evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: ELISA – Enzyme-Linked Immunosorbent Assay

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Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

- Immunoassays –RIA and ELISA
- ELISA-principle
- Analytes
- Antibodies
- Formats
- Detection
- Derived methods

History - RIA

- RIA-RadioImmunoAssay -
  - 1960 – developed Rosalyn Sussman Yalow and Solomon Berson
  - 1977 – Nobel Prize for Medicine for the development of the RIA for peptide hormones
  - displacement of antibody-bound I^{131}-insulin by serum-derived insulin
  - separation of antibodies and antigen-antibodies complex
    - paper radioelectrophoresis
    - precipitation and ultracentrifugation
    - chromatography
  - still used for small molecules
  - radioactive waste
  - time consuming

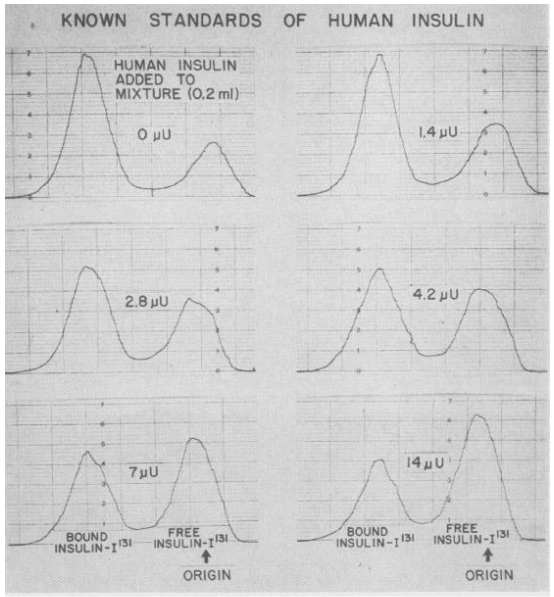


FIG. 2. A: RADIOCHROMATO-ELECTROPHORETOGRAMS OF ANTISERUM, INSULIN MIXTURES. Mixtures contained the same concentrations of guinea pig antibeef insulin serum and beef insulin-I^{131} but varying concentrations of human insulin as indicated.

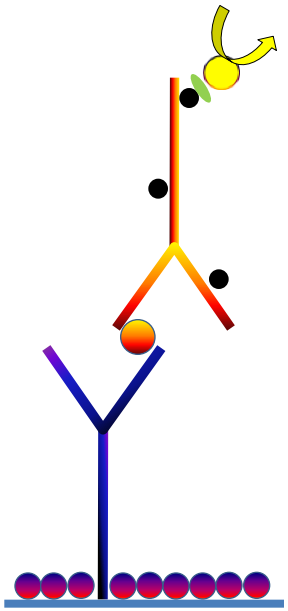
History - ELISA

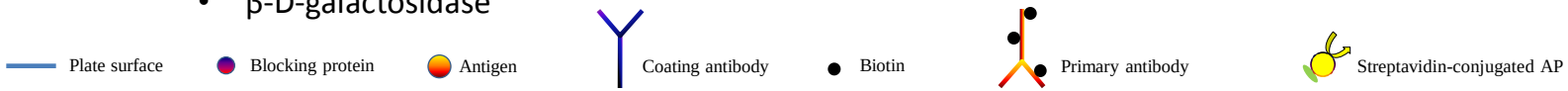
- ELISA - Enzyme-Linked Immunosorbent Assay or EIA – Enzyme ImmunoAssay
- **First ELISA:**
  - 1971 – E. Engwall, P. Perlman - Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G
  - 1971 - Van Weemen BK, Schuurs AH - Immunoassay using antigen-enzyme conjugates
  - 1974 – A. Voller, D. Bidwell, G. Huldt, E. Engwall – Microplate method of Enzyme-Linked Immunosorbent Assay and its application to malaria (96-well polystyrene plate)
- **Steps had to be invented:**
  - Enzyme-labeling of the antibody
    - 1966 – S. Avrameas and G. B. Pierce (independently)
    - early 1970s – ERIAC – scepticism. „*Too bulky, too large, sterical hindering*“
  - Adsorption surface
    - 1966 – L. Wide, J. Porath – sephadex-coupled antibodies
    - 1967 – antibody-coated tubes for RIA
    - 1951 – first microplate (acrylic) - Gyula Takátsy
- **Automation:**
    - 1976 – 96-well microtiter plate format
    - early 1980 – first commercial tests
    - 1980 – first fully automated instrument

