Biosensors in the meat chain continuum as a tool for animal health, food safety, food quality and food crime control

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Food on a Global Market - Opportunities and Threats

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1. Introduction – biosensors in the meat chain

1962 – first biosensor (glucose detection)

Platinum-oxygen electrode & glucose oxidase enzyme

(Clark and Lyons, 1962)

1975 – first practical application (glucose concentration)

Springs Instrument Company (USA)

1987 – first widely commercially available biosensor (glucose concentration)

1997 – IUPAC gives first definition:

"Device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signal".

Applications:

- Animal breeding
- Plant cultivation
- Food processing
- Food trade

1. Introduction – biosensors in the meat chain

- Meat chain composed of several modules
  (farm, abattoir, processing, distribution, retail)

- Consumers` awareness

✓animal health

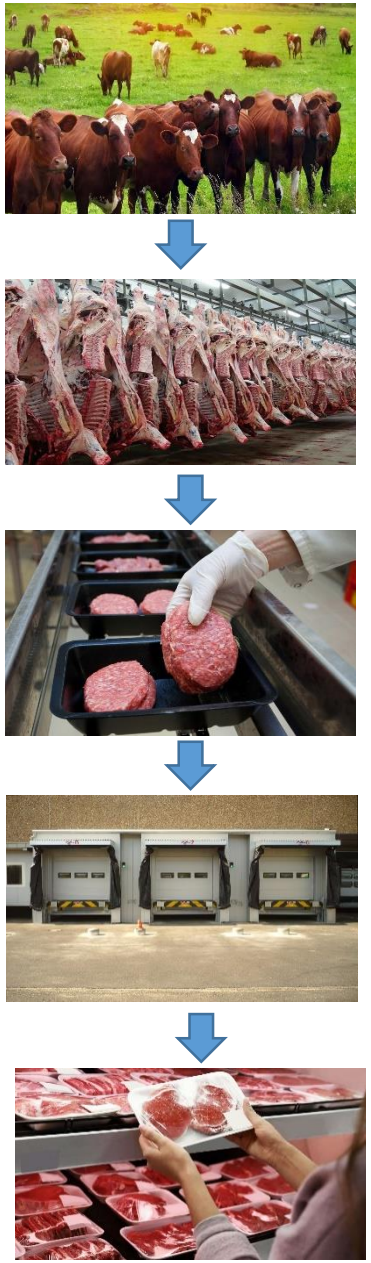
✓animal welfare

✓meat safety & quality

✓food labelling (food fraud)

✓bioterrorism

- Healthy animals essential precondition for a safe meat supply



- Zoonotic food (meat) borne pathogens
  (*Salmonella*, *Campylobacter*, *L. monocytogenes,* HP-STEC, *Yersinia*)

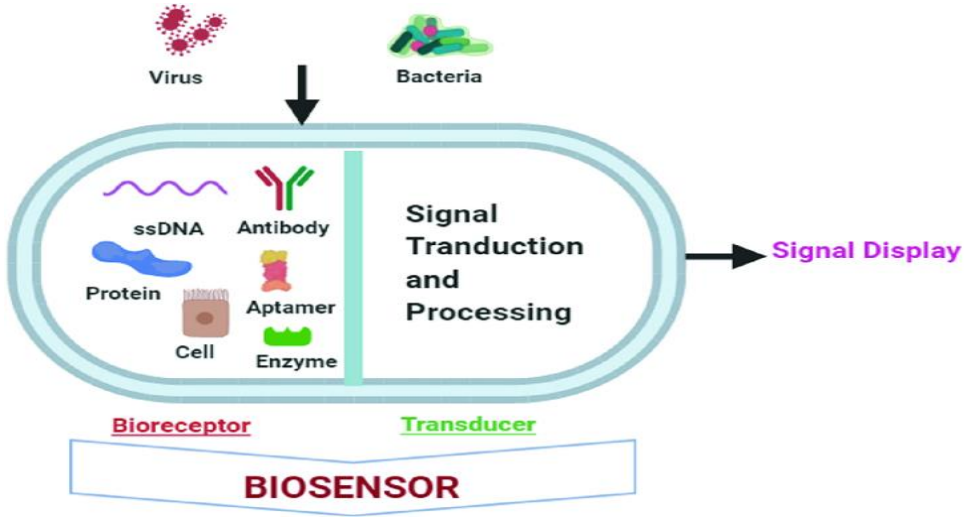
- Early detection of animal health, animal welfare and major food borne hazards of relevance for public health

- **Biosensors**

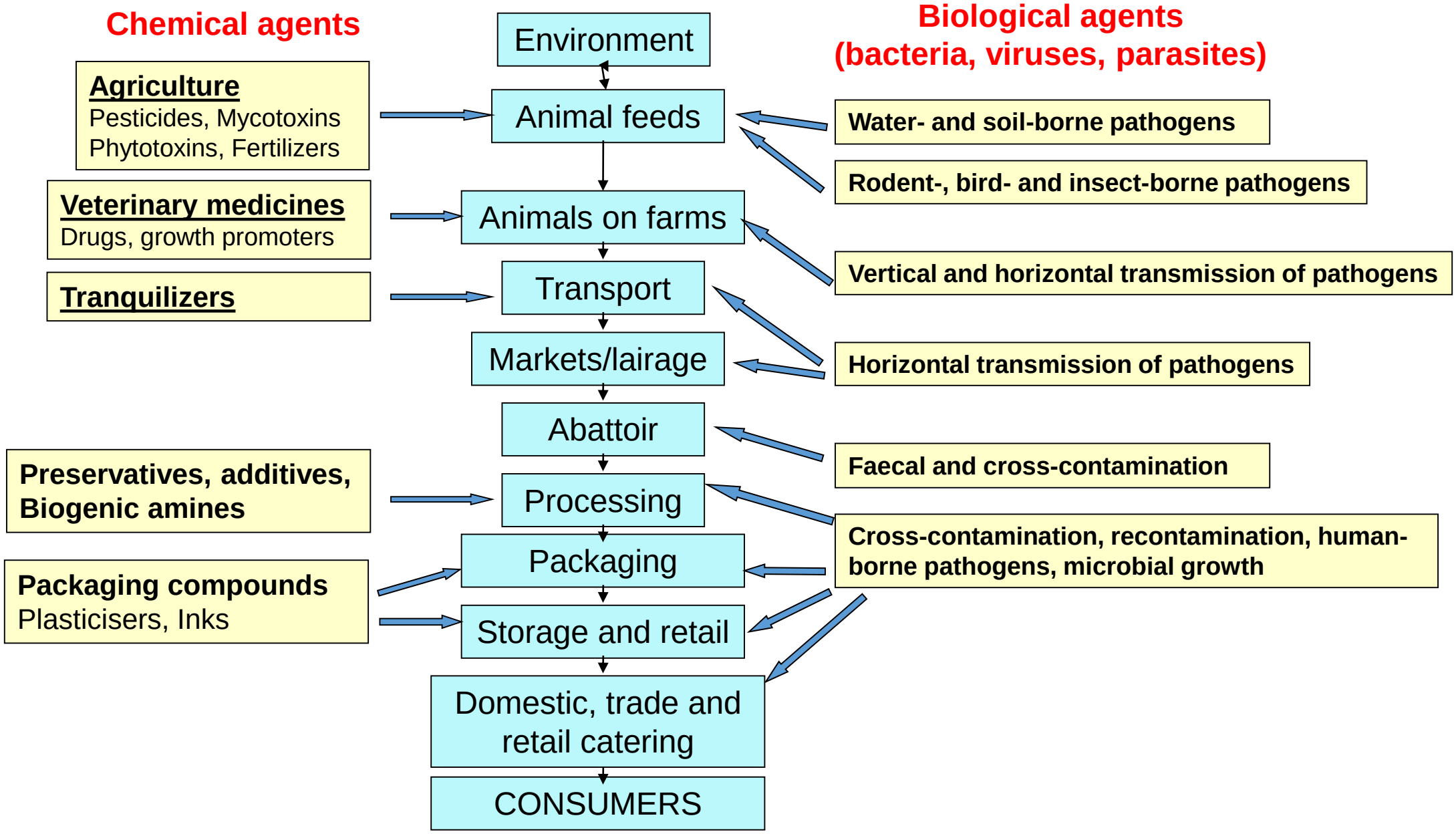
✓require little sample preparation

✓provide rapid (quantitative) detection of major pathogens and meat species

✓tool for effective food safety/food defence management.



