Electrochemistry in diagnostics

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biorecognition

- Life in molecular level is organized according to hierachy of recognition and non-recognition. Enzyme-substrate, nucleic acids, immune reactions, etc. – called ”bioaffinity”.
- Currently molecules in body fluids are predominantly analyzed by exploiting their bioaffinity.

Procedure

- Label = measurable marker molecule or atom
- Number of molecules **A** is determined with labelled antibodies binding to A (Ab-L):
- (X copies of) **A** + (Y copies of) Ab-L →
- (X) **A**-Ab-L + (Y-X) Ab-L ; (Y>X)
- Mixture is purified to contain pure **A**-Ab-L
- Number of **A**-Ab-L is measured = number of L

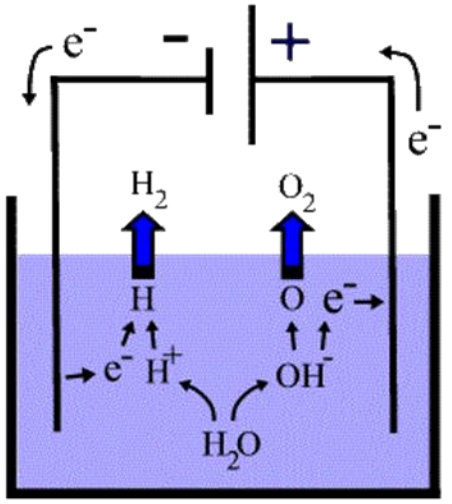
How L is measured?

- Fluorescent or luminescenct labels are currently used and an evident choice for future.
- L is induced or ”excited” to produce light emission.
- Excitation is done with light or with electrical pulses.

Basic electrochemistry

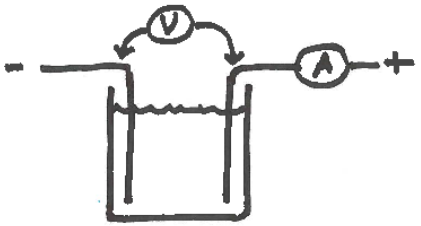
- Electrolyte: water solution containing salts. Salts form ions in water and allow electric current through water.
- Electrodes (metal or graphite) connect electricity source to electrolyte.
- Inert or reactive electrodes.
- Electric current from anode to cathode effects electrode reactions, charge or discharge of ions in solution.
- Water decomposition, example.

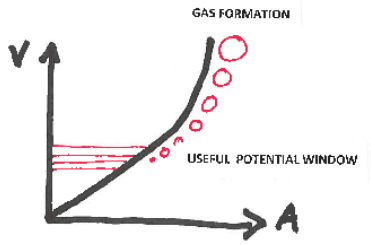
Electrolysis: Splitting water with electricity to produce hydrogen and oxygen:



Electrode reactions

- Different molecules are reduced or oxidized at different potentials on inert electrodes.
- Water decomposition starts at a certain voltage. Not possible to use this area for excitation of labels.
- Redox voltage of L must be less that that of water



