



**Grant Agreement no. 287596**

**d-LIVER**

**ICT-enabled, cellular artificial liver system incorporating personalized patient management and support**

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**OBJECTIVE: ICT-2011.5.1**

***D3.3: Improved sensor integration onto the platform for extended prototype platform***

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## Document History

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7 <sup>th</sup> May 2014	v0.2	Compilation of partner contributions
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## Table of Contents

<b>1. Executive Summary</b> .....	<b>4</b>
<b>2. Introduction</b> .....	<b>5</b>
<b>3. Biomolecule platform</b> .....	<b>6</b>
3.1. Description of the final biomolecule platform .....	6
<b>4. ISE platform</b> .....	<b>6</b>
4.1. Description of the platform .....	6
4.2. Fluidic sequence .....	8
4.3. Final characterisation and performance of creatinine sensor .....	8
<b>5. Clotting time platform</b> .....	<b>9</b>
5.1. Description of the final clotting time platform.....	9
5.2. Fluidic sequence .....	10
5.3. Final characterisation and performance.....	10
<b>6. Electronic board and signal processing</b> .....	<b>11</b>
6.1. BBI cartridge readout electronics .....	11
<b>7. Conclusions</b> .....	<b>13</b>

# 1. Executive Summary

Deliverable D3.3 is the third of the four deliverables due for WP3 in the d-LIVER project.

It describes the three sensor platforms in their final configuration.

With regard to the results and characterisation studies carried out on the different sensors, decisions have been made by the consortium since the production of deliverable D3.2. Overall, 6 sensors will be finally integrated within the fluidic cartridge: albumin, bilirubin, creatinine, sodium, potassium and blood clotting time. They are distributed in three independent platforms:

## 1. Biomolecule platform for albumin and bilirubin sensors

Albumin is detected by impedance measurements using a 3 electrode system and bilirubin is measured by amperometry also using a 3 electrode system. This platform will be assembled in the fluidic cartridge using double-sided adhesive tape.

## 2. ISE platform for sodium, potassium and creatinine sensors

Potassium and sodium ions are detected directly by potentiometry and creatinine is measured by indirect potentiometry. This platform will be assembled to the fluidic cartridge using double-sided adhesive tape.

## 3. Clotting time platform for clotting time sensor

Clotting time is measured optically by detecting the speckle figure due to the stop in motion of the red blood cells during coagulation. This platform is part of the fluidic channels in the fluidic cartridge.

The electronic readout has been specified for all the sensors and is presented in this document.

## 2. Introduction

This document is deliverable D3.3 of the d-LIVER project. It presents the extended prototype platforms. The extended prototype is finally composed of three platforms: the first one is for biomolecule sensors (albumin and bilirubin) developed by URV, the second for ion sensors (sodium and potassium) developed by CEA-LETI and the third for clotting time, also developed by CEA-LETI. This platform is part of the fluidic cartridge itself. The major evolution concerning these platforms compared with those described in deliverable D3.2 is the substitution of the ammonium sensor by the creatinine sensor developed by URV. Indeed, the ammonium sensor will not be integrated into the cartridge at this stage and the creatinine sensor is based on indirect potentiometric detection. Consequently the creatinine sensor will be implemented within the ion sensor platform.

The platforms have been developed in close collaboration between URV, CEA-LETI, SINTEF - in charge of the development of the electronic readout - and FhG-ICT-IMM - in charge of the development of the fluidic cartridge in order to integrate in the best way all the sensors in the fluidic cartridge. The platforms presented in this document are in their final integrateable versions.

In this report, the detection principles chosen for each sensor will be described and then the platforms and the associated fluidic sequence reported. Some final results on characterisation will also be presented where appropriate.

The clinical significance has already been reported in deliverable D3.2 and details on integration of the different platforms within the cartridge are presented in deliverable D4.5.

### 3. Biomolecule platform

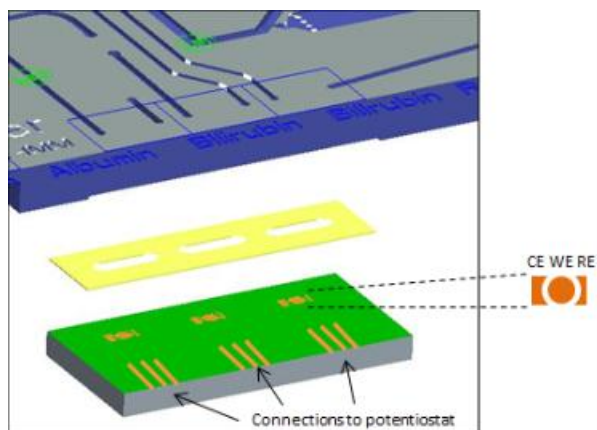
#### 3.1. Description of the final biomolecule platform

Albumin and bilirubin will be detected electrochemically in diluted serum.

