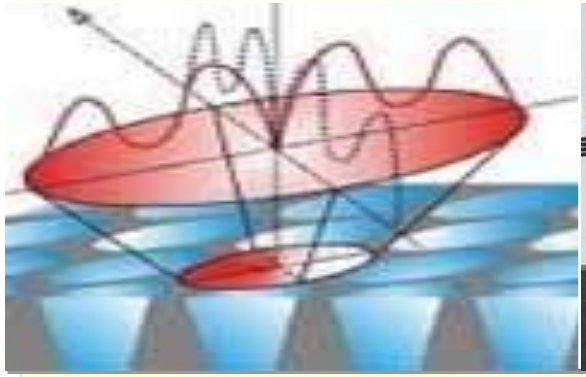
Biosensors for Heavy metals, Pesticides & Aflatoxin





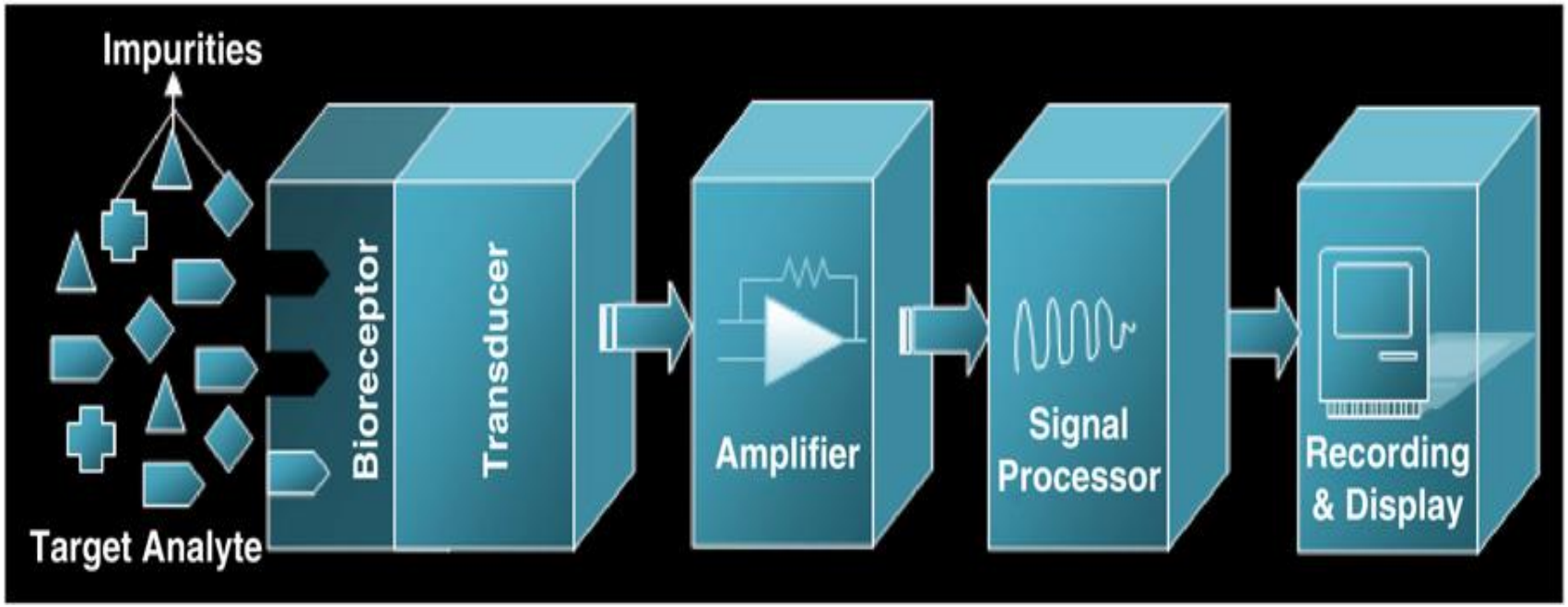


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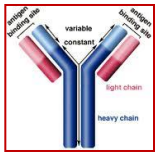
Biosensor

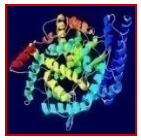
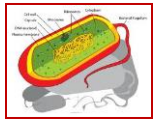
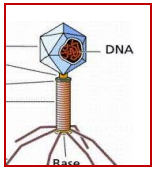
"A biosensor is an analytical device that consists of immobilized biocomponent in intimate contact with transducer which convert the biological signal into measurable electrical signal"

Birth of biosensor – Clark & Lyons, developed first glucose analyzer in 1962



Classification of Biosensors

- Biosensors
  - Biocomponent
    - 

      Antibody
    - 
      Enzyme
    - 
      Cell
    - 
      DNA
    - 

      Phage
  - Transducers
    - Optical
      - SPR
      - Fibre optic
    - Mass based
      - Piezoelectric
    - Electrochemical
      - Amperometric
      - Potentiometric
      - Conductometric

Heavy Metals

- Metals that have a density more than 5g/cm3 are known as heavy metals. There are 60 heavy metals.

- Some are essential for all forms of life at a very low concentration (Cu, Cr, Zn, Fe, Mo, Mn and Ni etc.)

- Heavy Metals e.g. Pb, Cd, Hg are already toxic in very low concentration, mostly behave as cumulative toxins.

- Causes disturbance to nervous, kidney & liver functions, damage to reproductive system, many metabolic deficiency systems caused by enzyme inhibition.

Conventional Tools

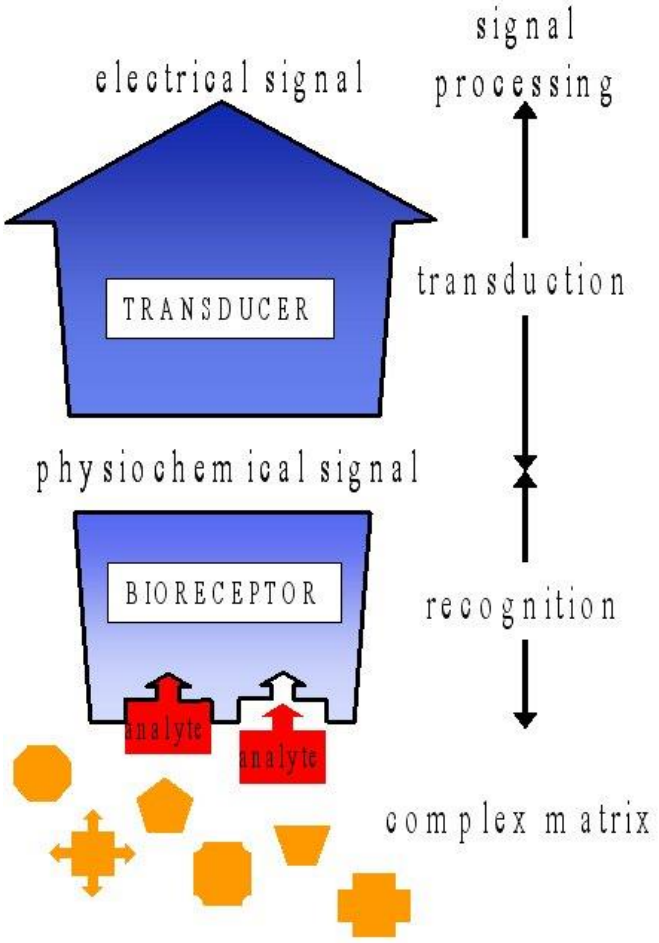
Analytical tool	Detection limit
Differential pulse polarography	100 ppb
Atomic absorption spectrophotometry (AAS)	25 ppb
Electrothermal AAS (ETAAS)	5 ppb
Inductively coupled plasma mass spectrometry (ICP-MS)	0.025 ppb
Dynamic reaction cell inductively coupled ICP-MS (DRC-ICP-MS)	0.5-1.5µg/kg
Differential pulse cathodic stripping voltametry (DPCSV)	0.01 ppb
Electrochemical metal analyzer	0 – 500 ppb