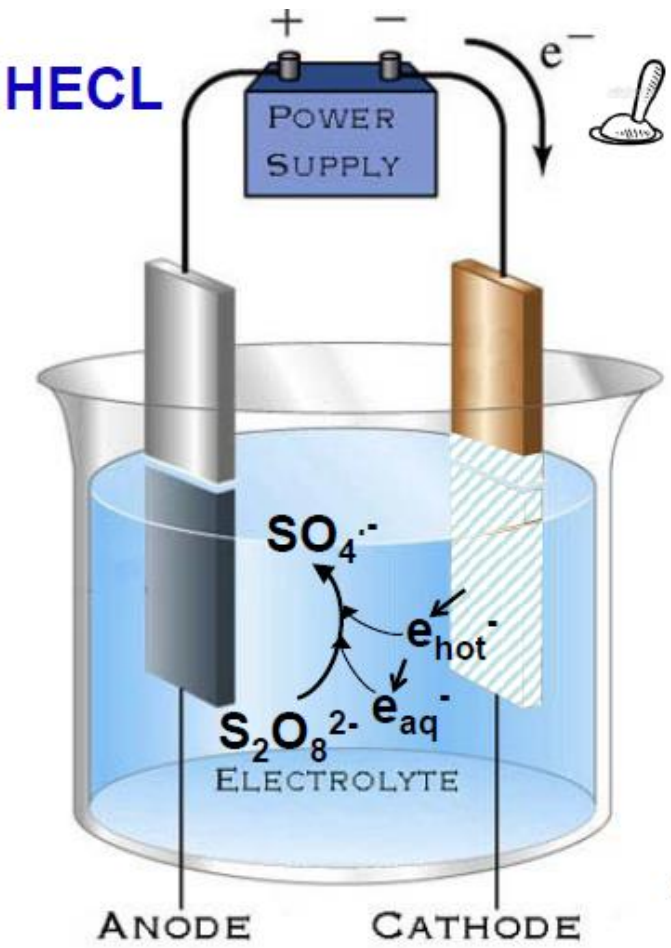


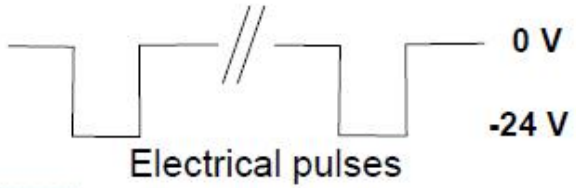
Potential window at inert anode is limited for exciting L

- Bioaffinity reaction must be done in water.
- Organic solvents can decrease decomposition of water and allow higher applied voltages to excite L.
- Still, limited number of L are measurable (Roche:rutheniumbipyridium)
- UV –excitation not possible

How to widen the potential window in water?

- Excitation of labels is carried out by very high energy, ”hot electrons” at CATHODE.
- Hot electrons are achieved by forcing electrons to ”jump” over an insulator barrier with pulsed voltage of 10-30 V.
- Normal electrons jump 1-2 nm BUT hot electrons jump up to 100-200nm distance from electrode. Gas evolution occurs only at high voltages →strong redox reactions possible in 100 times higher volume.





= insulation layer
e.g. SiO_2 layer, 3-4 nm

e_{hot}^- = hot electron

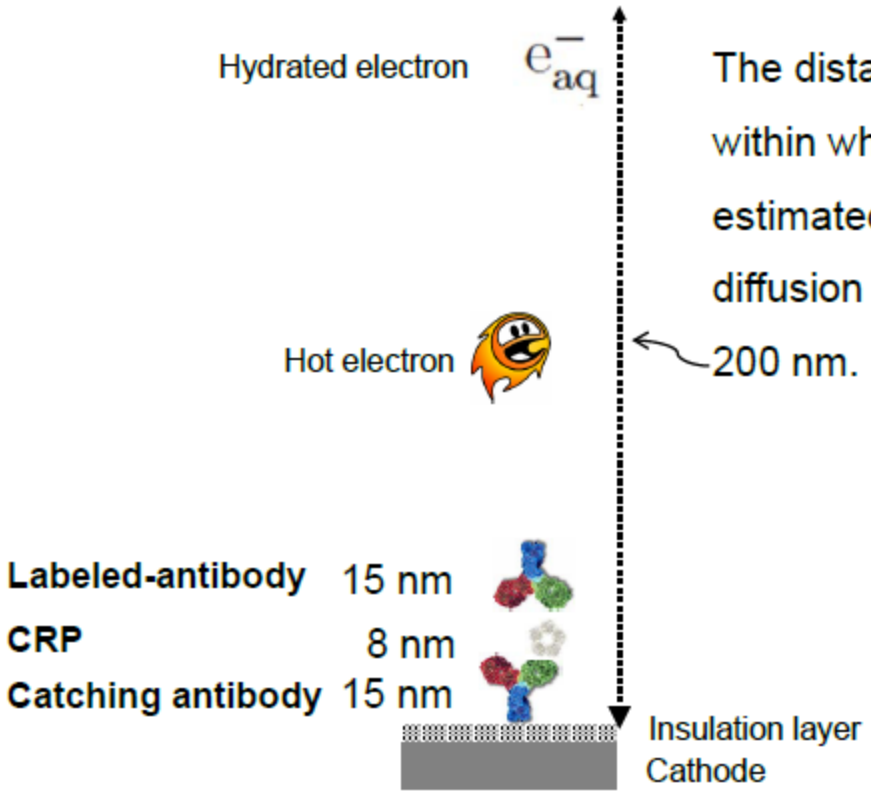
e_{aq}^- = hydrated electron

Highly reducing reactants

$S_2O_8^{2-}$ = peroxydisulfate ion — Coreactant

$SO_4^{\cdot -}$ = sulfate radical — Highly oxidizing reagent

Highly reducing and oxidizing conditions are simultaneously achieved by injection of hot electrons into aqueous solution from cathodically **pulse** polarized thin film-coated electrodes.



