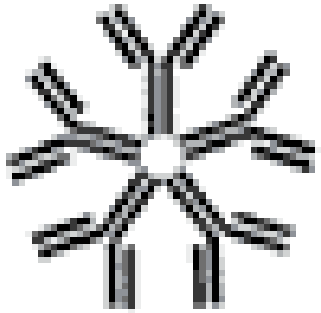
Principles of Immunochemistry



Immunoassay

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An immunoassay is an analytical method which uses antibodies as reagents to quantitate specific analytes

Immunoassays

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- **$6 Billion Industry Worldwide**
- **2.5 Billion Tests Sold Annually**
- **Highly Quantitative**
- **Regulatory Approved**
- **Flexible Test Formats**
- **Diverse Markets and Applications**

Clinical Diagnostic Immunoassays

- **In Use >30 Years**
- **Basis for Critical Human Health Decisions**
  - Disease diagnosis (AIDS, Hepatitis, PSA)
  - Therapeutic drug monitoring
  - Drugs of abuse screening
  - Over 70 clinical analytes tested by immunoassay
  - Home pregnancy tests
- **Highly Reliable**

Other Immunoassay Markets

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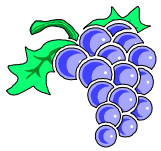
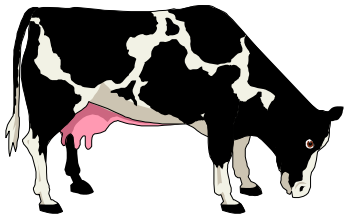
- Agricultural
- Environmental
- Food
- Industrial
- Pharmaceutical
- Veterinary
- Water Quality

Clinical vs. Environmental Immunoassay

- **The Sample**
  - Clinical
    - Urine, blood, saliva
  - Environmental, Agriculture
    - Water
    - Soil extracts
    - Plant extracts
    - Animal products/tissues - blood, urine, milk, meat
    - Food
    - Industrial processes and effluents





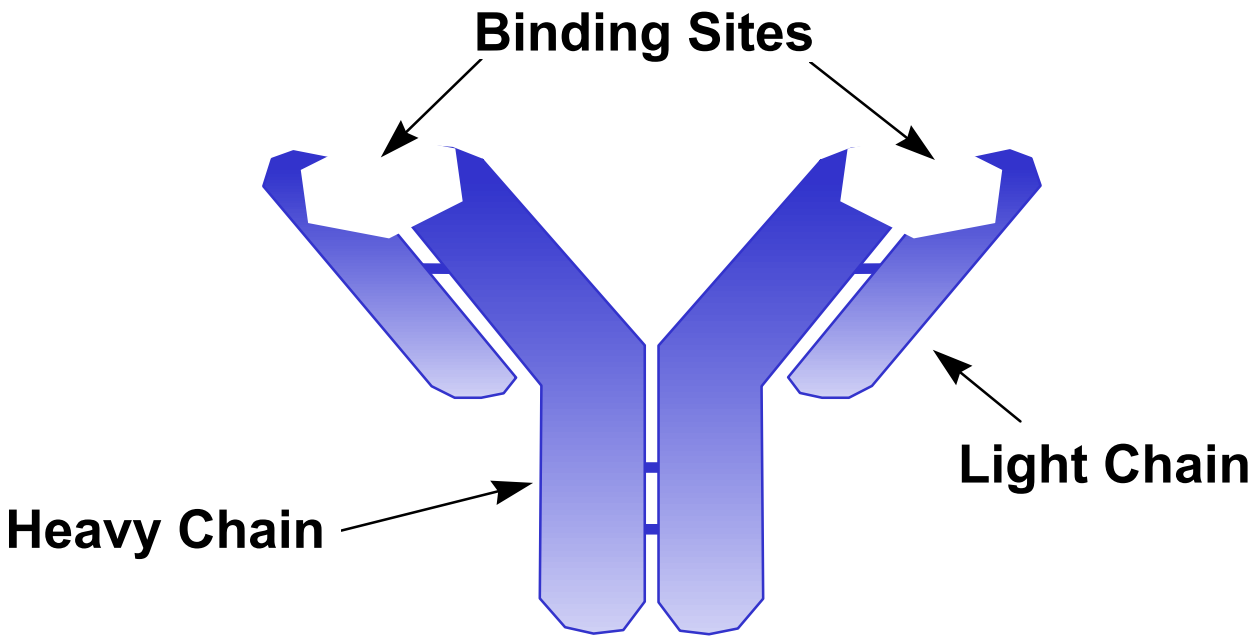
Antibodies

- **Key Reagents in All Immunoassays**
- **Proteins Produced by Immune System of Higher Animals**
  - Produced by specific white blood cells
  - In response to recognition of “foreign” substances
  - Examples:
    - Vaccinations
    - Response to natural infections (mumps, chicken pox)
- **Physically Bind to Antigens**