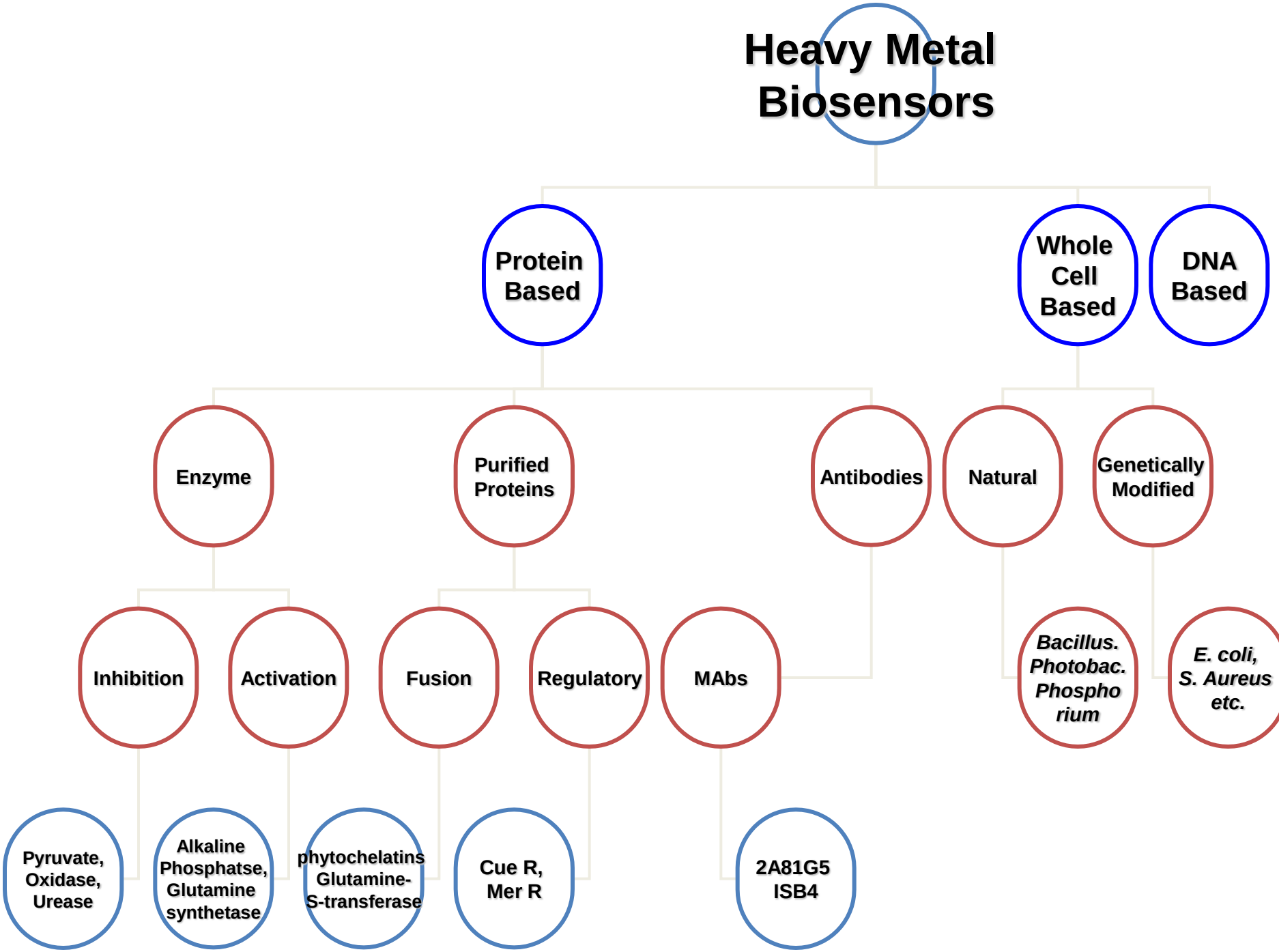
Limitations

- Expensive
- Time consuming
- Laboratory bound
- Demands expertise
- Needs pretreatment of the sample, making it a laborious task

Biosensor Technology

- Specificity
- Low manufacturing cost
- Higher limits of detection
- Fast response time
- Ease of use
- Portability
- Furnish continuous real time signals
- Provide toxicity level




Heavy Metal Biosensors
Protein Based
Whole Cell Based
DNA Based
Enzyme
Purified Proteins
Antibodies
Natural
Genetically Modified
Inhibition
Activation
Fusion
Regulatory
MAbs
Bacillus. Photobac. Phospho rium
E. coli, S. Aureus etc.
Pyruvate, Oxidase, Urease
Alkaline Phosphatse, Glutamine synthetase
phytochelatins Glutamine-S-transferase
Cue R, Mer R
2A81G5 ISB4

THE BIRTH OF THE BIOSENSOR

- The Biosensor was first described by Clark and Lyons in 1962, when the term enzyme-electrode was adopted.
- In the first enzyme electrode, an oxido-reductase GOD was held next to platinum electrode in a membrane sandwich.
- The platinum electrode polarized at + 0.6 V responded to the peroxide produced by the enzyme reaction with substrate.

$$Glucose + O_2 \rightarrow gluconic\ acid + H_2O_2$$

- The first glucose analyzer was developed for the measurement of glucose in whole blood. This 23YSI model appeared on the market in 1974.
- A key development in the YSI sensor was the employment of membrane technology to eliminate interference by other electro-active substance like ascorbic acid which polarizes at + 0.6 V.
- The enzyme layer was sandwiched between a cellulose acetate membrane and a nucleopore polycarbonate membrane.

Construction of a Biosensor

For the construction of successful Biosensor following requirements are to be fulfilled:

- ✓ Characterization of Bioassay principle.
- ✓ Compatibility of the bioassay principle with the transducer.