The distance from the working electrode, within which HECL occurs, has been estimated from e^-_{aq} reactivity and diffusion coefficients to be on the order of 200 nm.

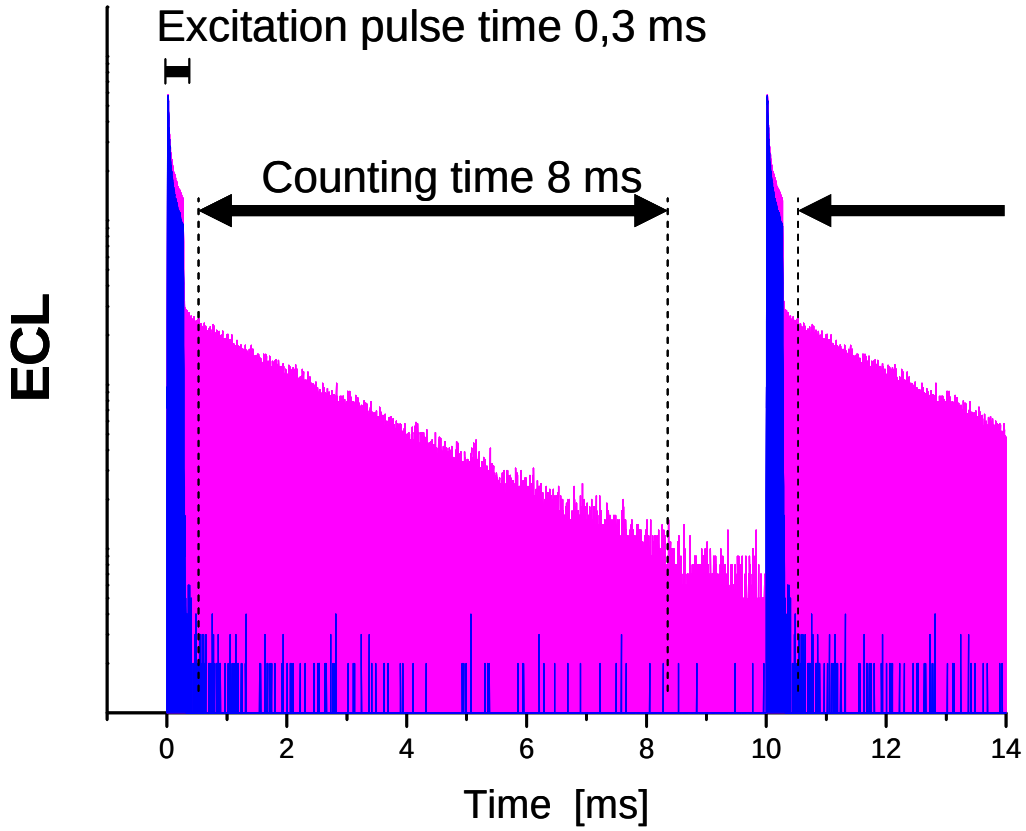
IPR

- Rosche Diagnostics owns patents on excitation on inert metal anode. Applied in practise worldwidely.
- Labmaster Ltd. Turku, owns patents on excitation of labels on insulator-covered cathode (>10 inventions).
- ”hot electron electrochemistry” - ”HECL”, development stage for POC-diagnostics. Very potential technology for future.

