Biosensors in Clinical Chemistry

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Introduction to Biosensors

"Biosensors are analytical devices composed of a biological recognition element (target analyte) and a physical and or chemical transducer. The sensing takes place either as a binding event or a biocatalytical event leading to a measureable change in physical or chemical property that the transducer converts into a quantifiable electrical signal".

- D'Orazio P, 2003, Instrumentation Laboratories-

Important Aspects for Biosensor Development

- **Sensitivity** – The ability of a biosensor to measure small variations of the concentration of the target analyte

- **Specificity** – The ability of a biosensor to detect the target analyte with minimal or no response signal generated from potentially interfering substances that may be co-present in the same sample matrix

- **Accuracy** – The closeness of the agreement between the biosensor measured value (concentration) and the true value the analyte in a test sample

- **Precision** – The closeness of agreement between independent measurements on the same sample

MEASUREMENT OF BLOOD GLUCOSE

Direct measurement of glucose is difficult
and thus indirect methods are used.

An indirect measurement of blood glucose converts glucose to another substance that is easily measured.

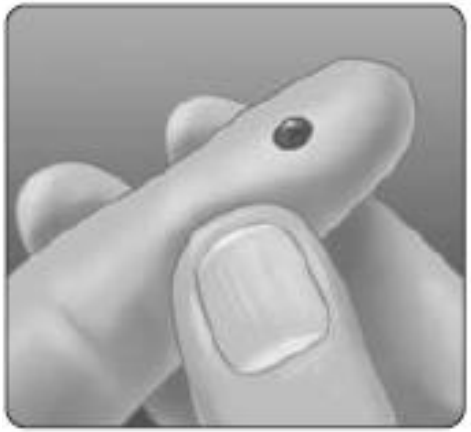
Common current blood glucose monitoring systems use one of two technologies:

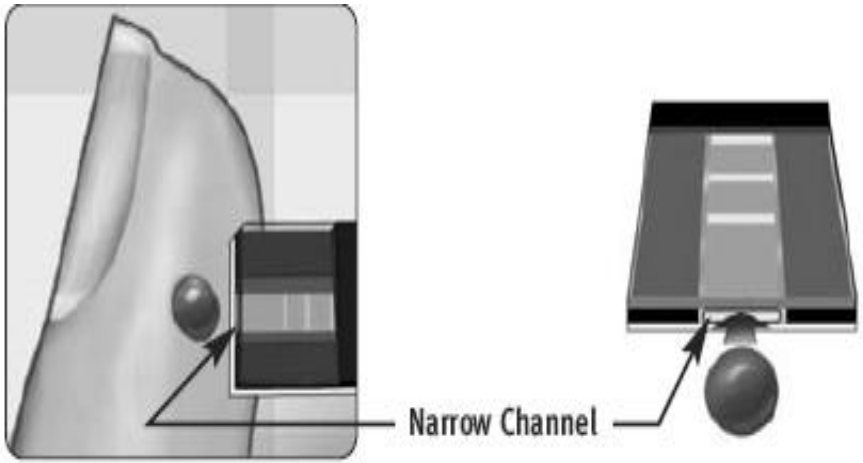
-reflectance photometry (measures amount of a colored product) formed.
-electrochemistry (measures electrical current).

104
10:35
5-18
PM
mg/dL

Puncture finger

Get a round drop of blood

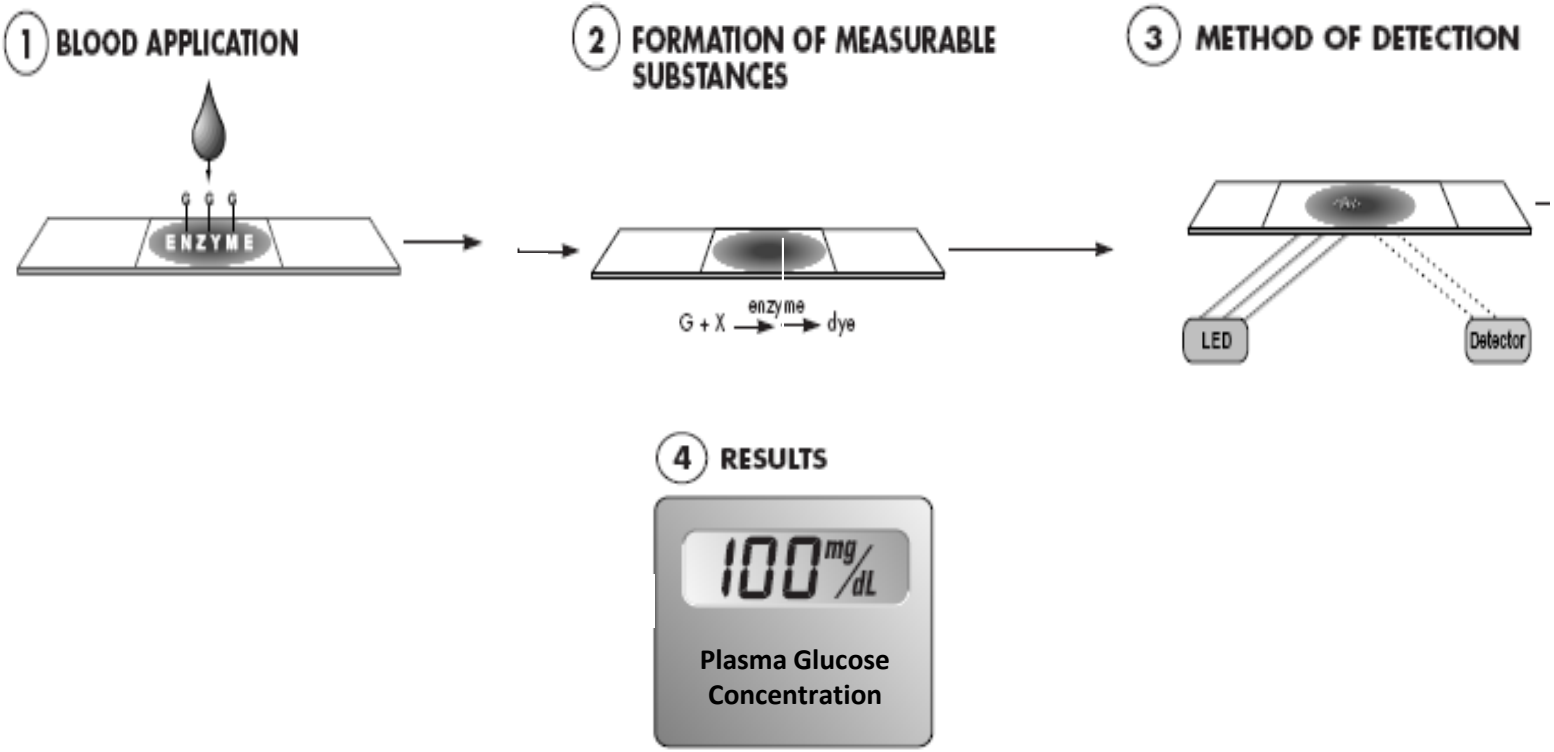



Narrow Channel

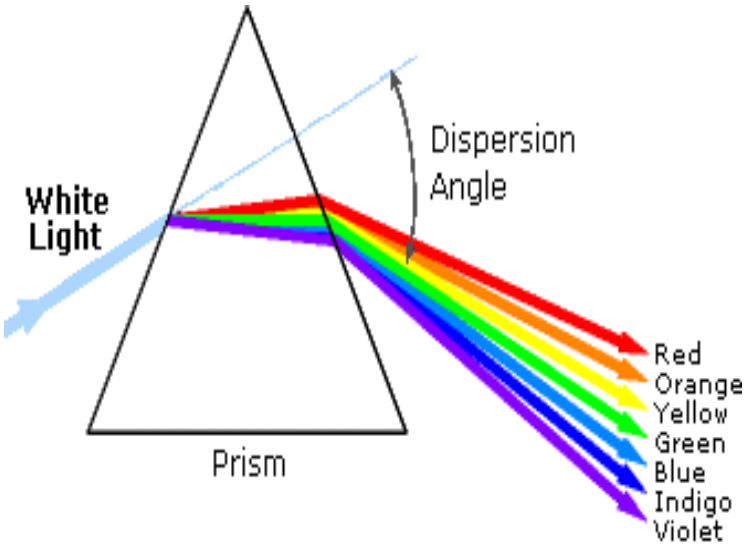
Primary Reaction: Glucose + O_2 + H_2O —(Glucose oxidase)→ Gluconolactone + H_2O_2

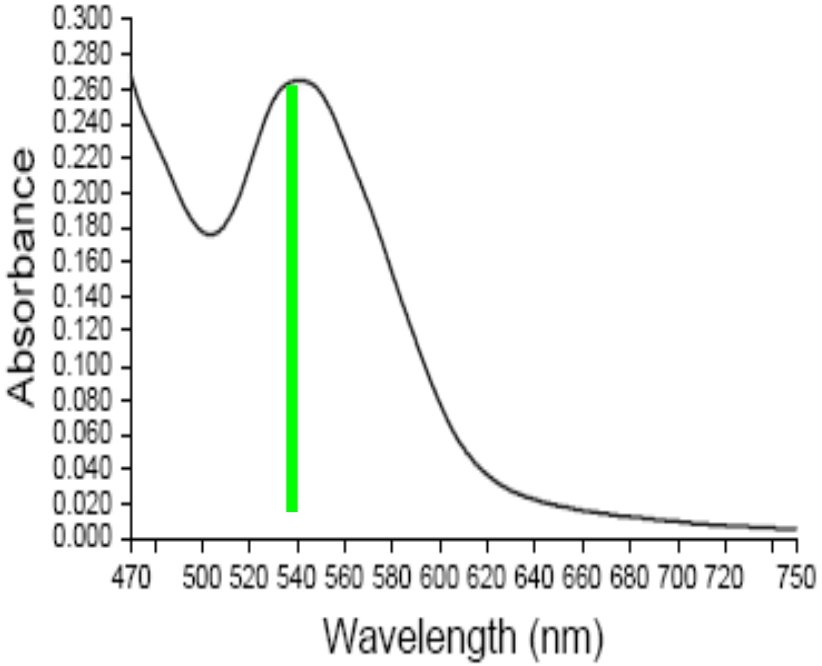
Measurement Signal Production

H_2O_2 + Reduced dye (colorless) —(Horseradish Peroxidase)→ Oxidized Dye (Colored) + H_2O