Albumin detection is based on changes in impedance measurements which are related to albumin concentration.

In the case of bilirubin, the sensor is based on amperometric measurement.

Both sensors are three-electrode systems on PCB with working, reference and counter electrodes made of gold and interfaced with the cartridge via double-sided adhesive tape (Figure 1).



*Figure 1: Biomolecule sensors integration consisting of a PCB (in green) with gold electrodes (three electrode cells) and an adhesive channel foil (in yellow), which connects the PCB to the cartridge to complete the microfluidic path.*

Fluidic sequences for both the albumin and bilirubin sensors in the biochemistry cartridge have been devised and are undergoing testing.

### 4. ISE platform

#### 4.1. Description of the platform

Sodium, potassium and creatinine will be detected by integrated Ion Selective Electrodes (ISEs) in diluted serum. The detection principle is described in more detail in deliverables D3.1 and D3.2.

Sodium and potassium are detected directly by the ISE based on the interaction between the respective ions and specific PVC membranes. Creatinine will be detected by indirect potentiometry.

The 3 sensors will have a common integrated reference electrode. The platform will be thus constituted of 4 electrodes.

The platform has already been described in deliverable D3.2. The major change for the last version is the positioning of the 4 electrodes to save space on the cartridge.

It is composed of 3 parts:

- A PCB with 4 gold electrodes and pads for connector (and a connector)
- A module with 4 chambers (one for each electrode) with one smaller for reference electrode
- A fluidic cover containing the fluidic chamber in which the sample will be introduced

A 3D view and a picture of the platform in its final shape are shown in Figure 2.

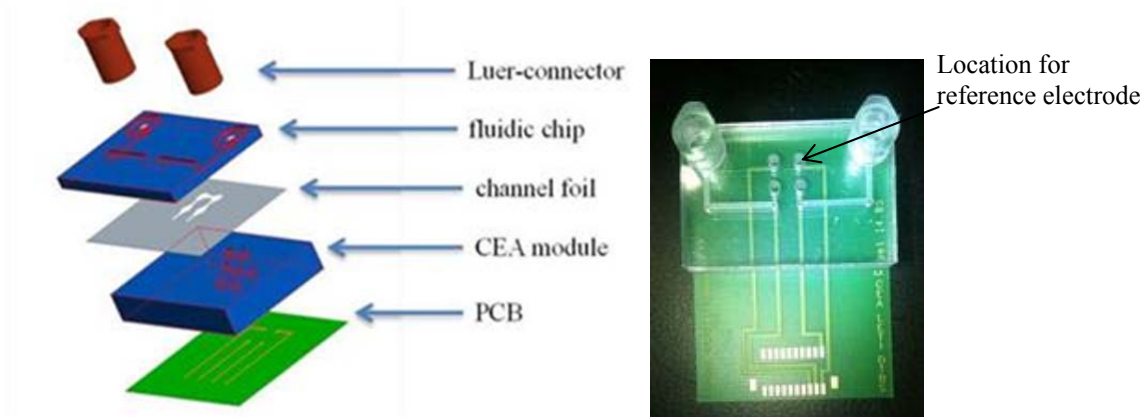


Figure 2: 3D view and picture of the final platform.

The PCB receives gold electrodes and connector. Gold electrodes have a diameter of 500  $\mu\text{m}$ . The 4 electrodes are covered with a silver pellet and then chlorinated.

The module, manufactured by FhG-ICT-IMM, could be made in either PVC or polycarbonate (PC). The platform will be interfaced with the fluidic cartridge via a double sided adhesive tape (Figure 3).