Advantages of cathodic excitation over old methods

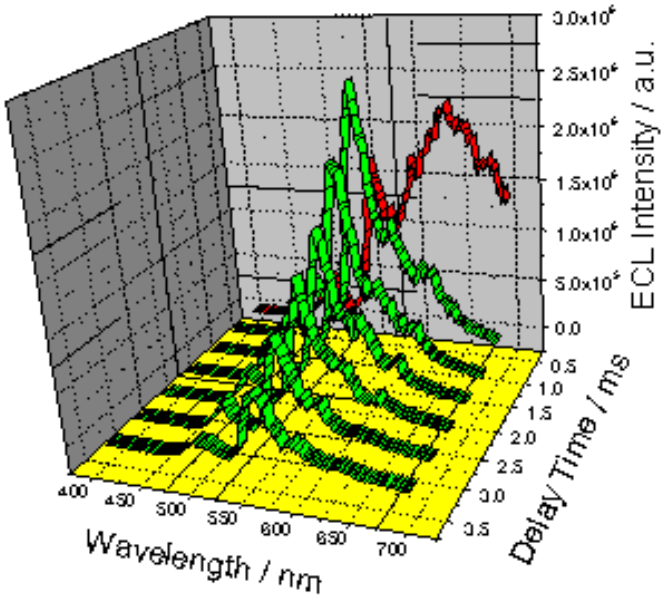
- Water solutions are used throughout, no need to change to organic ”measuring” solutions.
- Any kind of labels can be excited to produce emission from UV to IR range.
- ”Time-resolved” technology can be used to increase sensitivity (require UV excitation and long-life luminescence label).
- Internal calibration from insulator´s fluorescence signal.
- Cathode can be cheap metal because it is covered with inert layer →single used test sticks →POC
- Multiplexing easy
- Cheap simple measuring instrument, no optics

Time-resolved measuring principle

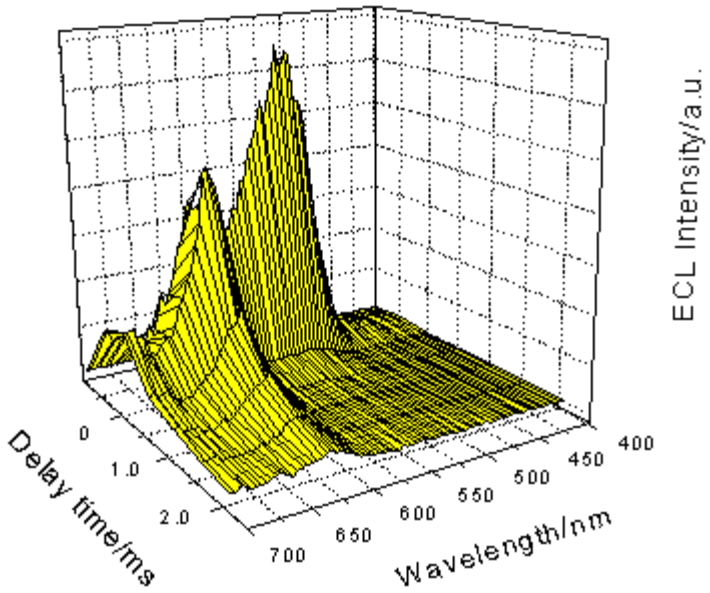


Simultaneous excitation of short-lived and long-lived luminescence displaying labels using HECL