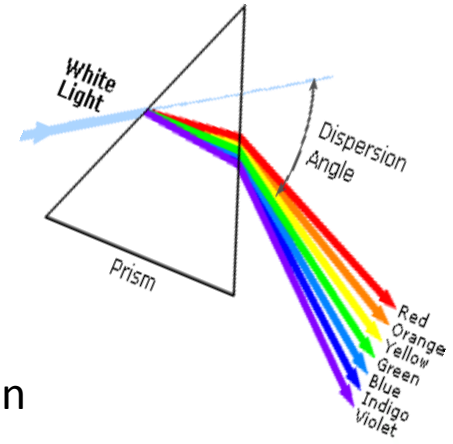


Visible Absorbance Spectrum of the Oxidized Dye

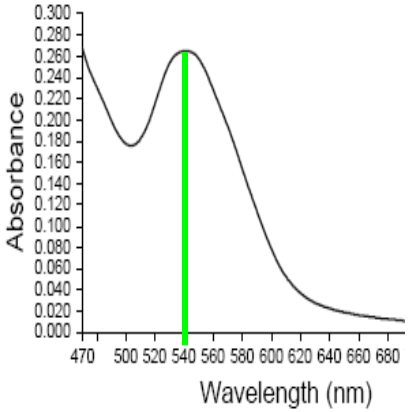


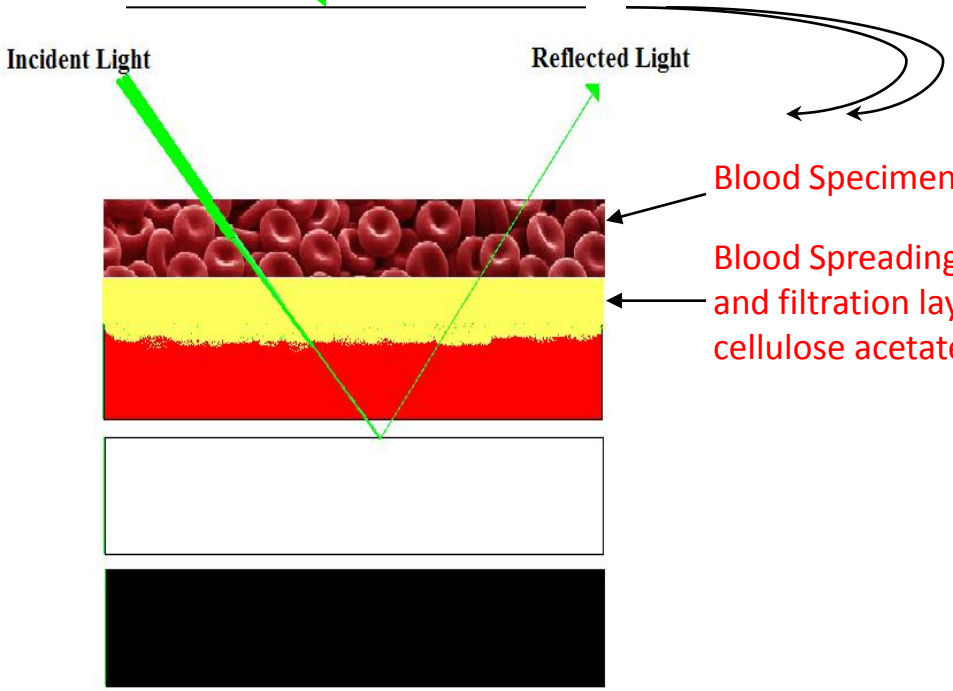


Glucose Measurement

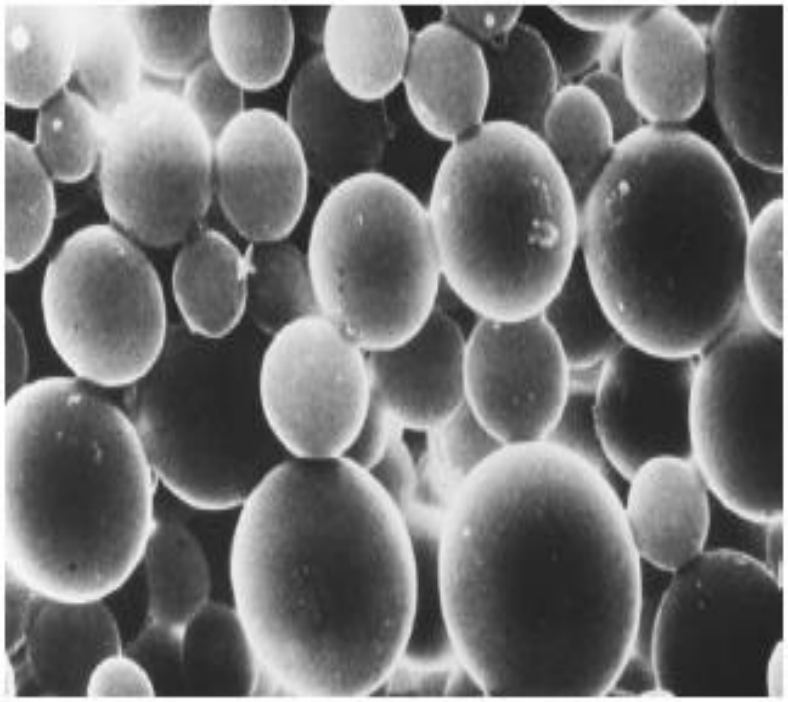


Visible Absorption Spectrum of **Oxidized Dye (Colored)**

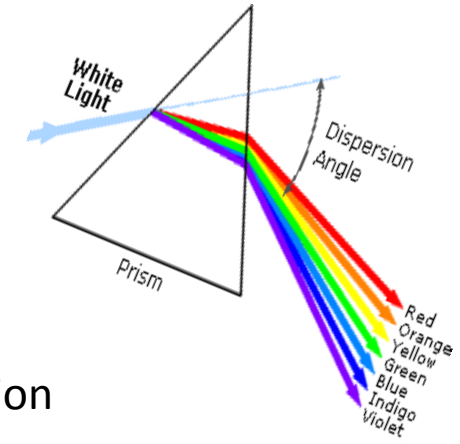




Photograph of TiO_2 – cellulose acetate layer used for blood sample spreading and filtration layer (average sphere size 1 micrometer)

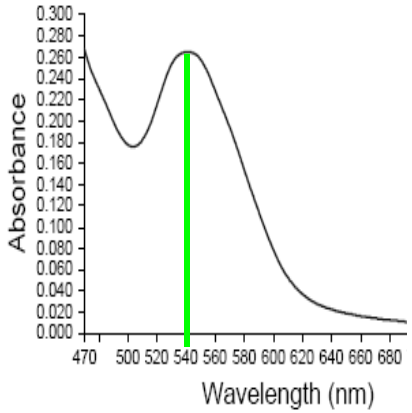


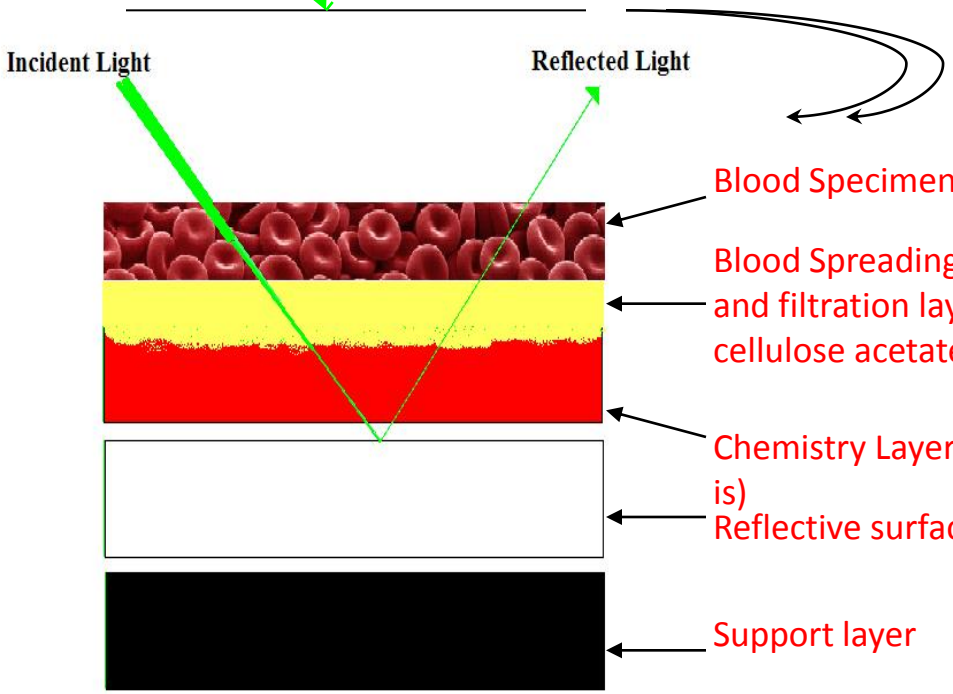
Glucose Measurement



Photodetector

Visible Absorption Spectrum of **Oxidized Dye (Colored)**





Primary Reaction:

Glucose Oxidase

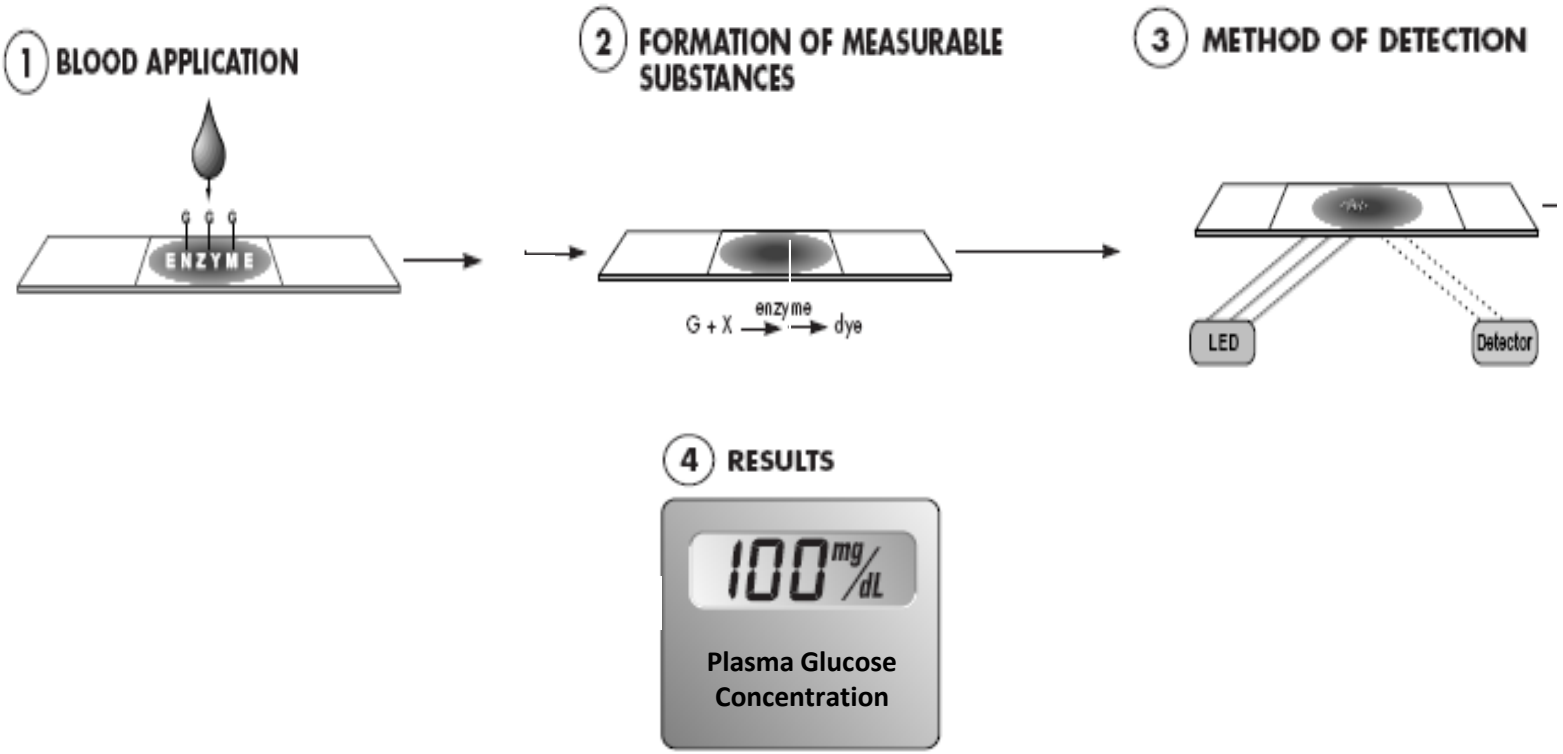
Glucose + O_2 + H_2O → Gluconolactone + H_2O_2

Cholesterol — Cholesterol Oxidase — Cholest-4-en-3-one

Measurement Signal Production

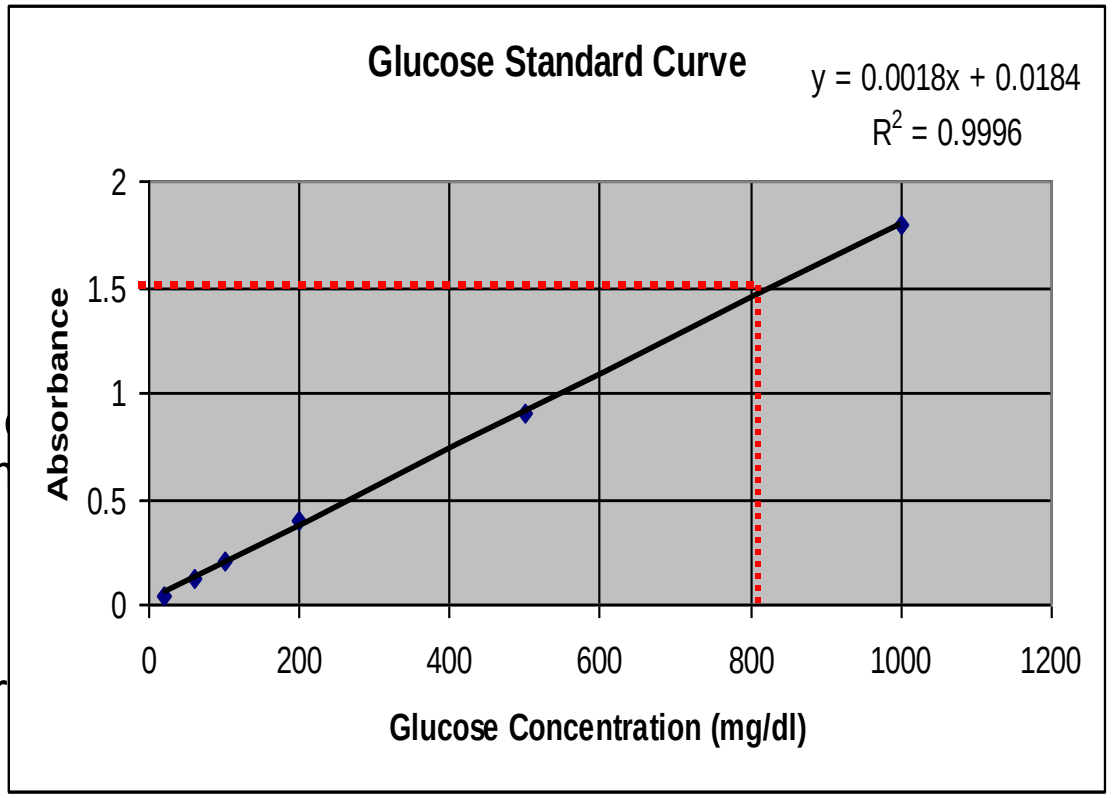
Horseradish Peroxidase

H_2O_2 + Reduced dye → Oxidized Dye (Colored) + H_2O



Glucose Standard Curve

- Insert sample into reading device (spectrophotometer)
- The result reported can be an absorbance value or a transmittance value
- Plot glucose standard concentration vs. absorbance
- When analyzing unknowns, rea absorbance value obtained fro the instrument
- Use the absorbance value to extrapolate the analyte concentration from the standar curve



What is the value for a glucose sample that produces an absorbance value of 1.5?

