

Figure 19. *Comparison of the blank corrected and uncorrected slope for the data showed in the previous figure. For the blank corrected signal the maximal slope is found at the beginning of the measurement, for the uncorrected signal the maximal slope is found around 17 seconds.*

5.3. REJECTION OF UNCERTAIN MEASUREMENTS

If the data do not fit well to the third order polynomial this is an indication that something is out of order, for instance noise or an interfering substance is affecting the measured signal. In order to reject this kind of measurements, two parameters, which measure how close the data points lies to the polynomial, are calculated.

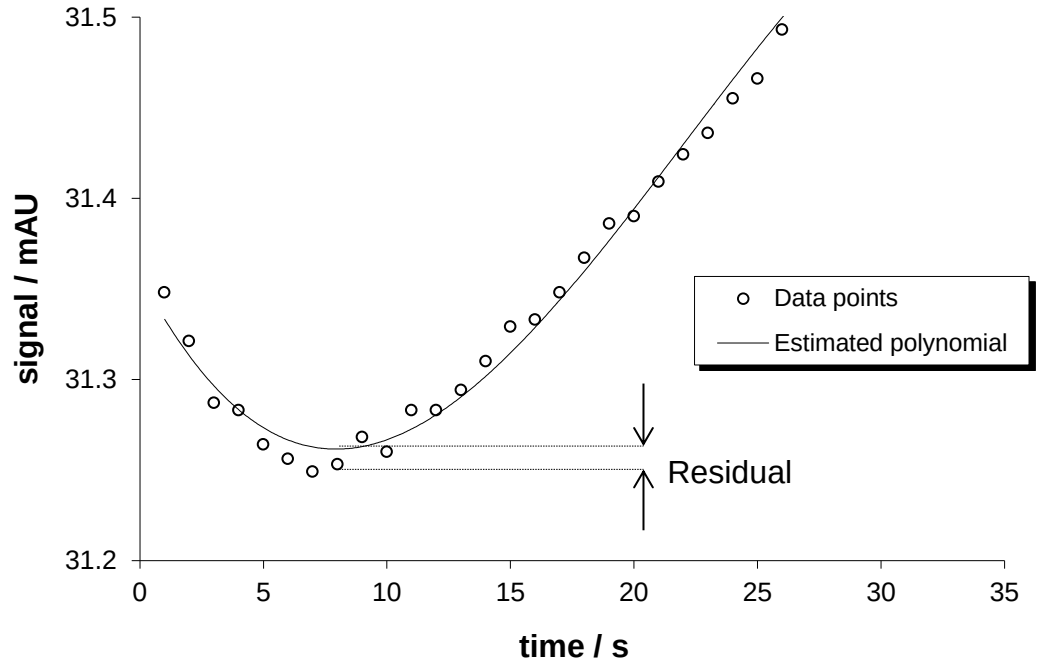

Figure 20. *The residual is the vertical distance between a data point and the estimated polynomial. Note that the last three data points are out of scale.*

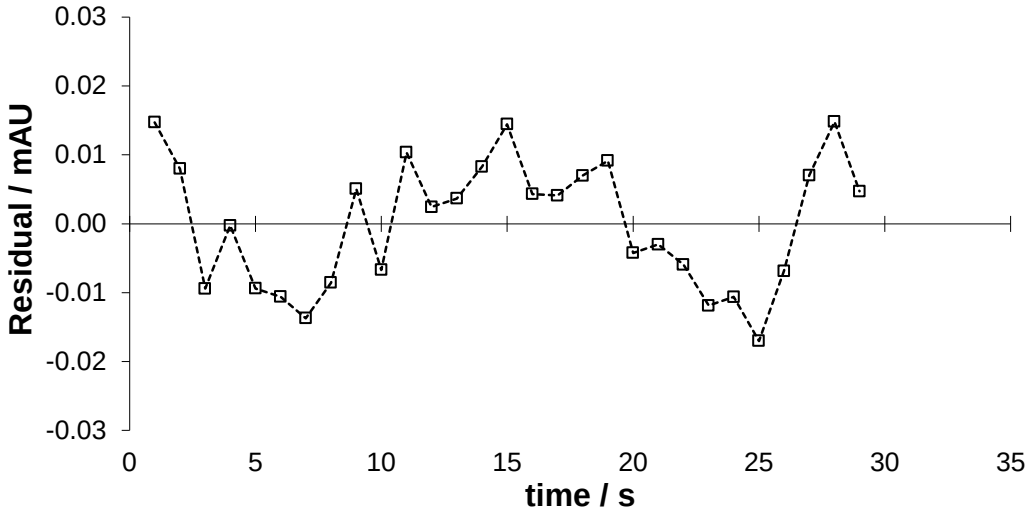

Figure 21. The residuals plotted versus time for the data shown in figure 20.

The residual square sum (*RSS*), which is the sum of the squared vertical distance between each data point and the estimated polynomial.

$$RSS = \sum (A_0 + A_1 \times t_i + A_2 \times t_i^2 + A_3 \times t_i^3 - y_i)^2$$

When the reaction rate is high, larger deviations can be accepted. Therefore, a parameter that is normalised with the slope is calculated as well, the normalised residual quadratic sum, *NormRSS*

$$NormRSS = \frac{\sqrt{RSS}}{Slope}$$

At least one of the following criteria must be fulfilled for the measurement to be accepted

$$RSS \leq 0.8$$

$$NormRSS \leq 4.0$$

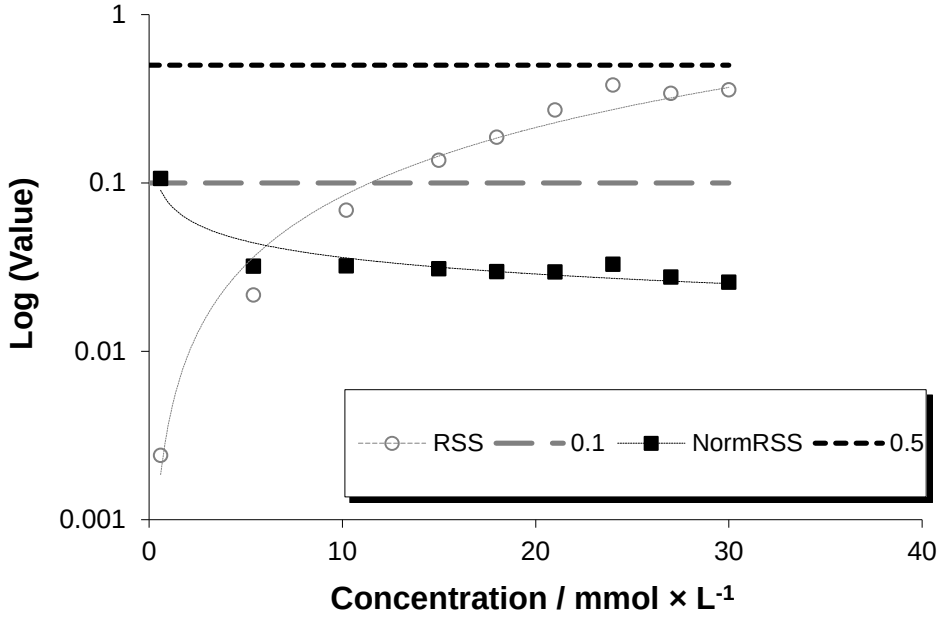

Figure 22. RSS and NormRSS plotted versus concentration for glucose.

These checks are done on both sample/calibrator and blank measurements (see section 2.7). If the measurement fails, the instrument will try to reanalyse the sample. If also the second attempt fails, a warning message is shown on the screen and the next analysis in the queue will be done.

5.4. CALIBRATION AND CALCULATION OF RESULTS

The analytical methods are calibrated by analysing a solution with known concentrations of the different analytes. Two replicates of the calibrator solution are analysed and the average slope from these measurements is used for calculating the concentration in the unknown samples.

$$c_{unknown} = \frac{slope_{unknown}}{average\ slope_{calibrator}} \times c_{calibrator}$$

5.4.1. Checks for calibrator measurements

A number of different checks are done on the calibrator measurements before a new calibration value is accepted.

1. Reject uncertain measurements according to the previous paragraph.
2. Find the root to the equation

   $(A_0 - B_0) + (A_1 - B_1) \times t = 0$,

   i.e. estimate the time, $t=t_0$, when the calibrator signal equals the blank signal. This time should be close to the methods delay time.

   The found value of t_0 shall be within limits specified in the analytical method specified for the respective reagent. If t_0 is outside these limits, the calibration is repeated. If also the second calibration fails, further measurements with this reagent are shut down. The failed calibration is indicated by a crossed over red dot in the status bar in the lower part of the screen.
3. Calculate the relative standard deviation for the calibrator measurements. The relative standard deviation shall be ≤ 5 %.

   If the deviation is too great, the calibration is repeated. If also the second calibration fails, further measurements with this reagent are shut down. The failed calibration is indicated by a crossed over red dot in the status bar in the lower part of the screen.

4. The calibration value is checked if it is within the acceptable limits defined in the analytical method.

   If the calibration value is outside these limits, the calibration is repeated. If also the second calibration fails, further measurements with this reagent are shut down. The failed calibration is indicated by a crossed over red dot in the status bar in the lower part of the screen.

