Biosensors: Selected applications

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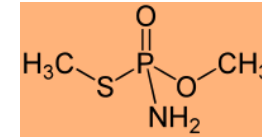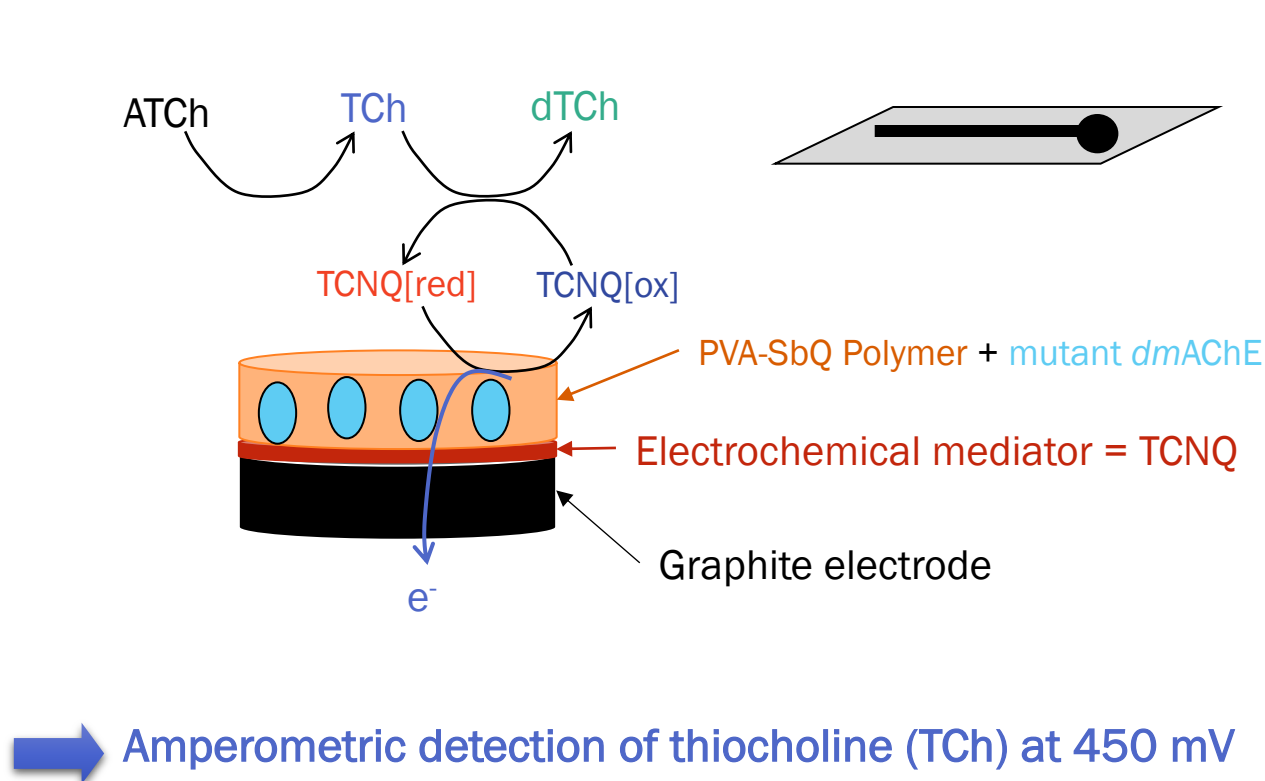Michele.salmain@sorbonne-universite.fr

Electrochemical biosensors

Examples of enzyme biosensors

Amperometric biosensor of methamidophos

Mono-enzymatic biosensor with acetylthiocholine (ATCh) as substrate

ATCh TCh dTCh

TCNQ[red] TCNQ[ox]

PVA-SbQ Polymer + mutant *dm*AChE

Electrochemical mediator = TCNQ

Graphite electrode

e⁻

Pesticide used for protection of rice cultures (China)

Biosensor response to Methamidophos

Amperometric detection of thiocholine (TCh) at 450 mV
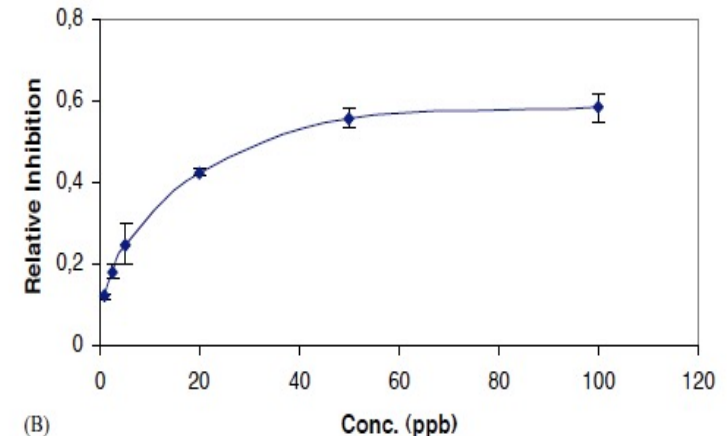
relative inhibition = $(I_0-I)/I_0$