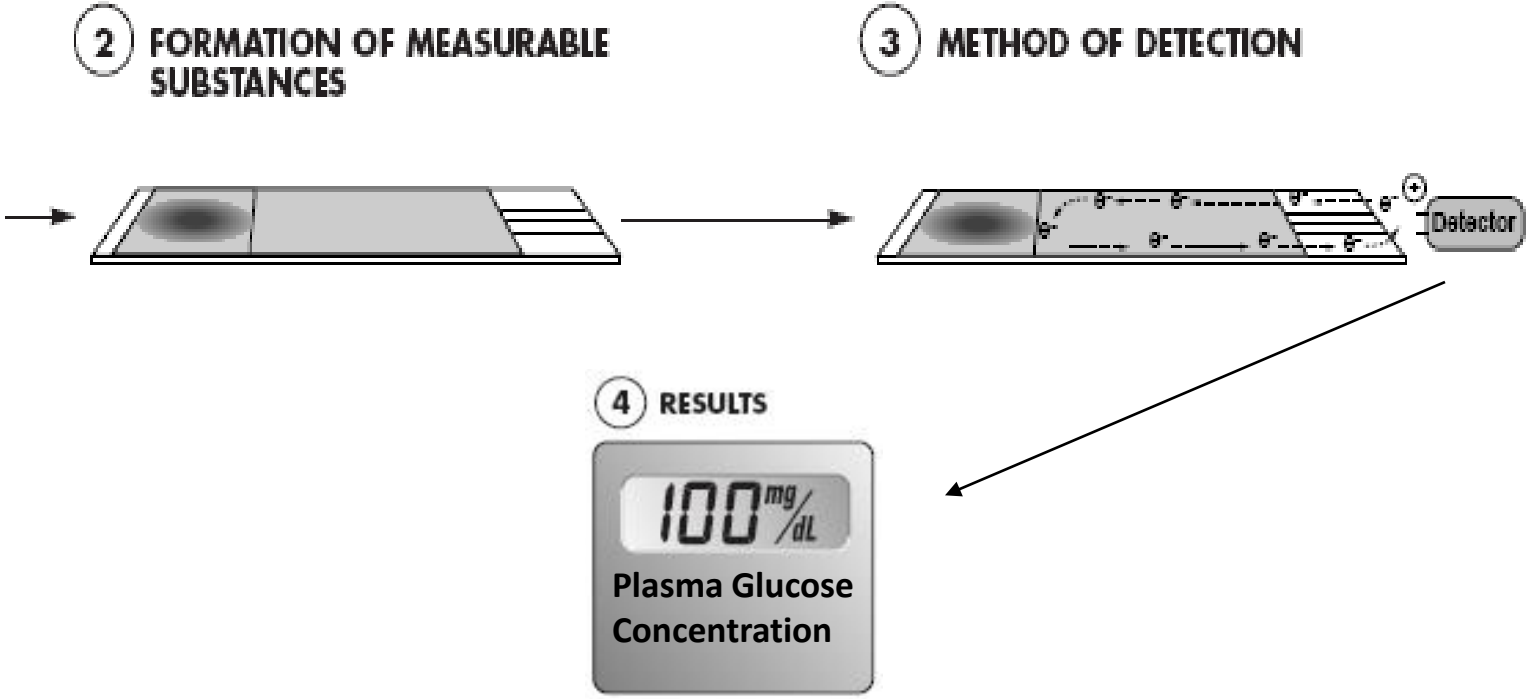
Clark Oxygen Electrode (Coulometric Detection)

Primary Reaction: $Glucose + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} Gluconolactone + H_2O_2$

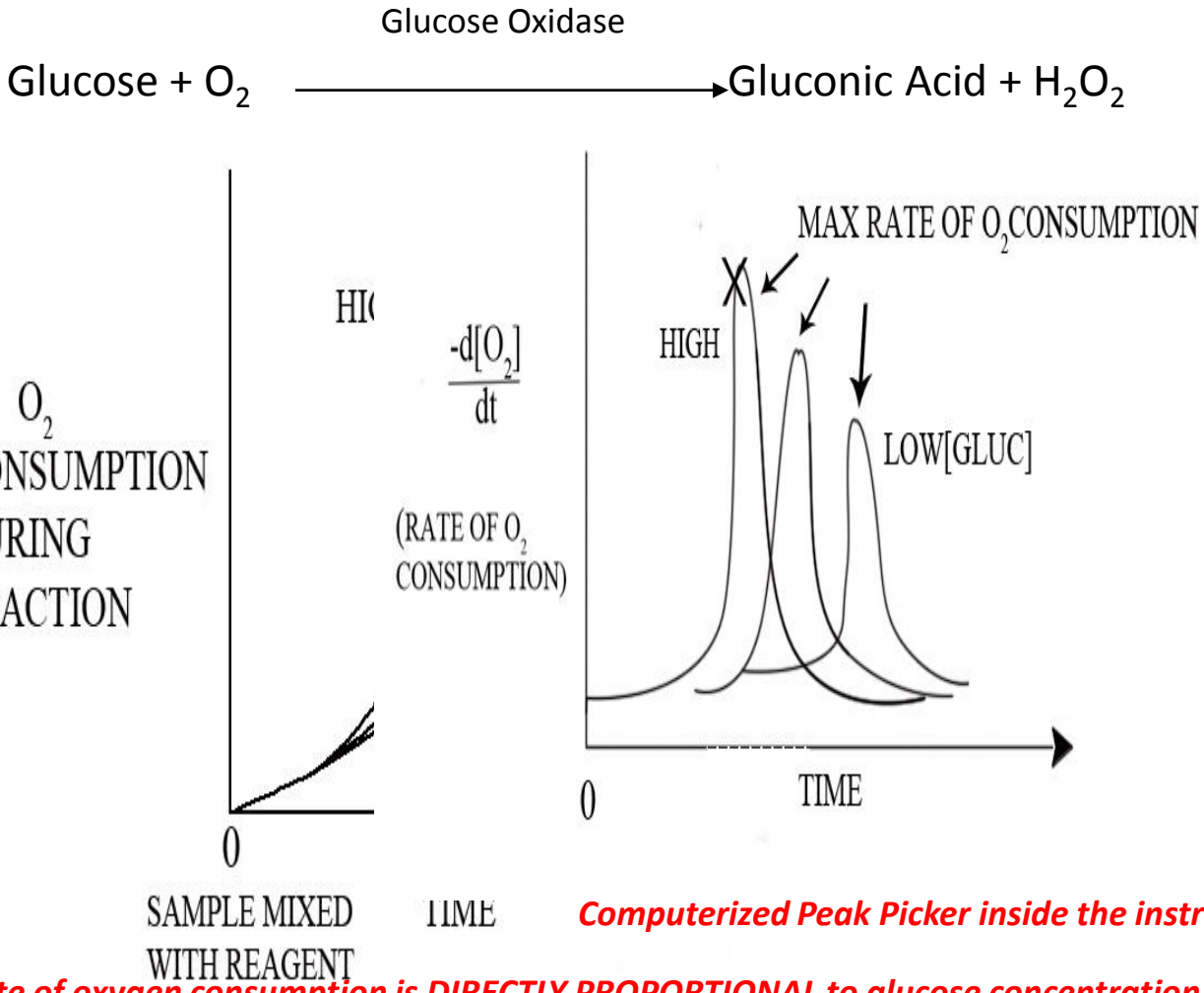
Measurement Signal Production

Silver Anode: $4Ag \rightarrow 4Ag^+ + 4e^-$

Rhodium Cathode: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$



Glucose Measurement by Glucose Oxidase and Clark Oxygen Electrode

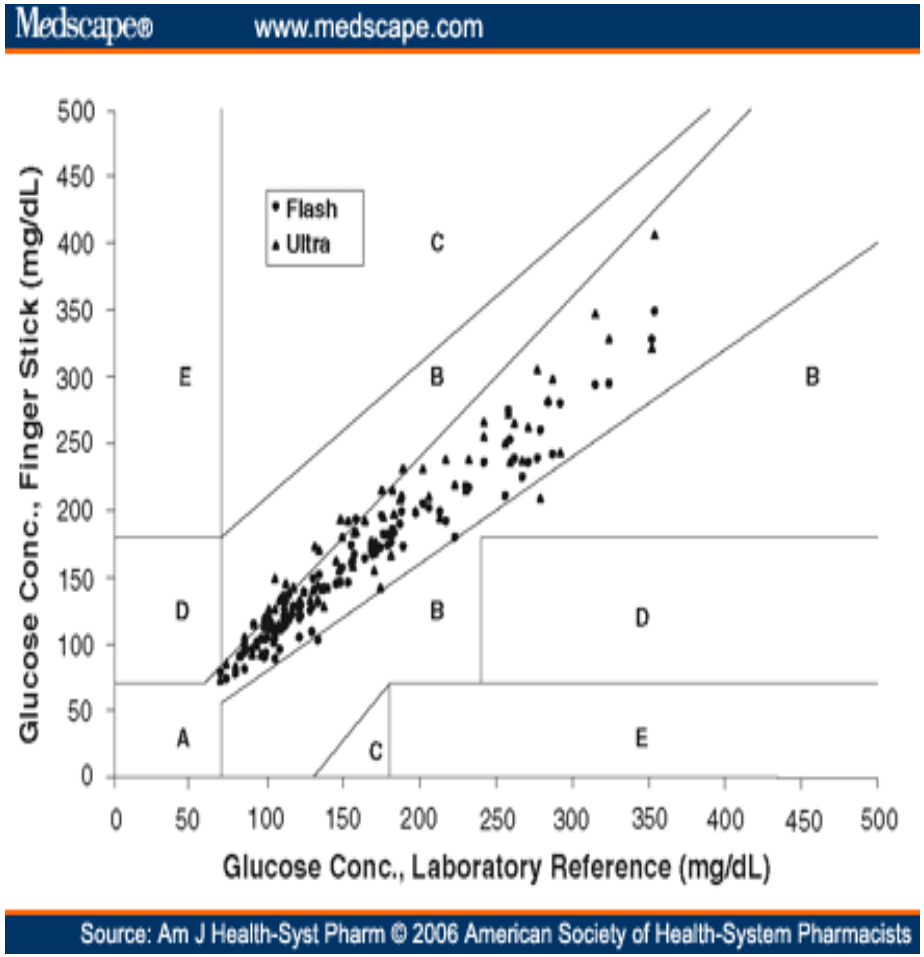


Computerized Peak Picker inside the instrument

Maximum rate of oxygen consumption is DIRECTLY PROPORTIONAL to glucose concentration