   The calibration value is checked if it deviates too much from previous calibrations with the same set of reagent and calibrator.

   If the deviation is too great, the calibration is repeated. If also the second calibration fails, further measurements with this reagent are shut down. The failed calibration is indicated by a crossed over red dot in the status bar in the lower part of the screen.

5.4.2. Checks for sample measurements

A number of checks are also done on the sample measurements:

1. Reject uncertain measurements as above.
2. If the calculated concentration is lower than the limit of detection, as defined in the analytical method for the analyte, the result is reported with a star (*) in front of the measured concentration to indicate that it is less than the limit of detection.
3. If the calculated result is above the upper limit of the linear range, as defined in the analytical method for the analyte the result is reported as > calculated concentration.

5.5. TREND ARROWS

A trend arrow reflects the overall trend in data. It is calculated by comparing the values according to the algorithm described below. The result from the calculations can be a positive trend, for which an arrow pointing upwards is presented, a negative trend for which an arrow pointing downwards is presented or no trend for which an arrow pointing to the right is presented. If there are fewer than 6 points, no trend at all is presented.

Calculation algorithm:

Each point is compared to each straggling point. Only the six last points are compared. If the ratio between a straggling point and the comparison point is larger than a predefined threshold, a counter is increased, if the ratio is lower than another predefined threshold the same counter is decreased.

After all comparisons have been made the up-/down-counter is compared with a predefined threshold for positive trend and a threshold for negative trend to determine what sort of trend arrow should be presented.

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6. INFORMATION STORAGE AND DATA HANDLING

6.1. COMMON DATA

Common data is stored in the internal database on the Smart Media card.

6.2. SAMPLE DATA

Sample data is stored in the internal database on the Smart Media card. No sample data is stored for more than 6 weeks.

Sample data is organised in recordings, which contain all sample data from one catheter and one substance.

The recordings are organised in admissions, which contain all recordings for one patient during a time period (when the patient is active and the machine is on).

The admissions are connected to patients, so that one patient can have several admissions.

If a writeable Secure Digital (SD) card or USB memory is inserted, data from the current patient admission will be automatically saved as new analyses are completed.

6.3. EXPORTING DATA TO EXTERNAL COMPUTER

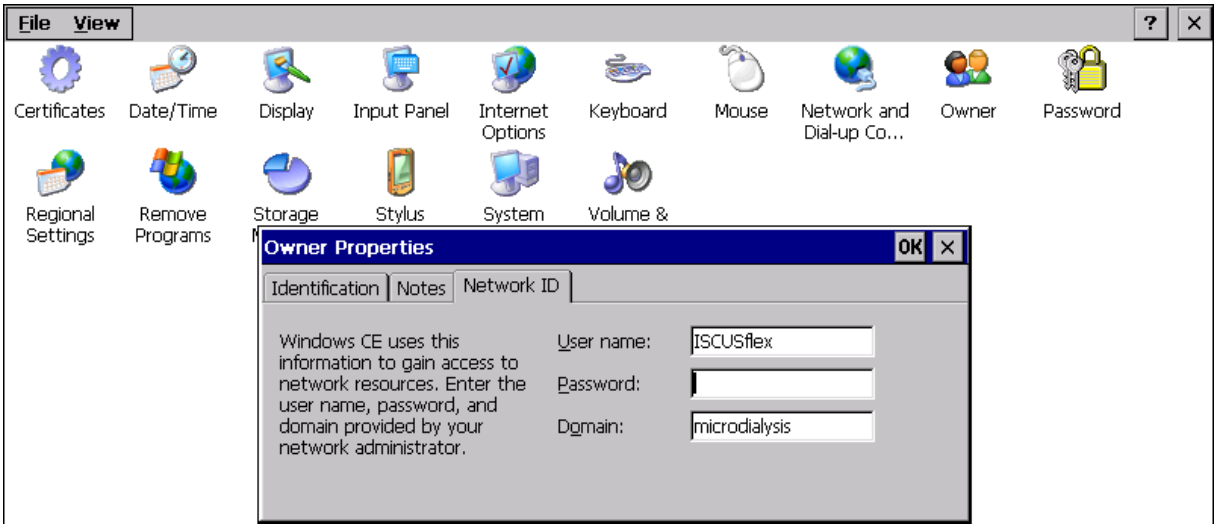
Sample data can be saved on the SD card, a USB-memory or a network share (for more information, see User's manual and below). Each admission is saved in a file called [GUID].xml, where [GUID] is a unique number assigned to the admission.

The file can be viewed in LABpilot, ICUpilot or, for instance, Microsoft Excel.

6.3.1. Network share

In order to save data in a network share the ISCUSflex has to be set up for network login. A user account has to be created in your network environment, with proper rights (Read/Write/Create/Modify) in the network share to be used. The network share URL has to be entered as the Network Storage location in User GUI/Settings/Data.

In order to complete the setup, please enter the Control Panel from the Service GUI. Select 'Owner' and enter the proper settings (User name/Password/Domain) under Network ID.



Close the dialog and return to the Service GUI to save the settings.

6.4. NETWORK DATA TRANSMISSION

Sample data can be transmitted using the network port on ISCUS*flex*. The network transmission must be activated in the Settings/Network tab.

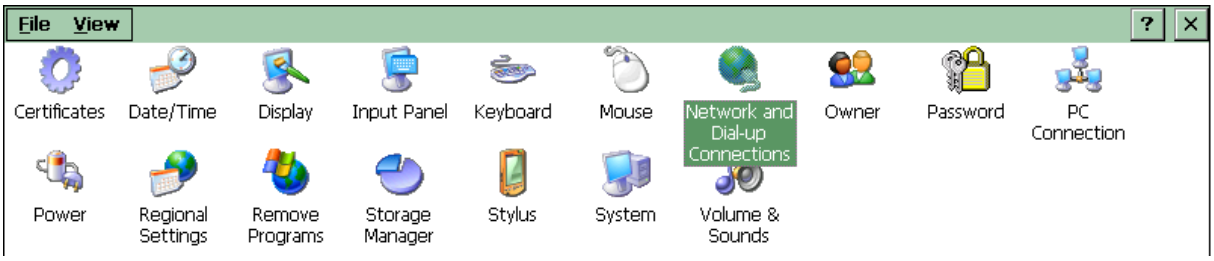
6.4.1. ISCUS*flex* network settings

Activate the network data transmission in Settings/Network. Specify listening computer name or IP-address as well as the communication port (default 13000).

6.4.2. IP-number

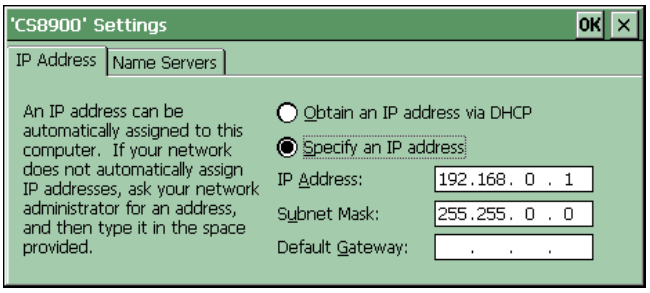
ISCUS*flex* must have an IP-number assigned. Default an IP-number is assigned if ISCUS*flex* is connected to a network with a DHCP-server, otherwise an explicit setting must be done using the Control Panel, which is reached from the Maintenance menu/Service GUI.

When setting an explicit IP-number please consult the IT-department to get a proper number. In the Control Panel, select *'Network and Dial-up Connections'*.



Select the active connection *'SC89001'*

Default *'Obtain an IP address via DHCP'* is selected. In this case, select *'Specify an IP address'* and enter the figures proposed by the IT-department. Touch OK to confirm the selection.



6.4.3. Connecting Iscus*flex* to a Windows computer with ICUpilot (example)

6.4.3.1.Cable

Use a crossed Ethernet cable (REF 8003117 KAB TP UTP CAT5 RJ45 5 m Cross-over) to connect Iscus*flex* with the computer.

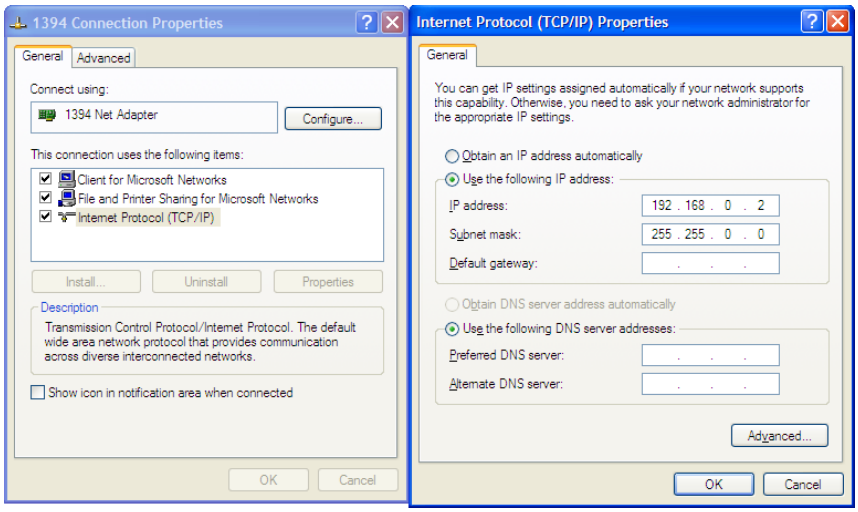
6.4.3.2.Iscus*flex* IP address

Specify the address 192.168.0.1 for Iscus*flex* according to paragraph 6.4.2 above.