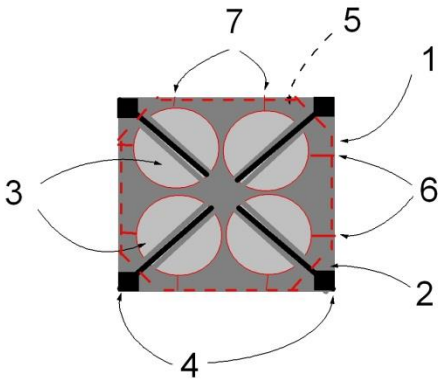
Tb(III) chelate + $Ru(bpy)_3^{2+}$

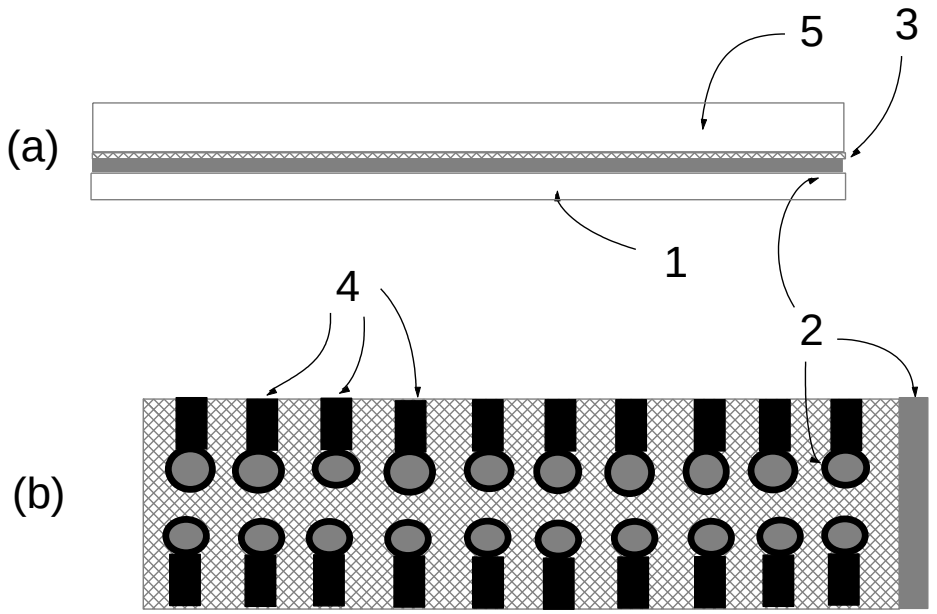


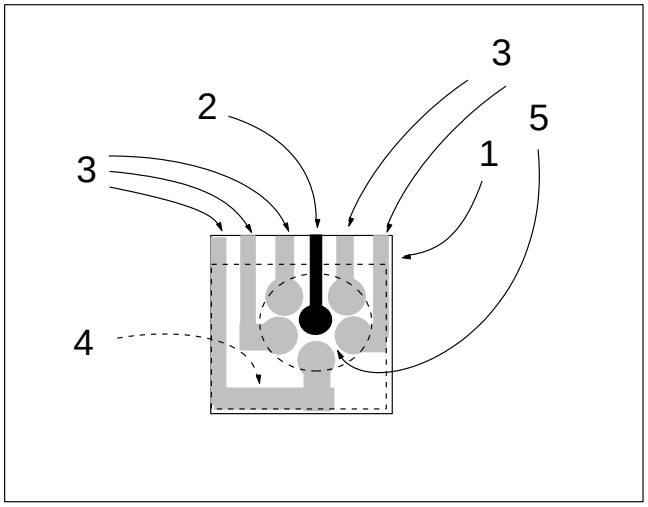
Eu(III) chelate + FITC

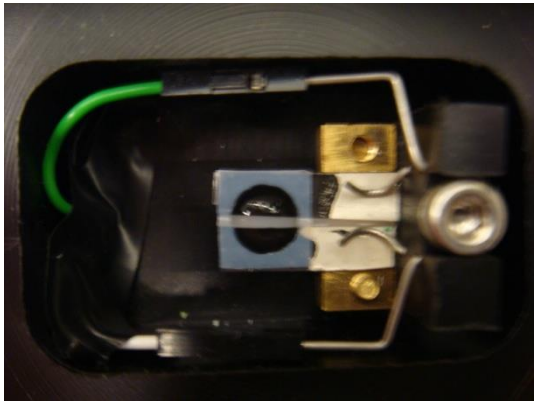


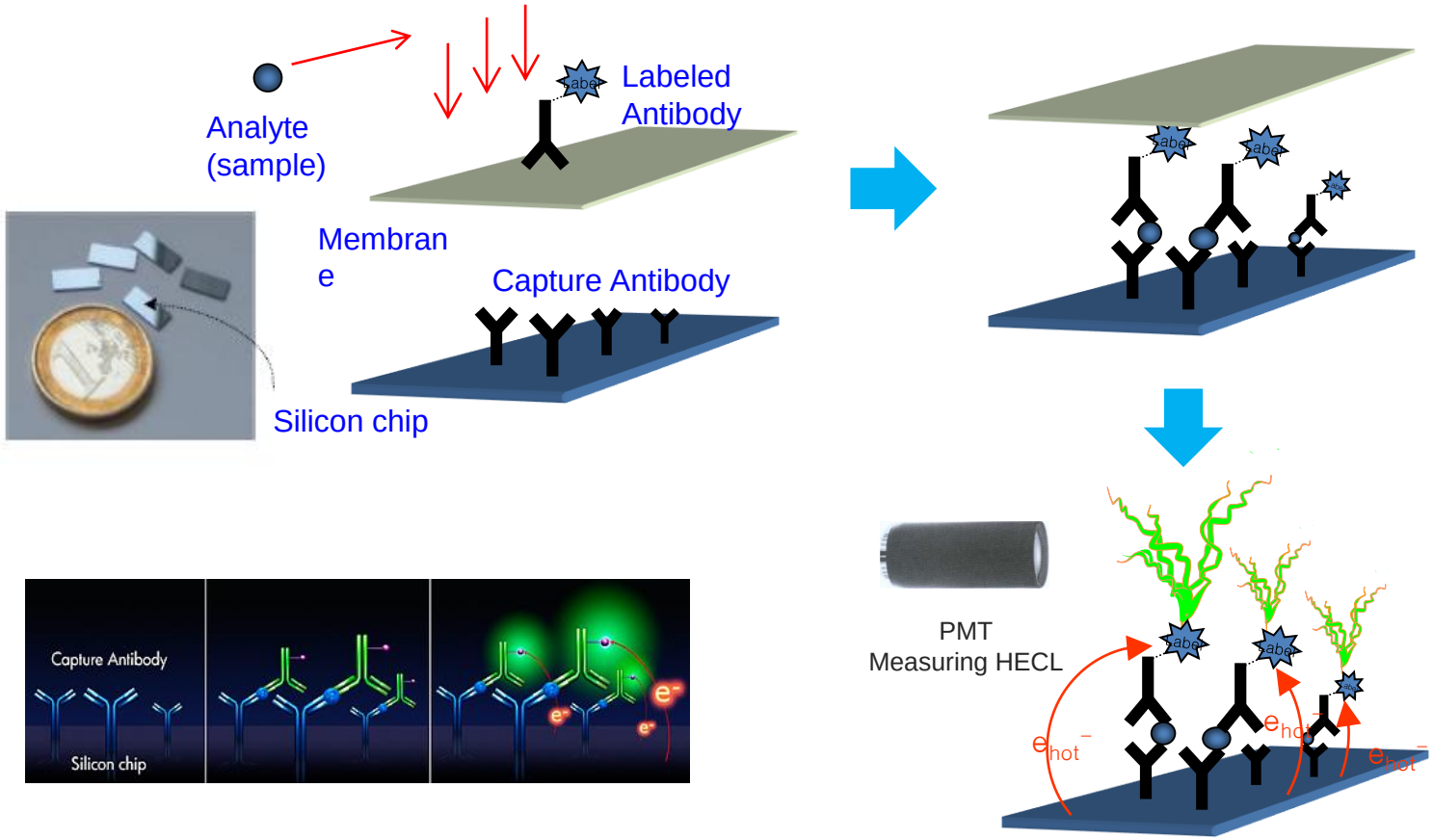