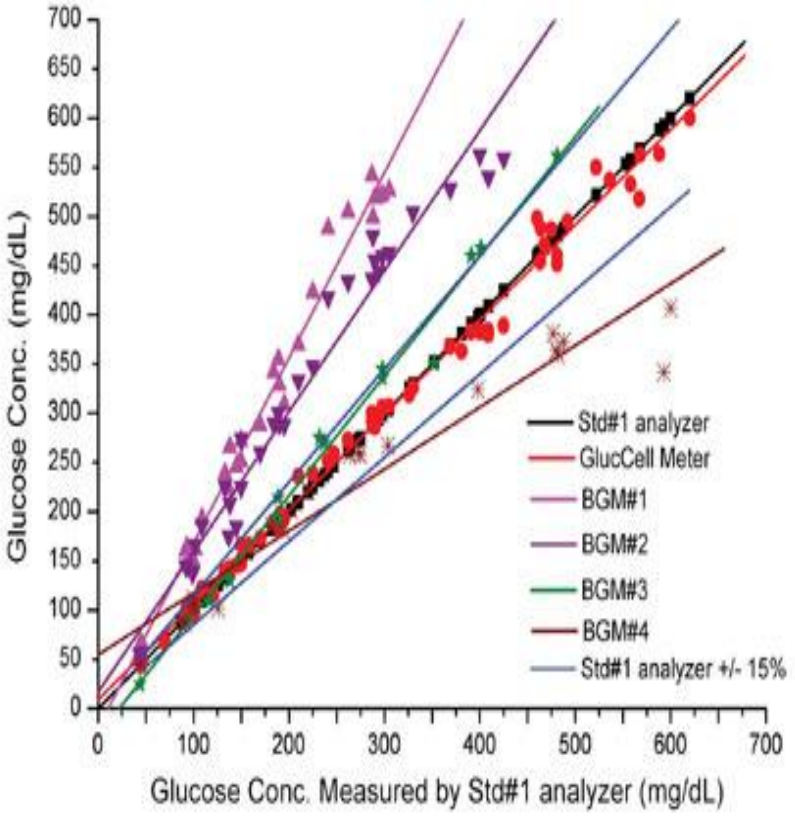
Clinical Error Grids (ie. glucose)

- Zone A: Values do not vary by more than 20%
- Zone B: Values vary by >20% from the reference method
- Zone C: Values could result in an overcorrection of blood glucose
- Zone D: Values represent a dangerous failure to detect and treat errors
- Zone E: Represents extremely erroneous treatment zones



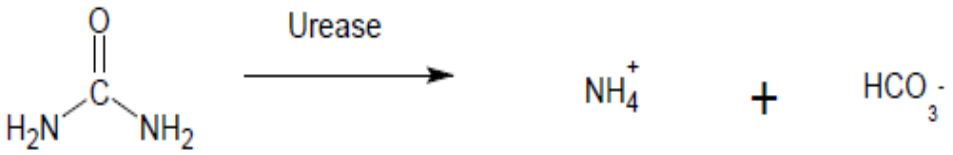
Imprecision

- The black line represents multiple glucose measurements measured by one method (Std#1 analyzer).
- All other lines represent measurements of the same glucose sample by different methods/analyzers.
- You can see great imprecision between the tested methods/analyzers.



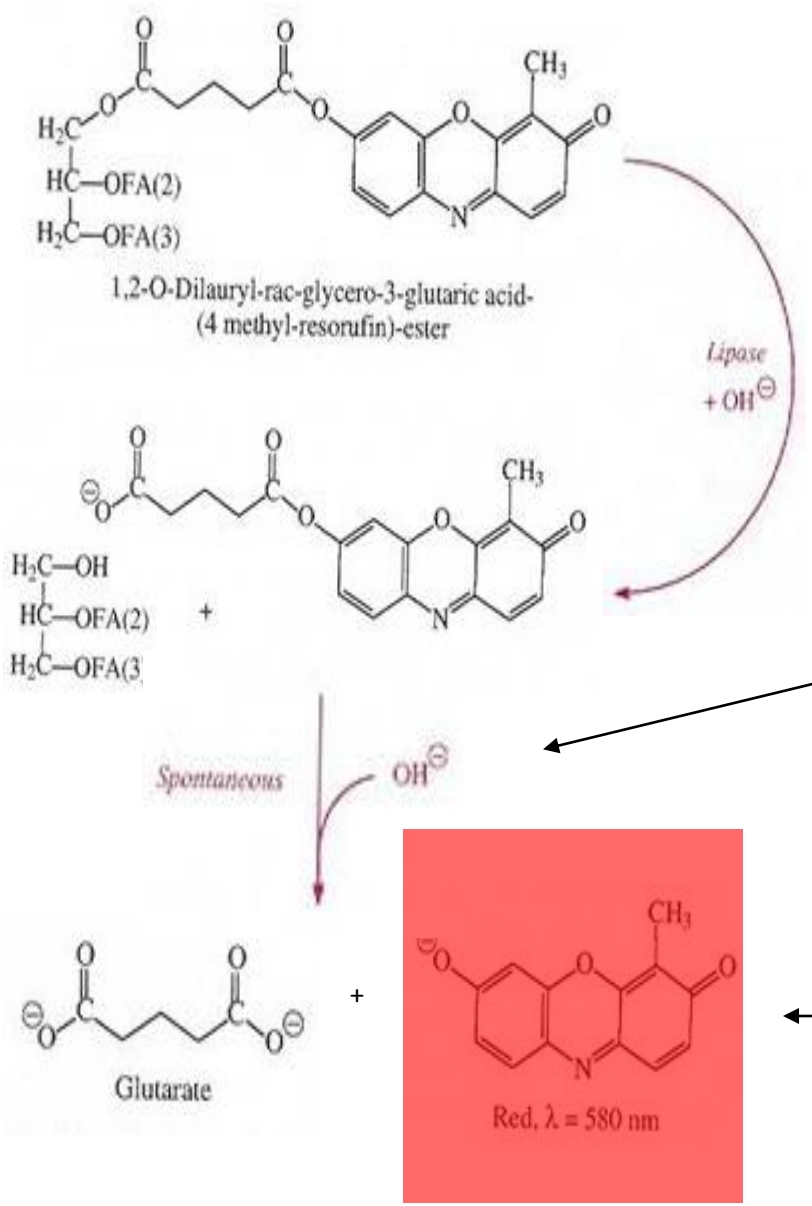
Conductometric Urea Biosensor

- Urea increases in blood in kidney disease. The higher the urea level the greater the degree of loss of kidney function



- The measured increase in conductivity during the reaction is proportional to the concentration of urea in the sample