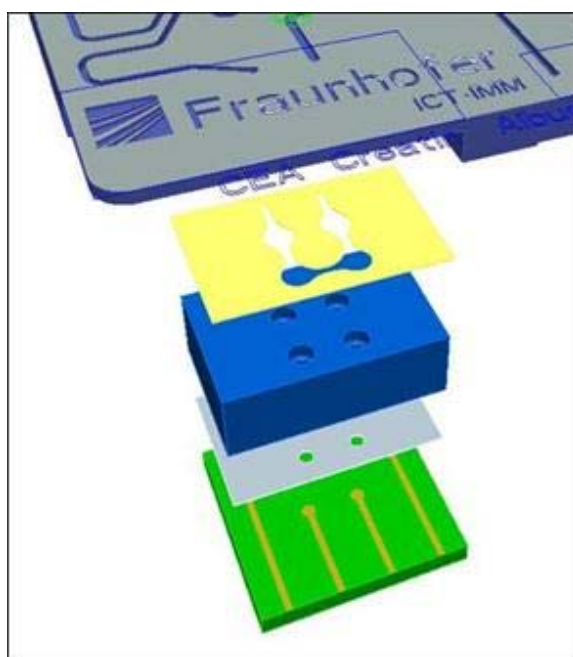
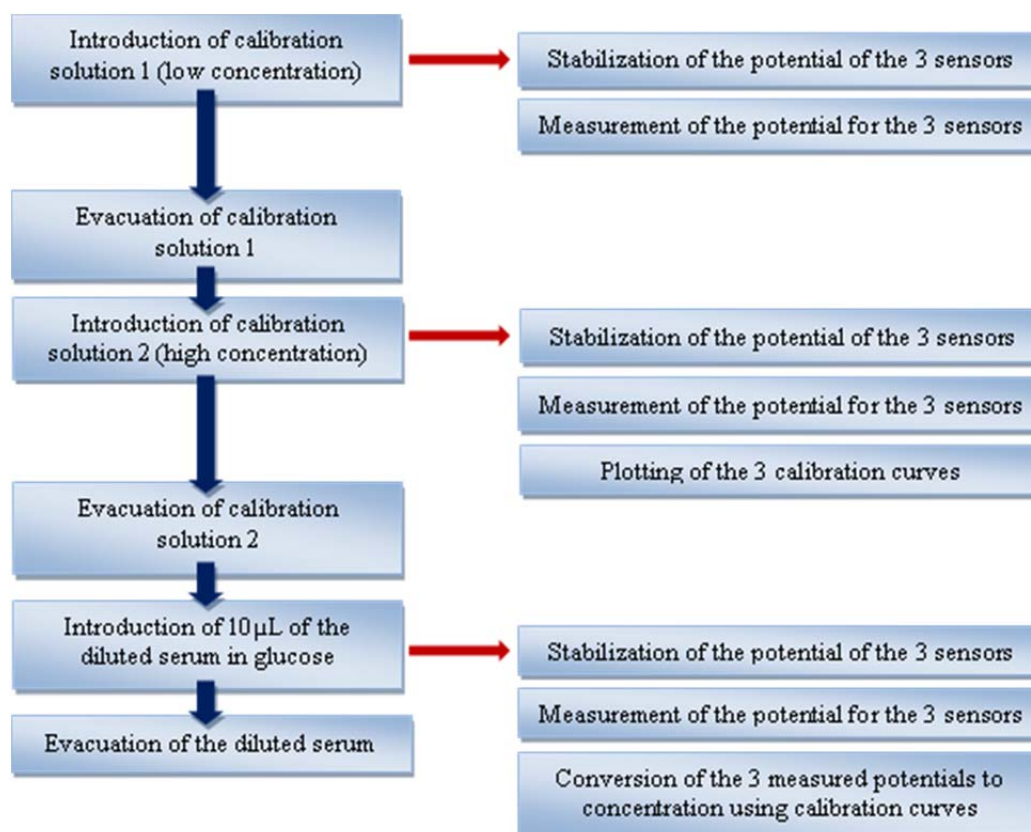


Figure 3: ISE sensors integration consisting of a PCB (in green) with gold electrodes, a first adhesive foil (in grey) to assemble a module (in blue) and a final adhesive channel foil (in yellow), which connects the whole assembly to the cartridge to complete the microfluidic path.

## 4.2. Fluidic sequence

The fluidic sequence for the ISE measurements is depicted below:



## 4.3. Final characterisation and performance of creatinine sensor

The sensors have been first characterised in a cone-shaped ISE format and then in a PCB format.