6.4.3.3.Computer IP address

Specify the address 192.168.0.2 for the computer (this will override any previous computer setting and disable the normal network connection). This is done in the Control Panel/Network Connections:

Select Properties for the connection, Select Internet Protocol (TCP/IP) properties and enter the IP address and Subnet mask.



6.4.3.4.Setup Iscus*flex* for sending data

Follow the instructions in the User's manual to 'Send data via network' in the Settings/Network tab. Enter 192.168.0.2 in the Remote Host field. Enter appropriate port number (default 13000 in the ICUpilot/Iscus module).

6.4.3.5.ICUpilot support for Iscus

Install the EXT MON ISCUS (REF 8002706) option and restart the computer.

6.4.4. Firewall issues

Any firewall between ISCUS*flex* and the listening computer must be properly configured. For a WinXP computer, the settings can be made in Control Panel/Security Center.

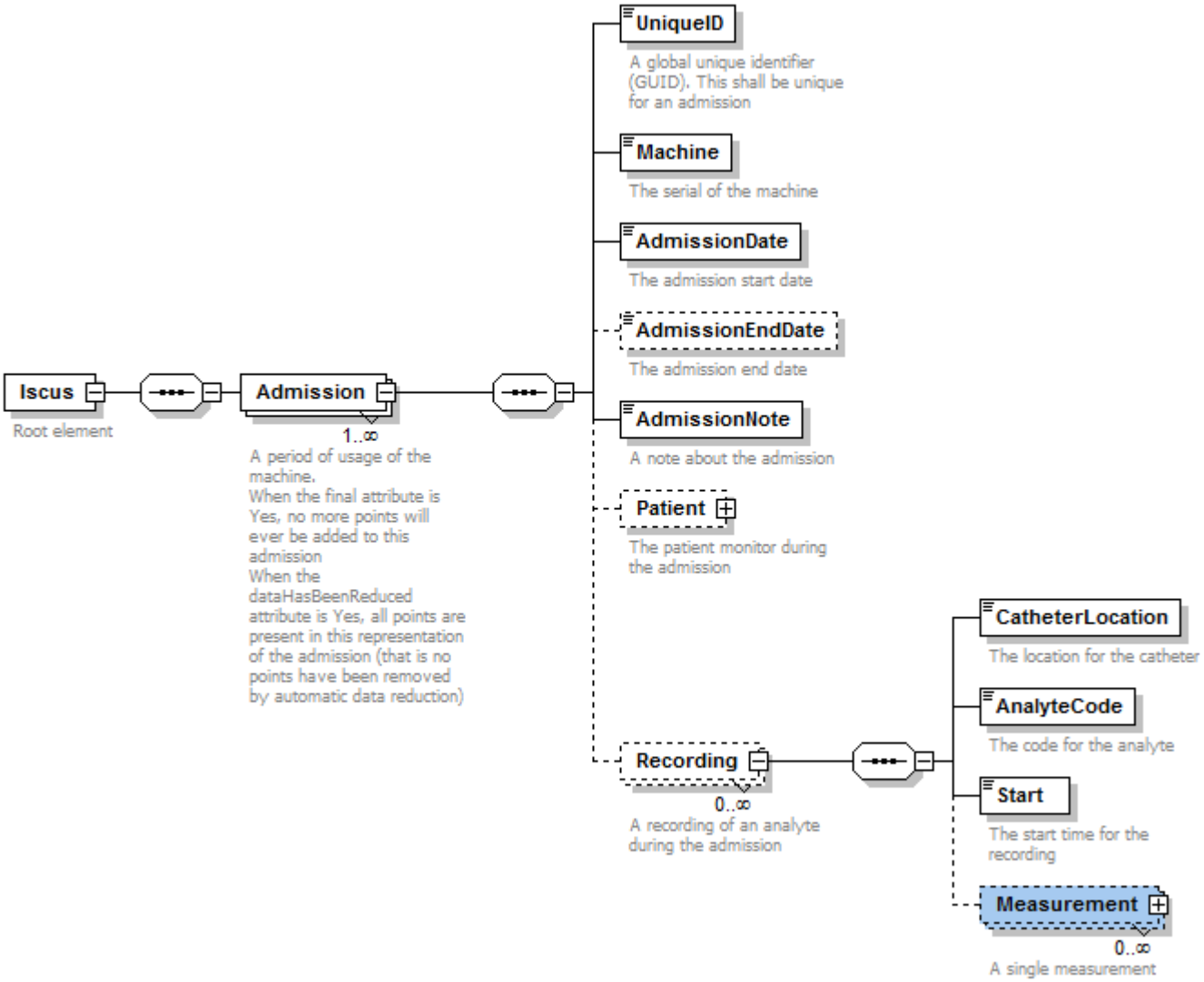
Three different settings will work with ISCUS*flex*:

1. Completely turn off the firewall
2. Allow access for the port number used (default is 13000). The port number is specified in ISCUS*flex* Settings/Network tab and in the listening application (e.g. ICUpilot/Iscus module).
3. Allow network communication for a certain program (e.g. ICUpilot/Iscus module).

6.5. FILE FORMAT

6.5.1. Description

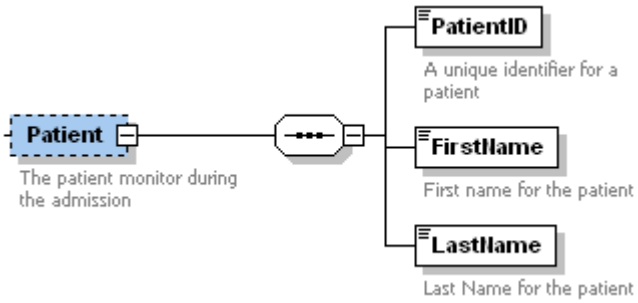
The data is stored in XML. The complete definition is given in 6.5.2 XML Schema. Below is an overview of the format.



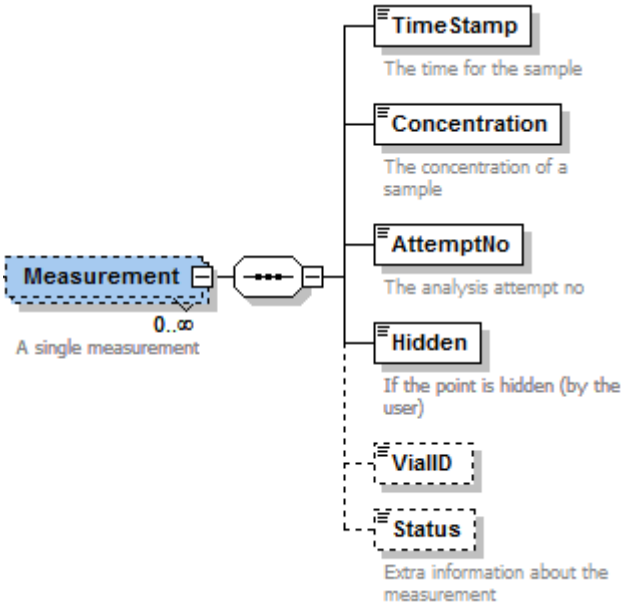
The document contains a number of admissions. These admissions contain information about the machine and optionally about a patient. Each admission contains a number of recordings. The recordings are a series of analysis of a single substance, for instance glucose for a given catheter location.

The admission optionally has an attribute, “final”, that indicates whether new samples can be added to the admission (at a later point) or not. After the “final” attribute is set to “Yes”, the admission will not change and it is thus possible to use the “UniqueID” to compare two admissions for equality.

The admission optionally has an attribute, “dataHasBeenReduced”, that indicates whether data has been reduced by ISCUS, and that all measurements for the admission are not present in this presentation of the admission.



The patients contain a unique id and optionally a first and last name.



The measurements contain a timestamp, a concentration, attempt number, if the point is hidden, optionally the vial identification number and optionally a status.