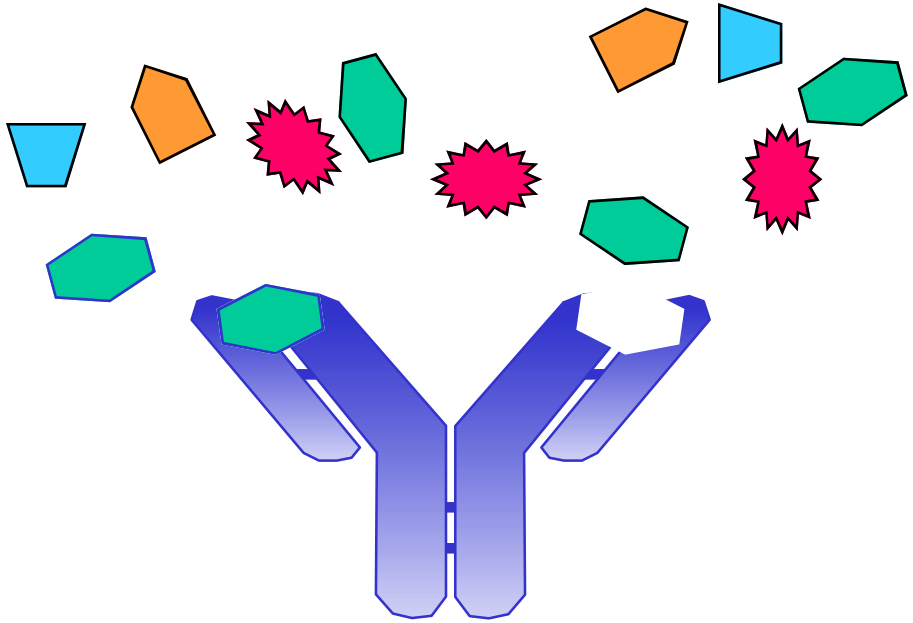
Antibodies

- **Tightly Bind Only to Substance Which Elicited Production**
- **Strength of Binding (Affinity) Determines Sensitivity of Method**
- **Specificity Allows Detection in Complex Matrix - Minimum Sample Preparation**

Antibody Structure



Antibody-Antigen Binding



Polyclonal Antibodies

- **Animals are injected with analytical target**
- **Many different antibody-producing cells make Polyclonal antibodies**
- **Polyclonal antibodies purified directly from blood**

Monoclonal Antibodies

- Mice are injected with the analytical target
- Antibody producing cells are taken from the animals
- Antibody-producing cells are fused with cells that grow continuously in culture to form Hybridomas
- A single hybridoma produces only one antibody
- A single hybridoma divides to produce a large population of clones all making the same Monoclonal antibody
- Living hybridomas are frozen indefinitely in liquid nitrogen
- Indefinite supply of uniform consistency reagent

Monoclonal vs. Polyclonal

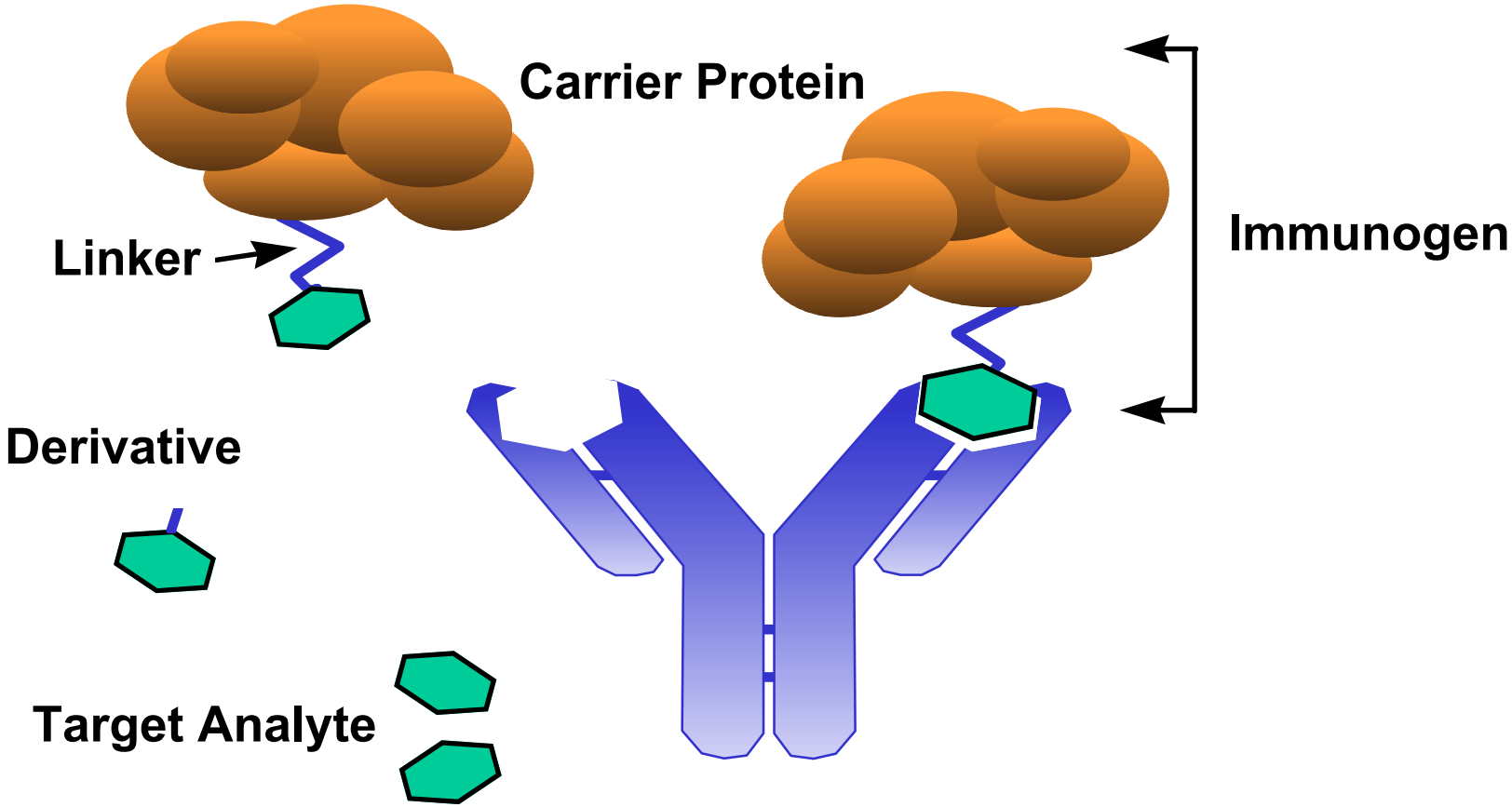
- **Monoclonal**
  - Lot-to-lot consistency
  - Indefinite supply
  - Highly specific
  - Longer lead time
  - Higher initial costs
- **Polyclonal**
  - Lot-to-lot variability
  - More broadly reactive
  - Often more sensitive
  - Shorter lead times
  - Lower initial costs