Meat (food) chain and associated hazards



2. Manufacturing and design of biosensors

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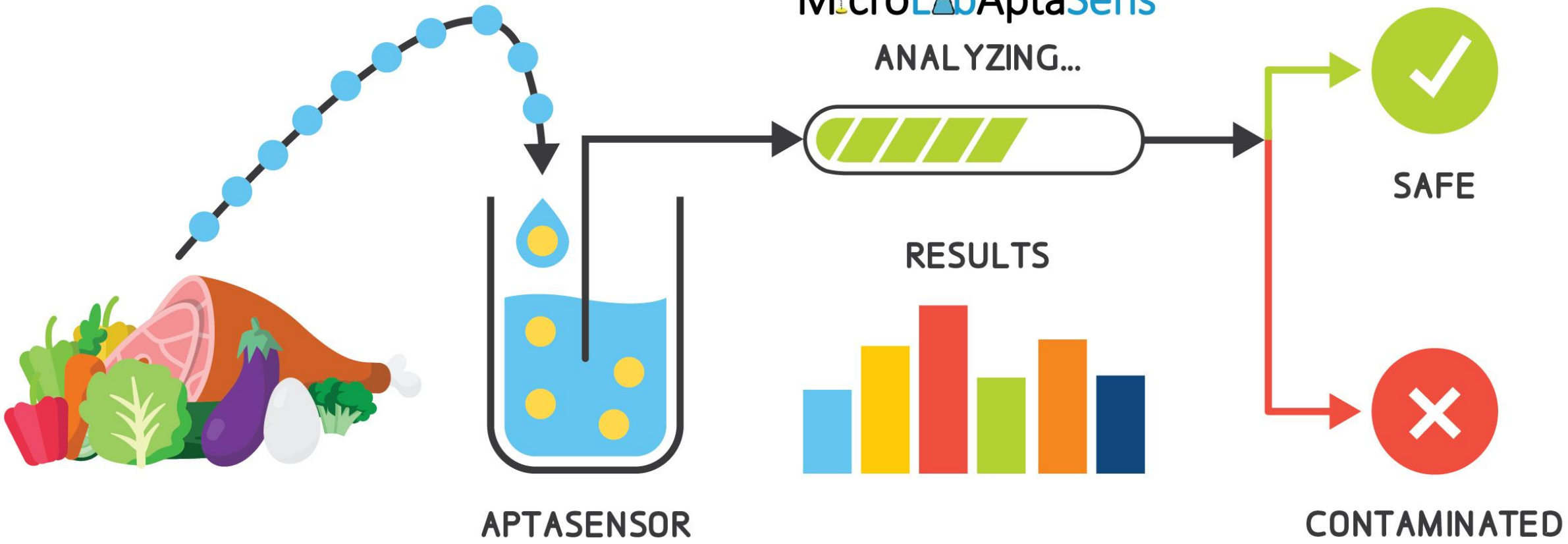


Biotechnology - Biosensors

Biosensor technology (BsT): the fastest-growing area of the modern science in recent years

- **Biosensors**, a **powerful alternative to conventional methods**

✓ culture techniques
✓ serological tests
✓ immunoassays

require costly lab equipment, trained personnel, and special conditions,

Biosensors: low-cost portable devices with good specificity and selectivity.

- **Current BsT research**

✓ **integration** of biosensors with microfluidic (MF) devices, optics, electronics, and signal readouts - Lab-on-a-Chip (LoC) concept

✓ **increase the biosensing capacity** and develop diagnostic tools - ASSURED criteria [Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Deliverable] *(Source: WHO)*

Biosensors: applications and fields

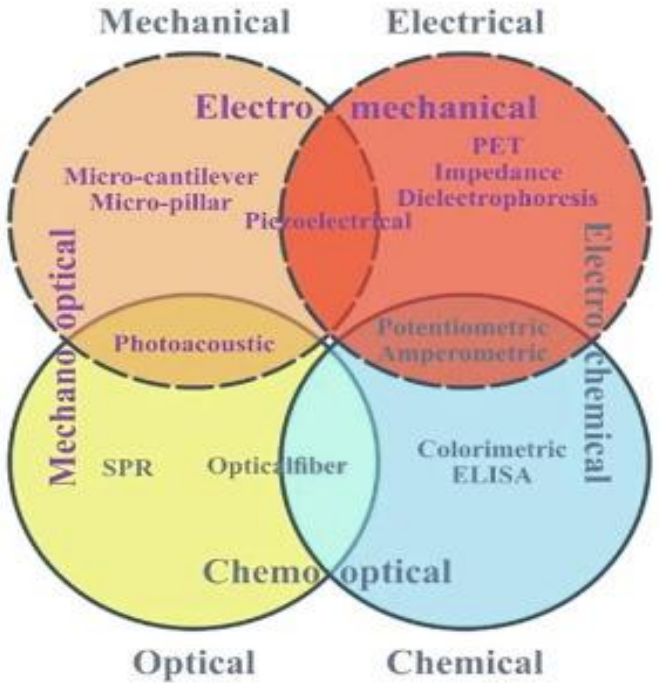
- **Biosensors categorized** into different fields