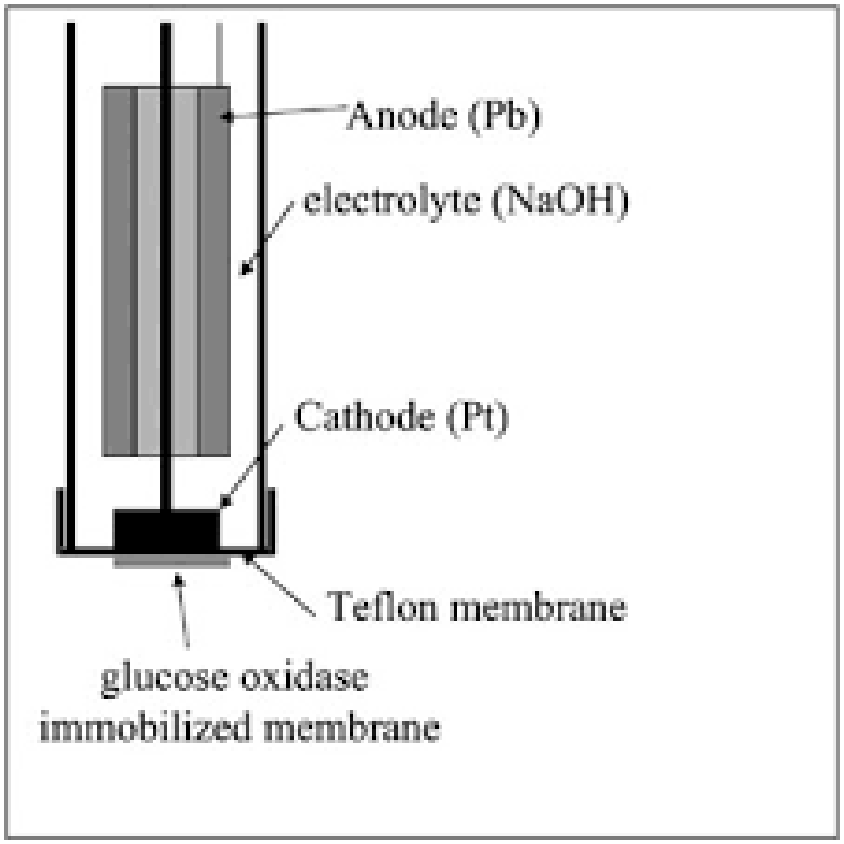


Fig 1: The Clark Enzyme Electrode

CLARK ELECTRODE

- **Monitoring Of Oxygen**

Ag Anode $4\,Ag + 4\,Cl^- \rightarrow AgCl + 4\,e^-$

Pt Cathode $O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O$

- **Monitoring of H_2O_2**

Pt Anode $H_2O_2 \rightarrow 2H^+ + 2e^- + O_2$

Ag Cathode $2\,AgCl + 2e^- \rightarrow 2\,Ag + 2\,Cl^-$

The current produced by the amperometric biosensor is related to the rate of reaction (vA))by the expression:

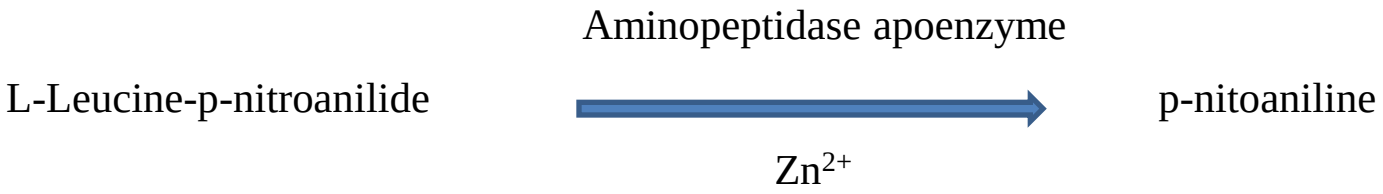
$$\mathbf{i = nFAvA}$$

APPLICATIONS OF BIOSENSOR FOR HEAVY METAL IONS

- **ENZYME ELECTRODE**
  - ✓ Heavy metal ions as **activator** for micro reactivation assay.
  - ✓ Heavy metal ions as **inhibitor**.

I Activator

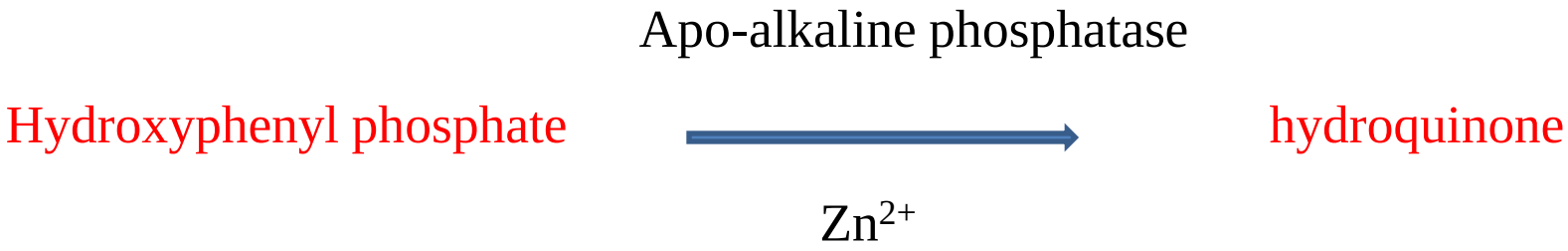
1. Assay of zinc ions



Fiber optic biosensor-cellulose pads impregnated with enzyme and reagent, 1 ppb of zinc can be detected.

2. Apo-alkaline phosphatase restores enzyme activity on addition of Zinc ions.

As determined by the formation of electroactive hydroquinone from hydroxy phenyl phosphate. As little as 0.8 micromole per liter of zinc ions could be detected.



II Inhibitor

- Reversible inhibition of urease, acetylcholinesterse, invertase, glucode oxidase, glutamate dehrdrogenase has been used to measure Hg, Cu, Pb, Cd etc.

Fiber Optic biosensor for detection of Cd ions in milk

Bio component: *Bacillus badius*

Detection limit: 0.1µg L^{-1}

Sample volume: 10 µl

Linear range of detection: 0.1 – 10 µg L^{-1}

Response time: 5 mins

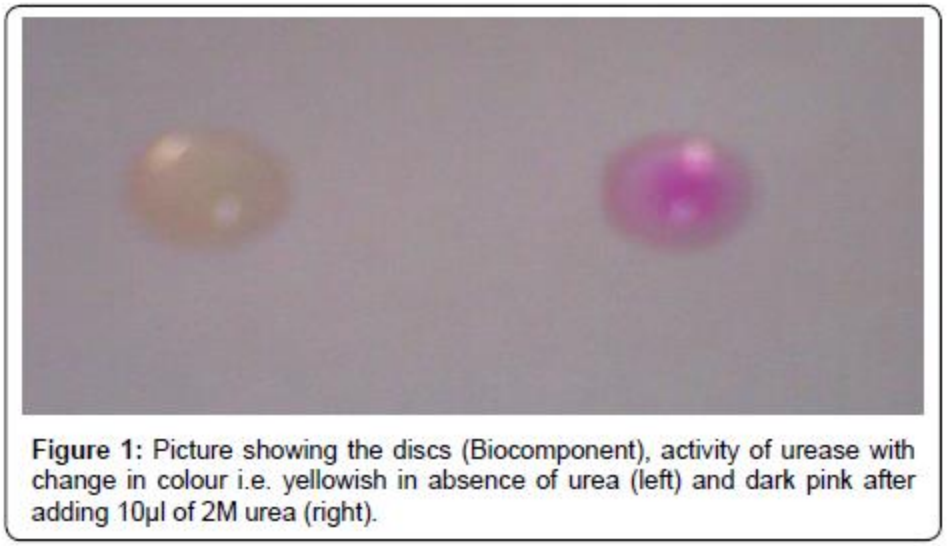


Figure 1: Picture showing the discs (Biocomponent), activity of urease with change in colour i.e. yellowish in absence of urea (left) and dark pink after adding 10µl of 2M urea (right).