Enzymatic biosensor for blood glucose assay

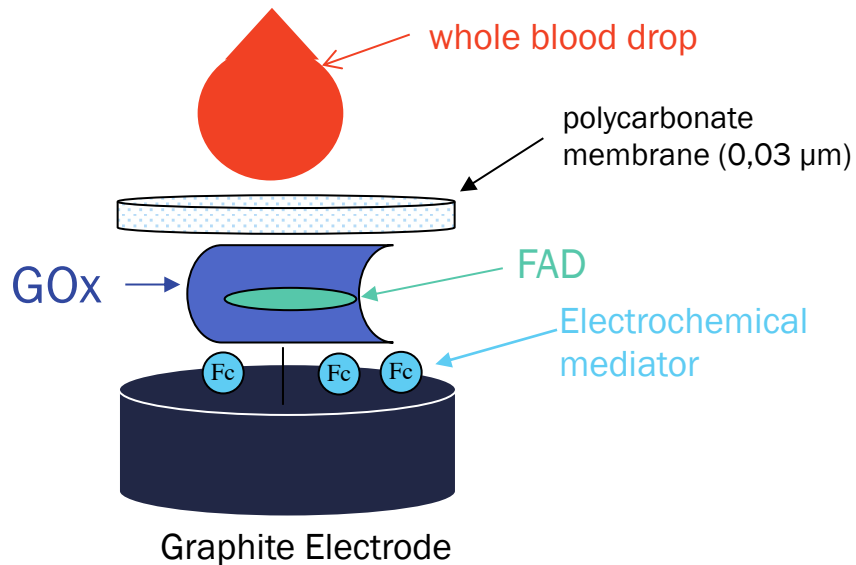Diabetes is a disease that affects a high number of people in the western world

Endocrine disease; glucose metabolism issue; results in high blood sugar levels which in turn results in other serious pathologies

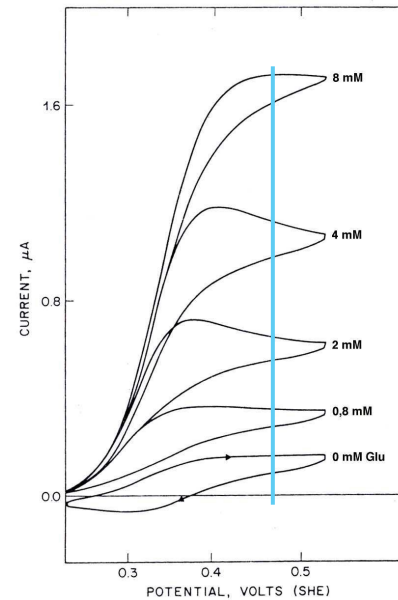Importance of blood sugar monitoring to optimize treatment

Self-control of blood sugar level by the patient with a simple device (« point-of-care testing »)
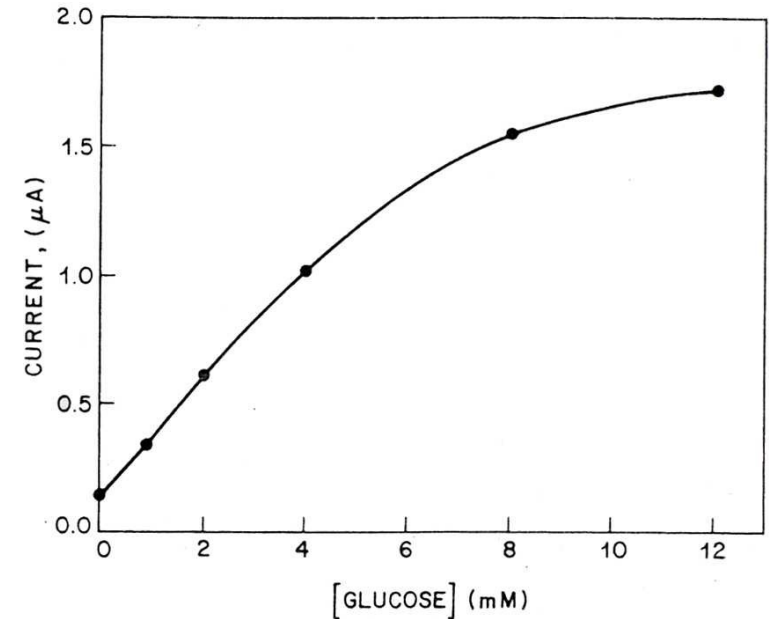
Assay of blood glucose by amperometric biosensor

ExacTech (Medisense) was marketed in 1987 on the basis of a work published in the scientific literature in 1984

whole blood drop

polycarbonate membrane (0,03 µm)

GOx

FAD

Electrochemical mediator

Fc   Fc   Fc

Graphite Electrode

cyclic voltammograms

8 mM

4 mM

2 mM

0,8 mM

0 mM Glu

CURRENT, µA

POTENTIAL, VOLTS (SHE)

Calibration curve

CURRENT, (µA)

[GLUCOSE] (mM)

Commercial devices

Lifescan OneTouch

1 ul sample of total blood

Measurement time 5 s

Range : 20 - 600 mg/dl

Accu-chek Aviva (Roche)