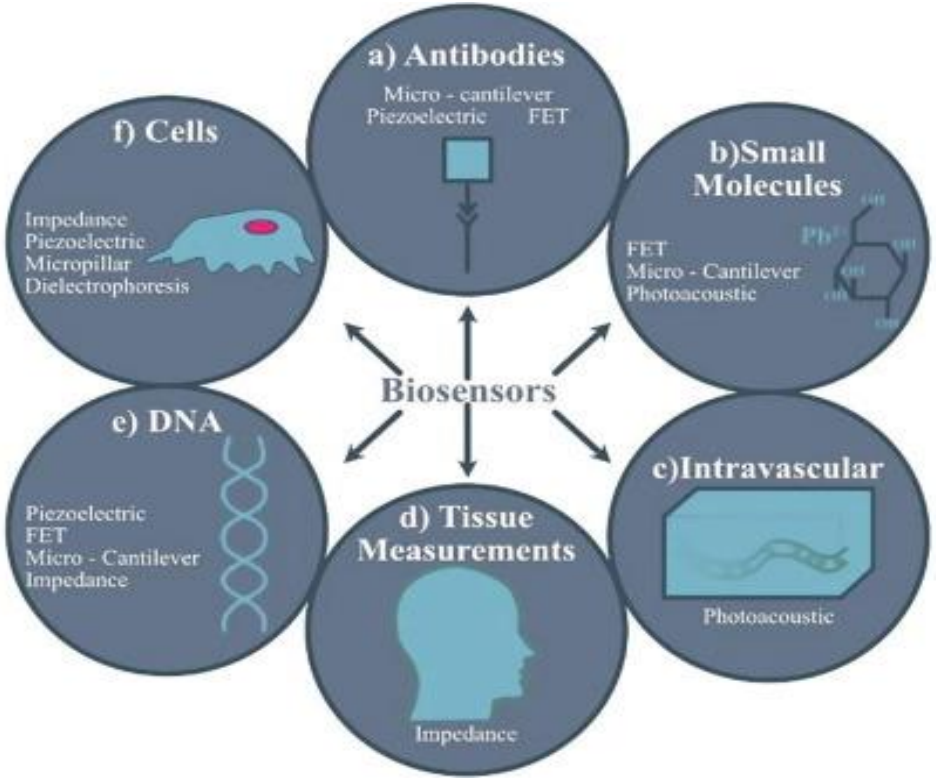
✓ detection methods

✓ signal transduction methods

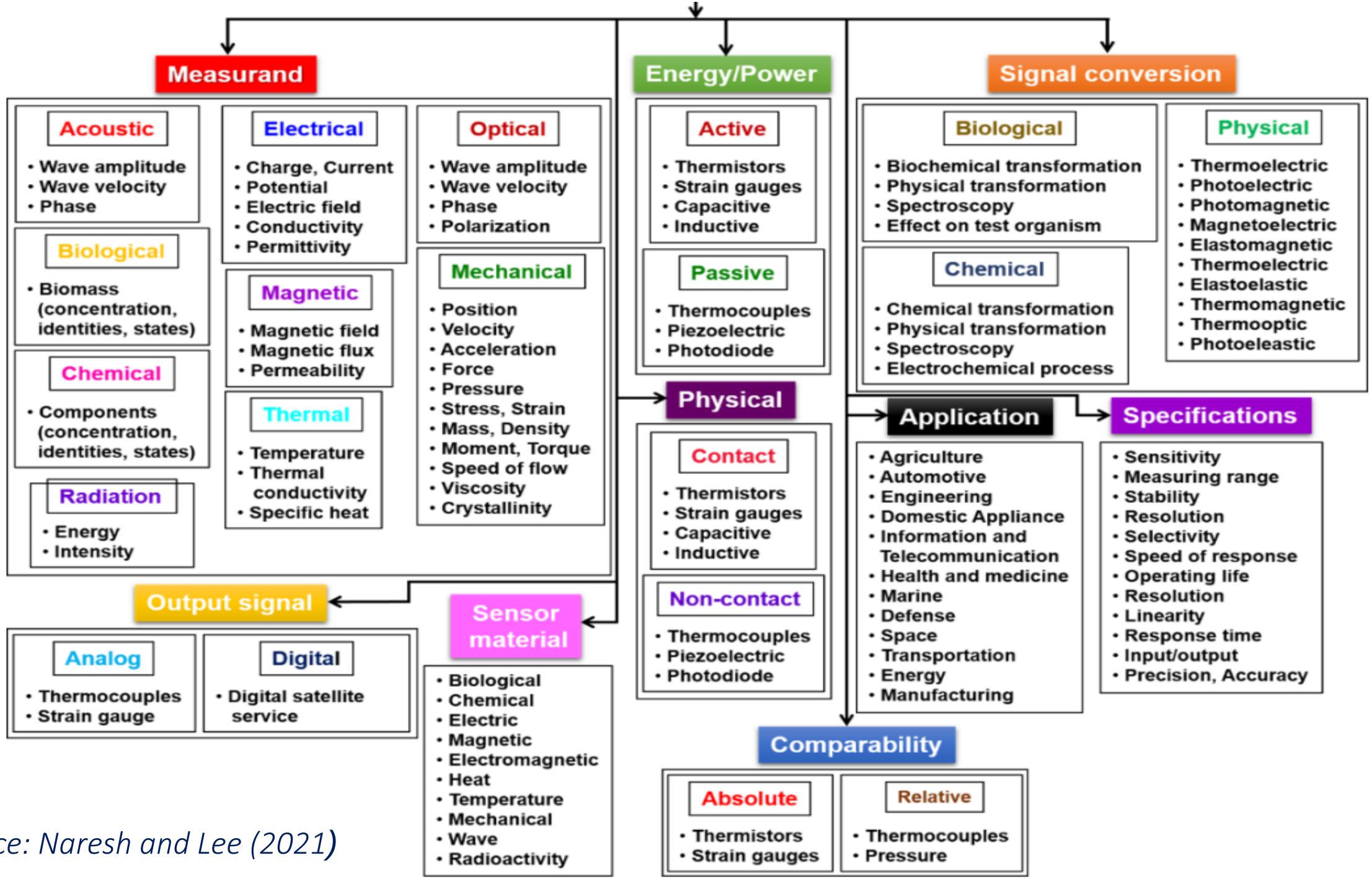
- **Biosensor applications**

✓ different analytes (antibody detection, small molecule detection, intravascular detection, full body measurements, DNA detection, or cell measurements)

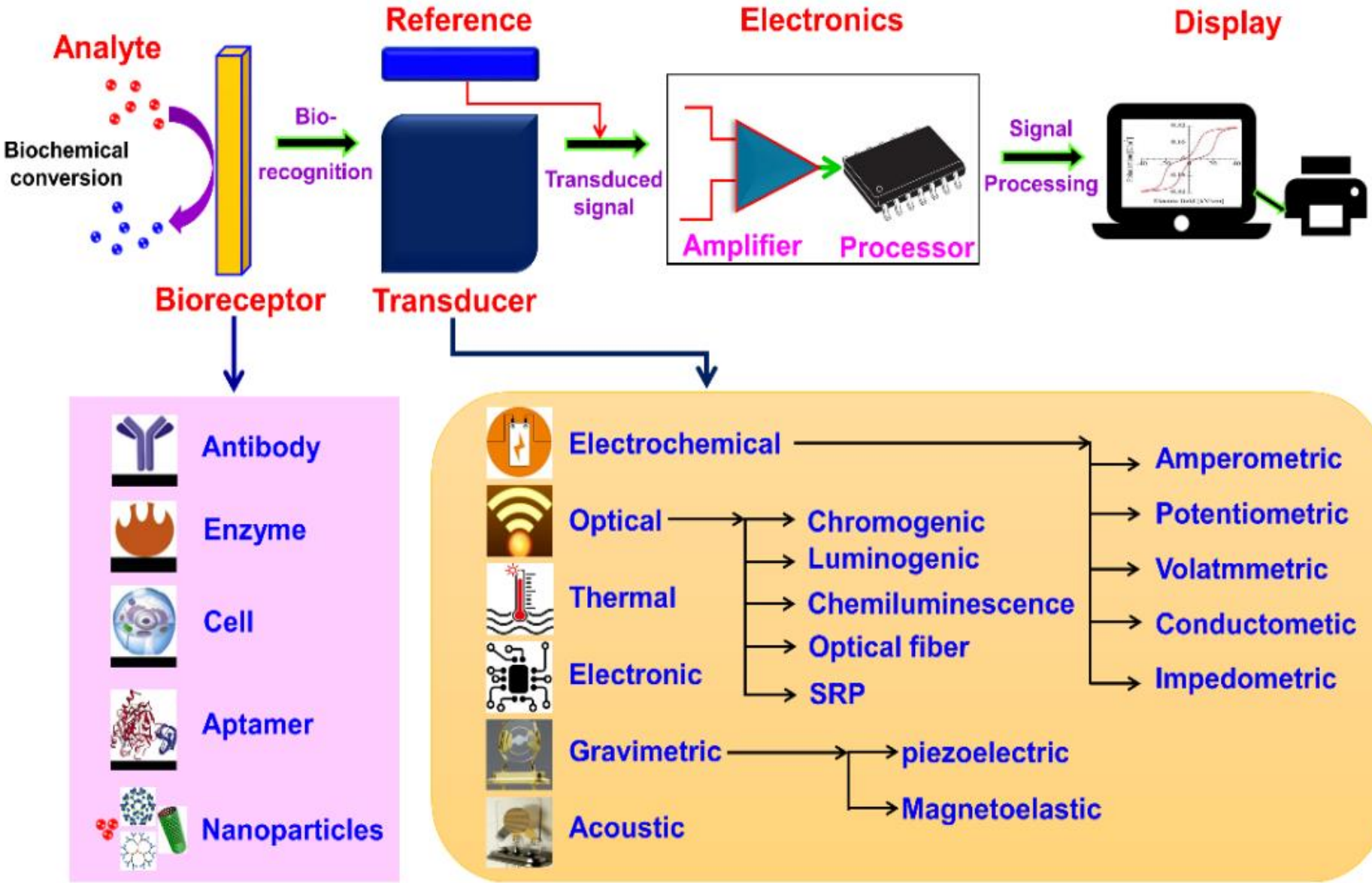


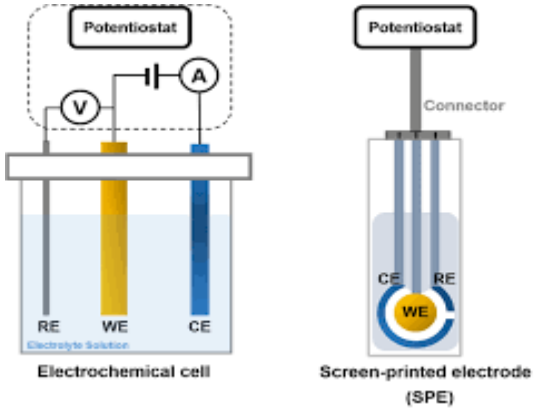


Source: Chadha et al. (2022)