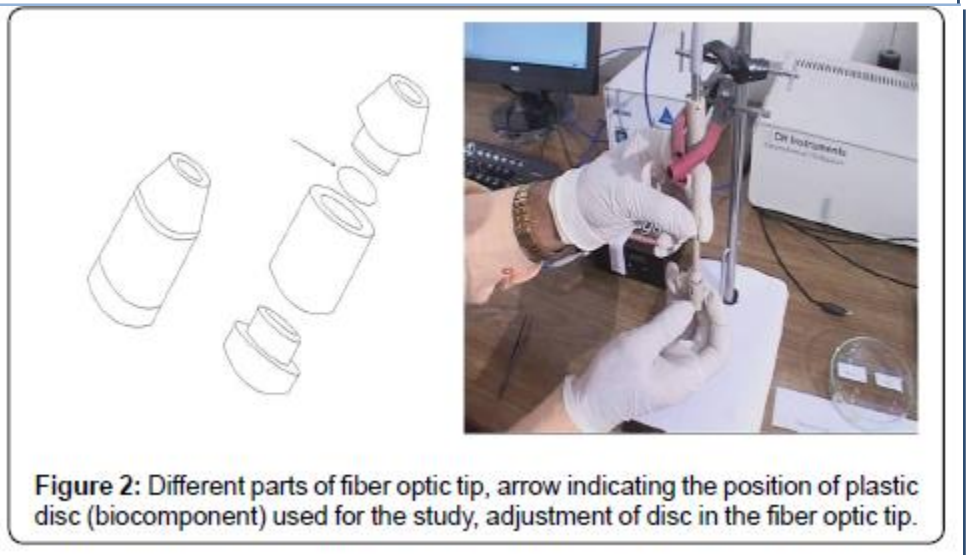
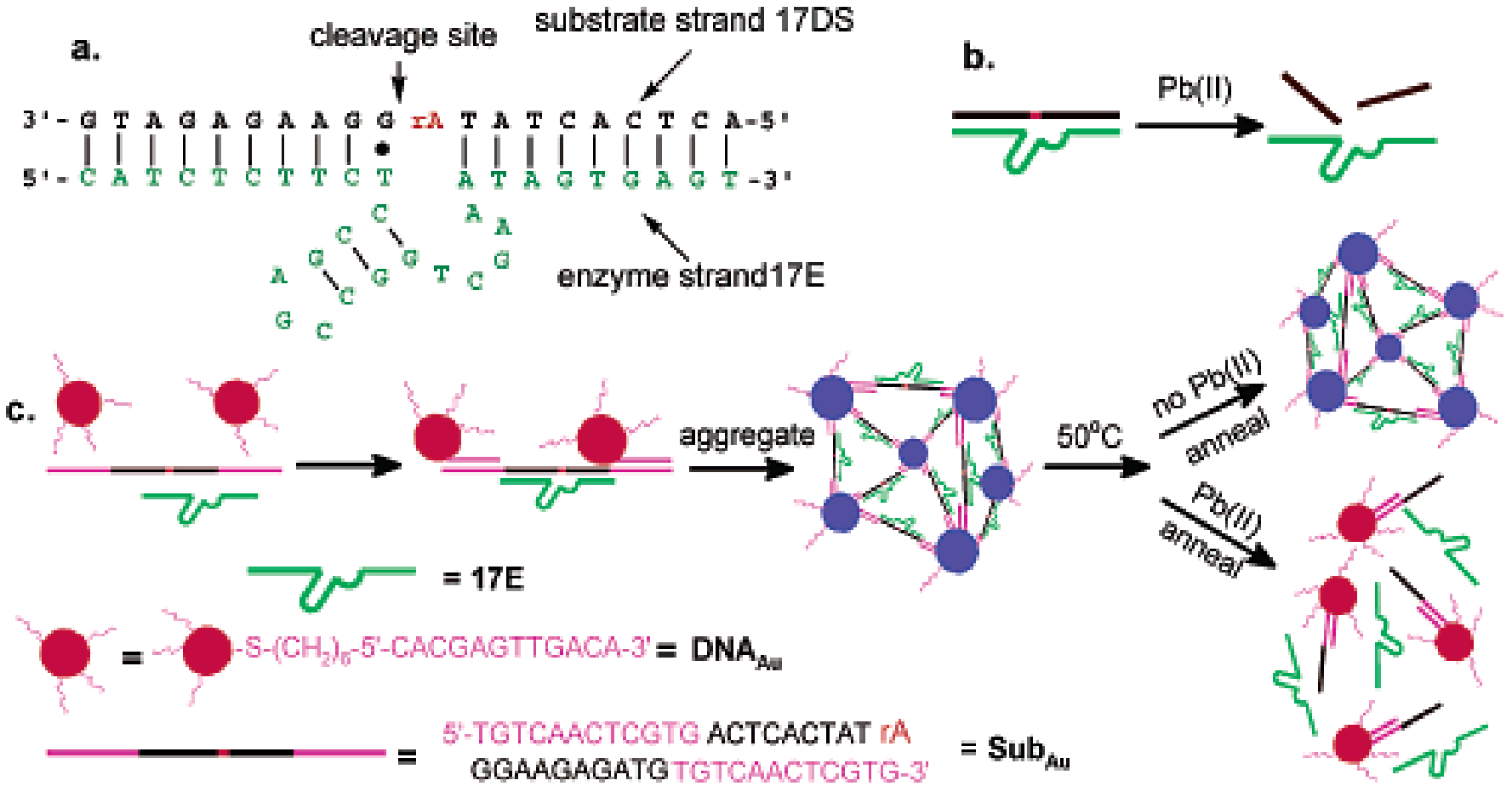


Figure 2: Different parts of fiber optic tip, arrow indicating the position of plastic disc (biocomponent) used for the study, adjustment of disc in the fiber optic tip.

Verma et al. 2011

DNA zyme based optical biosensor



(a) Secondary structure of the "8-17" DNAzyme system that consists of an enzyme strand (17E) and a substrate strand (17DS). The cleavage site is indicated by a black arrow. Except for a ribonucleoside adenosine at the cleavage site (rA), all other nucleosides are eoxyribonucleosides. (b) Cleavage of 17DS by 17E in the presence of Pb(II). (c) Schematics of DNAzyme-directed assembly of gold nanoparticles and their application as biosensors for metal ions such as Pb(II). In this system, the 17DS has been extended on both the 3¢ and 5¢ ends for 12 bases, which are complementary to the 12-mer DNA attached to the 13-nm gold nanoparticles (DNA Au).

Pesticides

- **Pesticides** are substances or mixture of substances intended for preventing, destroying, repelling any pest.
- Pests include insects, plant pathogens, weeds, birds, nematodes that destroy property, spread disease
- Many pesticides can be grouped into chemical families. Prominent insecticide families include
- Organochlorines,
- Organophosphates,
- Carbamates.
- Organochlorine hydrocarbons (e.g. DDT) operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bioaccumulate.

- Organophosphate and carbamates largely replaced organochlorines. Both operate through inhibiting the enzyme acetylcholinesterase, allowing acetylcholine to transfer nerve impulses indefinitely and causing a variety of symptoms such as weakness or paralysis.
- Prominent families of herbicides include pheoxy and benzoic acid herbicides (e.g. 2,4-D), triazines (e.g. atrazine), ureas (e.g. diuron), and Chloroacetanilides (e.g. alachlor).