enzyme = glucose-1-dehydrogenase

cofactor = PQQ (pyroquinoline quinone)
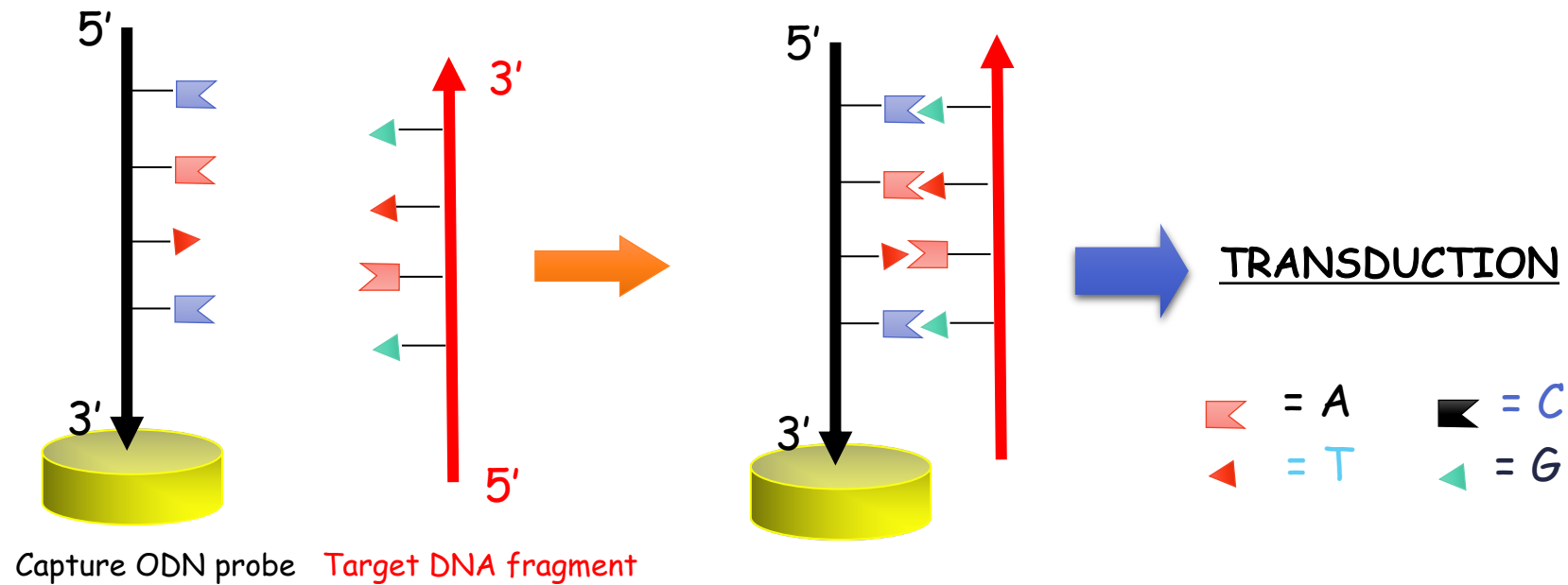
0.5 ul sample of total blood

Measurement time 5 s

range: 10 - 600 mg/dl

Optical biosensors

Examples of genosensors

Genosensors

Association (=hybridization) between capture ODN probe (= bioreceptor) immobilised
on the transducer and target DNA (= analyte)
Specificity brought by base complementarity

Capture ODN probe   Target DNA fragment

TRANSDUCTION

= A   = C
= T   = G

DNA chips (gene chips)

Solid support (glass or silicon) covered with DNA sequences as microspots

Capture DNA sequences are spotted on the solid support or synthesized directly on the support (glass or silicon)

Up to 1 million different capture probes on each chip

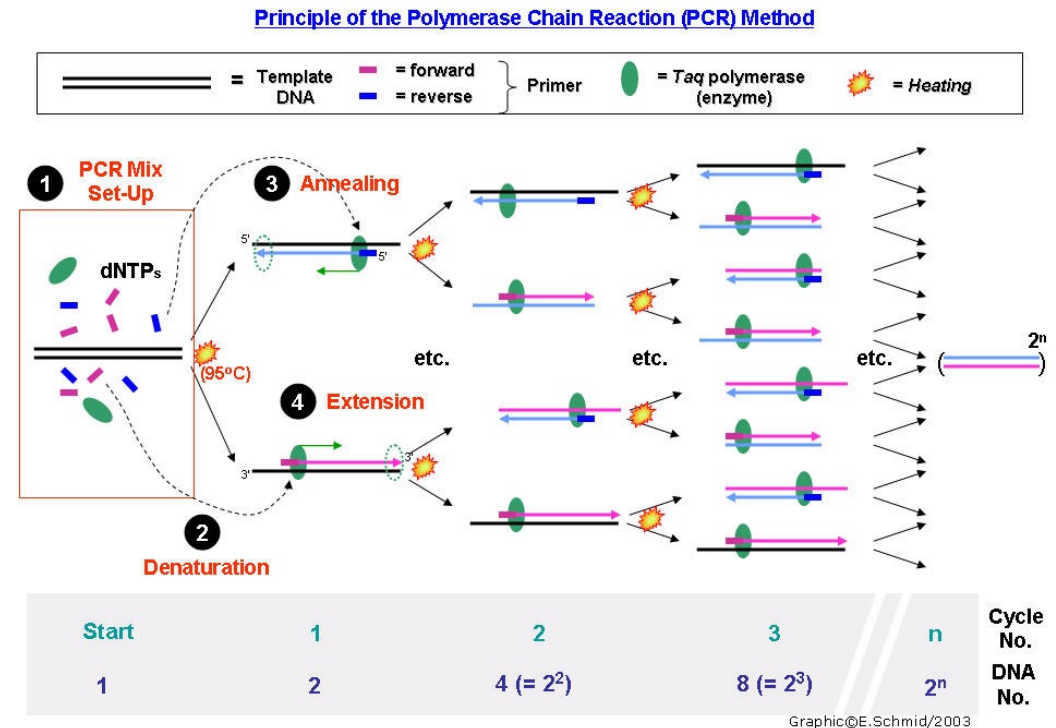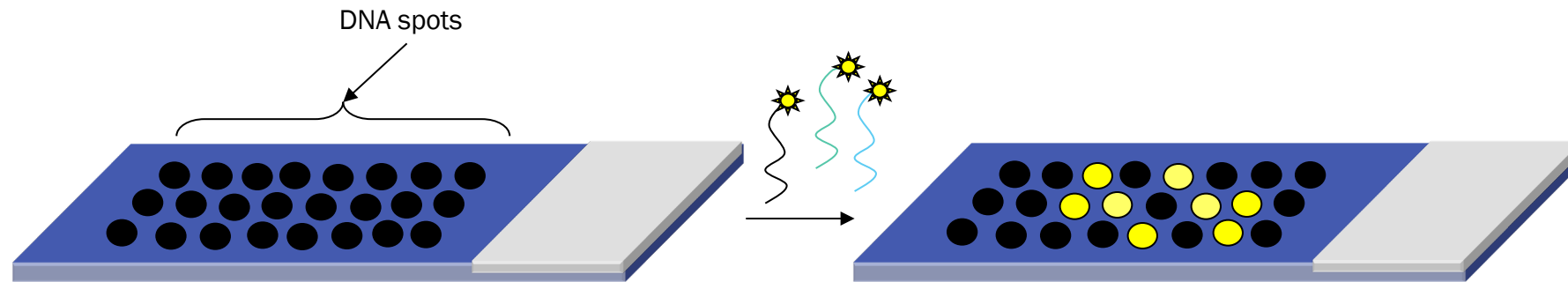https://www.youtube.com/watch?v=eSr5CxAdiww