Dr. Eva Engvall (Sweden), Dr. Anton Schuurs (The Netherlands), Dr. Peter Perlmann (Sweden), Dr. Bauke van Weemen (The Netherlands), ELISA inventors

Principles

- **Solid-phase adsorption** – polystyrene, polypropylene, PVC
  - uncoated
    - electrostatic interaction
  - avidin-coated
    - avidin-biotin technology
  - protein-A/G coated
    - immobilization of mammalian antibodies
  - biotinylated
    - avidin-biotin technology
  - organic
  - poly-Lys-, sulphydryl-, amine-
  - IMAC
    - His-tagged proteins
- **Antigen-antibody interaction**
- **Enzymatic activity detection**
  - primary antibody, or
  - secondary antibody, or
  - antigen labeled with
    - Alkaline phosphatase (AP)
    - Horseradish peroxidase (HRP)
    - β-D-galactosidase





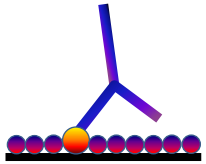
Analytes

- Antigens
  - Antibodies
    - disease diagnosis, e. g. anti-HIV antibodies
  - Proteins
  - Nucleic acids
    - autoimmune diseases
    - DNA damage
    - interaction with proteins
  - Polysaccharides
- Haptens
  - partial antigens
  - substances having a single antigenic determinant that can react with previously existing antibody but doesn't stimulate antibody production unless combined with other molecules
    - low-molecular compounds, e. g. pesticides, hormones, drugs, allergens, peptides
- Whole cells or virions
  - tumour cells
  - bacteria
  - viruses

Antibodies

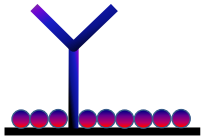
- *Polyclonal*
- *Monoclonal*
- *Recombinant*
- *Synthetic*

- **Primary**
  - interacts with antigen
  - un/labeled
- **Secondary**
  - interacts with primary antibody
  - labeled
- **Coating**
  - interacts with antigen
  - unlabeled
- **Conjugate**
  - labeled secondary antibody
  - avidin or biotin – conjugated enzyme
- **Tracer**
  - labeled antigen in competitive assays

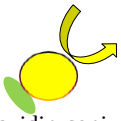


Primary antibody-antigen binding

Labeled secondary antibody



Coating the plate with antibody and blocking



Streptavidin-conjugated AP

Radioactively labeled insulin

Formats

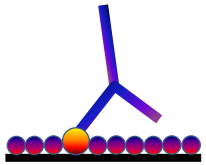
- Capture × Sandwich
- Direct × Indirect
- Competitive × Non-competitive

- Microplate
- Paramagnetic beads
- Microfluidics
- Immunosensors

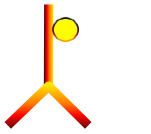
Non-competitive Indirect Capture ELISA:

Coating the plate with antigen and blocking

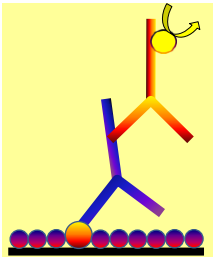
Primary antibody



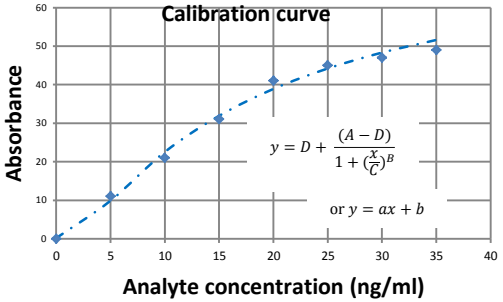
Antibody-antigen binding



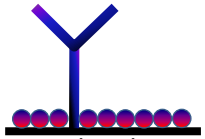
Labeled secondary antibody



Detection

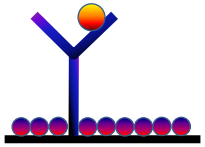


Non-competitive Direct Sandwich ELISA:

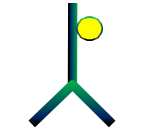


Coating the plate with antibody and blocking

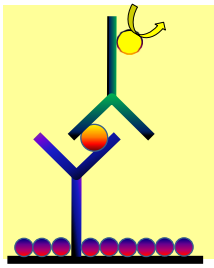
Antigen



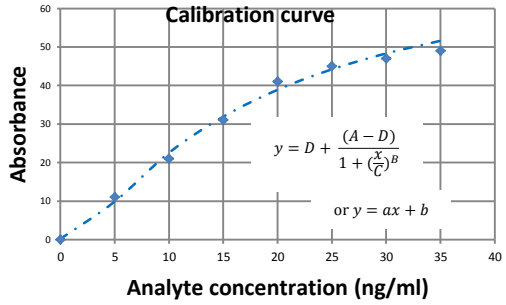
Antigen-antibody binding



Labeled detection antibody

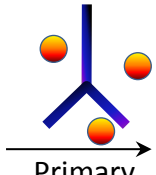


Detection

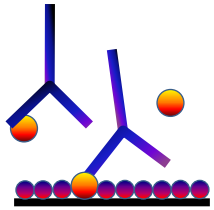


Competitive Indirect Capture ELISA:

Coating the plate with antigen and blocking

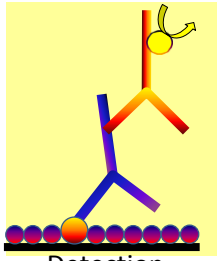


Primary antibody

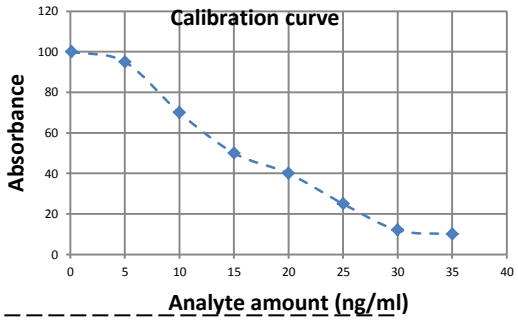


Antibody-antigen binding

Labeled secondary antibody



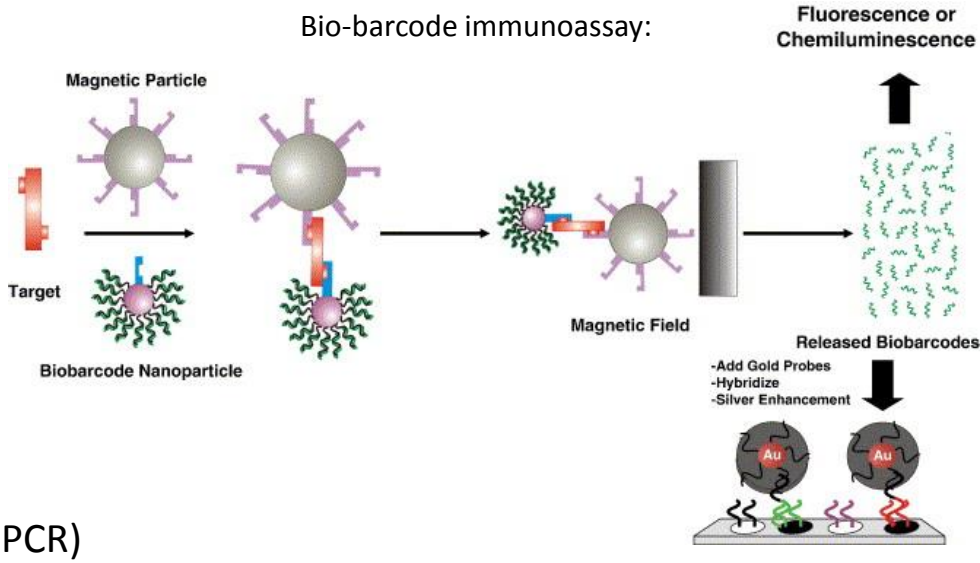
Detection



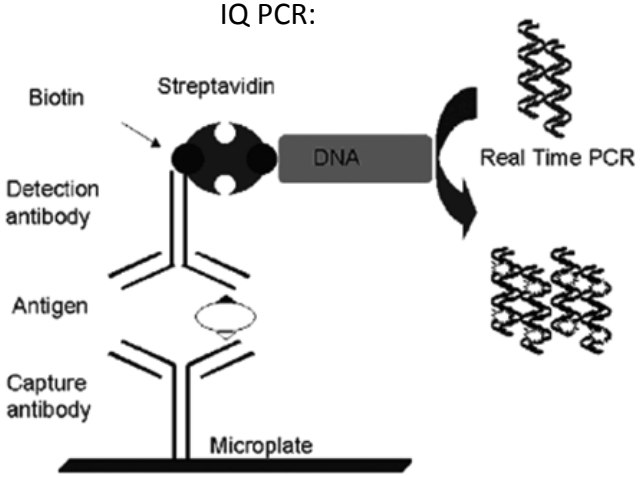
Detection

- Enzymatic activity
  - absorbance
    - chromogenic substrate
  - fluorescence
    - fluorogenic substrate
- Nanoparticles
  - QDs
- DNA reporters
  - Immunoquantitative Real-Time PCR (iqPCR)
- Label-free
  - SPR
- Fluorogenic reporters
- Electrochemiluminiscent tags

Bio-barcode immunoassay:



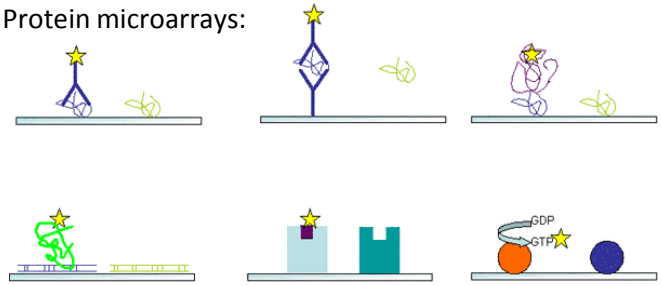