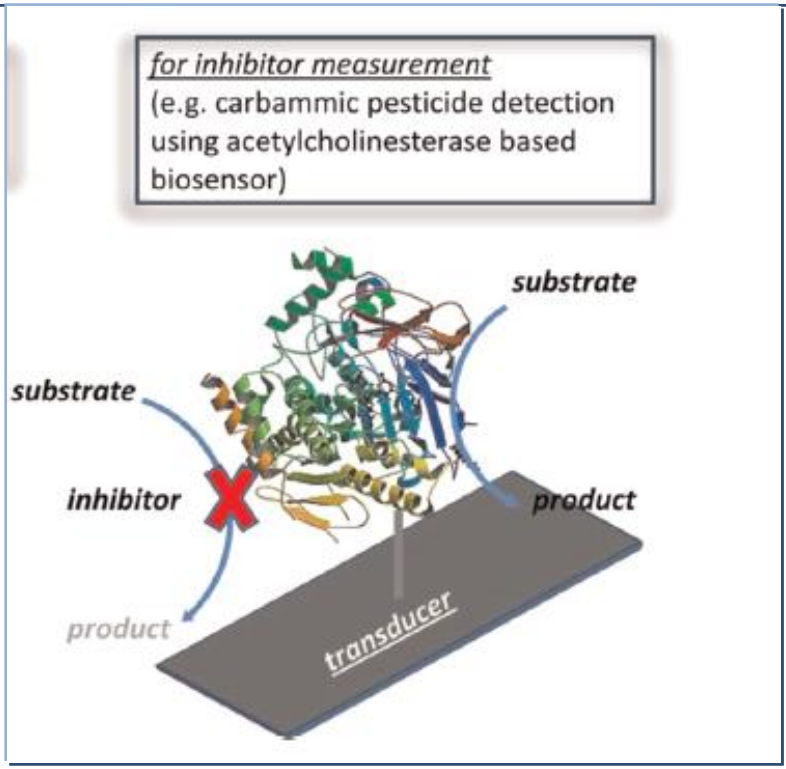
The analysis of pesticides is usually carried out by chromatographic techniques -liquid chromatography & HPLC with mass spectrometry.

Inhibition based amperometric biosensor of first generation

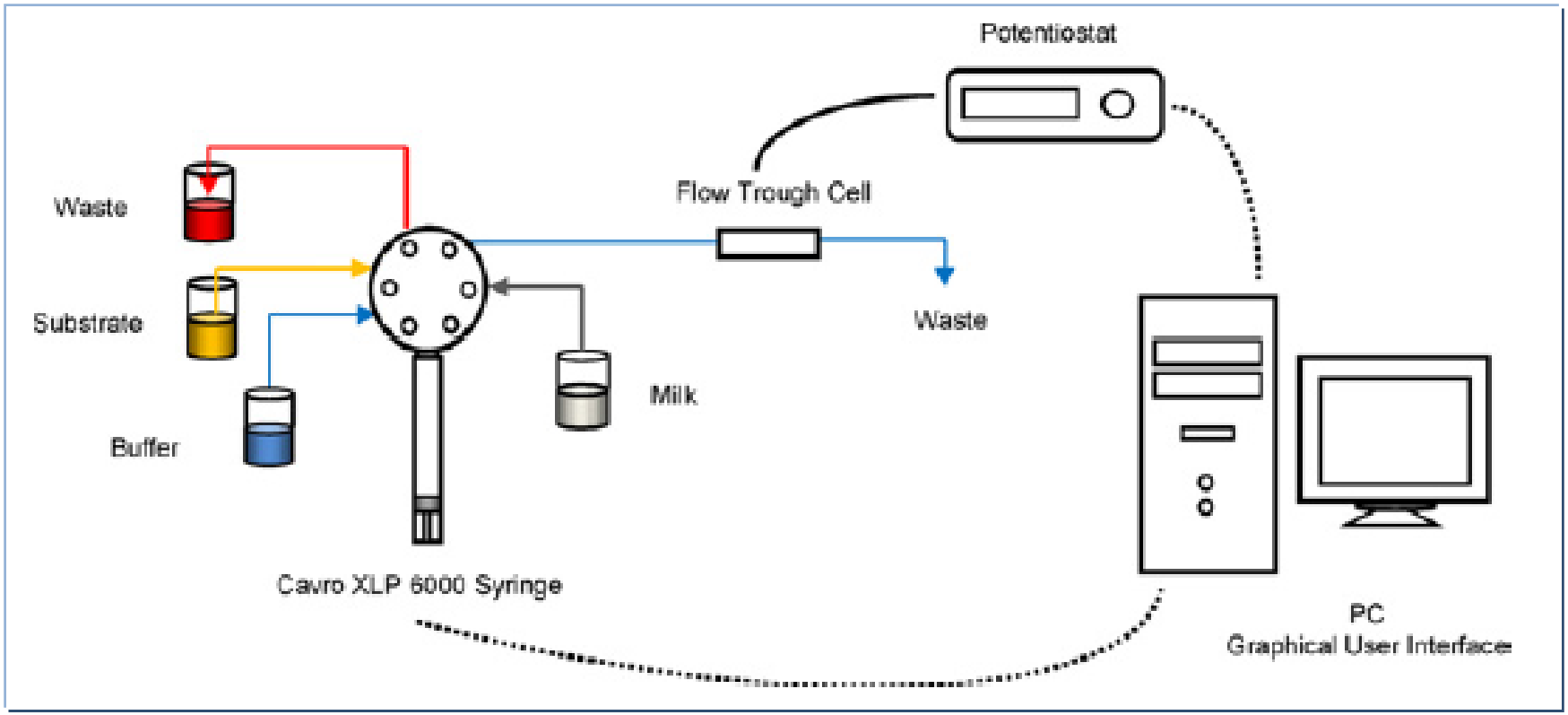
$$\text{R-choline} + H_2O \xrightarrow{\text{ChE}} \text{choline} + \text{R-COOH}$$

$$\text{choline} + 2O_2 + H_2O \xrightarrow{\text{ChO}} \text{betaine} + 2H_2O_2$$