Selection is based on application, time and money

Antibody Development

- **Immune System Responds Only to High Molecular Weight Immunogens**
  - M.W. Typically > 10,000
- **Agrochemicals and Environmental Pollutants Mostly Small Molecules**
  - M.W. Typically < 1,000
- **Agrochemicals Require Preparation of Suitable Immunogen**
  - Couple chemical to carrier protein

Immunogens


Carrier Protein
Linker
Immunogen
Derivative
Target Analyte

Immunoassay Visualization

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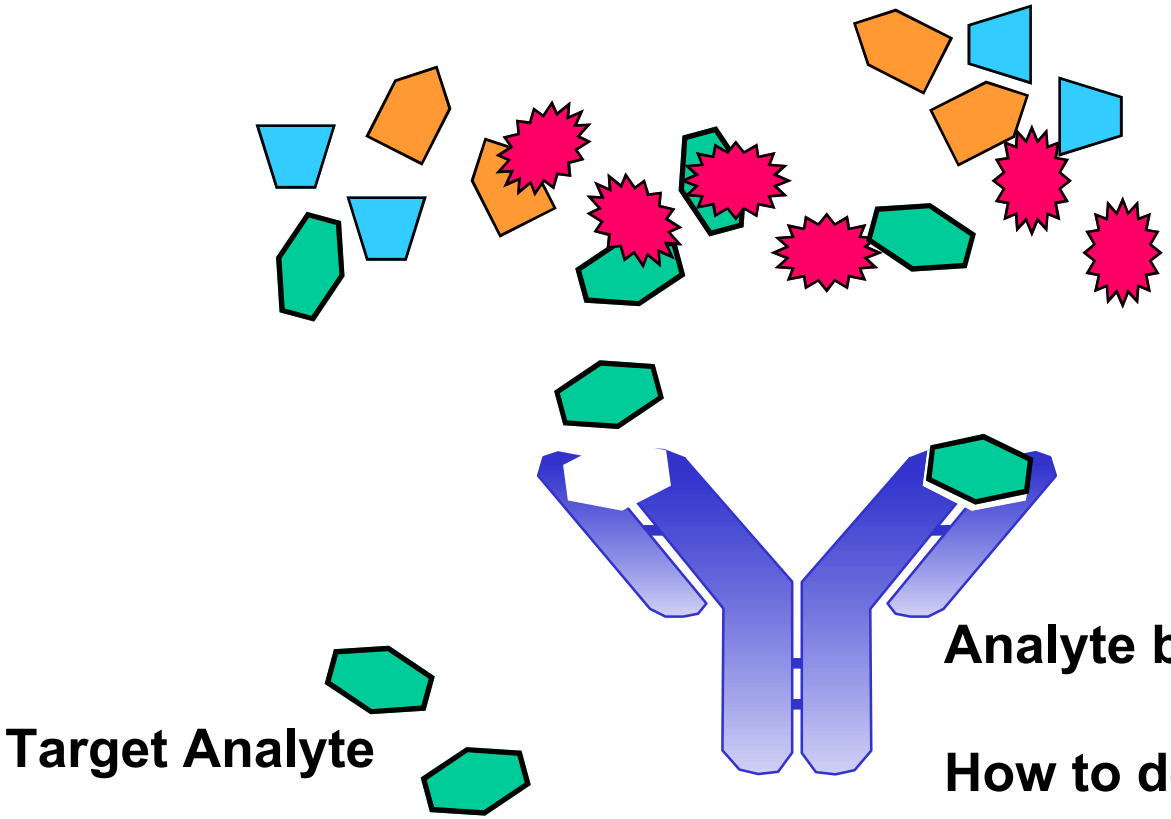


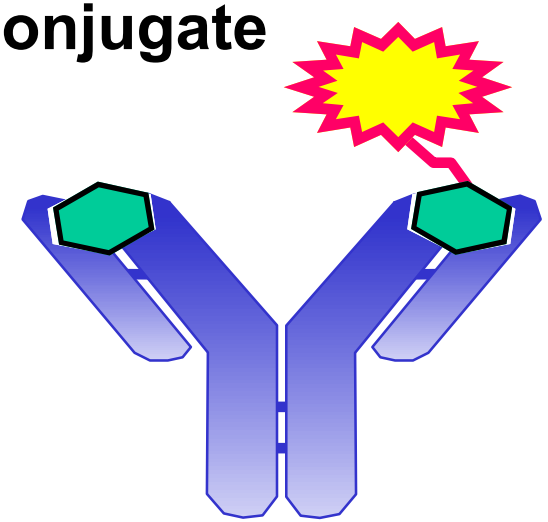
Target Analyte

Analyte bound by antibody

How to detect binding?