Measurement of Serum Lipase Activity for Diagnosis of Acute Pancreatitis

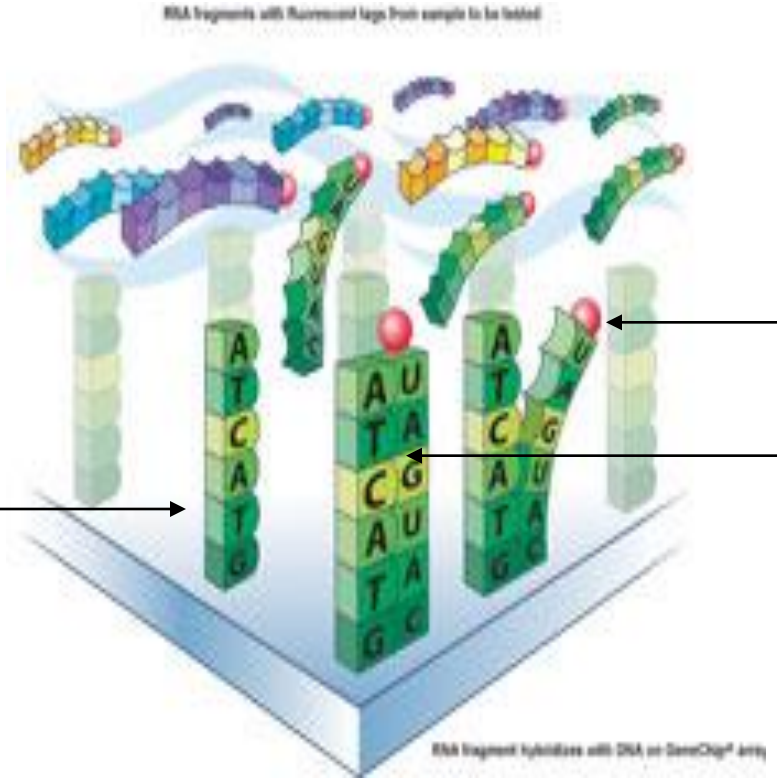


First hydrolysis of ester bond by serum lipase enzyme

Second hydrolysis of second ester bond by base

Red colored compound produced for quantitation

Gene Chip Layout



Fixed nucleic acid sequence

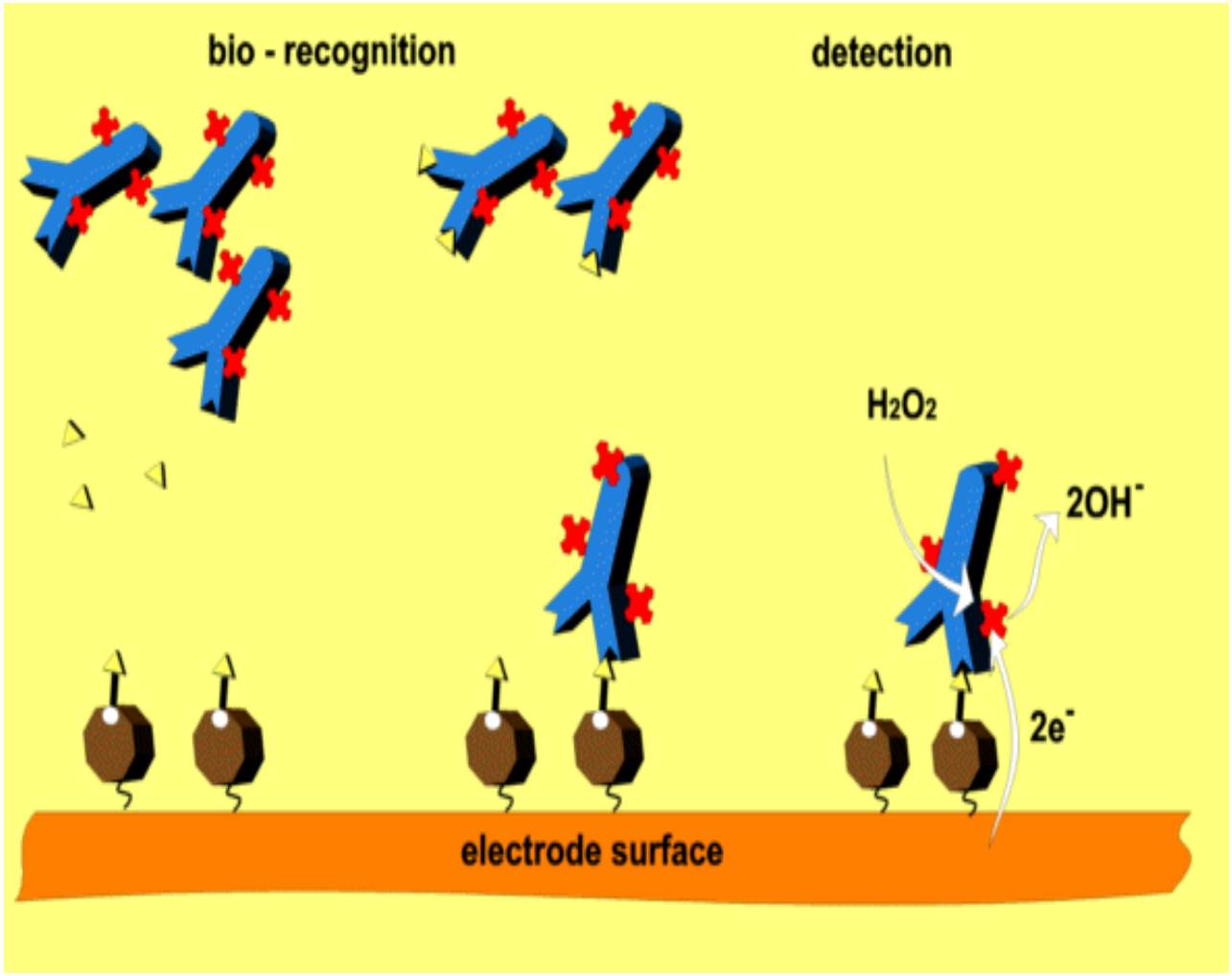
Fluorescent tagged nucleic acid sequence

Hybridization (visualized by fluorescence)

Immunosensors

- When antibodies are used as a reagent to recognize, bind to and detect target analytes

Amperometric Immunosensor Schematic



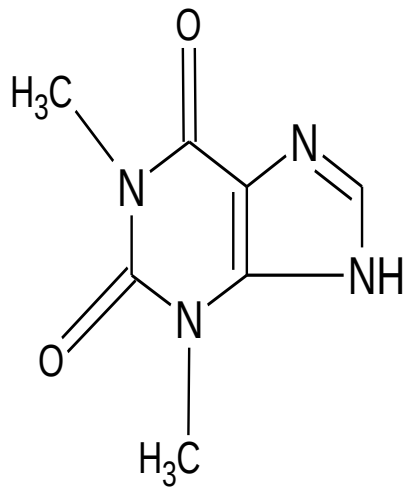
Assay Interferences (substances which if present in the sample may falsely alter measurement outcome)

- Chemical compounds that may also occur in the sample to be tested that possess similar chemical structures to the analyte of interest OR interfere with the reagents or the instrument (ie. Spectral)
  - Endogenous: Heterophilic human anti-mouse antibody development in cancer patients treated with mouse monoclonal antibodies
  - Exogenous: Ascorbic acid interferes with uric acid measurement, caffeine interferes with theophylline measurement, and lipemia interferes with photodetection

Significance of Assay Interferences

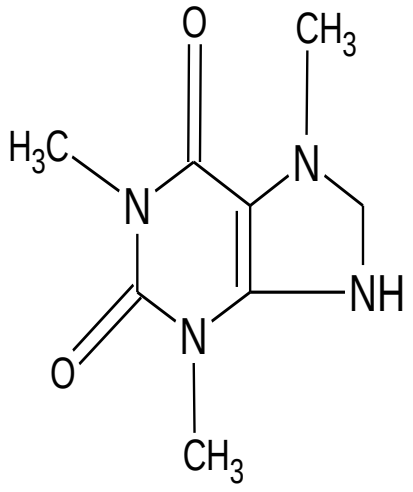
Interferent Strength	Interferent Occurance	Significance/Concern
Weak	Rare	+
Weak	Common	++
Strong	Rare	++
Strong	Common	++++

Example of Crossreactivity Issue in a Biosensor Immunoassay



THEOPHYLLINE

(antiasthmatic drug)



CAFFEINE

Crossreactivity

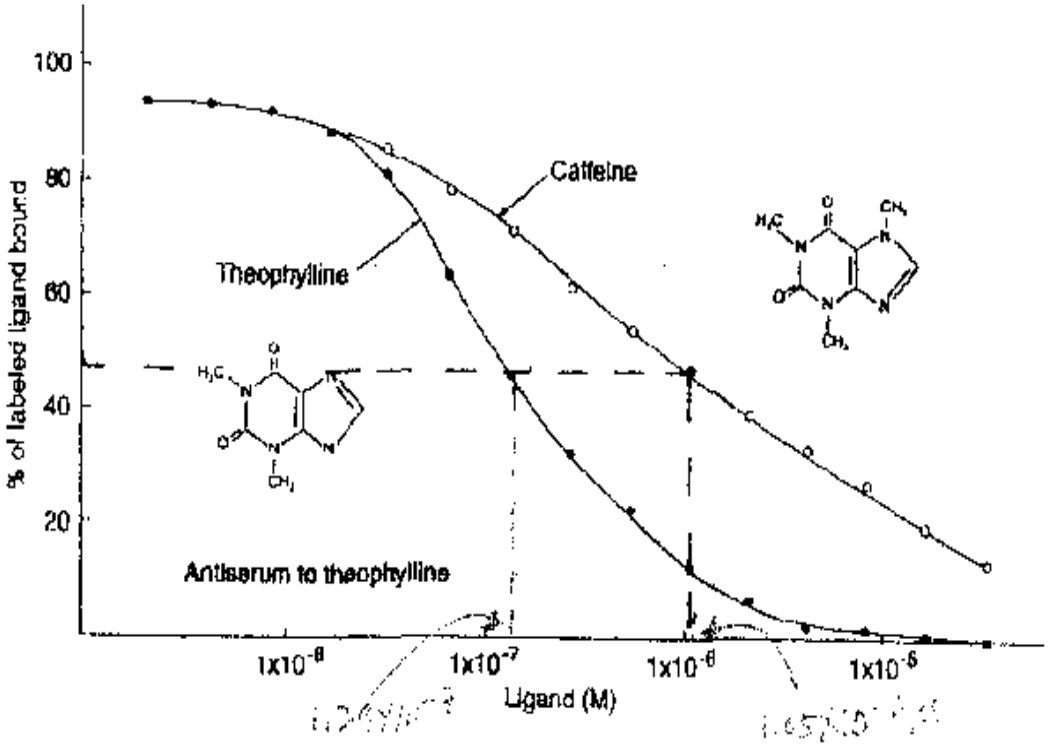


Fig. 13-2 Cross-reactivity of caffeine with a polyclonal antibody to theophylline in a homogeneous fluorescent immunoassay. Cross-reactivity is determined at concentrations of theophylline and caffeine required for 50% of the dose response. This is equivalent to 46.5% of the bound label. Refer to Table 13-7 for cross-reactivity data.