$$2H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

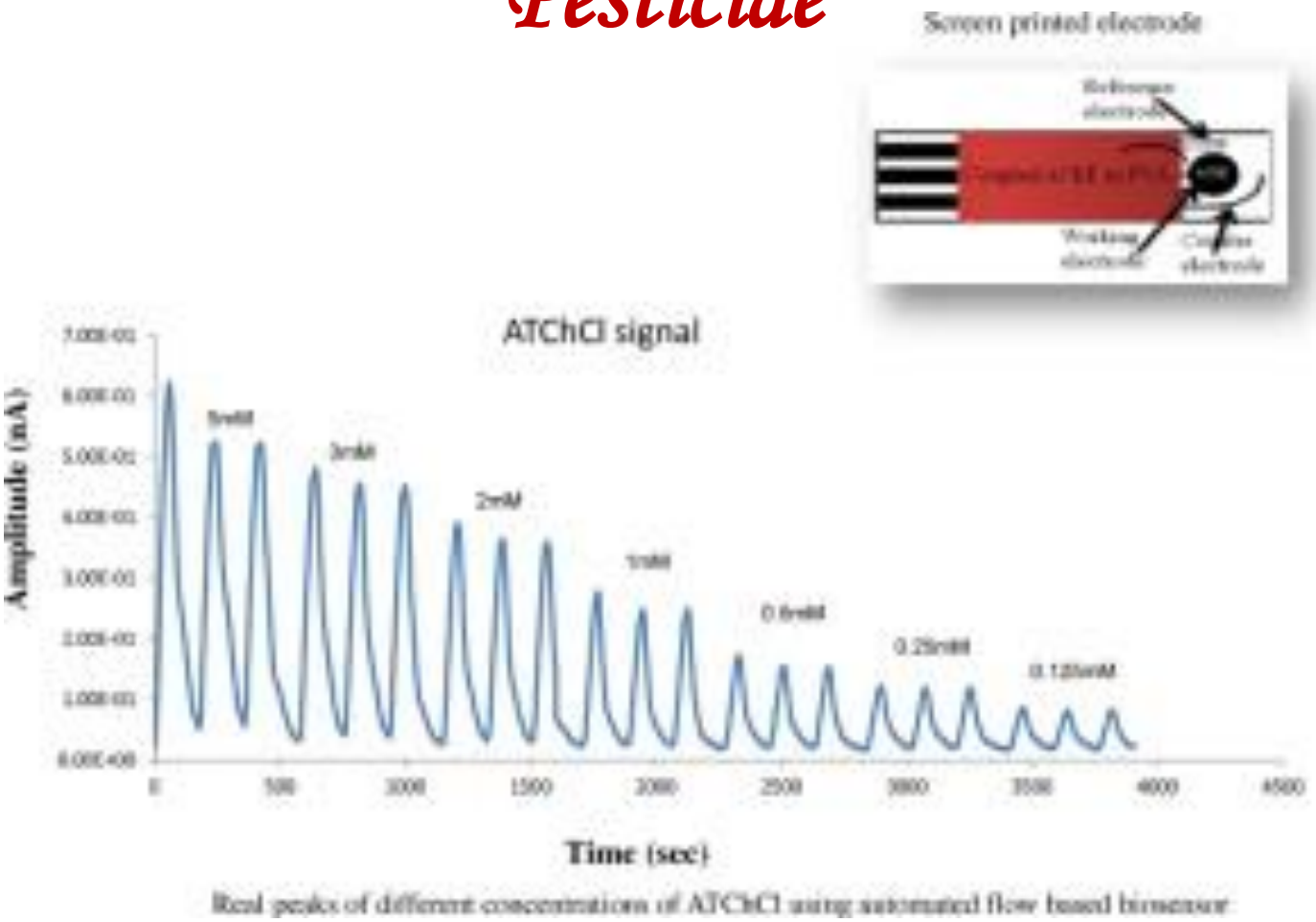


Automated flow based biosensor for Pesticide



Mishra et al. 2012

Automated flow based biosensor for Pesticide



Real peaks of different concentrations of ATChCl using automated flow based biosensor

Mishra et al. 2015

Electrochemical biosensor for pesticide

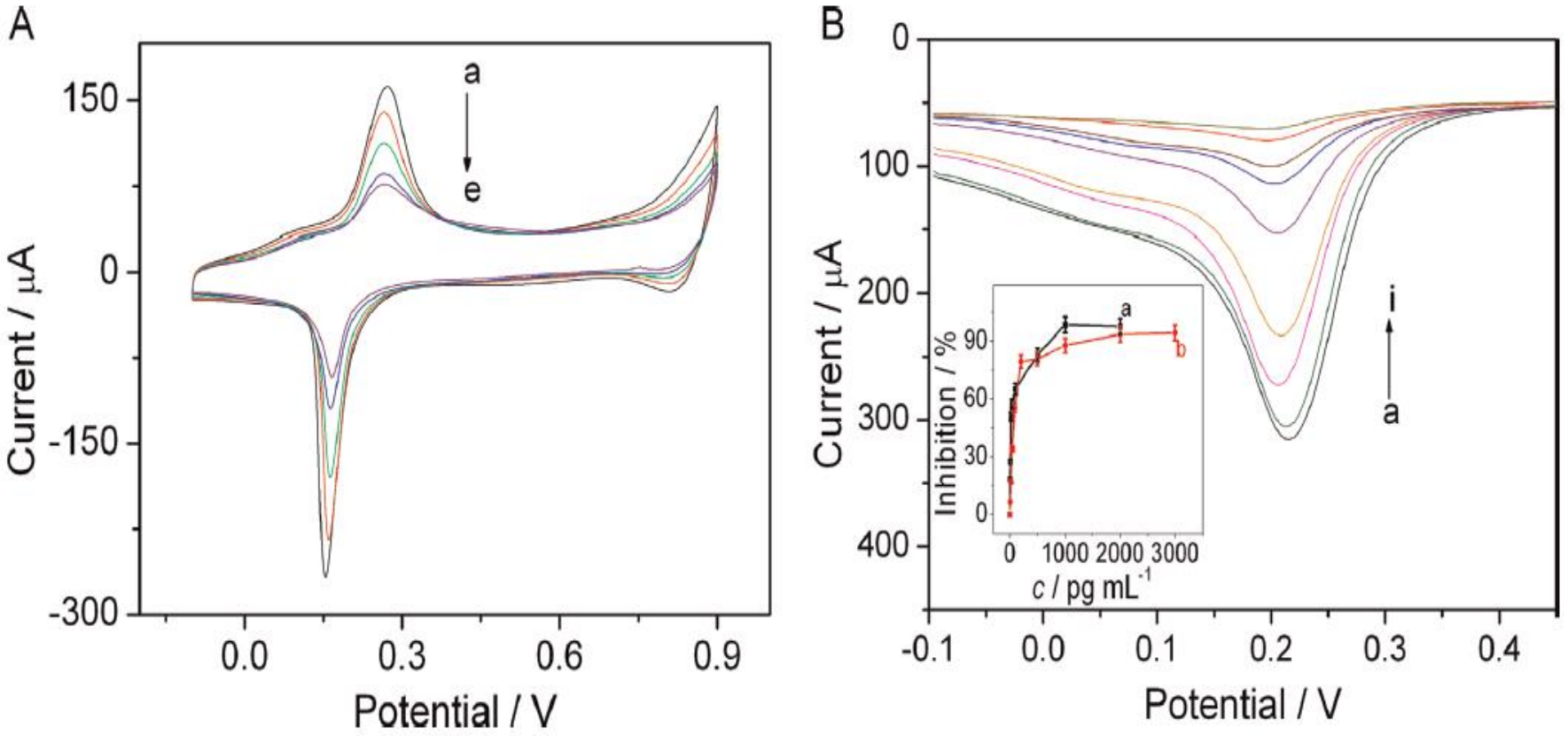
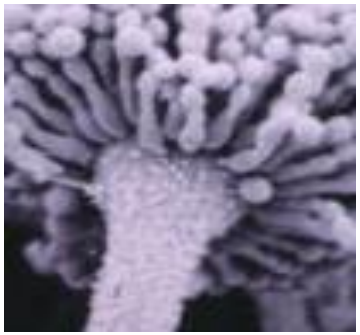


Fig. 5. CV (A) and DPV (B) of the biosensor in 0.1 M PBS (pH 6.5) containing 10.0 mM ATCl after inhibition with malathion for 10 min. Malathion concentration (A, a–e): 0, 12, 195, 390, 585 pg mL^{-1}, (B, a–i): 0; 7.98; 20; 40; 80; 198; 495; 1000; 2000; 3000 pg mL^{-1}. Inset (B), relationship between inhibition rate and malathion (a) and carbaryl (b) concentration.