Immunoassay Conjugates - Detecting Binding

Immunoassay Conjugate



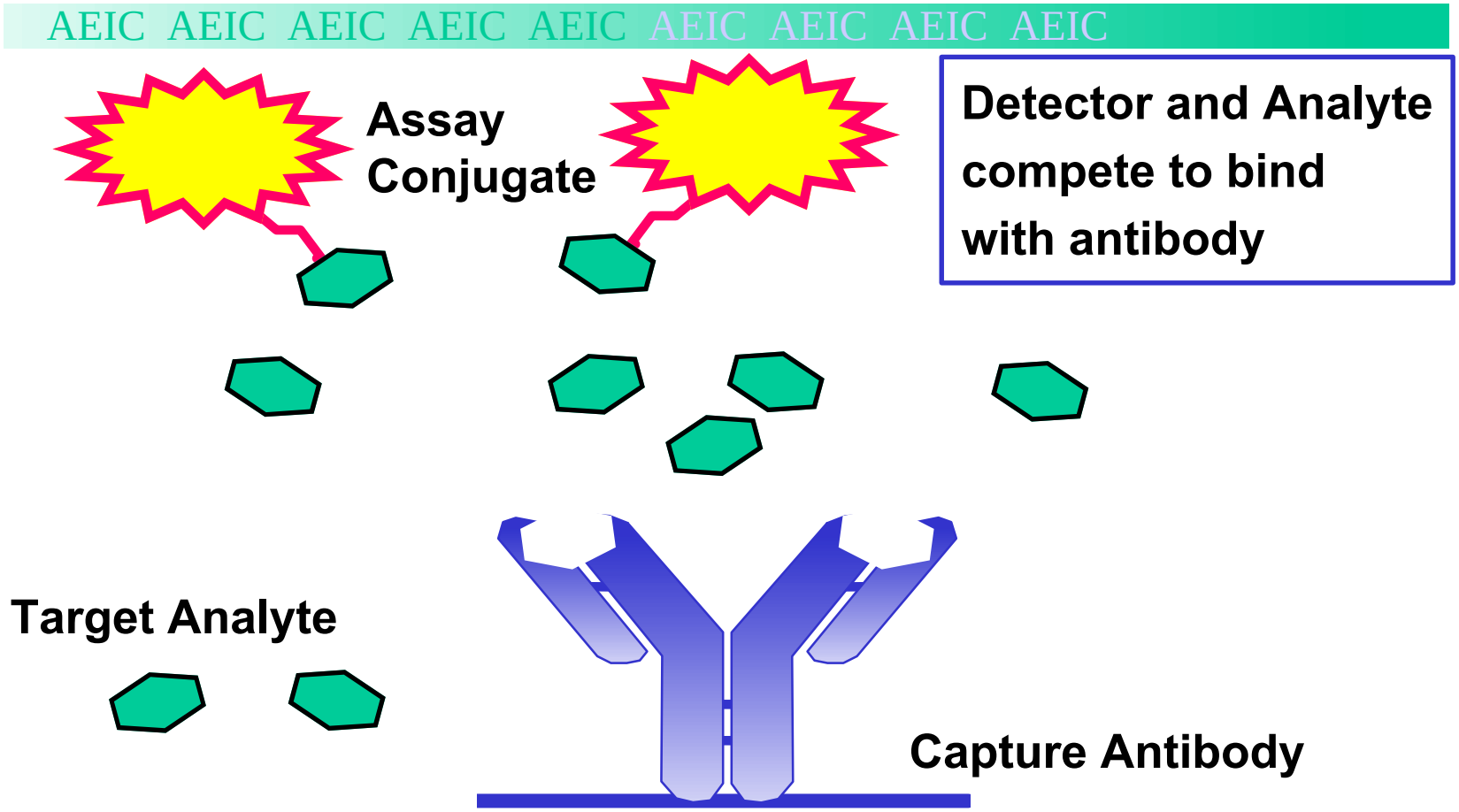
Detectable Label

- **Radiolabel (RIA)**
- **Enzyme (EIA)**
- **Fluorescence (FIA)**
- **Luminescence**
- **Electrochemical**
- **Visual**
  - **Colloidal gold**
  - **Colored latex**

Immunoassay Formats

- **Antibodies attached to a solid phase**
  - Plastic wells, tubes, capillaries
  - Membranes
  - Latex particles
  - Magnetic Particles
- **Solid phase used to separate bound from free Assay Conjugate (label)**
- **Choice of format determined by application**