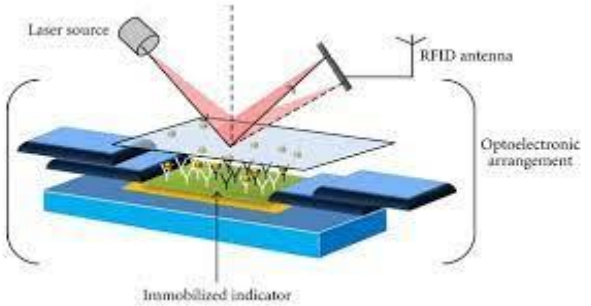


Source: Naresh and Lee (2021)

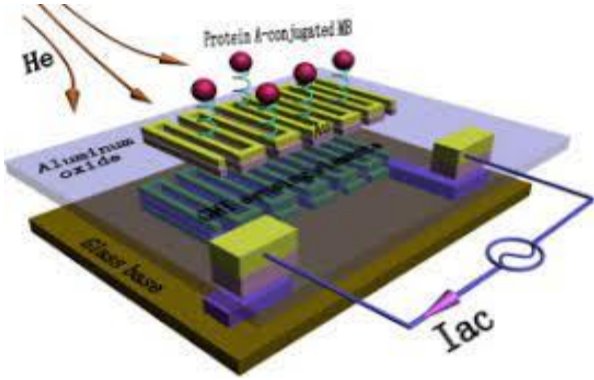




Electrochemical



Optical



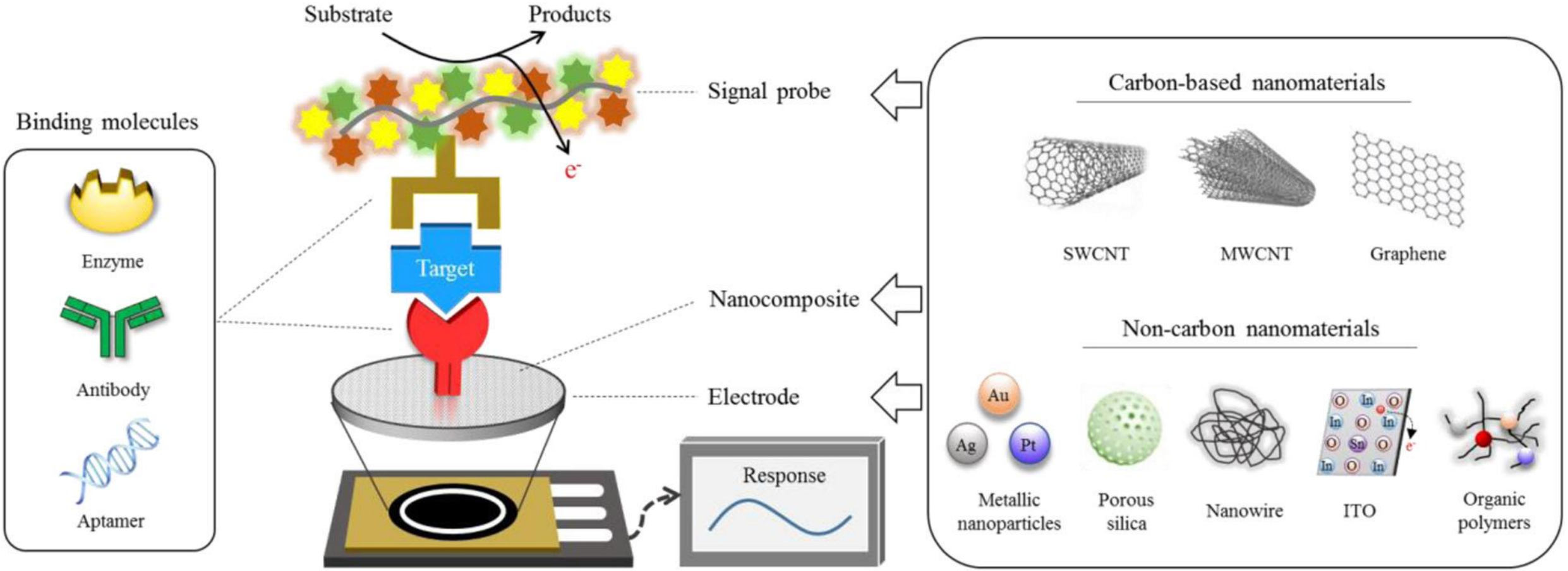
Magnetic

Source: Naresh and Lee (2021)

Electrochemical Biosensors

How to develop a biosensors?

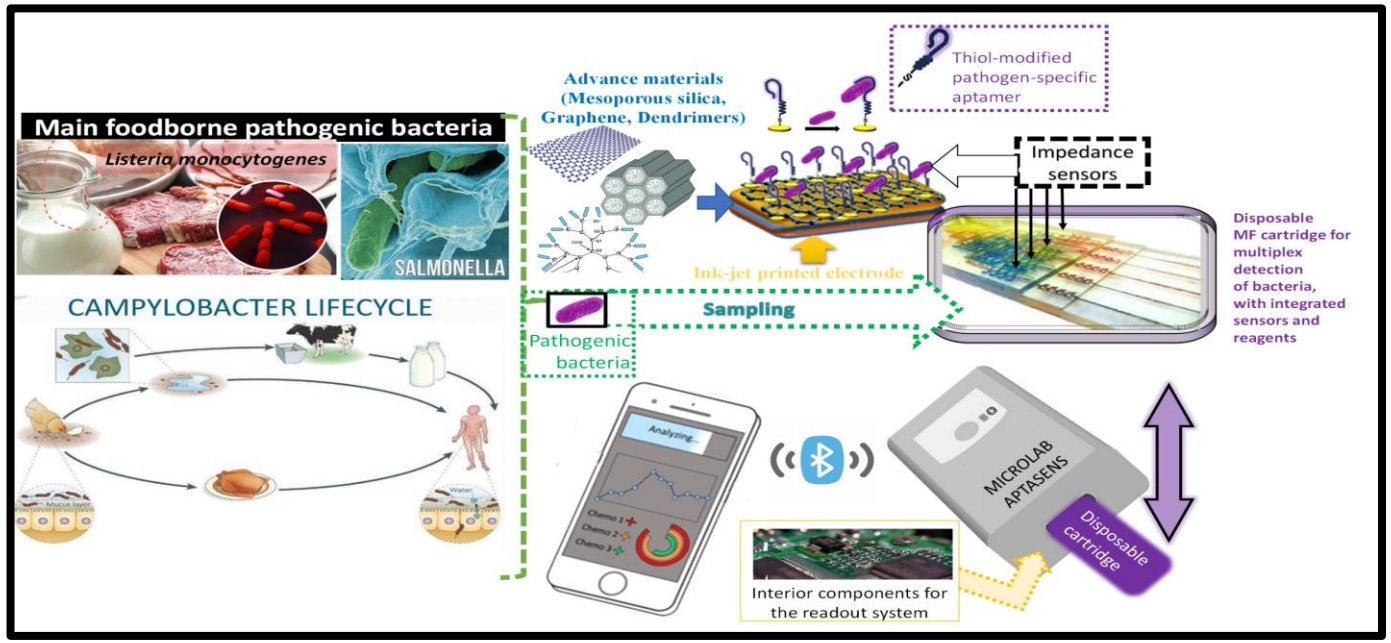
Electrodes fabrication >> Cleaning >> Nanomaterials >> Functionalization >> Testing and validation (TRL5 / TRL6)