DNA samples

- DNA extraction from cell samples

- mRNA extraction and inverse transcription with reverse transcriptase and dNTP -> cDNA

- Polymerase chain reaction (PCR) : amplification of DNA fragments

Mixture of DNA polymerase + dNTP + sense and anti-sense primers; denaturation cycles, primer annealing and extension
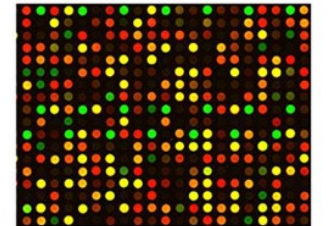
One of the strands can be preferentially amplified by asymmetric PCR (by playing on the relative proportion of the 2 primers)
Probes (labels) can be introduced during the PCR process by using labeled dNTP

Principle of the Polymerase Chain Reaction (PCR) Method

| | = Template DNA | | = forward | | Primer | | = Taq polymerase (enzyme) | | = Heating |

Cycle No.	Start	1	2	3	n
DNA No.	1	2	4 (= 2^2)	8 (= 2^3)	2^n

Graphic©E.Schmid/2003

Optical transduction

DNA spots

Target DNA is labeled with a **fluorophore** (during PCR)

The mixture of labeled target DNA fragments is deposited on the chip to allow **hybridization to occur**

After washing, the surface is analyzed by a **scanner**

➡ The spots where hybridization took place are bright under illumination

Agilent Scanner

DNA Microarray

Applications

- ✓ Detection of genetic suceptibility to diseases (cystic fibrosis, thrombosis, Alzheimer's disease, cancers) = <u>genotyping</u>

- ✓ Gene expression profile (= transcriptional <u>profile</u>)

- ✓ Detection of DNA variants resulting in variable responses to therapy (= <u>pharmaco-genomics</u>)

- ✓ Analysis of gene modifications in cancer cells

- ✓ Detection of pathogens associated to infectious diseases

- ✓ Detection of pathogenic agents in food or environment

- ✓ Detection of GMO in cultures

- ✓ Detection of biological weapons

Pharmacogenomics

AmpliChip CYP450 – Roche diagnostics

The family of cytochrome P450 enzymes is involved in the metabolism of xenobiotics (drugs) which conditions their therapeutic activity (when they are rapidly metabolized they are less active).

The AmpliChip CYP450 test allows genotyping of **genes coding for 2 CYP450** from human DNA samples so as to predict their enzymatic activity for a given patient. 29 polymorphisms of the CYP2D6 gene and 3 of the CYP2C19 gene can be differentiated.
It provides the clinician with a tool to choose the treatment and the most appropriate dose to be applied to each patient.

Applications in psychiatry

Food analysis

Affymetrix – Biomérieux Collaboration

FoodExpert-ID allows to assess the composition in animal species and the authenticity of food and feed, raw or transformed. The test is based on a high density DNA chip (GeneChip®) developed by Affymetrix, comprising 80 000 ODN probes complementary of gene fragments coding for cytochrome b.

The unique feature of FoodExpert-ID lies in the capacity to detect 33 different species of vertebrates and to simultaneously identify the presence of animal products in food samples at 2 levels of taxonomy : at the class level (mammal, bird or fish) and at the species level (beef, chicken, salmon).

It requires amplification by PCR prior to analysis

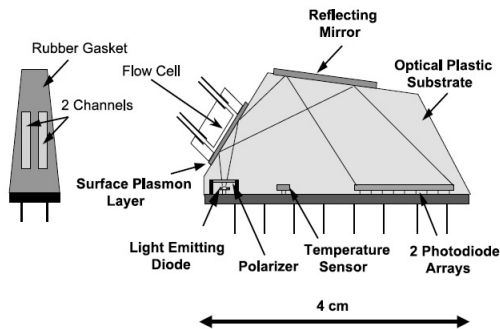# Label-free optical biosensors

Examples of immunosensors

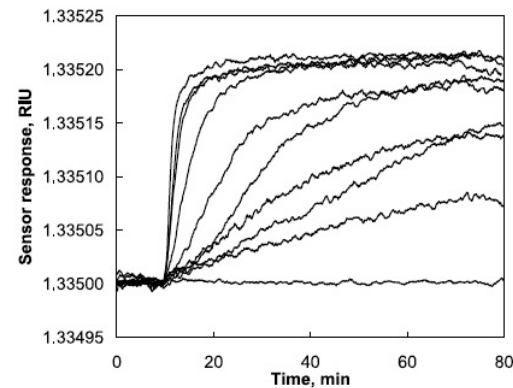# Assay of *S. aureus* enterotoxin B (SEB) by SPR with SPREETA
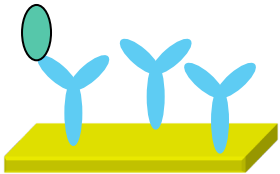
2-channel sensor : covered with polyclonal anti-SEB antibody and non specific antibody

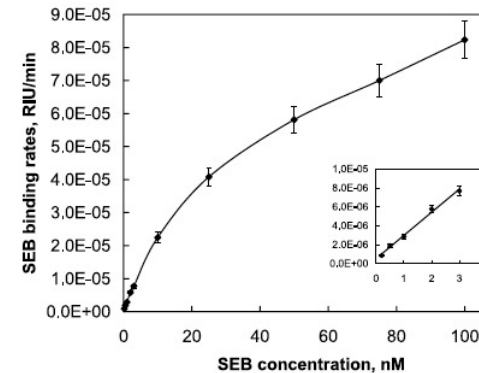Samples flown at 40 ul/min; sensor regeneration with 100 mM glycine pH 2