Competitive Immunoassay



Competitive Immunoassay

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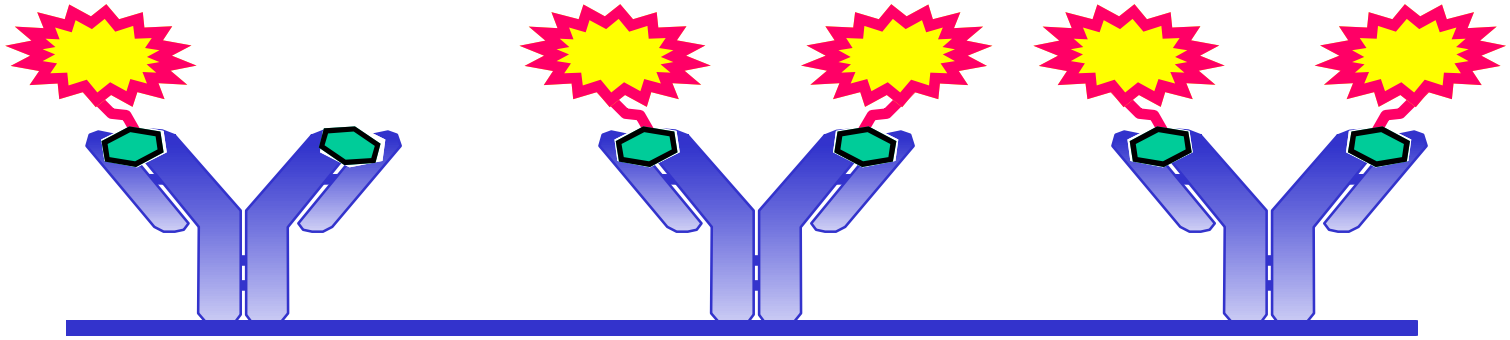
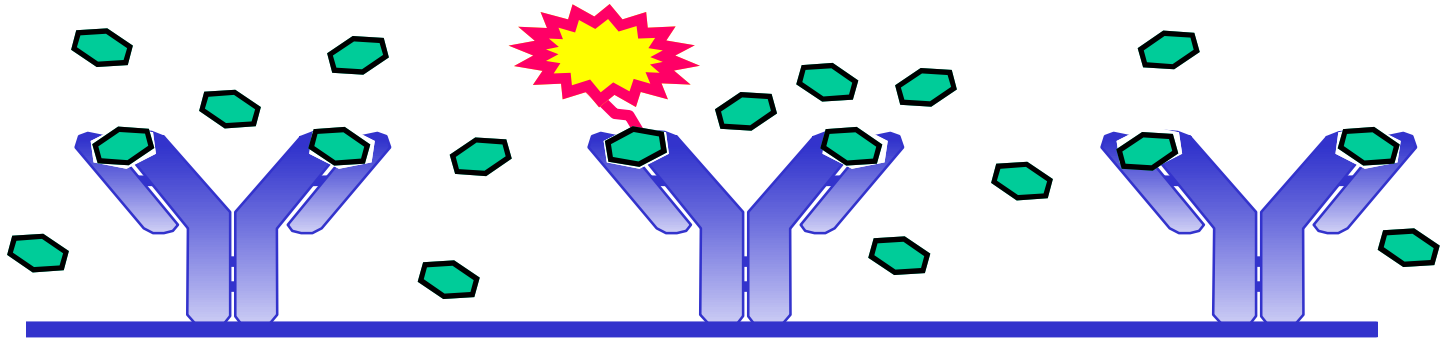
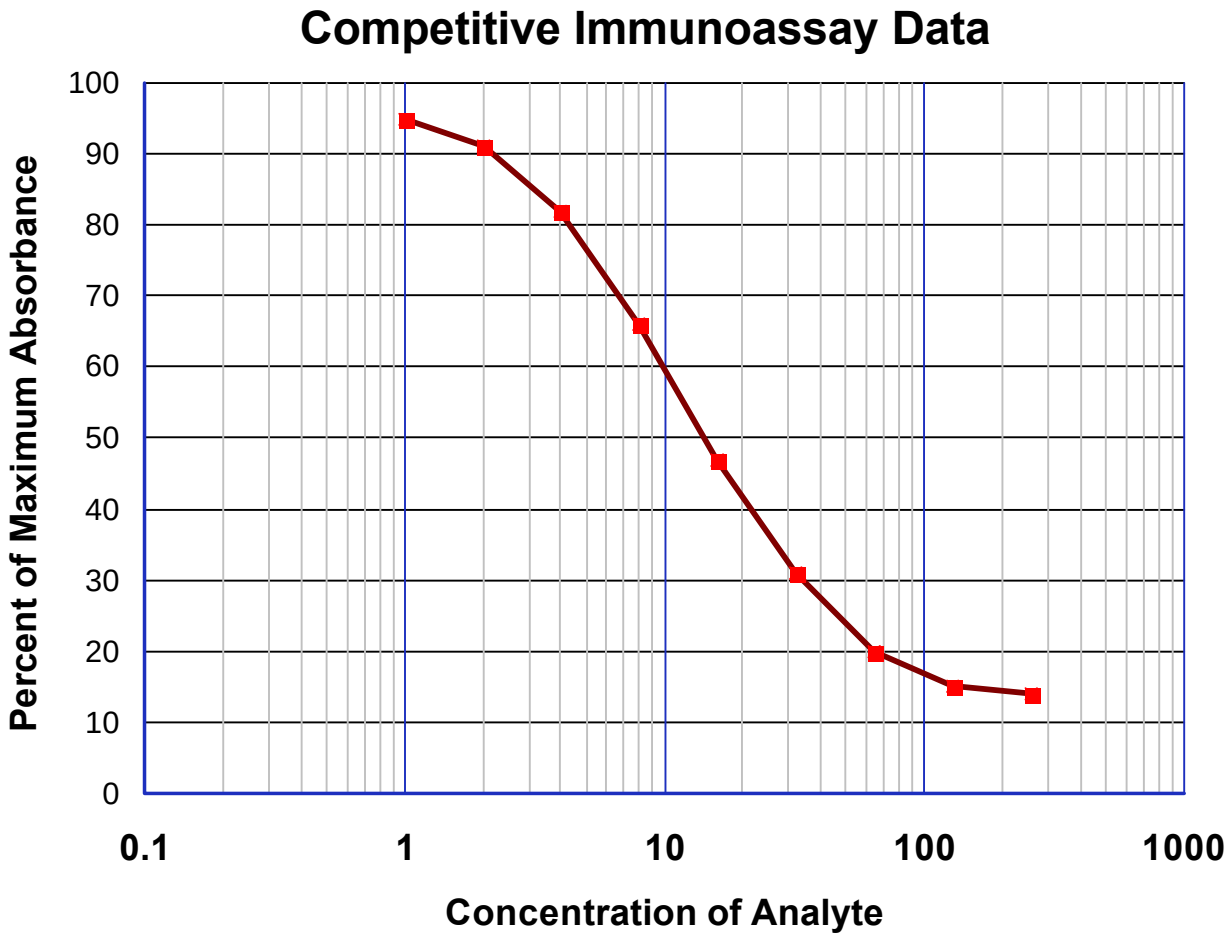
I. No analyte - high detection signal