Source: Cho et al. (2020)

Trends

- Improving the detection principle (more sensitive, eliminate noise, etc.)
- Electrode development (geometry, technology, etc.)
- Developed of new materials to improve sensitivity and selectivity: Graphene, MoS, MXens, etc.
- Incorporation of nanoparticles (golden, magnetic, etc.)
- Development of protocols for biofunctionalization (aptamers, antibody, DNA, dendrimers, etc.)
- Integration with MF or LoC
- Development of electronic read-out
- Long-term stability studies
- Protocols for sample preparation



3. Biosensors in animal health monitoring

***Biosensor*: target biomarker**

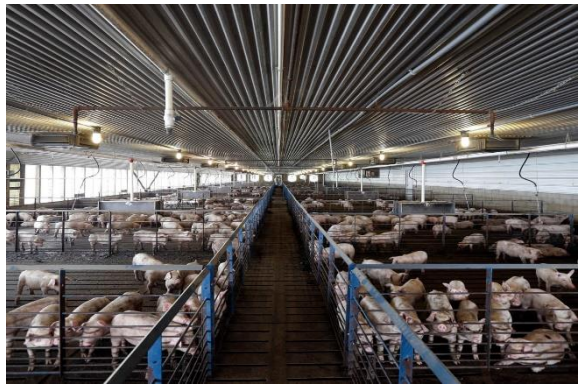
pathogen / animal welfare / animal health molecules

***Bioreceptor*: Immobilized sensing element**

monoclonal antibody/RNA, DNA/aptamer/glycan/lectin/enzyme/tissue/whole cell

***Challenge:* high level of sensitivity and specificity & quantitative detection of biomarkers in complex media**

farm environment / abattoir (dirt, faeces, saliva, blood, serum)



Biosensors on farm

Mechanical & metabolites` sensors

- Mechanical sensors (animal movement, bites/chews)
- Acoustic sensors (jaw movement, grazing behavior)
- Acceleration sensors (jaw movement, feeding behavior)
- Breath analysis sensors (VOCs)
- Perspiration metabolites` sensors (stress control)
- Tears sensor (glucose level)
- Progesterone sensor
- Salivary detection of metabolites (uric acid)

Animal diseases` sensor

- Bovine Respiratory Disease (BRD)
- Bovine Viral Diarrhoea (BVD)
- Avian Influenza virus (AIV)
- Foot and Mouth Diseases (FMD)
- Mastitis

Mechanical & metabolites` sensors

- *Mechanical sensors*

✓ Designed to be used specifically in pastures and stables

✓ noseband and an electronic interface connected to record, analyze and store data at 20 Hz at computer

✓ jaw movement, bites/chews

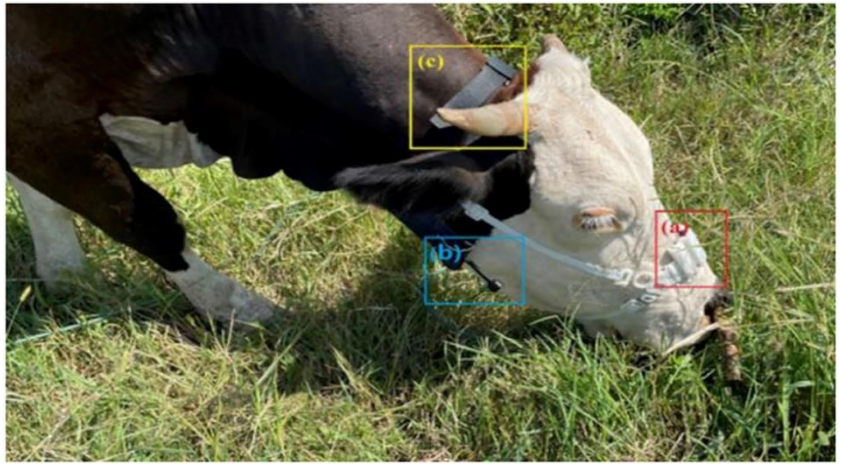
- *Acoustic sensors*

✓ jaw movement and grazing behaviour

✓ estimate food intake in cattle

- *Acceleration sensors*

✓ animal movement, jaw movement and feeding behaviour



Source: Chen et al., 2022

- *Breath analyses biosensors*

✓ non-invasive method

✓ disease diagnostics by detection and characterization of Volatile Organic Compounds (VOCs)

✓ VOCs: gasses, (e.g. hydrogen and methane) and fatty acids which all can be used as specific biomarkers for detection of metabolic and pathologic processes.

✓ Livestock: Bovine Respiratory Diseases (BRD), brucellosis, bovine tuberculosis, Johne`s diseases, ketoacidosis, foot and mouth (FMD) disease

- *Perspiration metabolites` biosensors*

✓Sweat analysis for sodium concentration and lactate levels

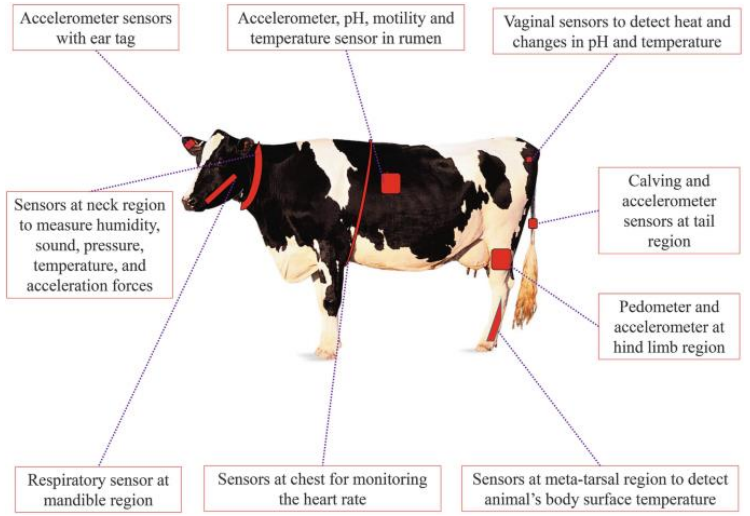
✓animal welfare control (physical stress)

- *Tears` analyses biosensors*

✓glucose sensor

- *Progesterone analyses biosensor*

✓aptamer specific for its binding properties with progesterone



Source: Zeineldin et al. (2021)

- *Salivary metabolites biosensor*

✓information on animal welfare and disease

✓biomarkers in saliva

Uric acid levels: metabolic syndrome, renal syndrome or physical stress

Cortisol level: level of animal stress

Sensors for detection of animal diseases

- *Bovine Respiratory Disease (BRD) biosensor*

✓sensitive and specific to anti-IgE in commercial anti-BHV_1 (Bovine Herpes Virus-1, causing BRD)

✓in serum samples from cattle

- *Bovine Viral Diarrhoea (BVD) biosensor*

✓detect a BVD antibodies in serum of cattle

✓detection limit of 10^3 CCID/ml in BVD samples

- *Avian Influenza virus (AIV) biosensor*

✓detection of immobilised H7N1 antibodies

- *Foot and Mouth Diseases (FMD) biosensor*

✓lateral flow immunocromatographic platform for detection of antibodies against FMD proteins

- *Mastitis biosensor*

✓on-line sensor system based on the automated California Mastitis Test (CMT)

✓haptoglobin (Hp) detection (Acute Phase Protein – inflammation biomarker

- Other, e.g. porcine reproductive and respiratory syndrome (PRRS)

4. Biosensors in food safety monitoring

Biosensors in slaughterhouses

- No wide commercial and routine use for meat safety monitoring
- Some are available

- Aptamer-based biosensors

✓ *Salmonella enteritidis* (10^2 CFU/ml) and *Escherichia coli* O157:H7 (10 CFU/ml)

- Antibody-based biosensors

✓ detection of *Escherichia coli* (1 to 10^3 CFU/ml)

- DNA-based sensor

✓ *Campylobacter* in meat (poultry) samples

✓ detection level 1.5×10^1 CFU/g

- Cell-based sensors

✓ *Clostridium perfringens* toxins

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Aptamer-based microfluidic biosensors