Sensor scheme
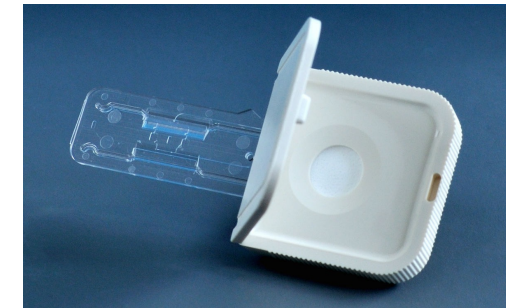

RIU vs time for various SEB concentrations

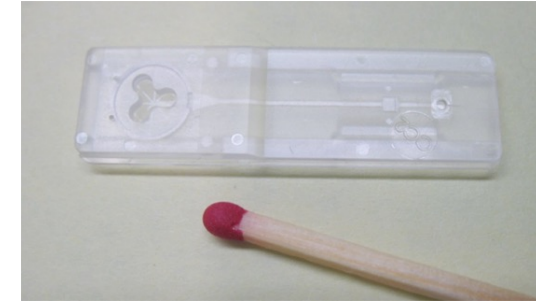**standard curve**

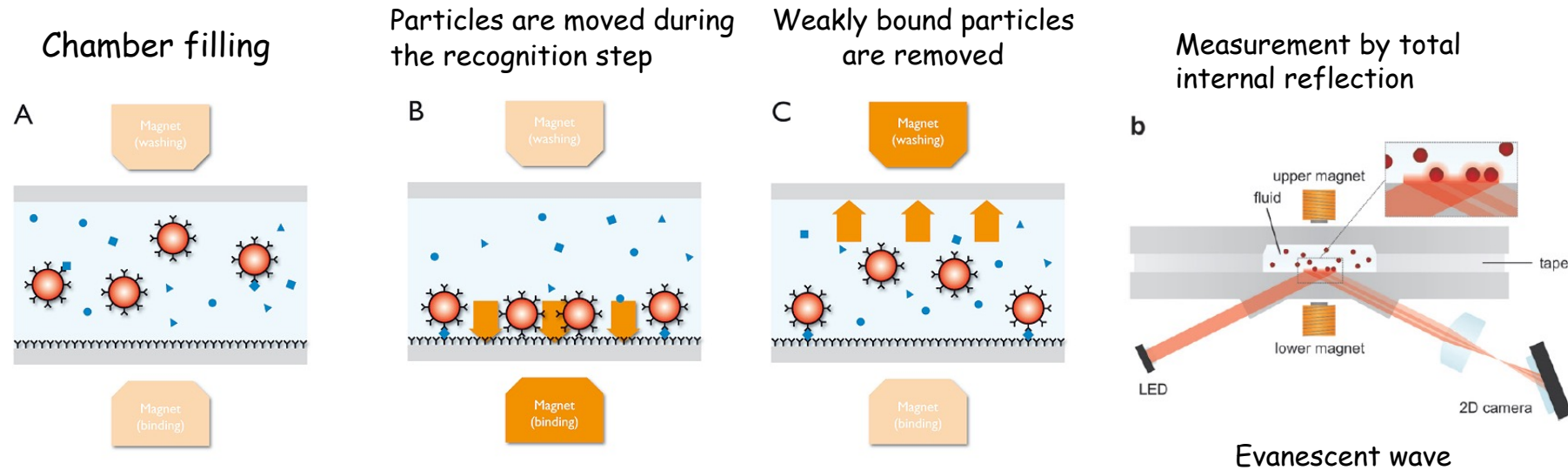Sensor response to SEB 5.6 -> 2100 ng/ml

Minicare I-20 – Philips diagnostics

- ✓ Immunosensor with optomagnetic transduction
- ✓ Application : biomedical area
- ✓ No manipulation of fluid other than sample
- ✓ Portable reader
- ✓ Single-use cartridge
- ✓ Fast measurement (10 min)

Principle

The process steps are controlled by electromagnets placed above and below the cartridge
Super-paramagnetic nanoparticles (500 nm) covered with capture antibody; chamber (1 µl) covered with revelation antibody

Chamber filling

Particles are moved during the recognition step

Weakly bound particles are removed

Measurement by total internal reflection

Evanescent wave

https://www.youtube.com/watch?v=vzytqmmLvec
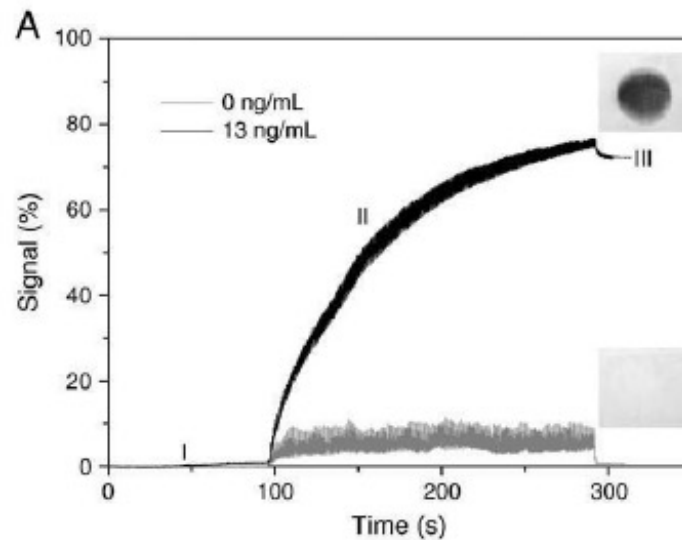
Cardiac troponin analysis