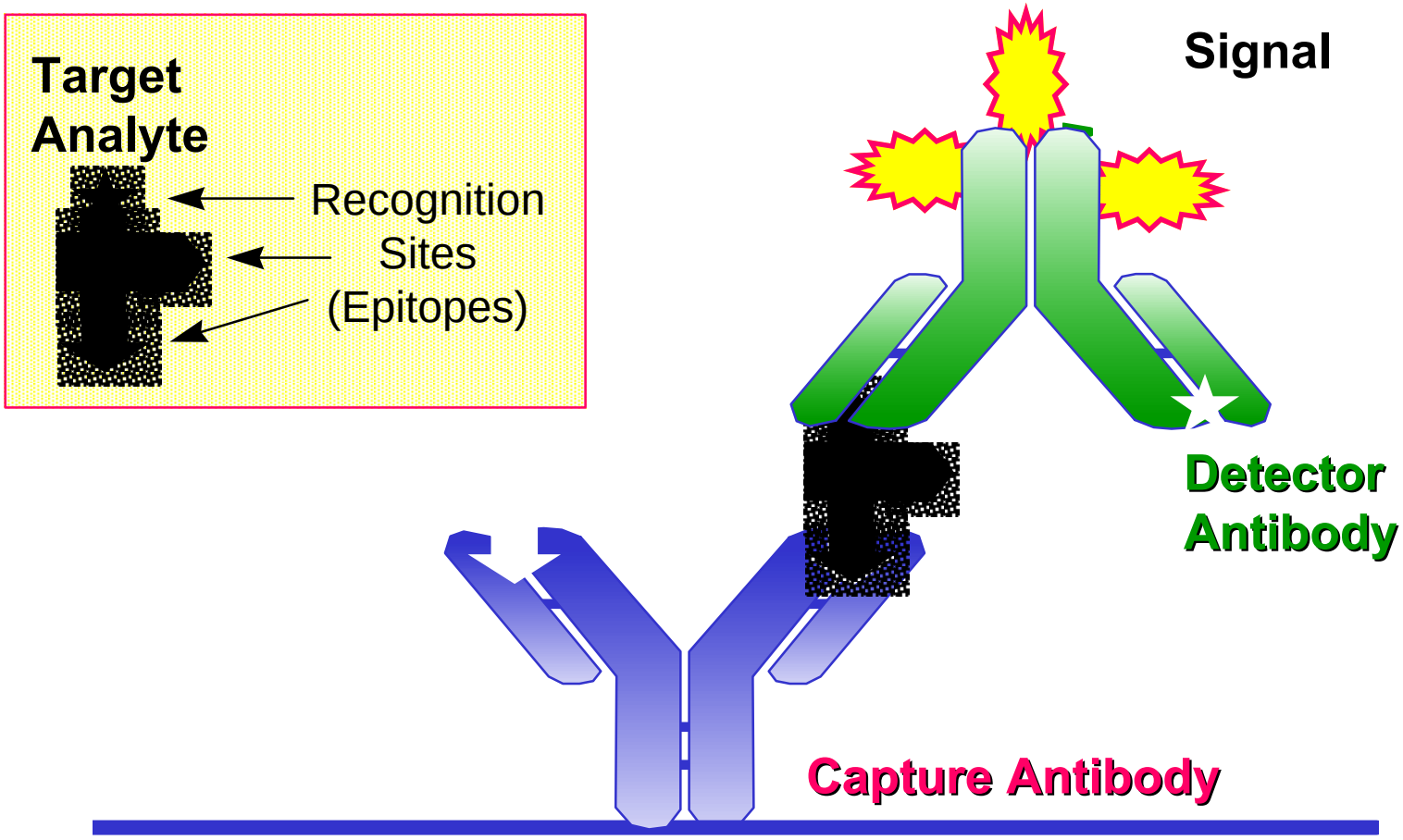
II. Analyte present - detection signal reduced



Competitive Immunoassay Data Format



Double Antibody Sandwich Immunoassay



Immunoassay Performance Characteristics

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- **Sensitivity (LOD, LOQ) - ppb to ppt (10^{-12}M)**
- **Specificity**
  - Families of chemicals vs. single compounds
  - Commercial products
  - Metabolites, degradation products
  - Process by-products, intermediates
- **Precision - Quantitative or qualitative**
- **Accuracy - Recovery and false negative/positive rates**
- **Matrix Effects/Interfering Substances**
- **Linear Range**
- **Stability, Reliability, Robustness**

Required Sensitivity of Some Environmental Immunoassays

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Compound		Detection Level		
		ppb	[M]	
Benzene	78	100	1.3E-06	8E+05
	106	100	9.4E-07	1E+06
TCE	137		7.3E-08	1E+07
PAH	202		5.0E-08	2E+07
PCB	324		3.1E-08	3E+07
RDX	222	1	4.5E-09	2E+08
TNT	227	1	4.4E-09	2E+08