Salmonella: meat chain
Campylobacter: poultry chain
L. monocytogenes: vegetables

Biosensors in environmental control (slaughterhouse wastewater)

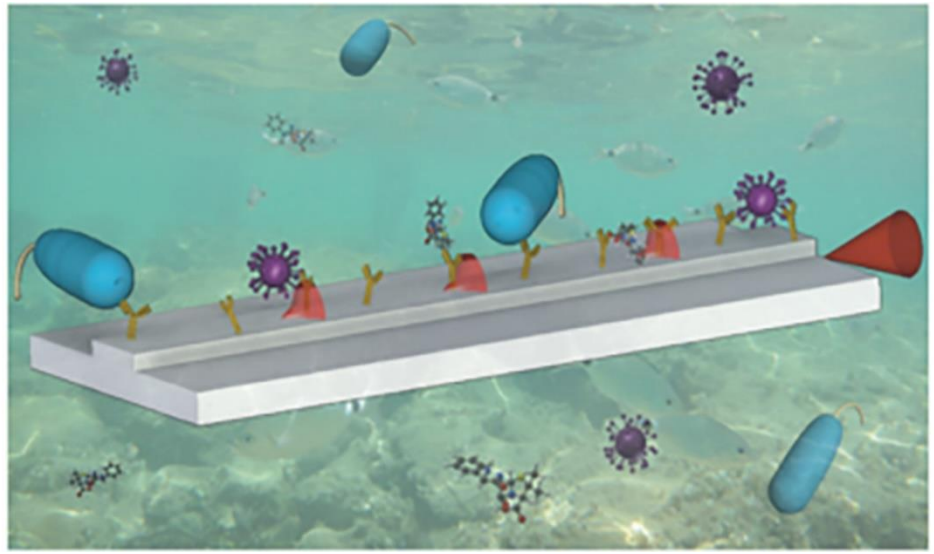
- Biochemical Oxygen Demand (BOD) biosensors

✓detection of refractory compounds

✓specific refractory compounds which microorganisms are not able to use and degrade within a short measuring time of biosensor

✓*Aeromonas hydrophila* in high fat and grease content wastewaters (service life 110 days)

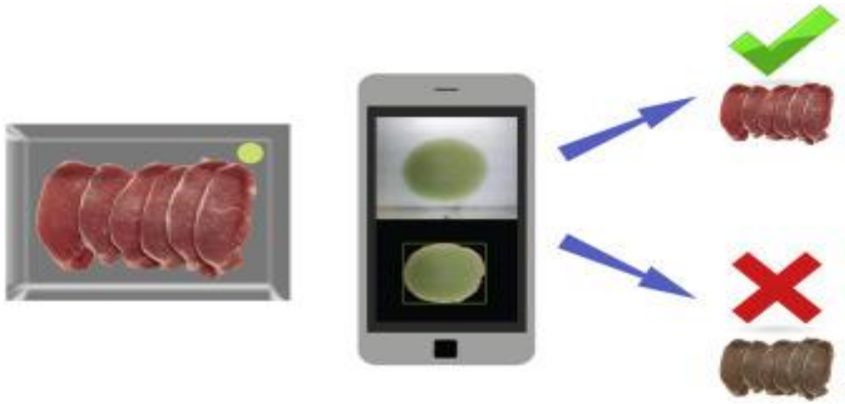
✓*Pseudomonas Fluorescens* (service life 115 days)



Source: Chocarro-Ruiz et al. (2017)

5. Biosensors in food quality monitoring

Smartphone based meat freshness detection



Source: De Vargas-Sansalvador et al. (2020)

Meat quality biosensors

- ✓ meat freshness
- ✓ beef tenderness
- ✓ pork quality traits

Based on metabolic transformation (markers and indicators)

Examples:
Amperometric - Pork meat freshness and ripening process
Optical & Fluorescence – Beef tenderness
Strip test (glucose & lactic acid) – Detection of meat extract pH
Strip test (swine blood) – Detection of pH
Pork meat drip loss (glucose) – Detection of pork meat with reduced quality

(Source: Sionek et al., 2021)

Biosensors

Sensory quality
- Visual texture
- Color
- Visible fat
- Natural drip

Heated meat
- Aroma
- Flavour
- Texture

Technological quality
- WHC
- pH
- Protein, lipid, connective tissue

Product safety
- Microbiological status
- Hormones
- Antibiotics
- Pesticides
- Heavy metals

6. Biosensors in food crime control

Definition

"Food crime as serious fraud and related criminality in food supply chains"

(UK Food Standards Agency, 2021)

- Food crime can occur in various ways

✓isolated acts of dishonesty by individual offenders

✓organised illegal activity co-ordinated by criminal networks

- Food crime can be reduced

✓denying offenders the means to commit offences

✓reducing the likelihood of individuals and groups becoming offenders in the first place

How biosensors can help to the National Food Crime Unit to identify rapidly the food crime?

- Pathogens of public health importance (zoonotic foodborne bacteria) enter the meat chain in multiple points

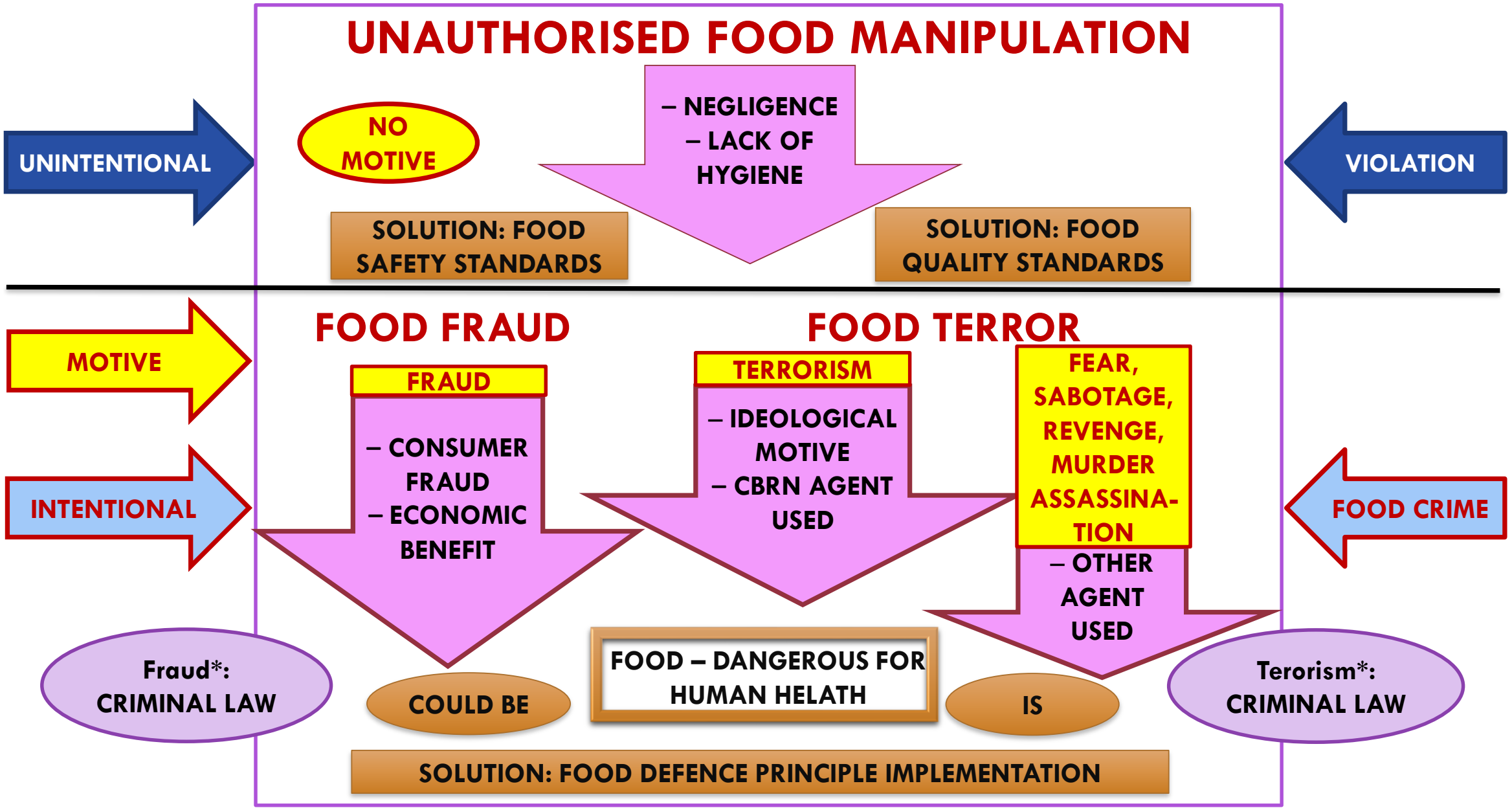
✓*unintentional* – poor biosecurity and hygiene