Marker of cardiac infarct
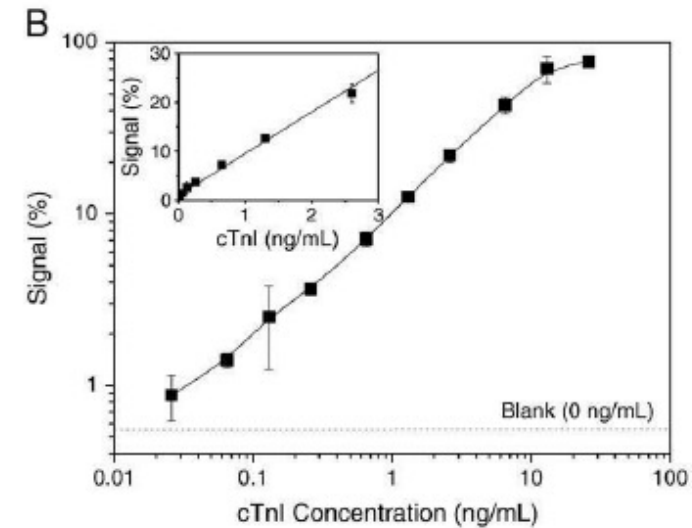Test on 25 µl of blood (taken on finger)
Rapid Test : 10 min
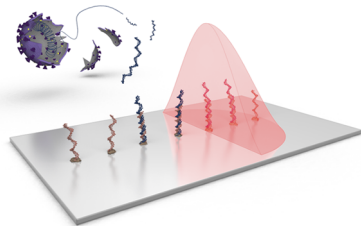Sensitive Test : limit of detection 0.03 ng/ml

Real-time measurement

standard curve

Diagnostic tests related to SARS-CoV 2

A Viral genomic analysis

Reference method: qRT-PCR (RNA –> cDNA then qPCR
Primer hybridization to viral RNA + amplification by PCR

https://www.youtube.com/watch?v=Vd38iS_W7ww

B Direct virus detection
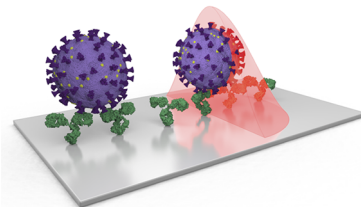
Recognition element = antibody
Specific detection of viral protein (N protein)
Lateral flow assay, answer in 15 min

https://diagnostics.roche.com/global/en/products/params/sars-cov-2-rapid-antigen-test.html

C Serology assay

Recognition element = viral antigen (N and S)
Specific detection of antibodies produced by host
Lateral flow assay; answer in 15 min from blood sample

Example of optical biosensor for SARS-CoV-2

- Sample Collection
- SARS-CoV-2 virus
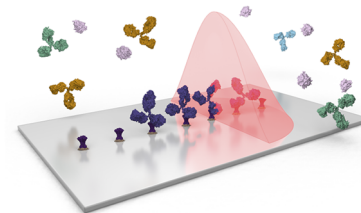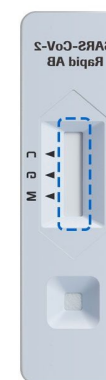- Viral RNA extraction
- Aggregated AuNPs
- Agglomerated AuNPs
- ASO capped AuNPs
- Viral RNA addition
- Selective 'naked-eye' detection of positive COVID-19 cases
- Precipitation
- RNaseH 5 min incubation at 65 °C
- 5 min incubation
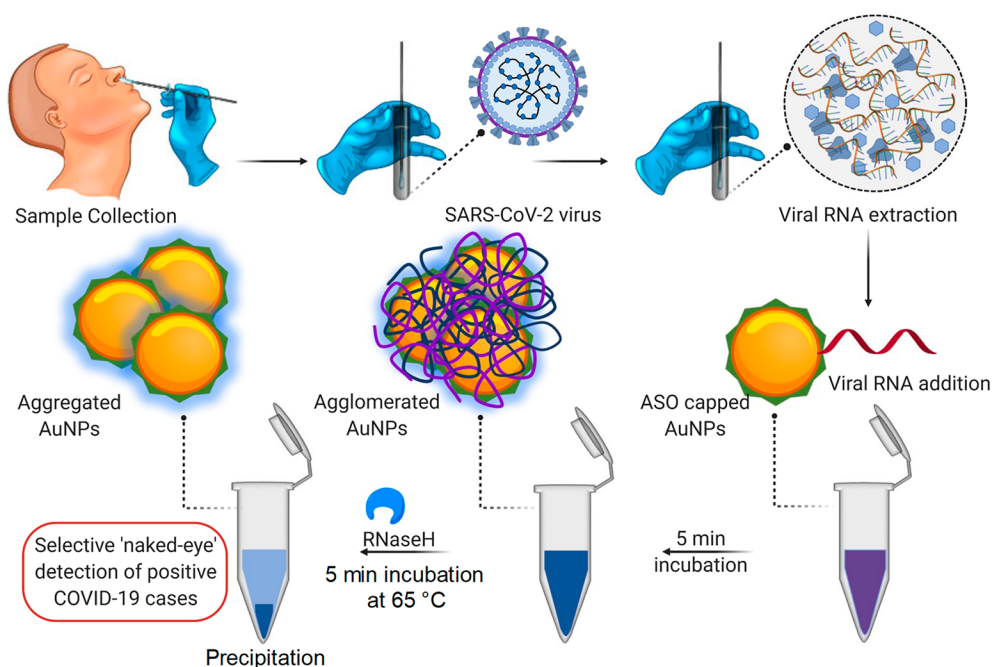
- Colorimetric—based, solution phase biosensor to detect gene coding for N protein

- Colloidal suspension of gold nanoparticles capped with 4 ≠ oligonucleotides complementary to target gene