### Sequential use of sensors

The sensors were tested for sequential use to simulate the measuring conditions in the cartridge. The sequence was as follows: PBS, 100 µM creatinine, PBS, 1000 µM creatinine, PBS and 100 µM creatinine. The reverse experiment was also performed (high-low-high concentration). As can be seen in Figure 4, the sensor responded to all these changes with good signal recovery ( $E_{\text{creatinine}} - E_{\text{PB}}$ ) of the initial concentration. The sensors could be used at least three times. The required stabilisation time was 10 min after addition.

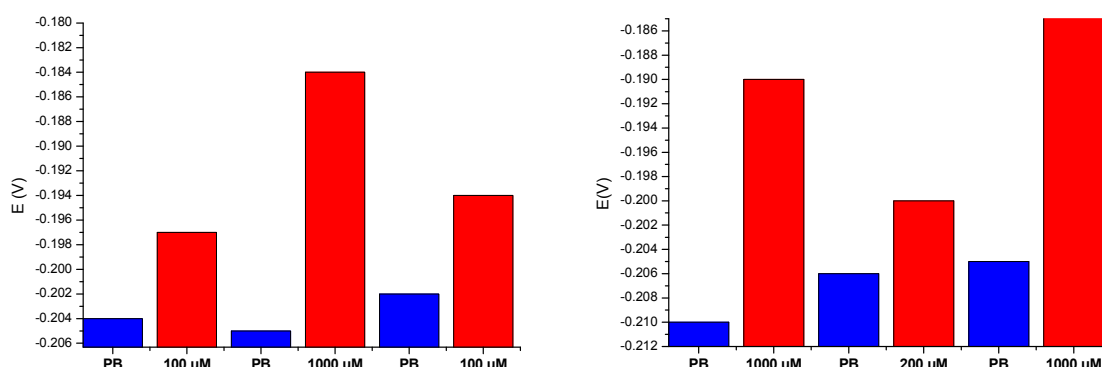


Figure 4: Potential changes for the creatinine sensor under sequential use in cone format.



This study has also been carried out on the PCB format sensor with the highest creatinine concentrations. Figure 5 illustrates the results:

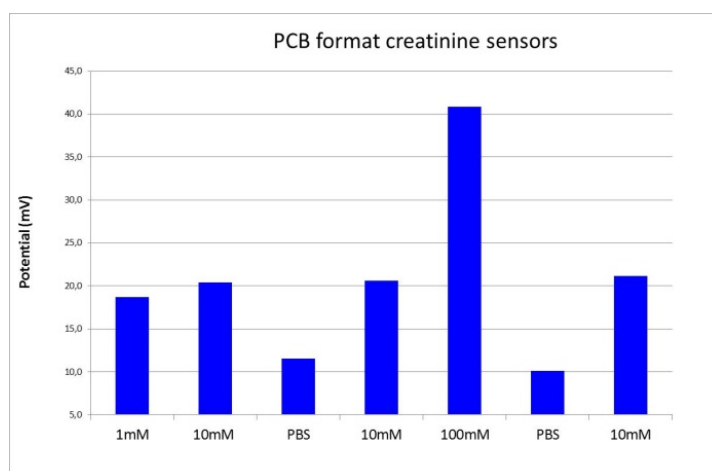


Figure 5: Potential changes for the creatinine sensor under sequential use in PCB format.

The reproducibility has been evaluated as +/- 8% off chip.

## 5. Clotting time platform

### 5.1. Description of the final clotting time platform

The decision was made to integrate the optical blood clotting sensor within the d-LIVER blood biochemistry instrument as a low-risk option. Results on electrical blood clotting sensor are promising but this approach would require significant further development.

The optical detection principle is based on imaging of red blood cell movement stopping when blood clots. It is described in more detail in deliverables D3.1 and D3.2. The sensor has already been described in D3.2. The major change for the last version of the sensor is the presence of a VCSEL (vertical-cavity surface-emitting laser) instead of a laser diode.

The sample has to be closed from the CMOS sensor and the VCSEL could be placed at about 15mm from the sample. Therefore using a VCSEL allows reducing distance between sample and light source in order to save place for the integration within the instrument.

The reference of the CMOS sensor used is MT9M001C12STM (Figure 6).