6.5.2. **XML Schema**

```
<?xml version="1.0" encoding="UTF-8"?>
<!-- edited by Andreas Broman (M Dialysis AB) -->
<xs:schema targetNamespace="http://www.mdialysis.com/XMLSchema/2012/PatientData.xsd"
xmlns:xs="http://www.w3.org/2001/XMLSchema" xmlns="http://www.mdialysis.com/XMLSchema/2012/PatientData.xsd"
elementFormDefault="qualified" attributeFormDefault="unqualified">
    <xs:element name="Iscus">
        <xs:annotation>
            <xs:documentation>Root element</xs:documentation>
        </xs:annotation>
        <xs:complexType>
            <xs:sequence>
                <xs:element name="Admission" maxOccurs="unbounded">
                    <xs:annotation>
                        <xs:documentation>A period of usage of the machine.
When the final attribute is Yes, no more points will ever be added to this admission
When the dataHasBeenReduced attribute is Yes, all points are present in this representation of the admission (that is no
points have been removed by automatic data reduction)</xs:documentation>
                    </xs:annotation>
                    <xs:complexType>
                        <xs:sequence>
                            <xs:element name="UniqueID">
                                <xs:annotation>
                                    <xs:documentation>A global unique identifier (GUID). This shall be unique for an
admission</xs:documentation>
                                </xs:annotation>
                                <xs:simpleType>
                                    <xs:restriction base="xs:string">
                                        <xs:pattern value="[0-9a-f]{8}-[0-9a-f]{4}-[0-9a-f]{4}-[0-9a-f]{4}-[0-9a-f]{12}"/>
```

```
            </xs:restriction>
        </xs:simpleType>
    </xs:element>
    <xs:element name="Machine">
        <xs:annotation>
            <xs:documentation>The serial of the machine</xs:documentation>
        </xs:annotation>
        <xs:simpleType>
            <xs:restriction base="xs:string">
                <xs:maxLength value="50"/>
            </xs:restriction>
        </xs:simpleType>
    </xs:element>
    <xs:element name="AdmissionDate" type="xs:dateTime">
        <xs:annotation>
            <xs:documentation>The admission start date</xs:documentation>
        </xs:annotation>
    </xs:element>
    <xs:element name="AdmissionEndDate" type="xs:dateTime" minOccurs="0">
        <xs:annotation>
            <xs:documentation>The admission end date</xs:documentation>
        </xs:annotation>
    </xs:element>
    <xs:element name="AdmissionNote">
        <xs:annotation>
            <xs:documentation>A note about the admission</xs:documentation>
        </xs:annotation>
        <xs:simpleType>
            <xs:restriction base="xs:string">
                <xs:maxLength value="50"/>
            </xs:restriction>
        </xs:simpleType>
    </xs:element>
    <xs:element name="Patient" minOccurs="0">
        <xs:annotation>
            <xs:documentation>The patient monitor during the admission</xs:documentation>
        </xs:annotation>
        <xs:complexType>
            <xs:sequence>
                <xs:element name="PatientID">
                    <xs:annotation>
                        <xs:documentation>A unique identifier for a patient</xs:documentation>
                    </xs:annotation>
                    <xs:simpleType>
                        <xs:restriction base="xs:string">
                            <xs:maxLength value="50"/>
                        </xs:restriction>
                    </xs:simpleType>
                </xs:element>
                <xs:element name="FirstName">
                    <xs:annotation>
                        <xs:documentation>First name for the patient</xs:documentation>
                    </xs:annotation>
                    <xs:simpleType>
                        <xs:restriction base="xs:string">
                            <xs:maxLength value="50"/>
                        </xs:restriction>
                    </xs:simpleType>
                </xs:element>
                <xs:element name="LastName">
                    <xs:annotation>
                        <xs:documentation>Last Name for the patient</xs:documentation>
                    </xs:annotation>
                    <xs:simpleType>
                        <xs:restriction base="xs:string">
                            <xs:maxLength value="50"/>
                        </xs:restriction>
                    </xs:simpleType>
                </xs:element>
            </xs:sequence>
        </xs:complexType>
```

```
                    </xs:element>
                    <xs:element name="Recording" minOccurs="0" maxOccurs="unbounded">
                        <xs:annotation>
                            <xs:documentation>A recording of an analyte during the
admission</xs:documentation>
                        </xs:annotation>
                        <xs:complexType>
                            <xs:sequence>
                                <xs:element name="CatheterLocation">
                                    <xs:annotation>
                                        <xs:documentation>The location for the catheter</xs:documentation>
                                    </xs:annotation>
                                    <xs:simpleType>
                                        <xs:restriction base="xs:string">
                                            <xs:maxLength value="50"/>
                                        </xs:restriction>
                                    </xs:simpleType>
                                </xs:element>
                                <xs:element name="AnalyteCode">
                                    <xs:annotation>
                                        <xs:documentation>The code for the analyte</xs:documentation>
                                    </xs:annotation>
                                    <xs:simpleType>
                                        <xs:restriction base="xs:string">
                                            <xs:enumeration value="Glucose"/>
                                            <xs:enumeration value="Lactate"/>
                                            <xs:enumeration value="Pyruvate"/>
                                            <xs:enumeration value="Glycerol"/>
                                            <xs:enumeration value="Glutamate"/>
                                            <xs:enumeration value="Urea"/>
                                        </xs:restriction>
                                    </xs:simpleType>
                                </xs:element>
                                <xs:element name="Start" type="xs:dateTime">
                                    <xs:annotation>
                                        <xs:documentation>The start time for the recording</xs:documentation>
                                    </xs:annotation>
                                </xs:element>
                                <xs:element name="Measurement" minOccurs="0" maxOccurs="unbounded">
                                    <xs:annotation>
                                        <xs:documentation>A single measurement</xs:documentation>
                                    </xs:annotation>
                                    <xs:complexType>
                                        <xs:sequence>
                                            <xs:element name="TimeStamp" type="xs:dateTime">
                                                <xs:annotation>
                                                    <xs:documentation>The time for the
sample</xs:documentation>
                                                </xs:annotation>
                                            </xs:element>
                                            <xs:element name="Concentration" type="xs:double">
                                                <xs:annotation>
                                                    <xs:documentation>The concentration of a
sample</xs:documentation>
                                                </xs:annotation>
                                            </xs:element>
                                            <xs:element name="AttemptNo" type="xs:integer">
                                                <xs:annotation>
                                                    <xs:documentation>The analysis attempt
no</xs:documentation>
                                                </xs:annotation>
                                            </xs:element>
                                            <xs:element name="Hidden" type="xs:boolean">
                                                <xs:annotation>
                                                    <xs:documentation>If the point is hidden (by the
user)</xs:documentation>
                                                </xs:annotation>
                                            </xs:element>
                                            <xs:element name="VialID" type="xs:string" minOccurs="0"/>
                                            <xs:element name="Status" default="OK" minOccurs="0">
                                                <xs:annotation>
```

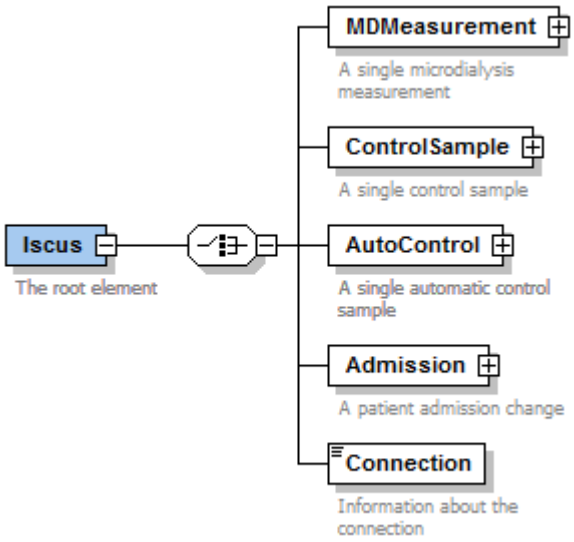
```
                                        <xs:documentation>Extra information about the
measurement</xs:documentation>
                                    </xs:annotation>
                                    <xs:simpleType>
                                        <xs:restriction base="xs:string">
                                            <xs:enumeration value="undefined"/>
                                            <xs:enumeration value="OK"/>
                                            <xs:enumeration value="under detection limit"/>
                                            <xs:enumeration value="above linearity interval"/>
                                            <xs:enumeration value="below reportable range"/>
                                            <xs:enumeration value="above reportable range"/>
                                            <xs:enumeration value="outside reportable range"/>
                                            <xs:enumeration value="failed"/>
                                        </xs:restriction>
                                    </xs:simpleType>
                                </xs:element>
                            </xs:sequence>
                        </xs:complexType>
                    </xs:element>
                </xs:sequence>
                <xs:attribute name="Unit" use="required">
                    <xs:simpleType>
                        <xs:restriction base="xs:string">
                            <xs:enumeration value="mmol/L"/>
                            <xs:enumeration value="mM"/>
                            <xs:enumeration value="µmol/L"/>
                            <xs:enumeration value="µM"/>
                            <xs:enumeration value="g/dL"/>
                            <xs:enumeration value="mg/dL"/>
                            <xs:enumeration value="mg/L"/>
                            <xs:enumeration value="mg/mL"/>
                            <xs:enumeration value="µg/L"/>
                            <xs:enumeration value="µg/mL"/>
                        </xs:restriction>
                    </xs:simpleType>
                </xs:attribute>
            </xs:complexType>
        </xs:element>
    </xs:sequence>
    <xs:attribute name="final" use="optional" default="Yes">
        <xs:simpleType>
            <xs:restriction base="xs:string">
                <xs:enumeration value="Yes"/>
                <xs:enumeration value="No"/>
            </xs:restriction>
        </xs:simpleType>
    </xs:attribute>
    <xs:attribute name="dataHasBeenReduced" use="optional" default="Yes">
        <xs:simpleType>
            <xs:restriction base="xs:string">
                <xs:enumeration value="Yes"/>
                <xs:enumeration value="No"/>
            </xs:restriction>
        </xs:simpleType>
    </xs:attribute>
</xs:complexType>
</xs:element>
</xs:sequence>
<xs:attribute name="version" use="required">
    <xs:simpleType>
        <xs:restriction base="xs:string">
            <xs:enumeration value="1.0"/>
        </xs:restriction>
    </xs:simpleType>
</xs:attribute>
</xs:complexType>
</xs:element>
</xs:schema>
```