Crossreactivity

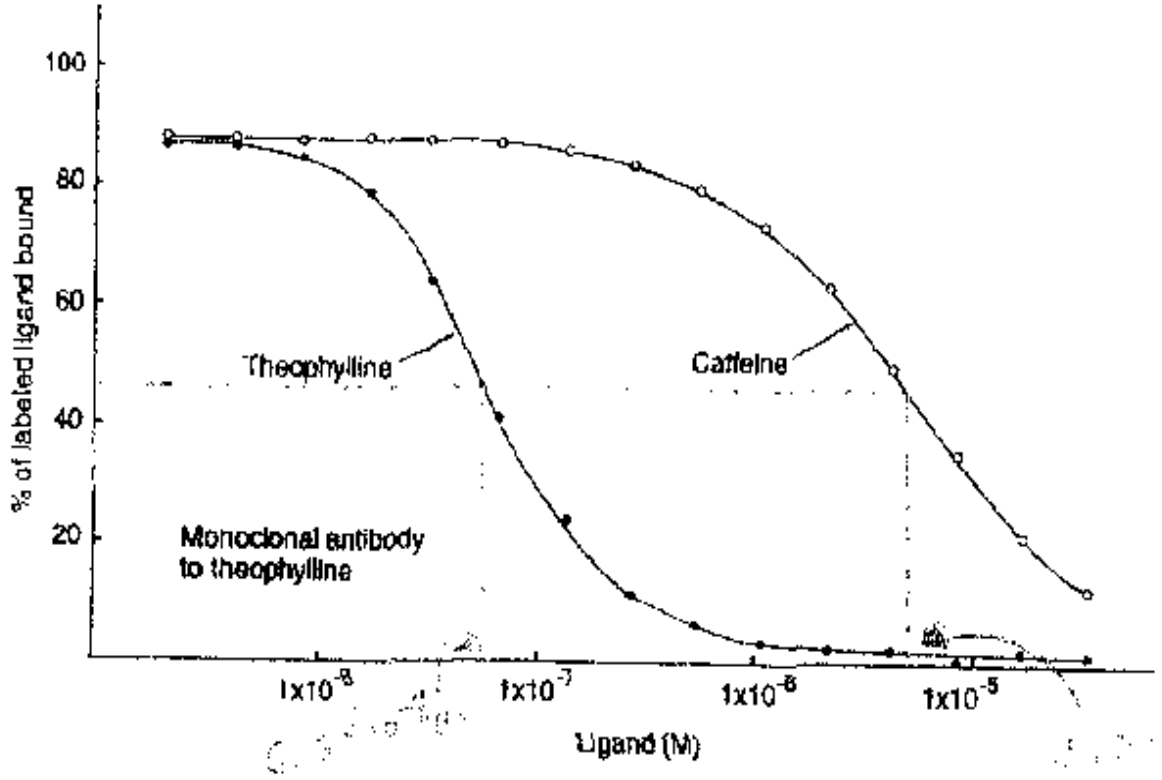
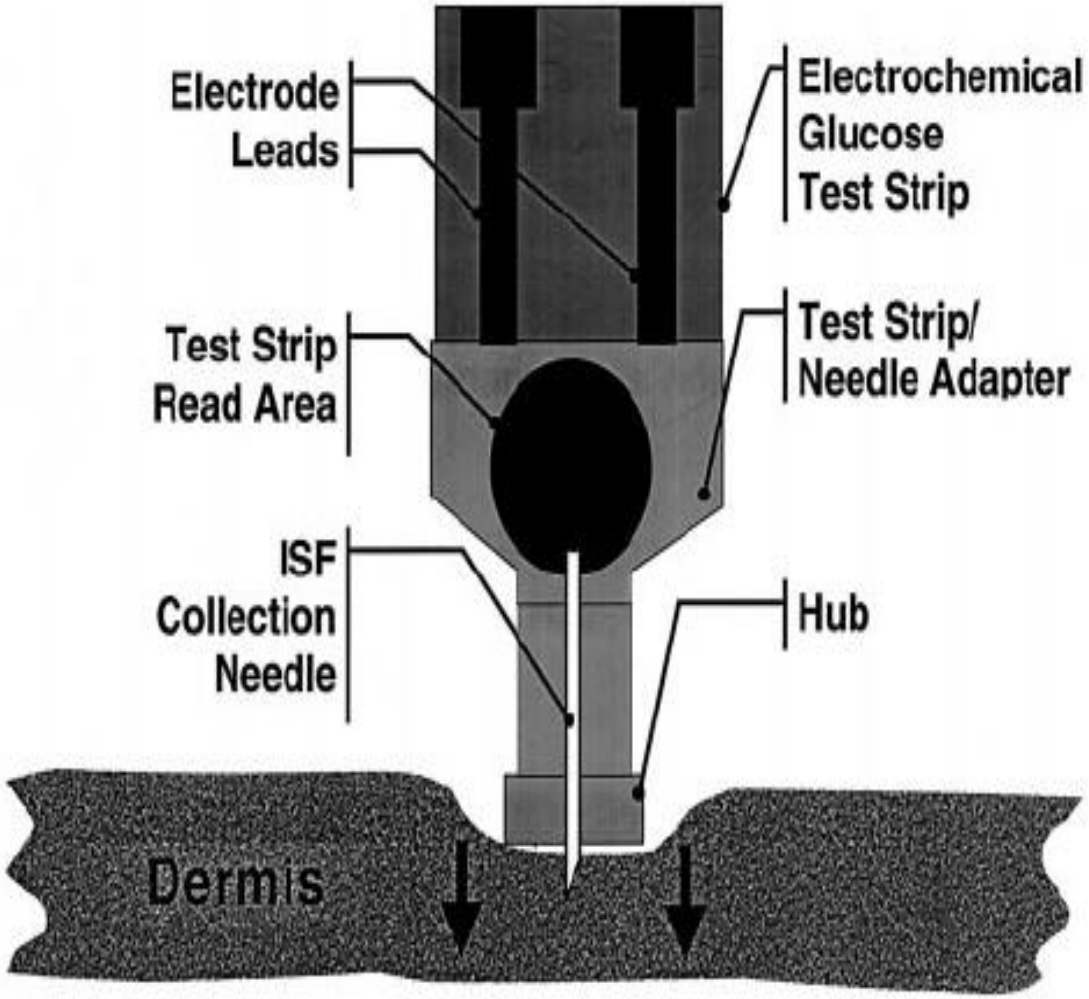


Fig. 13-3 Cross-reactivity of caffeine with a monoclonal antibody to theophylline in the same immunoassay. Cross-reactivity is determined at 43.2% of bound label.

ISF Glucose Monitoring



Interstitial Fluid (ISF) Glucose Monitoring

- Uses a single-use, electrochemical glucose test strip with a 30-gauge needle
  - Permits direct ISF sampling from the dermis
  - Permits real-time sample transfer to the test strip
- Electrochemical test strip that uses controlled working and counter electrodes in combination with glucose oxidase enzyme and ferricyanide electron transfer mediator reagents
- Requires a sample volume between 0.5 – 1.0 microliters

Elusive Goal in Biosensor Research:

***Development of Non-Invasive Transcutaneous Biosensors
for Critical Biochemical Analytes

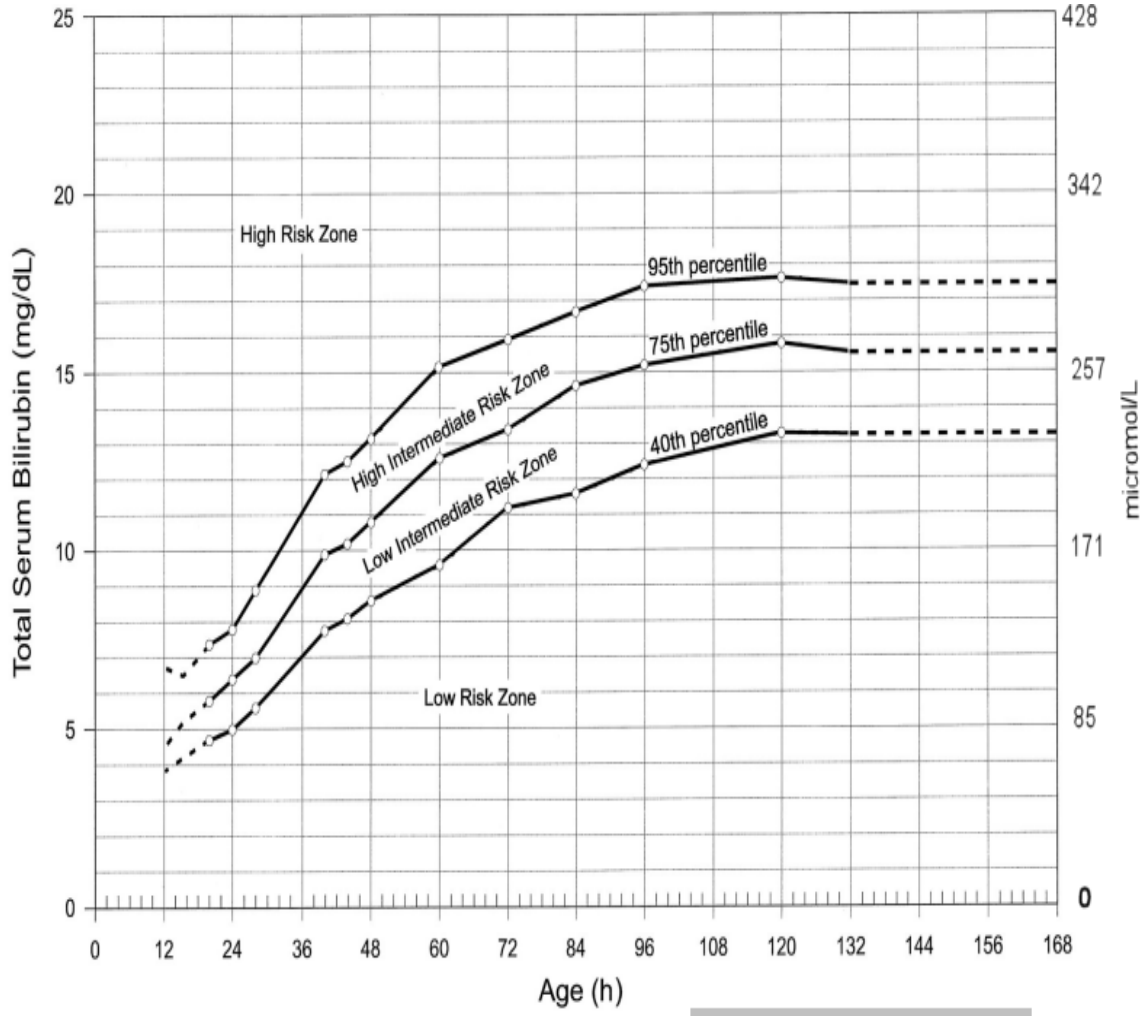
Limited Success To Date:

PO_2 – Partial pressure of oxygen (oxyhemoglobin) in blood

PCO_2 – Partial Pressure of carbon dioxide (carboxyhemoglobin) in blood

Bilirubin

Serum Bilirubin Nomogram

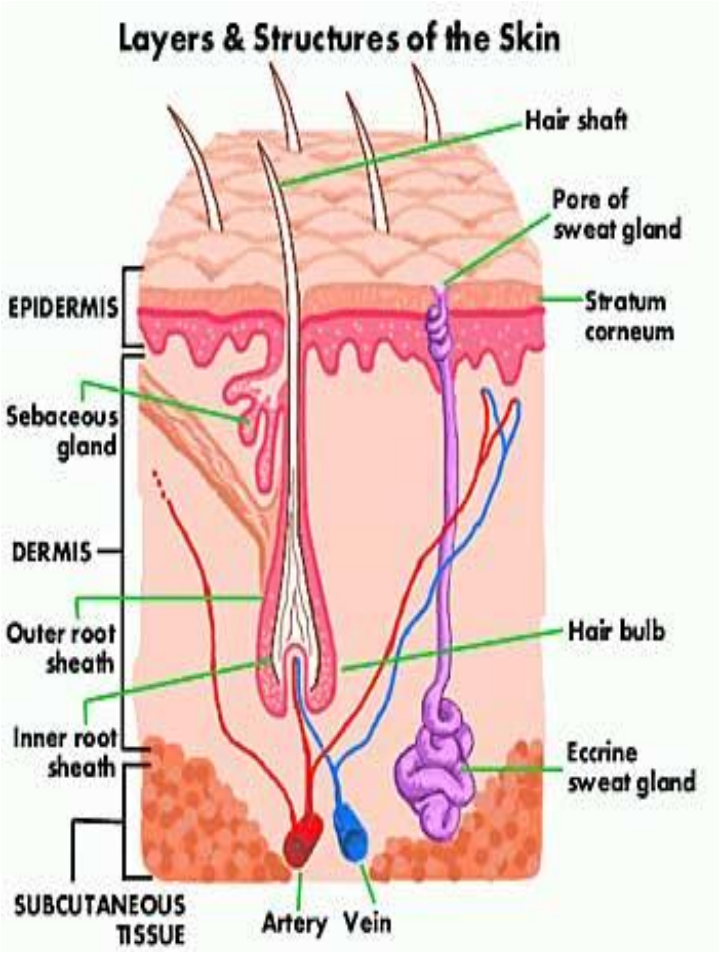


1mg/dL = 17.1 micromol/L

Transcutaneous Bilirubinometer

Transcutaneous Bilirubinometer Factors

- The skin components involved with the spectral reflectance are melanin, dermal maturity, hemoglobin, and bilirubin



Transcutaneous Bilirubinometer

- Non-invasive sensor (Bilimed) to detect neonatal jaundice
- Microprocessor controlled device
- Contains 10 LED's
  - 3 green, 3 yellow, 2 blue, 2 red
- Minimum of two readings for a result
- Skin color is factored into the result (use of different wavelengths)
- Distance between skin and sensor remains constant

Transcutaneous Bilirubinometer Measurement

- Direct light into the neonate's skin
- Measure the intensity of a specific wavelength that is returned
- The meter analyzes the spectrum of optical signal reflected from the subcutaneous tissue
- Optical signals are converted to an electrical signal by a photocell

Phototheraphy

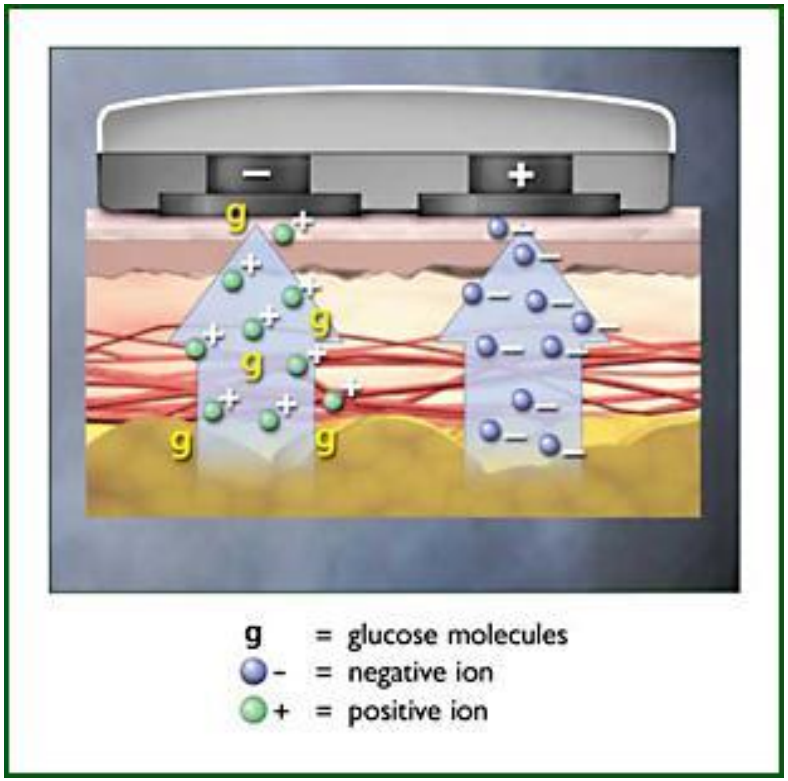
- Ultraviolet light is used to break down bilirubin into a water soluble form in the newborn.
- Note the protective eyewear designed to prevent ultraviolet radiation from damaging the newborn's eyes.

Glucowatch

First attempt at a “non-invasive” transcutaneous glucose measurement

Glucowatch Technology

- Non-invasive form of glucose testing
- Use reverse ionophoresis principle
  - A low current is applied to the skin in order to extract glucose molecules



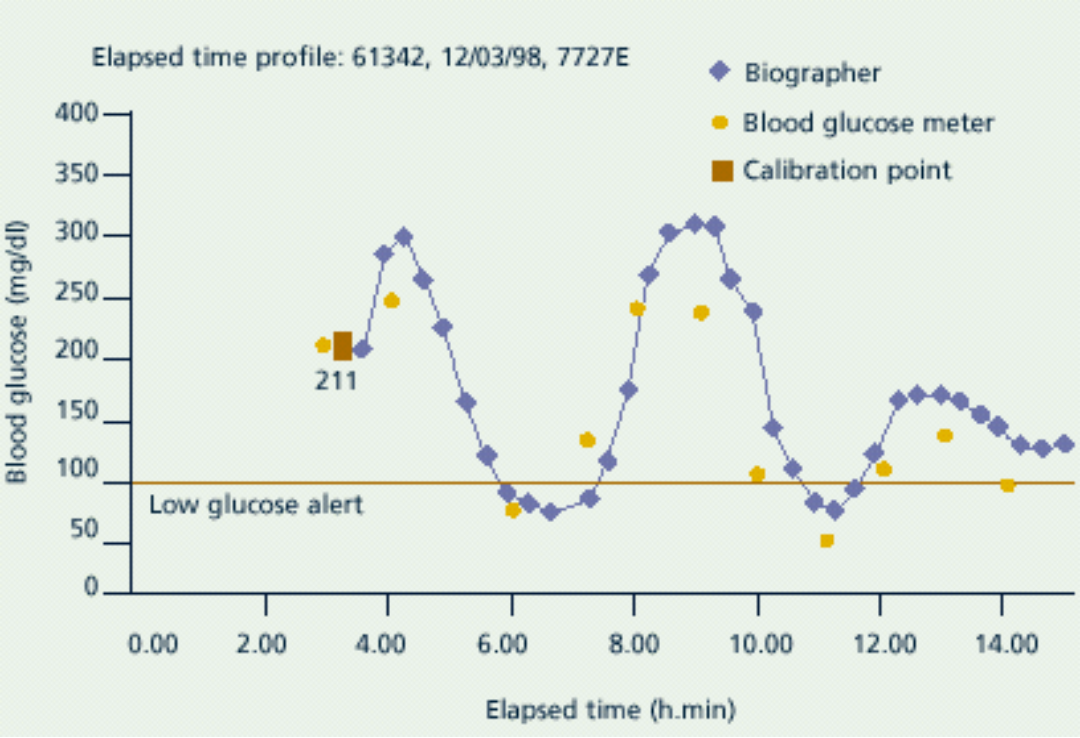
Glucowatch Technology

- Two hour warm up time required
- Sensor takes six glucose measurements per hour for thirteen hours
  - Then, the analytical components must be replaced
- Calibration is performed by correlation to a fingerstick blood glucose concentration
- Designed to supplement a finger stick, not replace it

Due to limitations such as a long lag time between sensor and actual blood glucose, poor sensitivity at low levels, and skin irritation from electrodes

Imprecision

- Measure blood glucose with a conventional meter and a glucowatch
- Compare results of both instruments
- Note differences in results between the two methods.



In Summary

Great Opportunity in developing continuous monitoring non-invasive biose

Trancutaneous
Saliva testing

Targets for Non-Invasive Testing:

Glucose, urea, potassium, hormones (ie. Insulin, thyroxine, cortisol, vit D) e

Critical Factors for Biosensor Development require combining:

Engineers: Chemical, Mechanical, Electrical and Computer Science skills and knowledge

AND

Clinical Laboratory Scientists: Knowledge of important target analytes, limitations of current assay approaches, possible interferences with new sens

Thank You