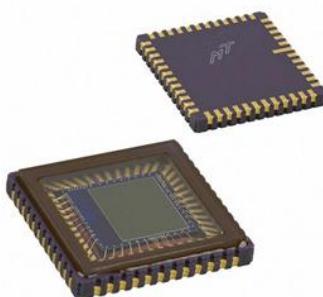


Figure 6: Picture of the CMOS sensor.

The CMOS and VCSEL will part of the instrument and the clotting chamber will be part of the fluidic cartridge. A picture of the configuration of the sensor is shown in Figure 7.

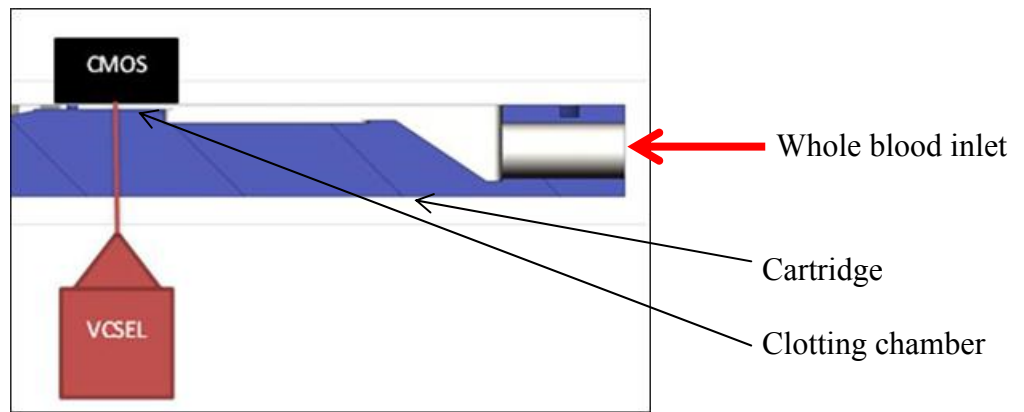
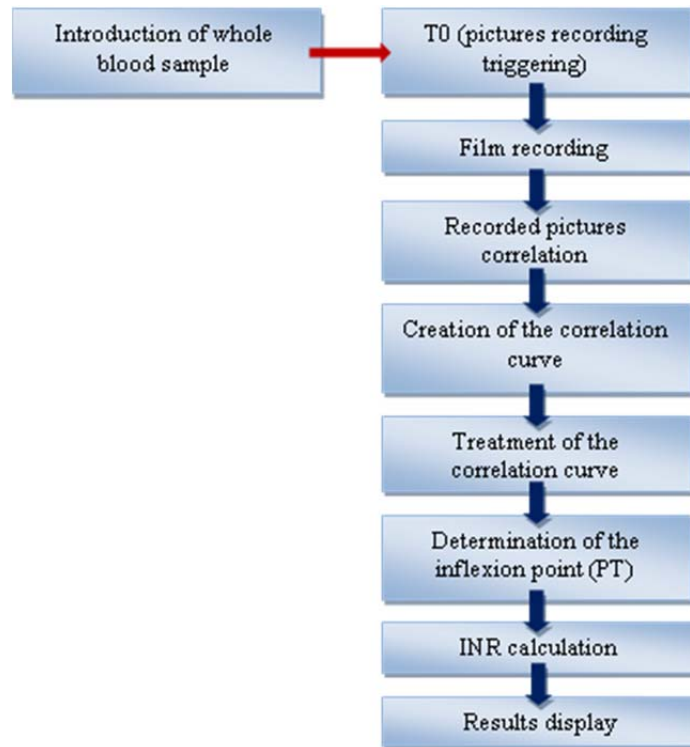


Figure 7: Clotting sensor integration consisting of a light source (in red), a light detector (in black) and a clotting chamber which is part of the cartridge.

## 5.2. Fluidic sequence

The complete measurement sequence has been defined as follows:



## 5.3. Final characterisation and performance

The issue here is to have a clotting chamber with good optical quality. Indeed the parts of the cartridge which will be crossed by the light need to be minimally diffusive. That means surface has to be smooth. If the surface is too diffusive the signal will be lost within diffusive noise and the accuracy of the measurement will deteriorate.

Experiments on cartridges showed that the surface condition is much improved when using injection moulded cartridges compared with micro-machined ones.

In the final version of the cartridge surface treatment after injection moulding will further improve the optical quality.

## 6. Electronic board and signal processing

SINTEF is responsible for the development of the electrical readout circuitry from the BBI cartridges. It is also likely that parts of this technology will be employed to make ammonia measurements linked with the BAL reactors.