6.6. CONTINOUS DATA EXPORT FORMAT

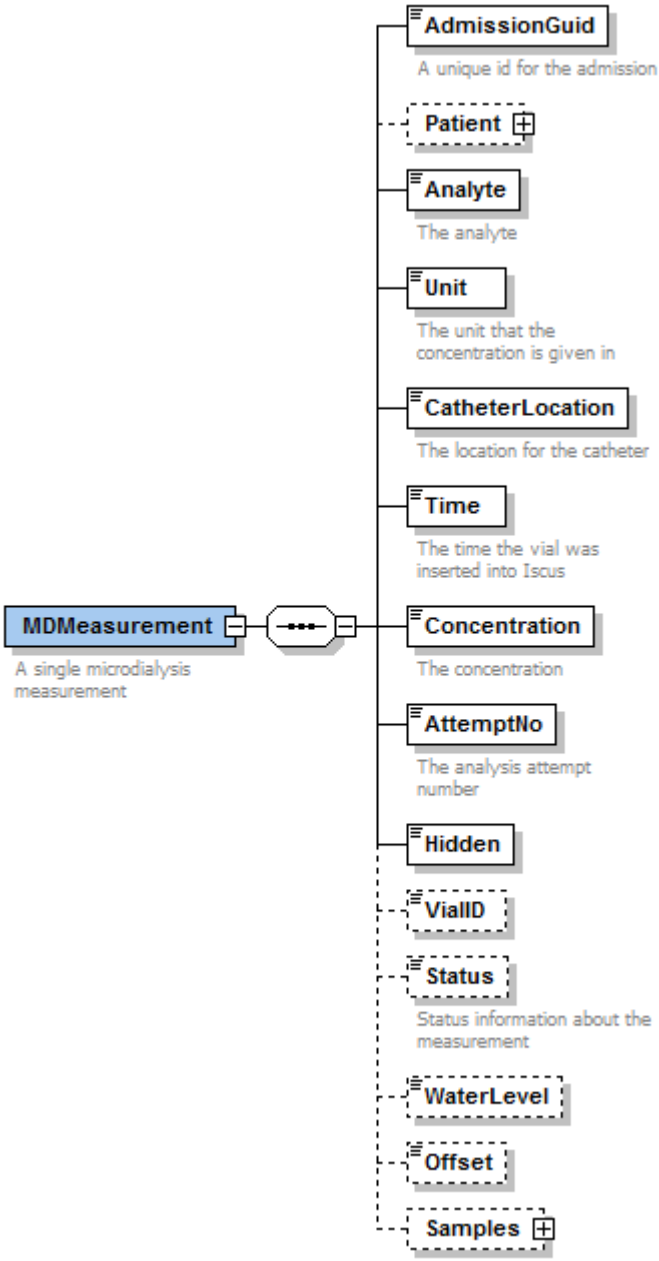
The machine can optionally be setup to send out data over TCP/IP when new values are analysed (see User's manual). One of three data export formats; XML, ASTM and CMAExt can be used. CMAExt is a format defined for the CMA 600 analyser and is kept for compatibility reasons.

6.6.1. XML Description

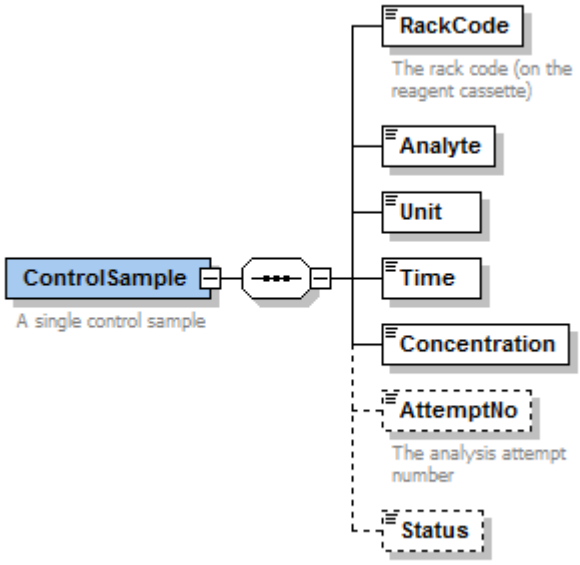
The data is stored in XML. The complete definition is given in 6.5.2 XML Schema. Below is an overview of the format.



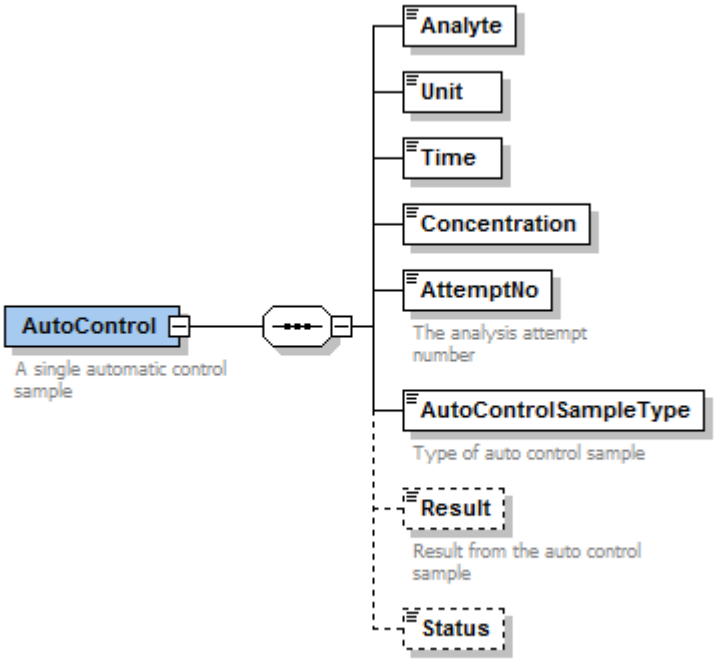
The root element is always Iscus. The root element can contain one of MDMeasurement, ControlSample, AutoControl, Admission or Connection.



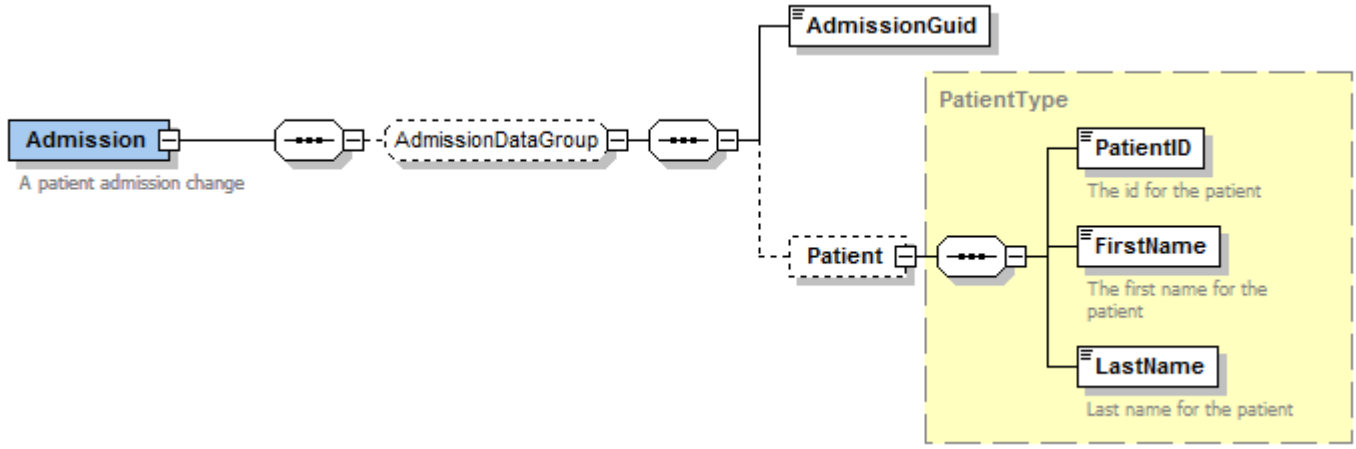
MDMeasurement describes a single Microdialysis measurement and contains information that identifies the patient admission and the patient (if known). This information is sent out when a microdialysis sample has been analyzed.



ControlSample describes a control sample. This information is sent out when a control sample has been analyzed.



AutoControl describes auto-control samples. This information is sent when an automated control sample has been analyzed.



Admission describes the current patient admission. This information is sent out every time the patient admission changes.