✓*intentional* – food fraud and food terrorism

- International trade - sourcing of raw materials from different regions

- Fraudulent practices in manufacturing of added-value meat products (meat species different from the labelling statement)

Source: Jurica et al. (2019)



(Source: Jurica et al., 2021)

Biosensors for detection adulteration of meat products

- Food authenticity

✓ mixture of different animal species

✓ Mislabeling

- Technical limitations

✓ 81% of developed prototypes of biosensors require extensive purification and/or amplification steps

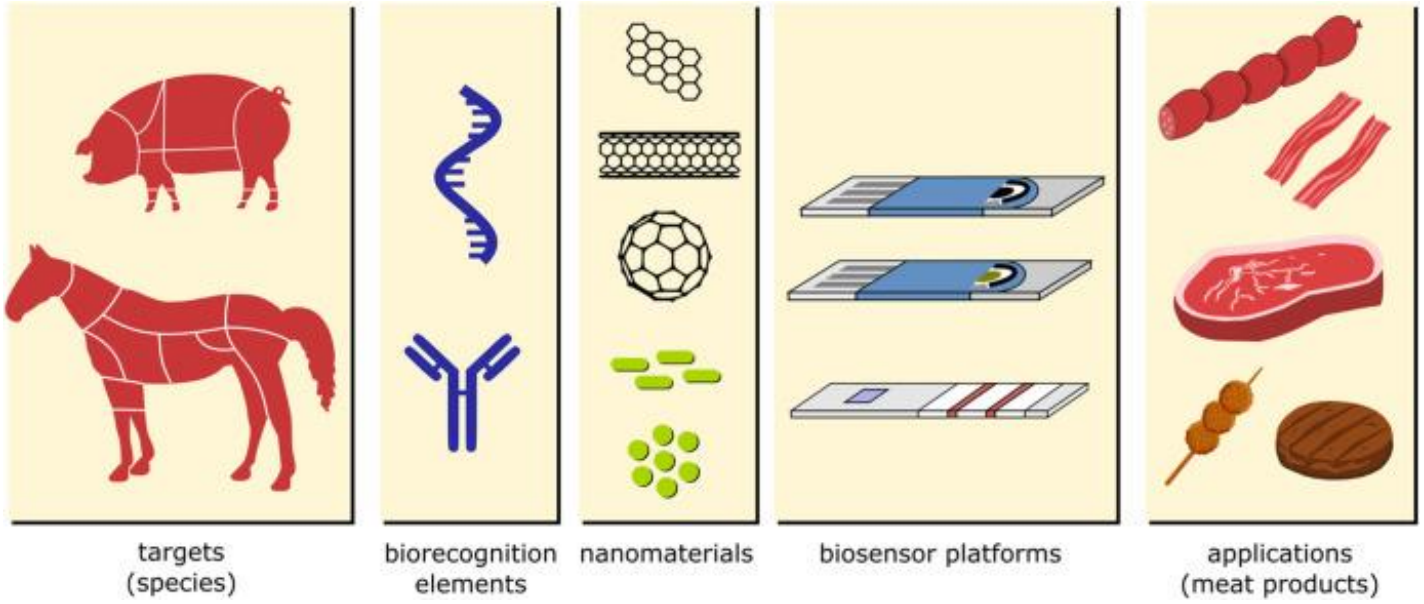
✓ Not suitable for point-of-care (POC) use

✓ Long-term stability studies needed

Mostly 2 types of biosensors

Electrochemical

Optical



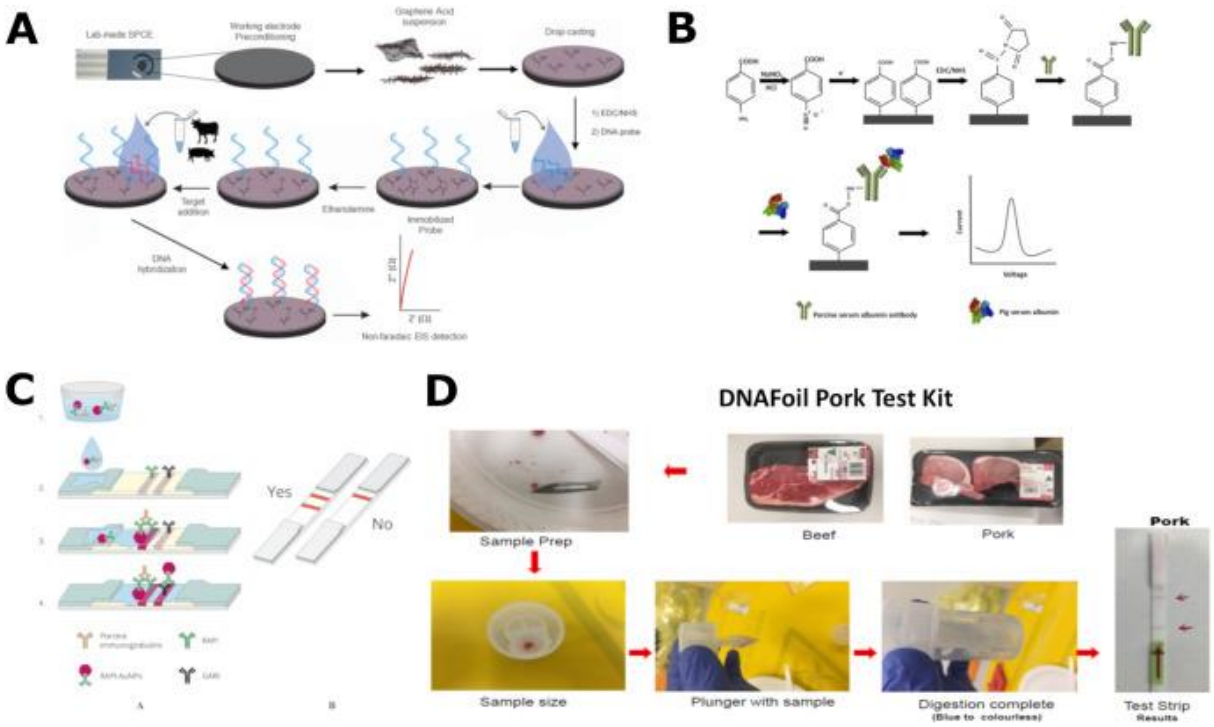
Source: Flauzino et al (2022)

Electrochemical biosensors (`genosensors`)

- Detection limits

weight by weight percentage (% w/w)

Concentration unit of detected nucleic acid (mol L^{-1} / g L^{-1})



Example:

Genosensor for identification of pork in meat products

(cytochrome b gene in mitochondrial DNA)

(Source: Flauzino et al., 2022)

Biosensor for identification of 13 meat species

(enzyme-free isothermal strand displacement amplification):

Cattle, sheep, pig, horse, donkey, dog, fox, rabbit, mouse, rat, chicken, duck, goose)

(*Source: Zhang et al., 2020*)

Optical biosensors

- Based on optical properties changes

(biomolecules, nucleic acids, antibodies)

- Colorimetry (by naked eye)