Zhao et al. 2015

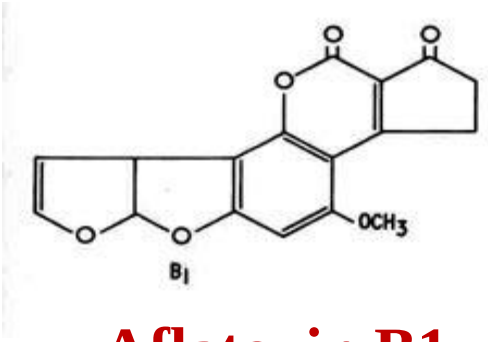
Aflatoxin background

- **Aflatoxins -** **produced by *Aspergillus flavus* and *A. parasiticus***



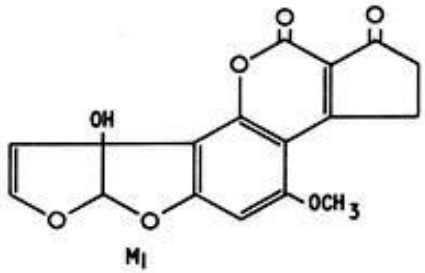
Conidiophores of *Aspergillus*

- **Types - Aflatoxin B1,B2, G1,G2**
- **Aflatoxin B1 - Group 1, carcinogen (IARC, 2002)**
- **Aflatoxin M1- hydroxylated form of aflatoxin B1**



Aflatoxin B1



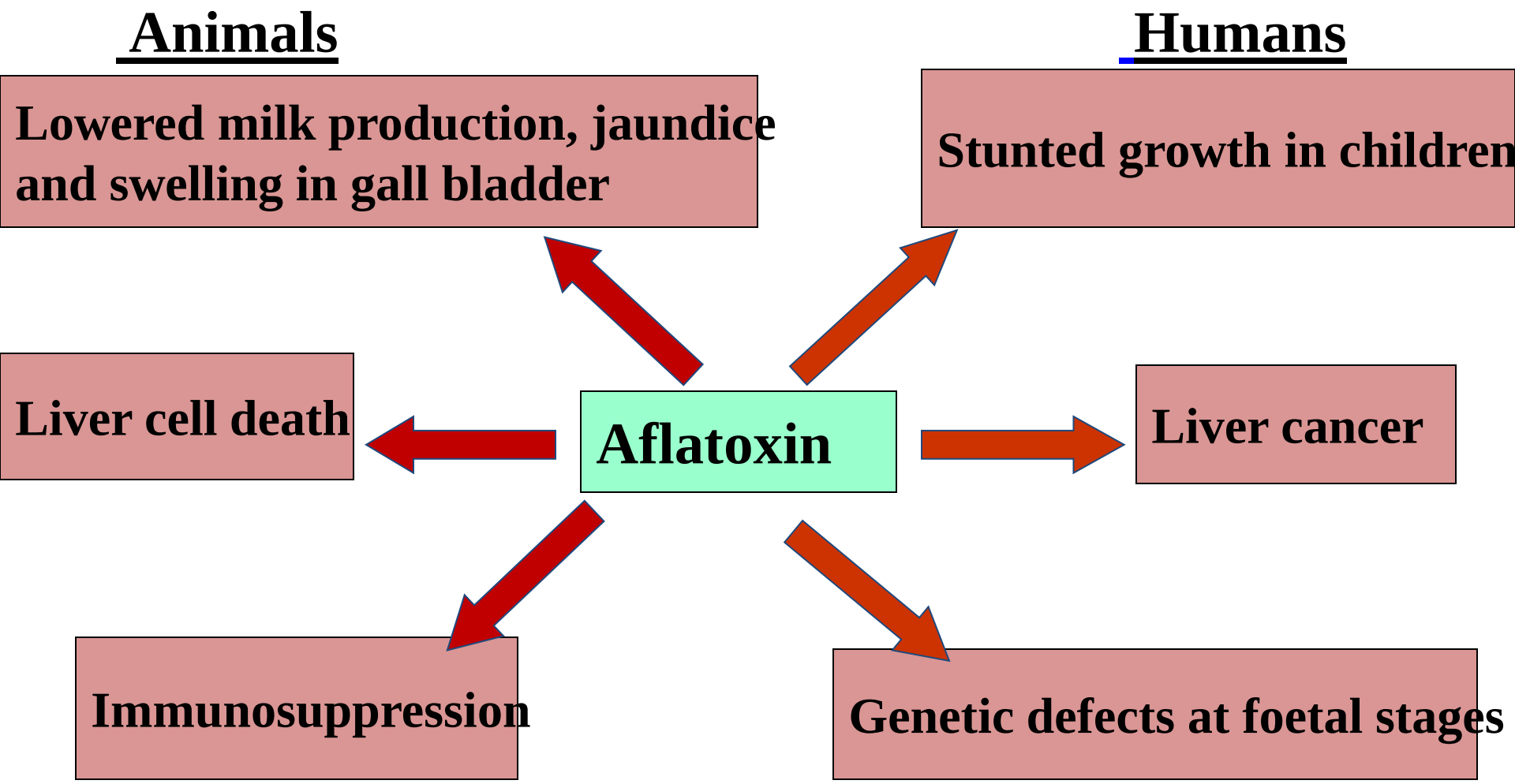


Aflatoxin M1

Permissible limits for Aflatoxin M1 in milk

- **United States Food Drug Administration (USFDA) - 0.5 ppb**
- **Codex Alimentarius Commission (CAC) - 0.5 ppb**
- **European Union (EU) – 0.05 ppb**