- Addition of viral DNA causes particle agglomeration resulting in a change of color from violet to dark blue that can be visually detected within 10 min

Limit of detection = 0.18 ng/ml (Abs @ 660 nm)
Dynamic range = 0.2 – 3 ng/ml

Ref: Moitra et al. ACS Nano 2020, 14, 7617

Market studies

Global market, technologies, applications,
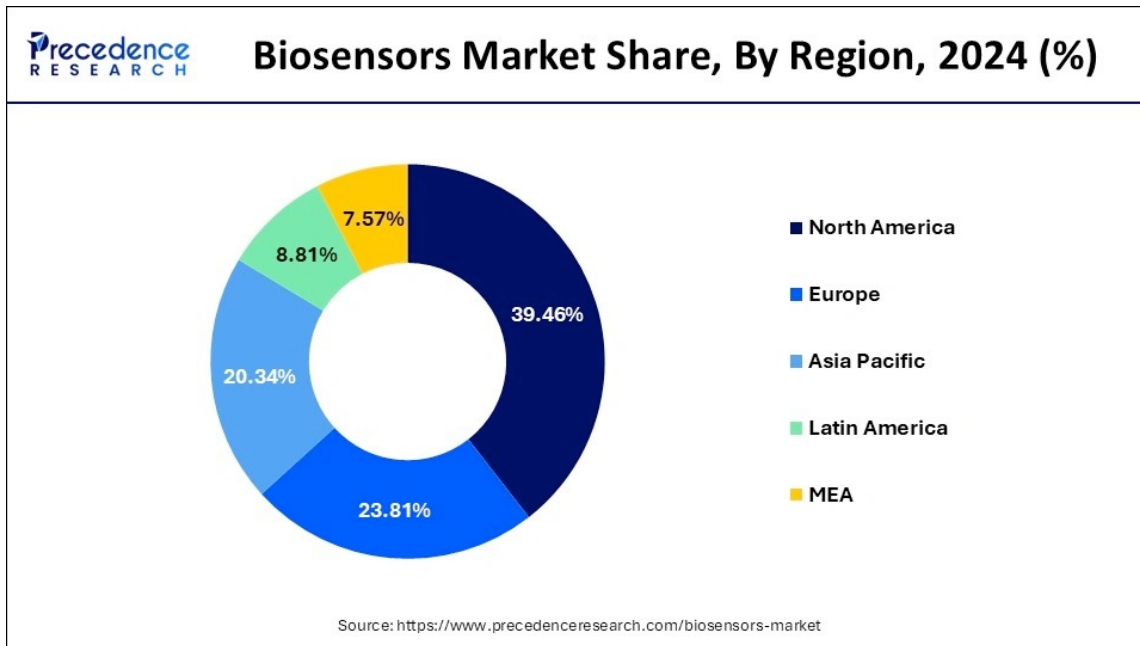
Market study

Global market : 18.2 billion $ in 2018 and 33.2 billion $ in 2025

Precedence RESEARCH

Biosensors Market Share, By Region, 2024 (%)

- North America — 39.46%
- Europe — 23.81%
- Asia Pacific — 20.34%
- Latin America — 8.81%
- MEA — 7.57%

Source: https://www.precedenceresearch.com/biosensors-market

Expected fastest rate of development in Europe:
- High number of cases of diabetes
- Growing prevalence of life related diseases
- Population getting older

- Growing need for non-invasive diagnostic tools
- Increased fundings for startups and research institutions
- Environmental monitoring, food safety and chronic disease management
- More stringent regulations

Market study

Biosensors Market Share, By Technology, 2024 (%)

Precedence RESEARCH

Thermal	Electrochemical	Piezoelectric	Optical
7.48%	70.09%	5.61%	16.82%

Source: https://www.precedenceresearch.com/biosensors-market

Biosensors Market Share, By Application, 2024 (%)

Precedence RESEARCH

Medical	Food Toxicity	Bioreactor	Agriculture	Environment	Others
65.79%	4.19%	6.57%	10.39%	8.77%	4.30%

Source: https://www.precedenceresearch.com/biosensors-market

Optical biosensors are emerging as the fastest growing technology due to their advanced capabilities in real-time, label-free detection.

They are increasing being adopted in medical diagnostics, environmental monitoring, and food safety testing owing to their non-invasive nature and miniaturization potential.

Furthermore, the integration of optical biosensors with smartphones and wearable health devices is enabling remote diagnostics and personal health monitoring,
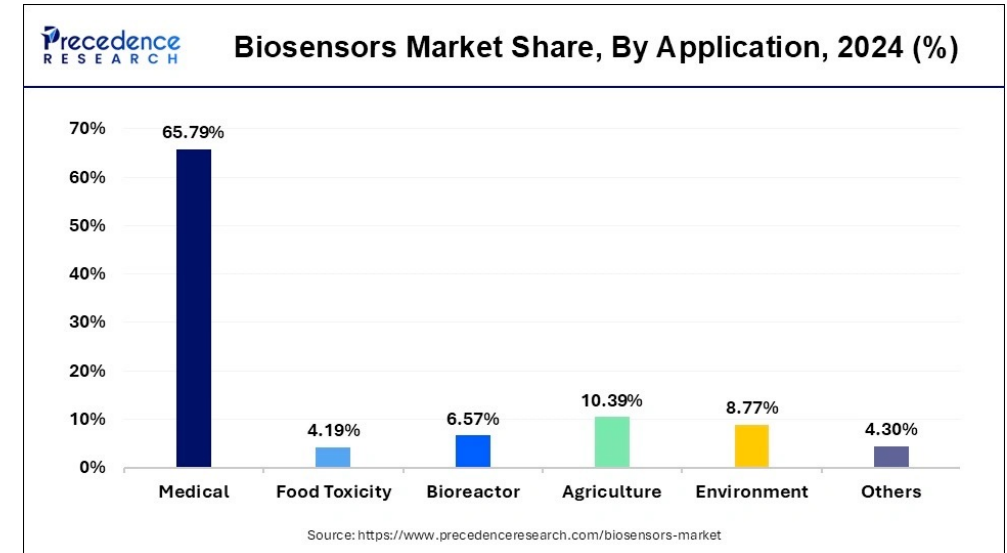
The agricultural sector is expected to witness the most rapid growth