6.6.2. **XML Schema**

```
<?xml version="1.0" encoding="UTF-8"?>
<!-- edited by Andreas Broman (CMA/Microdialysis AB) -->
<xs:schema xmlns:xs="http://www.w3.org/2001/XMLSchema" elementFormDefault="qualified">
    <xs:simpleType name="GuidType">
        <xs:annotation>
            <xs:documentation>A unique identifier</xs:documentation>
        </xs:annotation>
        <xs:restriction base="xs:string">
            <xs:pattern value="[0-9a-f]{8}-[0-9a-f]{4}-[0-9a-f]{4}-[0-9a-f]{4}-[0-9a-f]{12}"/>
        </xs:restriction>
    </xs:simpleType>
    <xs:simpleType name="AnalyteType">
        <xs:annotation>
            <xs:documentation>An analyte</xs:documentation>
        </xs:annotation>
        <xs:restriction base="xs:string">
            <xs:enumeration value="Glucose"/>
            <xs:enumeration value="Lactate"/>
            <xs:enumeration value="Pyruvate"/>
            <xs:enumeration value="Glycerol"/>
            <xs:enumeration value="Glutamate"/>
            <xs:enumeration value="Urea"/>
            <xs:enumeration value="LPRatio"/>
            <xs:enumeration value="LGRatio"/>
        </xs:restriction>
    </xs:simpleType>
    <xs:simpleType name="UnitType">
        <xs:annotation>
            <xs:documentation>A unit</xs:documentation>
        </xs:annotation>
        <xs:restriction base="xs:string">
            <xs:enumeration value="mmol/L"/>
            <xs:enumeration value="mM"/>
            <xs:enumeration value="µmol/L"/>
            <xs:enumeration value="µM"/>
            <xs:enumeration value="g/dL"/>
            <xs:enumeration value="mg/dL"/>
            <xs:enumeration value="mg/L"/>
            <xs:enumeration value="mg/mL"/>
            <xs:enumeration value="µg/L"/>
            <xs:enumeration value="µg/mL"/>
            <xs:enumeration value=""/>
        </xs:restriction>
    </xs:simpleType>
    <xs:simpleType name="DataString">
        <xs:annotation>
            <xs:documentation>A string</xs:documentation>
        </xs:annotation>
```

```
        <xs:restriction base="xs:string">
            <xs:maxLength value="50"/>
        </xs:restriction>
    </xs:simpleType>
    <xs:simpleType name="StatusType">
        <xs:annotation>
            <xs:documentation>Information about the measurement such as ok, failed and below detection
limit</xs:documentation>
        </xs:annotation>
        <xs:restriction base="xs:string">
            <xs:enumeration value="undefined"/>
            <xs:enumeration value="OK"/>
            <xs:enumeration value="under detection limit"/>
            <xs:enumeration value="above linearity interval"/>
            <xs:enumeration value="failed"/>
        </xs:restriction>
    </xs:simpleType>
    <xs:complexType name="PatientType">
        <xs:annotation>
            <xs:documentation>A patient</xs:documentation>
        </xs:annotation>
        <xs:sequence>
            <xs:element name="PatientID" type="DataString">
                <xs:annotation>
                    <xs:documentation>The id for the patient</xs:documentation>
                </xs:annotation>
            </xs:element>
            <xs:element name="FirstName" type="DataString">
                <xs:annotation>
                    <xs:documentation>The first name for the patient</xs:documentation>
                </xs:annotation>
            </xs:element>
            <xs:element name="LastName" type="DataString">
                <xs:annotation>
                    <xs:documentation>Last name for the patient</xs:documentation>
                </xs:annotation>
            </xs:element>
        </xs:sequence>
    </xs:complexType>
    <xs:group name="AdmissionDataGroup">
        <xs:sequence>
            <xs:element name="AdmissionGuid" type="GuidType"/>
            <xs:element name="Patient" type="PatientType" minOccurs="0"/>
        </xs:sequence>
    </xs:group>
    <xs:element name="Iscus">
        <xs:annotation>
            <xs:documentation>The root element</xs:documentation>
        </xs:annotation>
        <xs:complexType>
            <xs:choice>
                <xs:element name="MDMeasurement">
                    <xs:annotation>
                        <xs:documentation>A single microdialysis measurement</xs:documentation>
                    </xs:annotation>
                    <xs:complexType>
                        <xs:sequence>
                            <xs:element name="AdmissionGuid" type="GuidType">
                                <xs:annotation>
                                    <xs:documentation>A unique id for the admission</xs:documentation>
                                </xs:annotation>
                            </xs:element>
                            <xs:element name="Patient" type="PatientType" minOccurs="0"/>
                            <xs:element name="Analyte" type="AnalyteType">
                                <xs:annotation>
                                    <xs:documentation>The analyte</xs:documentation>
                                </xs:annotation>
                            </xs:element>
                            <xs:element name="Unit" type="UnitType">
                                <xs:annotation>
                                    <xs:documentation>The unit that the concentration is given in</xs:documentation>
```

```
                    </xs:annotation>
                </xs:element>
                <xs:element name="CatheterLocation">
                    <xs:annotation>
                        <xs:documentation>The location for the catheter</xs:documentation>
                    </xs:annotation>
                    <xs:simpleType>
                        <xs:restriction base="xs:string">
                            <xs:maxLength value="50"/>
                        </xs:restriction>
                    </xs:simpleType>
                </xs:element>
                <xs:element name="Time" type="xs:dateTime">
                    <xs:annotation>
                        <xs:documentation>The time the vial was inserted into Iscus</xs:documentation>
                    </xs:annotation>
                </xs:element>
                <xs:element name="Concentration" type="xs:double">
                    <xs:annotation>
                        <xs:documentation>The concentration</xs:documentation>
                    </xs:annotation>
                </xs:element>
                <xs:element name="AttemptNo" type="xs:int">
                    <xs:annotation>
                        <xs:documentation>The analysis attempt number</xs:documentation>
                    </xs:annotation>
                </xs:element>
                <xs:element name="Hidden" type="xs:boolean"/>
                <xs:element name="VialID" type="xs:string" minOccurs="0"/>
                <xs:element name="Status" type="StatusType" default="OK" minOccurs="0">
                    <xs:annotation>
                        <xs:documentation>Status information about the measurement</xs:documentation>
                    </xs:annotation>
                </xs:element>
                <xs:element name="WaterLevel" type="xs:unsignedInt" minOccurs="0"/>
                <xs:element name="Offset" type="xs:unsignedInt" minOccurs="0"/>
                <xs:element name="Samples" minOccurs="0">
                    <xs:complexType>
                        <xs:sequence>
                            <xs:element name="Sample" type="xs:unsignedInt" maxOccurs="unbounded"/>
                        </xs:sequence>
                    </xs:complexType>
                </xs:element>
            </xs:sequence>
        </xs:complexType>
    </xs:element>
    <xs:element name="ControlSample">
        <xs:annotation>
            <xs:documentation>A single control sample</xs:documentation>
        </xs:annotation>
        <xs:complexType>
            <xs:sequence>
                <xs:element name="RackCode" type="xs:string">
                    <xs:annotation>
                        <xs:documentation>The rack code (on the reagent cassette)</xs:documentation>
                    </xs:annotation>
                </xs:element>
                <xs:element name="Analyte" type="AnalyteType"/>
                <xs:element name="Unit" type="UnitType"/>
                <xs:element name="Time" type="xs:dateTime"/>
                <xs:element name="Concentration" type="xs:double"/>
                <xs:element name="AttemptNo" type="xs:int" minOccurs="0">
                    <xs:annotation>
                        <xs:documentation>The analysis attempt number</xs:documentation>
                    </xs:annotation>
                </xs:element>
                <xs:element name="Status" type="StatusType" default="OK" minOccurs="0"/>
            </xs:sequence>
        </xs:complexType>
    </xs:element>
    <xs:element name="AutoControl">
```

```
            <xs:annotation>
                <xs:documentation>A single automatic control sample</xs:documentation>
            </xs:annotation>
            <xs:complexType>
                <xs:sequence>
                    <xs:element name="Analyte" type="AnalyteType"/>
                    <xs:element name="Unit" type="UnitType"/>
                    <xs:element name="Time" type="xs:dateTime"/>
                    <xs:element name="Concentration" type="xs:double"/>
                    <xs:element name="AttemptNo" type="xs:int">
                        <xs:annotation>
                            <xs:documentation>The analysis attempt number</xs:documentation>
                        </xs:annotation>
                    </xs:element>
                    <xs:element name="AutoControlSampleType">
                        <xs:annotation>
                            <xs:documentation>Type of auto control sample</xs:documentation>
                        </xs:annotation>
                        <xs:simpleType>
                            <xs:restriction base="xs:string">
                                <xs:enumeration value="AutoControlLow"/>
                                <xs:enumeration value="AutoControlMedium"/>
                                <xs:enumeration value="AutoControlHigh"/>
                            </xs:restriction>
                        </xs:simpleType>
                    </xs:element>
                    <xs:element name="Result" minOccurs="0">
                        <xs:annotation>
                            <xs:documentation>Result from the auto control sample</xs:documentation>
                        </xs:annotation>
                        <xs:simpleType>
                            <xs:restriction base="xs:string">
                                <xs:enumeration value="Unknown"/>
                                <xs:enumeration value="OK"/>
                                <xs:enumeration value="TooHigh"/>
                                <xs:enumeration value="TooLow"/>
                            </xs:restriction>
                        </xs:simpleType>
                    </xs:element>
                    <xs:element name="Status" type="StatusType" default="OK" minOccurs="0"/>
                </xs:sequence>
            </xs:complexType>
        </xs:element>
        <xs:element name="Admission">
            <xs:annotation>
                <xs:documentation>A patient admission change</xs:documentation>
            </xs:annotation>
            <xs:complexType>
                <xs:sequence>
                    <xs:group ref="AdmissionDataGroup" minOccurs="0"/>
                </xs:sequence>
            </xs:complexType>
        </xs:element>
        <xs:element name="Connection">
            <xs:annotation>
                <xs:documentation>Information about the connection</xs:documentation>
            </xs:annotation>
            <xs:simpleType>
                <xs:restriction base="xs:string">
                    <xs:enumeration value="Open"/>
                    <xs:enumeration value="Close"/>
                    <xs:enumeration value="Alive"/>
                </xs:restriction>
            </xs:simpleType>
        </xs:element>
    </xs:choice>
    <xs:attribute name="version" use="required">
        <xs:simpleType>
            <xs:restriction base="xs:string">
                <xs:enumeration value="1.1"/>
            </xs:restriction>
```

```
                    </xs:simpleType>
                </xs:attribute>
            </xs:complexType>
        </xs:element>
</xs:schema>
```