Sensitivity of Small Molecule Immunoassays

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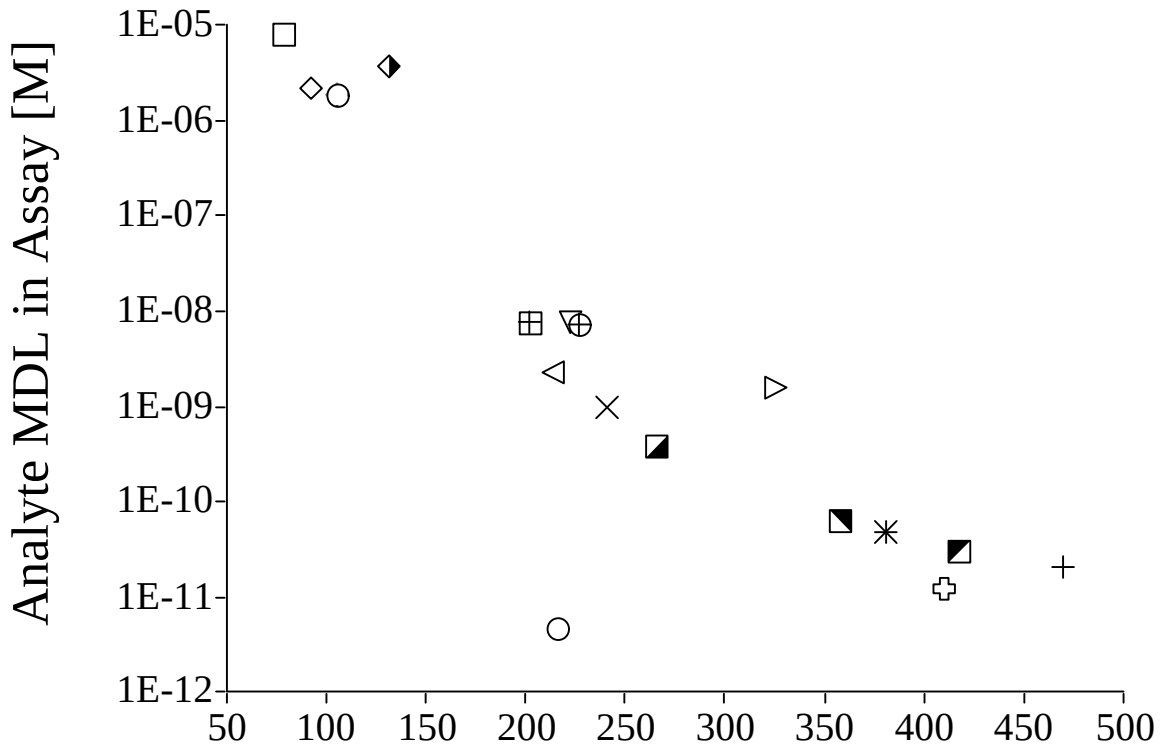
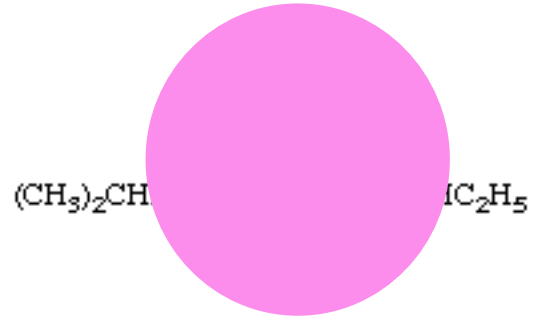


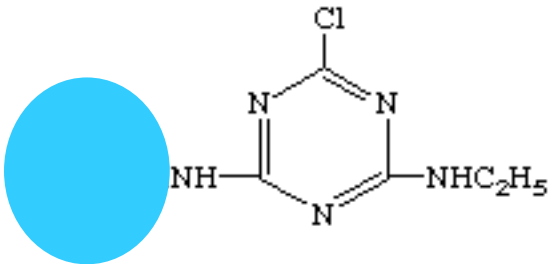
Specificity Considerations for Triazine Herbicides

Common to Class

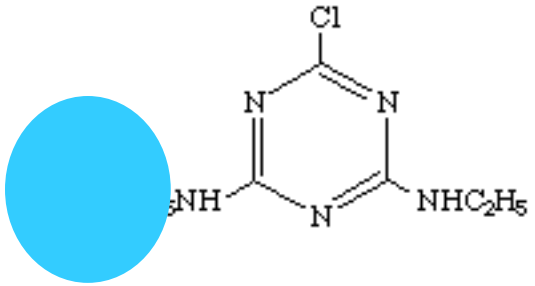
Compound Specific



Atrazine



Simazine



Immunoassay Development Process

- **Define Performance Characteristics**
  - Sensitivity and specificity are determined by the antibody and the assay conjugate
  - Format determined by application
- **Development Process**
  - Antibody and assay conjugate design and development
  - Test format development and optimization
  - Validation
  - Controlled production, QA/QC
- **1 to 2 Years**