Health hazards of aflatoxin M1



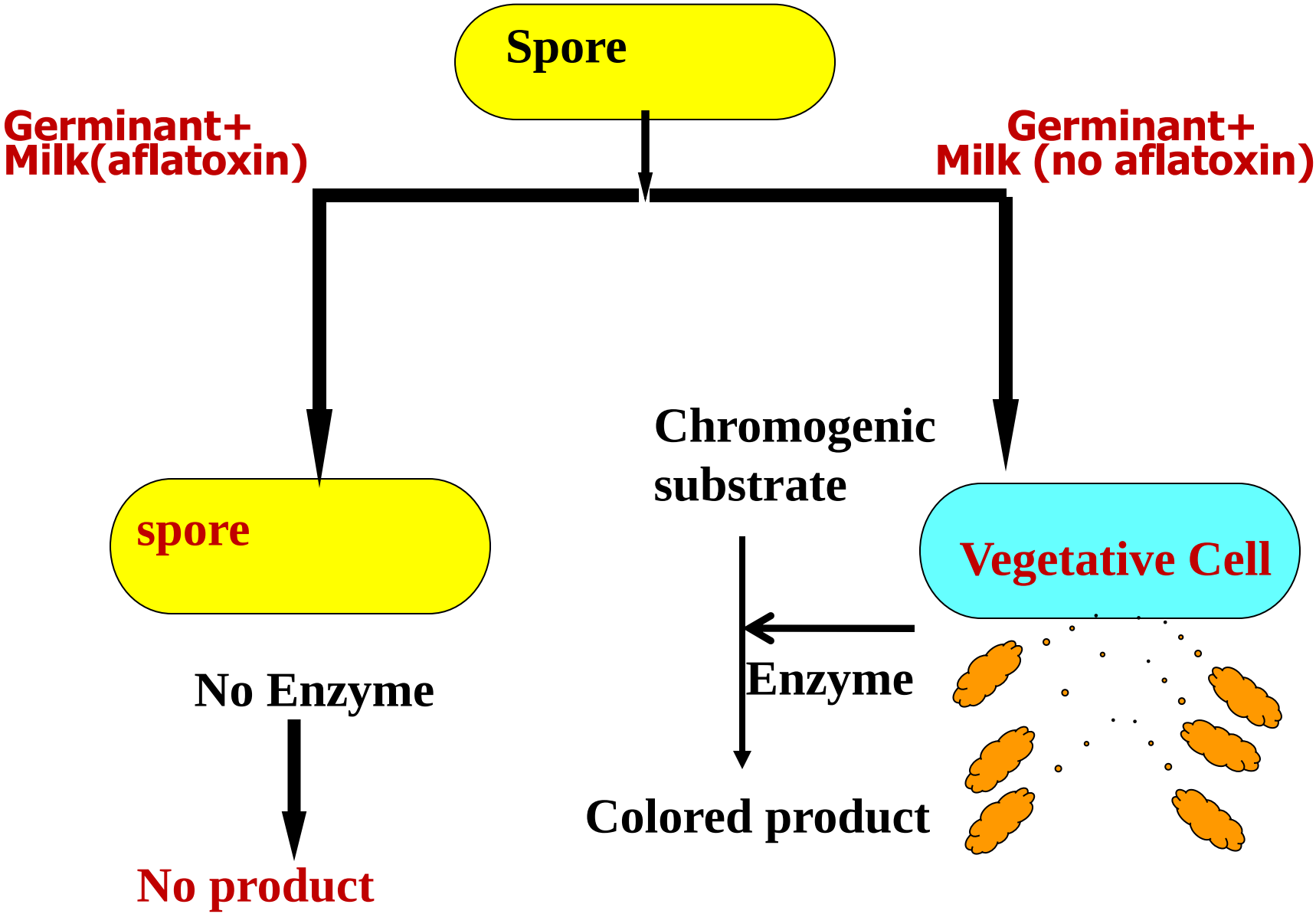
Conventional methods for aflatoxin M1

- **Radioimmunoassay (RIA)**
- **Lateral Flow assay**
- **Enzyme linked immuno sorbent assay (ELISA)**
- **High Performance liquid chromatography (HPLC)**

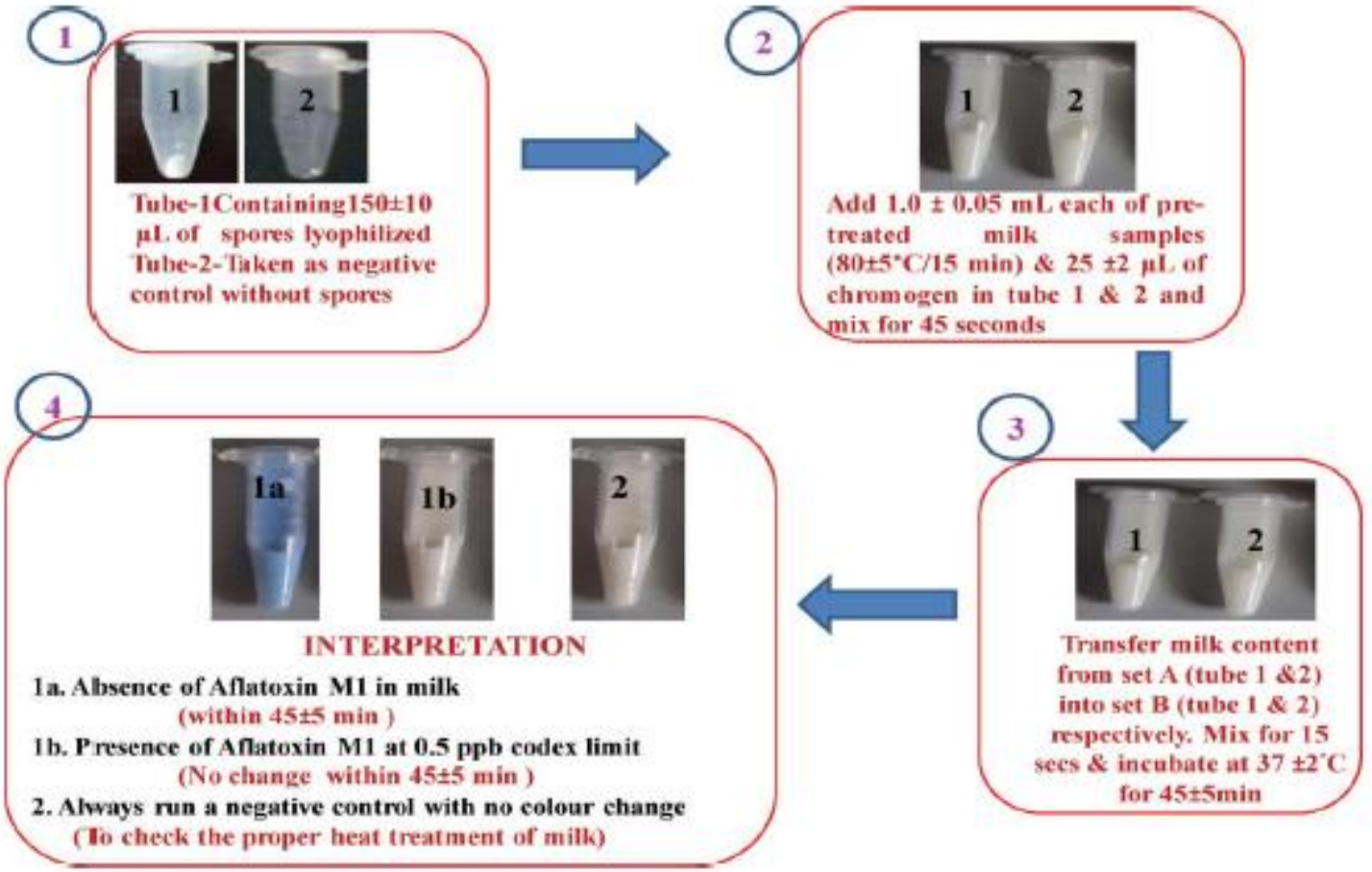
Limitations of conventional methods

- **Extraction problem in Chromatography**
- **Expensive, huge infrastructure**
- **Need experienced personnel**
- **Laboratory bound**

Principle for Aflatoxin detection

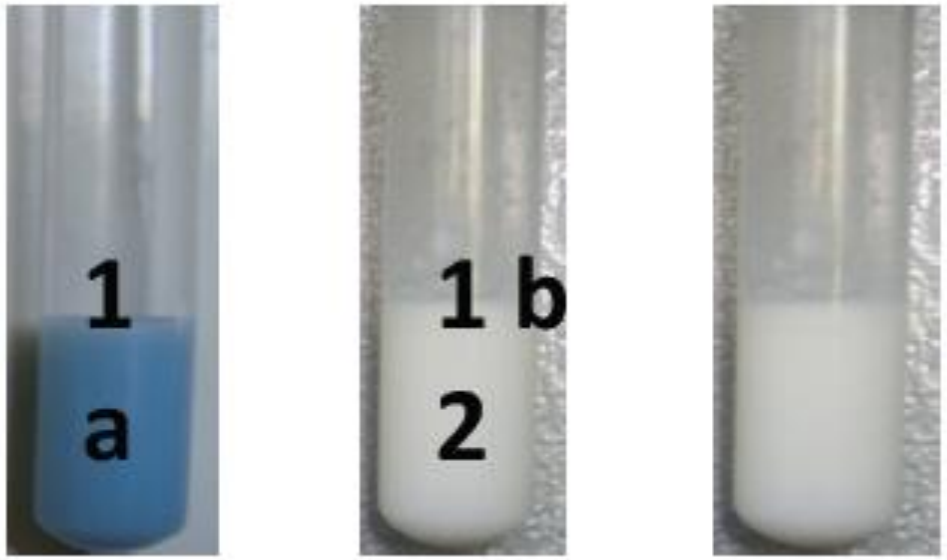


Spore germination based bioassay for aflatoxin



Singh et al. 2013

Chromogenic assay for Aflatoxin detection



[1a]Absence of Aflatoxin M1 in milk; [1b]Presence of Aflatoxin M1 at 0.5 ppb codex limit

[2]Control without spore with no color change (To check the proper heat treatment of milk)

Figure 5: Chromogenic assay.

Verma et al. 2013

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Thank you!