Cells for multiplexing and printable electrodes











Rapid Ultra Sensitive CRP Assay (2)

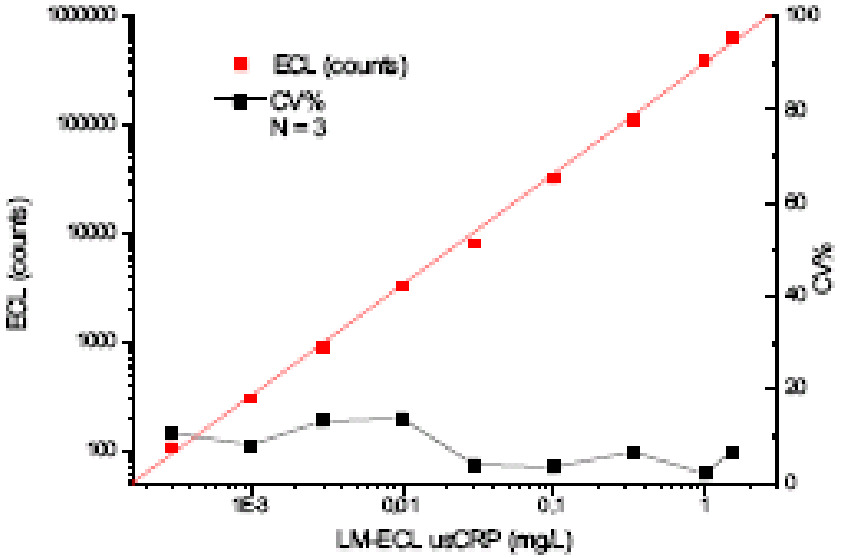


Figure 1. Linearity and variation of standards usCRP assay performed by PIA ECL analyser.