Specific developments concerning the ammonium sensor for the bioreactor are components of WP5 and are therefore reported in the associated deliverables.

### 6.1. BBI cartridge readout electronics

A decision was made to design one PCB for the readout electronics. This decision was made after discovering the possibility of placing the readout electronics PCB vertically. This allowed larger board dimensions and made it possible to combine all electronics in one single PCB while still being able to place the sensitive Analog front-end in close proximity to the sensors. For this to work a new connector type was needed, and a solution was found in a low pitch right angle spring loaded connector from Preci-dip (Figure 8).

8PC-28-0014-01-828

Right angle SLC connector, solder tail, single row

Standard number of contacts: 14

Please consult for other pin number or partially equipped connectors

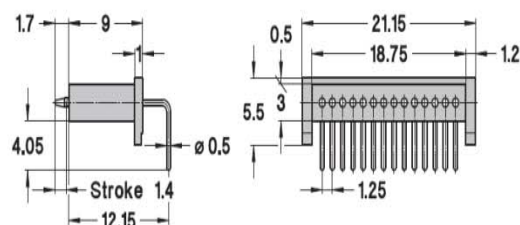


Figure 8 Preci-dip connector.

The readout PCB will contain:

- Analog front-end which will be placed in close proximity to the sensors and which will output low impedance value (buffered) signals from the sensitive sensor cells
- Readout electronics which will carry out the digital signal processing (DSP).

The sensor cells have very high impedance values and as a consequence they are very vulnerable to noise and discharging (i.e. leakage currents). Active shielding will therefore be implemented as a direct result of this.

Interfaces between the cartridge and the readout electronics will be accomplished using two Preci-dip connectors, one for each measurement technique. These connectors are spring loaded and will connect to the cartridge when the mechanical slider pushes it into place.

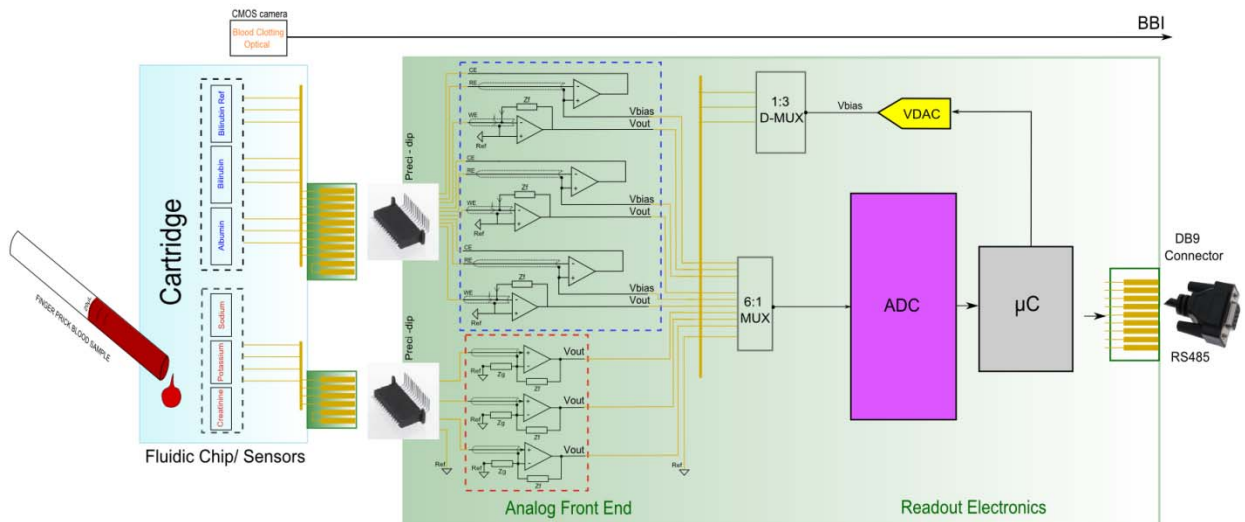


Figure 9: Top level block diagram of readout electronics.

Sensors to be read out are; creatinine, potassium, sodium, albumin and bilirubin. The first three will be sensed using Open Circuit Potential (OCP) measurements, whereas albumin will be sensed using an impedimetric approach and bilirubin using amperometry, for which a small current will be measured using a potentiostat in a three electrode cell.