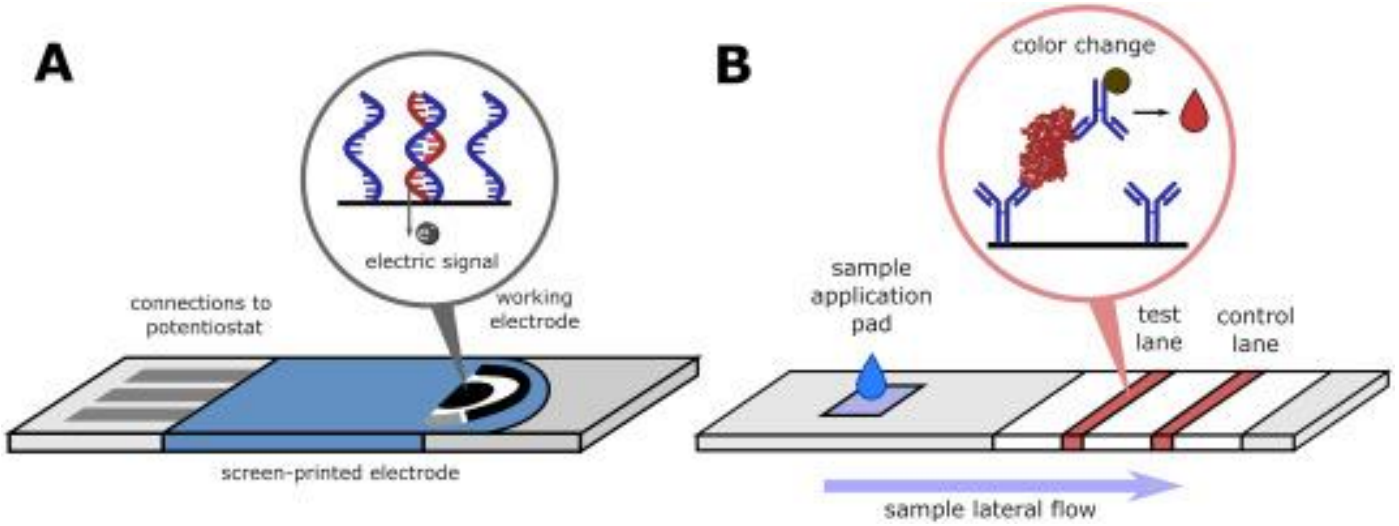
  gold nano-particles

  SERS (surface-enhanced Raman scattering)

  SPR (surface plasmon resonance)

  FS (fluorescence spectroscopy)

(Source: Chen and Wang, 2020)



7. Conclusions

- Biosensors: valuable tool for control of animal health, meat safety & quality and food fraud/crime
- Lab on chip, point-of care, user-friendly, cheap and reliable (sensitivity & specificity)
- Require little sample preparation
- Effective food safety/food defence management.
- Fraudulent practices in manufacturing of added-value meat products (meat species different from the labelling statement)
- International trade - sourcing of raw materials from different regions

Biosensors and globalization of food trade

"The World on your Plate"

Chicken Kiev

Herb butter:	Salted butter	- Ireland
	garlic puree	- China, USA, Spain
	garlic salt	- China, USA, Spain
	lemon	- USA
	parsley	- France, UK
	pepper	- Indonesia
	water	- Ireland
Chicken breast:	Chicken	- Ireland, Belgium UK, France etc.
Batter:	Flour	- Belgium, France
	Water	- Ireland
Bread crumb:	Bread crumb	- Ireland, UK
	Rape-seed oil	- EU, Australia
		- Eastern Europe

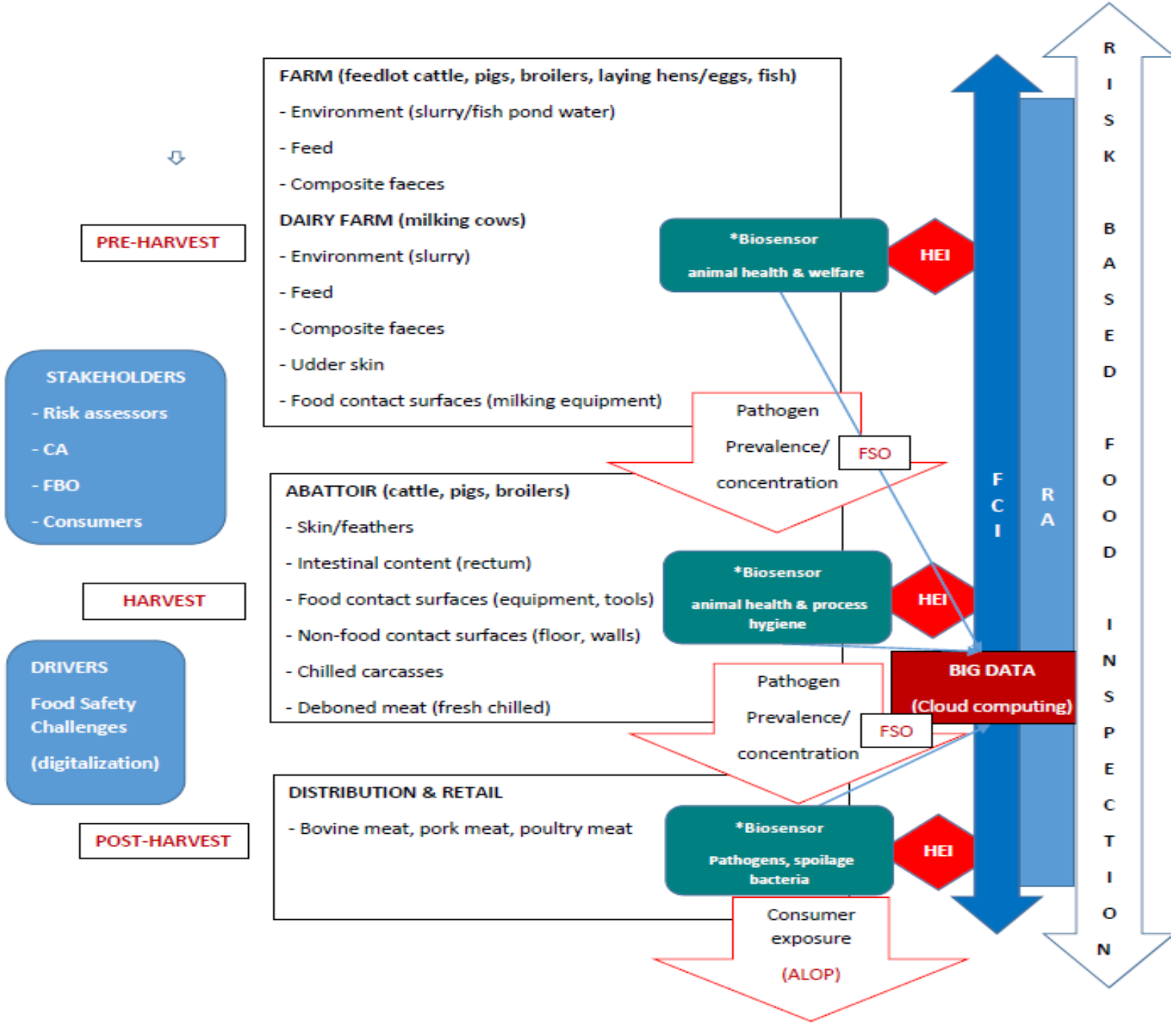
Food Safety Authority of Ireland

M:\lewis\ppt\Gresham Lecture - 3 December 08

Source: FSAI, 2008

Model system
Application of biosensors in the meat chain continuum

Source: Nastasijevic et al. (2021)



Indicators:
Pathogens (e.g. Salmonella, Campylobacter)
Animal Health (Acute Phase Proteins)
Animal Welfare (hormones)

CA – Competent Authorities
FBO – Food Business Operators
RA – Risk Analysis (risk assessment, risk management, risk communication)
FSO – Food Safety Objective
ALOP – Appropriate Level of Consumer Protection
HEI – Harmonized Epidemiological Indicators
FCI – Food Chain Information

Thanks for your attention!

Questions?