plasma samples

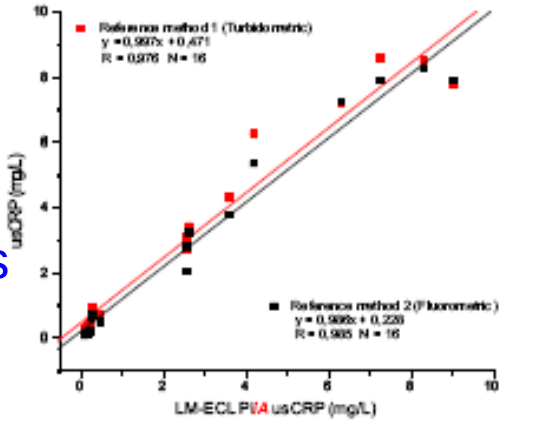


Figure 2. Correlation between usCRP assay performed by PIA ECL analyser and two reference methods using plasma samples; concentration range < 10 mg/L.

whole blood samples

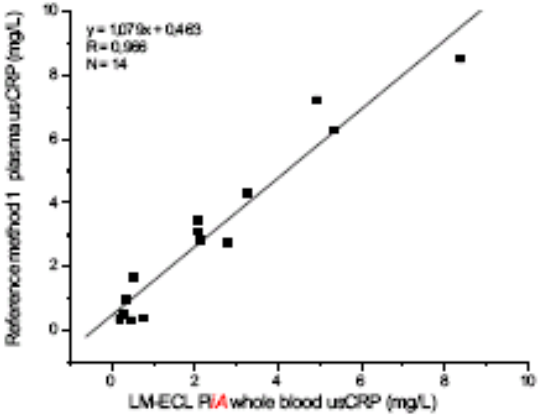


Figure 3. Correlation between whole blood and heparin plasma samples. Whole blood samples have been corrected for hematocrit.

The comparison methods: **Roche Hitachi 917 Tina-Quant® CRP** (latex)

high sensitive assay and **Innotrac Aio! usCRP** immunofluorometric assay.

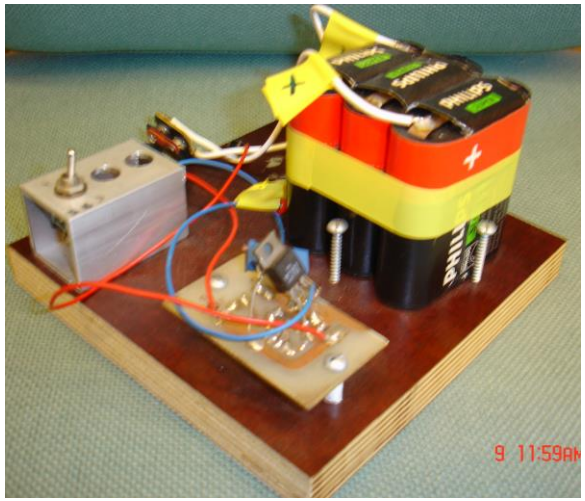
What is needed for using HECL and Time-Resolved Detection in Analysis ?

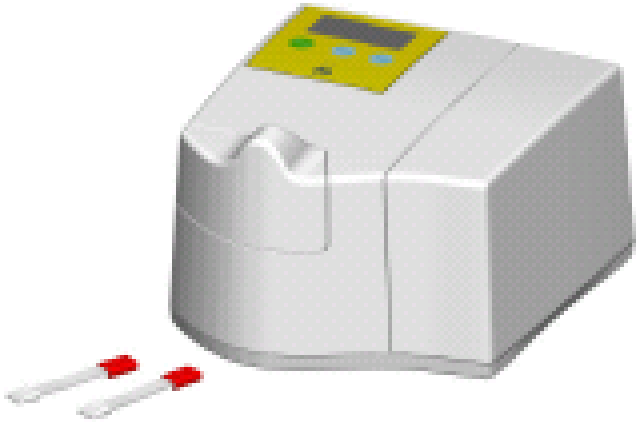
- A **pulse generator**

- **electrochemical cell (e.g. a casette)**

- **photon counter**

- A laptop **computer** or internal **microprocessor**





PiiA ECL analyser

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Thank you!
ご清聴ありがとうございました