.

6.6.3. **CMAExt Description**

6.6.3.1.Communication wrapper

All commands given can be enclosed in a communication wrapper. To define this communication wrapper the StartSequence and EndSequence registry values must be defined. Default a wrapper is defined with the StartSequence 0x0B (FF) and the EndSequence 0x1C0D (FS CR).

6.6.3.2.Protocol details

Any result is output in the following format:

123,”Lastname”,”Surname” ,”PatientID”,”Catheternаme”,”1A”,”Analyte”,”Date Time”,”Value”,”Unit”,Checksum <CRLF>

Where the different items are

- 123 – Total number of points, which is the same as the order number if the result is a direct sample. When it is a batch result, the figure is simply the total number of points (max 3 chars). When it is the LPRatio, it is always 0.
- Lastname - Last name of the patient (max 20 chars)
- Firstname – First name of the patient (max 20 chars)
- PatientID – ID of patient.
- Catheternаme - Name for catheter (max 15 chars)
- 1A - Patient/Catheter location, can be 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B or 3C (2 chars)
- Analyte - Analyte name = “Glucose”, “Lactate”, “Pyruvate”, “Glutamate”, “Glycerol” or “Urea”. Can also be “LPRatio” or “LGRatio” with the unit “”. N.B. always in English.
- Date Time- Date and Time for the result in the format
  ‘YYYY-MM-DD hh:mm:ss’, e.g. 1998-06-09 17:00:00 (19 chars)
  N.B. this is the format regardless of the Windows 95 setting.
- Value - The numerical value of the result.
- Unit - The unit used for displaying the result (max 10 chars)
- Checksum - A byte checksum calculated on all characters in the message from the beginning to the character immediately preceding the Checksum byte (max 3 chars)

6.6.3.3.Change wrapper

By using the tool CMAExtWrapper.Exe, it is possible to create the Iscus files necessary to re-define the communication wrapper. Create the files on a SD-card or USB memory stick and have it inserted in the Iscus while starting the Iscus. The new definition will be copied and used for the Iscus.

6.6.3.4.Communication examples

Without wrapper

169,"Hansson","Hans","123456-7890","CNS le worse","1A","Glucose","2005-07-28 00:04:00","1,763395457","mM",101
170,"Hansson","Hans","123456-7890","CNS le worse","1A","Glycerol","2005-07-28 00:04:00","203,7714531","µM",3
171,"Hansson","Hans","123456-7890","CNS le worse","1A","Lactate","2005-07-28 00:04:00","14,27827142","mM",62
172,"Hansson","Hans","123456-7890","CNS le worse","1A","Pyruvate","2005-07-28 00:04:00","377,8976346","µM",63
0, ,"Hansson","Hans","123456-7890","CNS le worse","1A","Pyruvate","2005-07-28 00:04:00","377,8976346","µM",63
173,"Hansson","Hans","123456-7890","CNS ri better","1B","Glucose","2005-07-28 00:11:00","6,124465861","mM",184
174,"Hansson","Hans","123456-7890","CNS ri better","1B","Glycerol","2005-07-28 00:11:00","449,1599853","µM",126
175,"Hansson","Hans","123456-7890","CNS ri better","1B","Lactate","2005-07-28 00:11:00","6,402524583","mM",162
176,"Hansson","Hans","123456-7890","CNS ri better","1B","Pyruvate","2005-07-28 00:11:00","373,7653962","µM",153
177,"Hansson","Hans","123456-7890","Sc","1C","Glucose","2005-07-28 00:11:00","3,21628266","mM",183
178,"Hansson","Hans","123456-7890","Sc","1C","Glycerol","2005-07-28 00:11:00","188,377917","µM",126
179,"Hansson","Hans","123456-7890","Sc","1C","Lactate","2005-07-28 00:11:00","2,452910412","mM",207
180,"Hansson","Hans","123456-7890","Sc","1C","Pyruvate","2005-07-28 00:11:00","127,9274791","µM",196

With wrapper

[0B]202,"Cortez","Ricardo","364356-4","Blk R-Periventr","1A","Glucose","2006-05-16 07:01:00","0.174","mM",160[1C0D]
[0B]170,"Cortez","Ricardo","364356-4","Blk R-Periventr","1A","Lactate","2006-05-16 07:01:00","1.135","mM",142[1C0D]
[0B]211,"Cortez","Ricardo","364356-4","Blk R-Periventr","1A","Pyruvate","2006-05-16 07:01:00","36.705","uM",111[1C0D]
[0B]0,"Cortez","Ricardo","364356-4","Blk R-Periventr","1A","LPRatio","2006-05-16 07:01:00","30.921","",126[1C0D]

6.6.4. **ASTM Description**

6.6.4.1.Supported ASTM messages

Data is sent without handshaking at every completed microdialysis vial (see communication example below).

6.6.4.1.1. Message Header Record:

Field nr	Field name	Description
1	Record type ID. Character 'H'.	Defines that it is a Message Header Record. Immediately followed by the field separator (normally '|').
2	Delimiter Definition. Normally \^&|	Defines the characters to be used as separators for the rest of the message. The last character is the field separator which also serves as such in this part of the message.
5	Sender Name or ID	Contains two strings; analyzer and system ID, e.g. ISCUSflex^T12345-01
13	Version #	Contains version nr (1.1)
14	Date and Time of Message	e.g. 20151018081500

6.6.4.1.2. Patient Information Record

Field nr	Field name	Description
1	Record type ID. Character 'P'.	Defines that it is a Patient Information Record. Immediately followed by the field separator (normally '|').
4	Patient ID	Contains the patient ID as entered in the patient ID field in ISCUS^{flex}. Empty if QC data.
6	Patient name	Contains the patient first name and last name as entered in the patient dialog. Empty if QC data. <Last name>^<First name>

6.6.4.1.3. Test Order Record

Sequence	Field name	Description
1	Record type ID. Character 'O'.	Defines that it is a Test Order Record. Immediately followed by the field separator (normally '|').
4	Instrument Specimen ID	The text 'Sample #', the actual nr (vial ID) and the attempt nr, e.g. Sample #^A01^1
		If calibration 'CAL #', 'Normal' or 'Low', 1, e.g. CAL #^Normal^1
		If auto control 'QC #', 'Elevated' or 'Low', 1, e.g. QC #^Elevated^1
8	Date/Time of observation	Date and time of measurement of the parameter in ISO format (YYYYMMDDHHMMSS).
11	Operator identity	The operator ID as defined in the CLIA module. If CLIA is not used the text UNKNOWN is sent. If Calibration or auto control the text ISCUSflex.
16	Specimen Descriptor	**Sample:** The text MD Sample and the catheter name, e.g. MD Sample^CNS Worse
		Calibration: The calibrator name and the lot numbers for the reagent and the calibrator, e.g. Calibrator A^T25145^T24905 (Calibrator A^*Reagent Lot*^*Calibrator Lot*)
		Auto Control: The text AutoControl and the control lot, e.g. AutoControl^T25055

6.6.4.1.4. Result Record

Sequence	Field name	Description
1	Record type ID. Character 'R'.	Defines that it is a Result Record. Immediately followed by the field separator (normally '|').
3	Parameter Name	Parameter name as the fourth parameter, e.g. ^^^Glucose^
4	Data Measurement value	Result, e.g. 5.55^
5	Units	Contains unit information. Note: If calibration the unit is mAbs.
9	Observation result status	**Sample:** OK – Value is OK and within the analysers linearity interval DL – Value is under the detection limit. The reported value is the value actually measured LL – Value is above the linearity limit and the real value is probably higher that reported value due to the non-linearity above the linearity limit. Less than – Ratio status when denominator is above linearity limit. above reportable range – Value is above defined reportable range. below reportable range – Value is below defined reportable range. outside reportable range – Value is outside reportable range.
		Calibration: OK – Calibration is valid and used by the analyser CalibInjectionsDeviateTooMuch – The two calibrator injections in a calibration deviate more than 5%. The calibration is NOT used. NewCalibDeviatesTooMuch – The new calibration result deviates too much from previous calibrations and is NOT used. <*Other text*> – Calibration failed and is NOT used by the analyser.
		Auto control: OK – Auto control is ok and within limits Too low – Value is below acceptable range Too high – Value is above acceptable range <*Other text*> – Auto control is not OK.
12	Date/Time of observation	Date and time of measurement of the parameter in ISO format (YYYYMMDDHHMMSS).