Additional pads to verify connectivity are also included on each side of one connector to ensure that the cartridge and analog front-end have established a secure electrical connection. The clotting time sensor (optical) is considered an external device and signals from this sensor will be forwarded directly to the main BBI instrument control independent of the read-out electronics described herein.

For read-out electronics, digital signal processing and communication with the BBI main instrument control will take place via RS485 serial communication. The command set with instructions and data transfer is being developed and the system will communicate via a 12 pin ERNI SMC cable assembly.

A suggestion for pin-out and layout for the ion sensors has been proposed and a datasheet with the recommended mating footprint provided to the biosensor development partners (Figure 10).

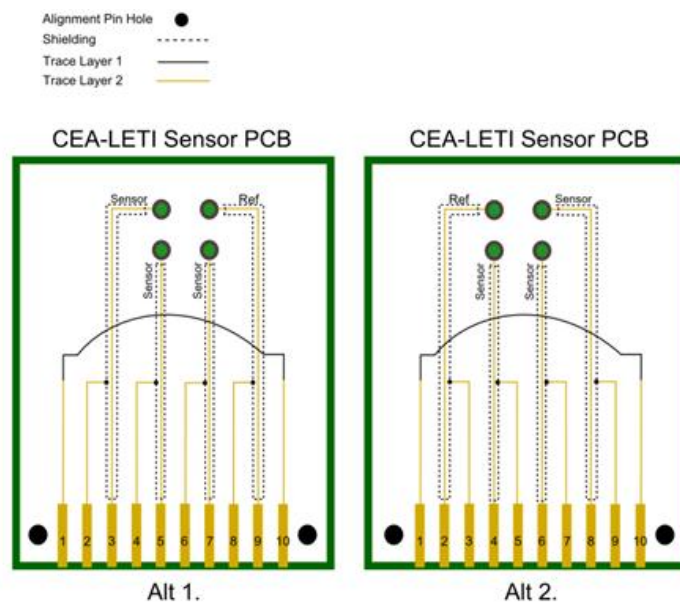


Figure 10: Suggested pin-out for ion sensors

## 7. Conclusions

A total of 6 sensors will be finally integrated in the fluidic cartridge developed in WP4: albumin, bilirubin, creatinine, sodium, potassium and clotting time. The sensors will be divided into 2 PCB platforms interfaced with the cartridge and able to be connected to the BBI.

Table 1 summarizes the measurement principle that will be used for each sensor.

Table 2 reports the performance characteristics achieved.

*Table 1 Measurement strategies for each sensor*

<b>Albumin</b>	<b>Impedance</b>
<b>Creatinine</b>	<b>Indirect Potentiometry</b>
<b>Bilirubin</b>	<b>Amperometry</b>
<b>Sodium</b>	<b>Potentiometry</b>
	Ion selective electrode
<b>Potassium</b>	<b>Potentiometry</b>
	Ion selective electrode
<b>Clotting time</b>	<b>Optical detection</b>
	Detection of the immobilization of red blood cells

*Table 2 Sensor performance characteristics*

Sensor	Number of electrodes	Chip interface	Dilution buffer	Measurement range	Reproducibility intra-sensor
<b>Albumin</b>	3 (working, reference and counter)	Double sided tape	Water + glucose (5%) + NaCl (0.9%)	0.5-32g/L	+/- 14%
<b>Bilirubin</b>	3 (working, reference and counter)	Double sided tape	Water + glucose (5%) + NaCl (0.9%)	1-500µmol/L	+/- 8%
<b>Creatinine</b>	2 (working and reference)	Double sided tape	Water + glucose (5%)	0-10mmol/L	+/-8% off chip
<b>Sodium</b>	2 (working and reference)	Double sided tape	Water + glucose (5%)	50-500mmol/L	+/- 1%
<b>Potassium</b>	2 (working and reference)	Double sided tape	Water + glucose (5%)	0.5-10mmol/L	+/- 1%
<b>Clotting time</b>	N/A	Part of the chip	Whole blood	10-120s	+/- 1%

For each sensor, the fluidic sequence has been established and a global fluidic sequence was approved by all the partners during a review meeting in Mainz in February 2014.

The performance characteristics of the sensors will be evaluated now after integration within the fluidic cartridge and compared with reference methods. This will be reported in deliverable D3.4.