IQ PCR:



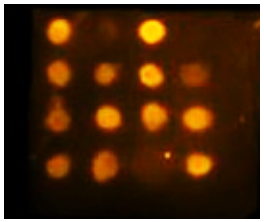
Derived methods

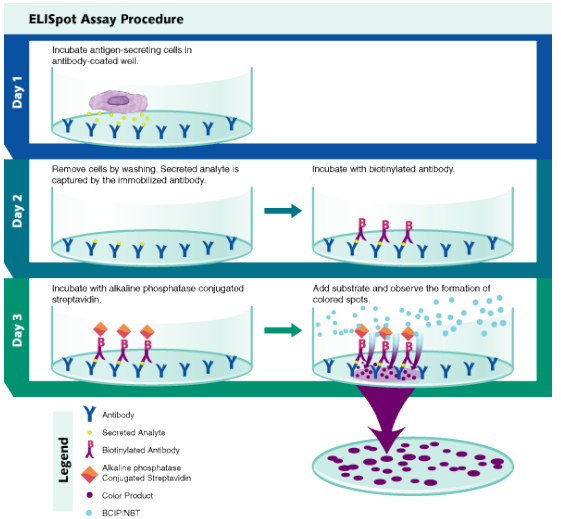
- LFIA, Dipsticks
- Magnetic immunoassays
- Immunosensors
- Protein arrays
- In cell ELISA (ICE)
  - in permeabilized cells
- Elispot
  - for molecules secreted by cells
- PCR-ELISA
  - ELISA-based detection of a PCR product
- ELIMSA
  - Enzyme linked immuno mass spectrometric assay
- MELISA
  - Multianalyte Enzyme-Linked Immunosorbent Assay

Protein microarrays:

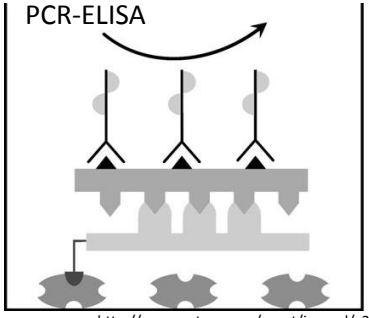


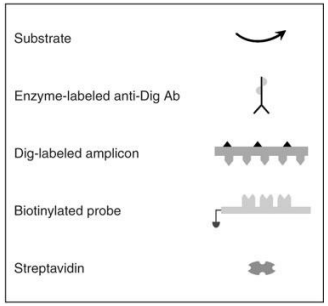
http://www.microarraystation.com/protein-microarray/





http://www.rndsystems.com/product_detail_objectname_ELISpotAssayPrinciple.aspx





http://www.nature.com/nprot/journal/v2/n10/fig_tab/nprot.2007.311_F1.html

Thank you for your attention!

DOES ANYONE HAVE THE STOP SOLUTION FROM THE ELISA KIT??!!

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Laboratory of Metallomics and Nanotechnologies

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