ASTM communication examples:

Sample

```
H|\^~&|||ISCUSflex^T16558-01||||||||1.1|20151002072454<CR>
P|||5708120133||Hedberg^Magnus<CR>
O|||Sample #^MHTest^1||||20151002072306|||1|||||MD Sample^I<CR>
R||^^^Glucose^|26.0|mg/dL||||OK|||20151002072306<CR>
R||^^^Lactate^|0.837|mmol/L||||OK|||20151002072306<CR>
R||^^^LGRatio^|0.580|||||OK|||20151002072306<CR>
R||^^^Pyruvate^|43.8|µmol/L||||OK|||20151002072306<CR>
R||^^^LPRatio^|19.1|||||OK|||20151002072306<CR>
```

Calibration

```
H|\^~&|||ISCUSflex^T16558-01||||||||1.1|20151001141827<CR>
P|<CR>
O|||CAL #^Normal^1||||20151001140524|||ISCUSflex|||||Calibrator A ^T24824^T24905<CR>
R||^^^Lactate^|0.783|mAbs||||OK|||20151001141827<CR>
```

Auto Control

```
H|\^~&|||ISCUSflex^T16558-01||||||||1.1|20151001144001<CR>
P|<CR>
O|||QC #^Elevated^1||||20151001141829||||||||AutoControl^T25055<CR>
R||^^^Lactate^|10.7|mmol/L||||OK|||20151001144001<CR>
```

7. MAINTENANCE

7.1. CANNULA

7.1.1. Replacing or checking the sampling cannula

1. Press the "*Change Cannula*" button.
2. Follow the instructions on screen. Check that the cannula is straight.

7.2. SYSTEM SHUT DOWN

To shut down ISCUS*flex*, use the Shut-down procedure. It is advisable to empty the Rinsing Fluid/Waste bottles and remove the Microvials and Reagent Vials/Cassette. When you follow the procedure, the system will automatically lock the sample cannula, which prevents instrument damages.

7.3. SYSTEM RESTART

1. Press On/Off button
2. Fill the Rinsing Fluid bottle.
3. Prepare new reagents.
4. Register the separate reagents or the reagent cassette (by manually entering the unique code written on the label on the cassette) in the system.

8. EMC - ELECTROMAGNETIC COMPATIBILITY

The use of ACCESSORIES, transducers and cables other than those specified, with the exception of transducers and cables sold by M Dialysis AB as replacement parts for internal components, may result in increased EMISSIONS or decreased IMMUNITY of ISCUS*flex*.

ISCUS*flex* should not be used adjacent to or stacked with other equipment. If adjacent or stacked use is necessary, ISCUS*flex* should be observed to verify normal operation in the configuration in which it will be used.

List of cables:

Network cable – Max length 5 meters

Power cable – Max length 1.8 meters

Guidance and manufacturer's declaration – electromagnetic emissions		
ISCUS*flex* is intended for use in the electromagnetic environment specified below. The customer or the user of ISCUS*flex* should assure that it is used in such an environment.		
Emissions test	Compliance	Electromagnetic environment – guidance
RF emissions CISPR 11	Group 1	ISCUS*flex* uses RF energy only for its internal function. Therefore, its RF emissions are very low and are not likely to cause any interference in nearby electronic equipment.
RF emissions CISPR 11	Class B	ISCUS*flex* is suitable for use in all establishments, including domestic establishments and those directly connected to the public low-voltage power supply network that supplies buildings used for domestic purposes.
Harmonic emissions IEC 61000-3-2	Class A	
Voltage fluctuations/ flicker emissions IEC 61000-3-3	Complies	

Guidance and manufacturer's declaration – electromagnetic immunity			
ISCUS*flex* is intended for use in the electromagnetic environment specified below. The customer or the user of ISCUS*flex* should assure that it is used in such an environment.			
Immunity test	IEC 60601 test level	Compliance level	Electromagnetic environment ─ Guidance
Electrostatic discharge (ESD) IEC 61000-4-2	±6 kV contact ±8 kV air	±6 kV contact ±8 kV air	Floors should be wood, concrete or ceramic tile. If floors are covered with synthetic material, the relative humidity should be at least 30 %.
Electrical fast transient/burst IEC 61000-4-4	±2 kV for power supply lines ±1 kV for input/output lines	±2 kV for power supply lines ±1 kV for input/output lines	Mains power quality should be that of a typical commercial or hospital environment.
Surge IEC 61000-4-5	±1 kV differential mode ±2 kV common mode	±1 kV differential mode ±2 kV common mode	Mains power quality should be that of a typical commercial or hospital environment.
Voltage dips, short interruptions and voltage variations on power supply input lines IEC 61000-4-11	<5 % U_T (>95 % dip in U_T) for 0,5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles <5 % U_T (>95 % dip in U_T) for 5 sec	<5 % U_T (>95 % dip in U_T) for 0,5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles <5 % U_T (>95 % dip in U_T) for 5 sec	Mains power quality should be that of a typical commercial or hospital environment. If the user of ISCUS*flex* requires continued operation during power mains interruptions, it is recommended that ISCUS*flex* be powered from an uninterruptible power supply or a battery.
Power frequency (50/60 Hz) magnetic field IEC 61000-4-8	3 A/m	3 A/m	Power frequency magnetic fields should be at levels characteristic of a typical location in a typical commercial or hospital environment.
NOTE U_T is the a.c. mains voltage prior to application of the test level.			

Guidance and manufacturer's declaration – electromagnetic immunity

ISCUS^{flex} is intended for use in the electromagnetic environment specified below. The customer or the user of ISCUS^{flex} should assure that it is used in such an environment.

Immunity test	IEC 60601 test level	Compliance level	Electromagnetic environment ─ guidance
			Portable and mobile RF communications equipment should be used no closer to any part of ISCUS^{flex}, including cables, than the recommended separation distance calculated from the equation applicable to the frequency of the transmitter. Recommended separation distance
Conducted RF IEC 61000-4-6	3 Vrms 150 kHz to 80 MHz	3 Vrms	$d = 0{,}12\sqrt{P}$
Radiated RF IEC 61000-4-3	3 V/m 80 MHz to 2,5 GHz	3 V/m	$d = 0{,}12\sqrt{P}$ 80 MHz to 800 MHz $d = 0{,}23\sqrt{P}$ 800 MHz to 2,5 GHz where *P* is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer and *d* is the recommended separation distance in meters (m). Field strengths from fixed RF transmitters, as determined by an electromagnetic site survey, [a] should be less than the compliance level in each frequency range. [b] Interference may occur in the vicinity of equipment marked with the following symbol: 

NOTE 1 At 80 MHz and 800 MHz, the higher frequency range applies.

NOTE 2 These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.

[a] Field strengths from fixed transmitters, such as base stations for radio (cellular/cordless) telephones and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast cannot be predicted theoretically with accuracy. To assess the electromagnetic environment due to fixed RF transmitters, an electromagnetic site survey should be considered. If the measured field strength in the location in which ISCUS^{flex} is used exceeds the applicable RF compliance level above, ISCUS^{flex} should be observed to verify normal operation. If abnormal performance is observed, additional measures may be necessary, such as reorienting or relocating ISCUS^{flex}.

[b] Over the frequency range 150 kHz to 80 MHz, field strengths should be less than 3 V/m.

Recommended separation distances between portable and mobile RF communications equipment and ISCUS*flex*

ISCUS*flex* is intended for use in an electromagnetic environment in which radiated RF disturbances are controlled. The customer or the user of ISCUS*flex* can help prevent electromagnetic interference by maintaining a minimum distance between portable and mobile RF communications equipment (transmitters) and ISCUS*flex* as recommended below, according to the maximum output power of the communications equipment.

Rated maximum output power of transmitter W	Separation distance according to frequency of transmitter m		
	150 kHz to 80 MHz $d = 0,12\sqrt{P}$	80 MHz to 800 MHz $d = 0,12\sqrt{P}$	800 MHz to 2,5 GHz $d = 0,23\sqrt{P}$
0,01	0,12	0,12	0,23
0,1	0,37	0,37	0,74
1	1,2	1,2	2,3
10	3,7	3,7	7,4
100	12	12	23

For transmitters rated at a maximum output power not listed above, the recommended separation distance *d* in meters (m) can be estimated using the equation applicable to the frequency of the transmitter, where *P* is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer.

NOTE 1 At 80 MHz and 800 MHz, the separation distance for the higher frequency range applies.

NOTE 2 These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.