➢ Real-time monitoring of soil health, crop conditions, and pathogen presence.
➢ Detection of contaminants and toxins in agricultural produce, to assure food safety from farm to table

Market study

Biosensors Market Share, By End User, 2024 (%)
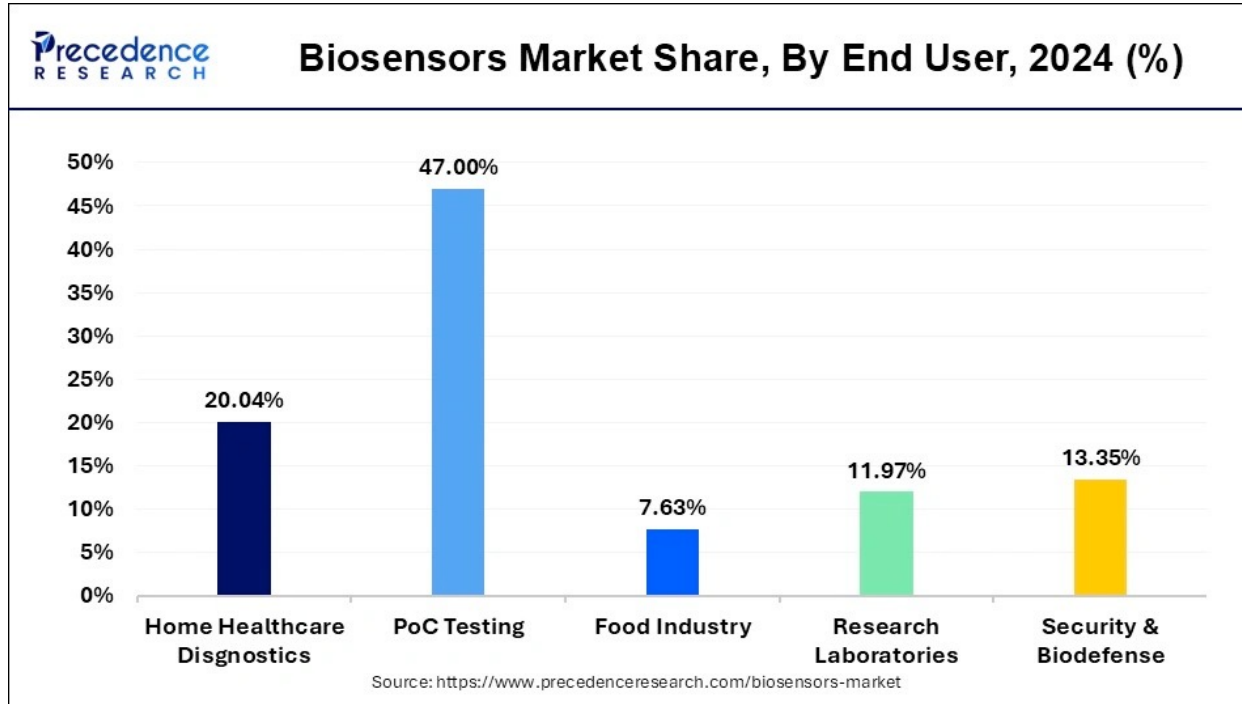
PoC = point of care

The food industry is rapidly adopting biosensors for applications across safety testing, quality assurance, and supply chain monitoring.

The manufacturers are prioritizing the detection of pathogens, allergens, and chemical contaminants in food products.
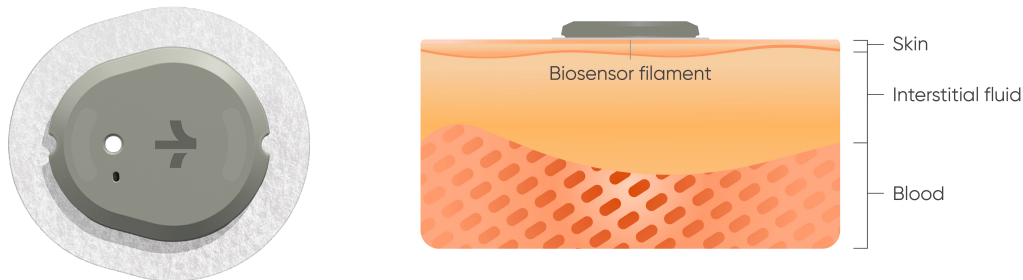
Current trends in biosensor development

Point-of-care testing systems

- miniaturization
- use of smartphone for readout
- Lab-on-chip technology

Wearable biosensors

Ex. Stelo continuous glucose biosensor

The filament that sits under the skin is coated with glucose oxidase. When the glucose oxidase enzyme comes into contact with glucose in the interstitial fluid (the fluid surrounding cells), glucose is converted to gluconic acid and hydrogen peroxide, which generates an electrical signal due to electron transfers that occur as a result of this chemical reaction. This signal communicates the level of glucose molecules in the blood, giving a reading of glucose levels.

The sensor transmits glucose levels to the patient's phone so he can see his levels 24/7, and a graph shows how the blood glucose fluctuates throughout the day.

Conclusions

Biosensors are currently making a breakthrough in the analysis of traces in various matrices and in several application domains

Their development takes advantage of discoveries in molecular biology but also in electronics and miniaturization

They are well suited for rapid analyses in difficult contexts or operated by non qualified manipulators for which the smallest possible number of manipulations is required