Designing Antibodies and Assay Conjugates

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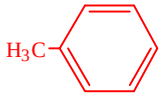
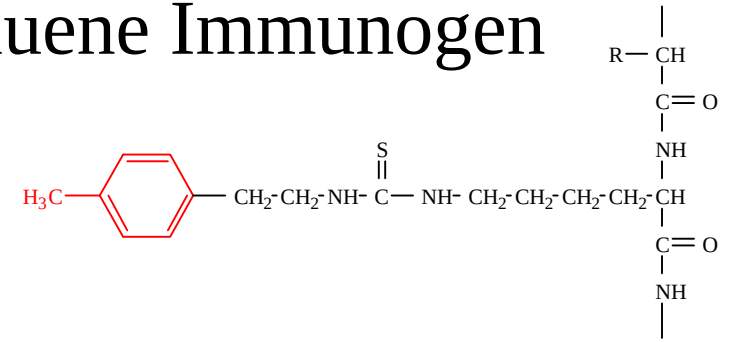
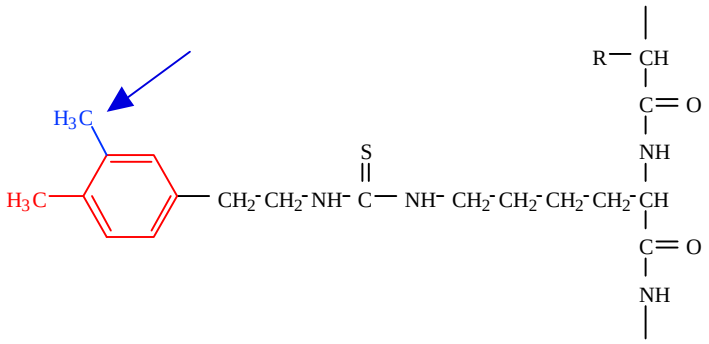
Toluene

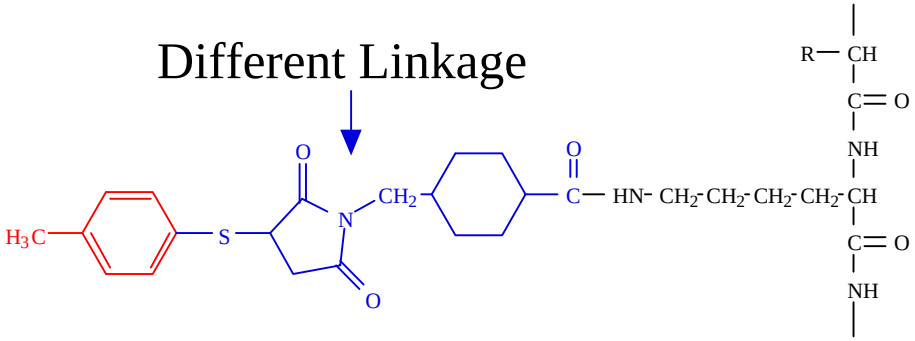


Toluene Immunogen





Different Linkage



Selecting an Assay Conjugate for Sensitivity

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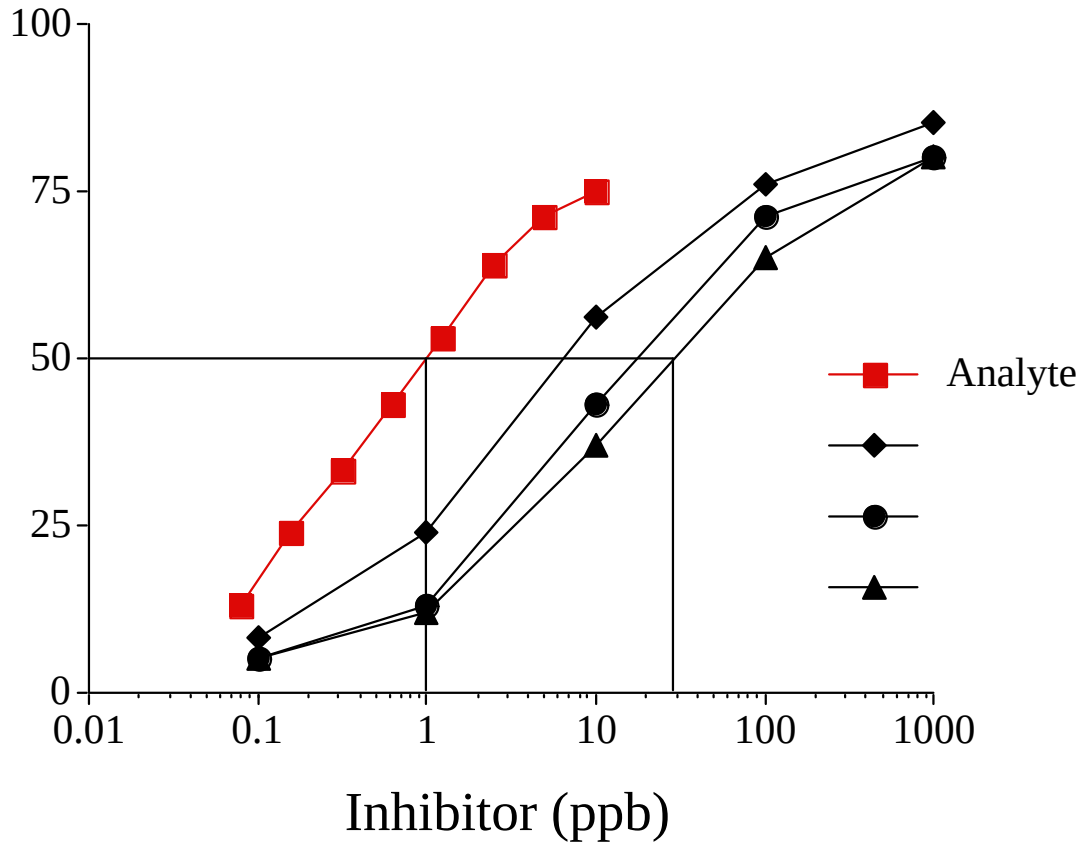

*AEIC, 1998

Assay Conjugate Effect on Sensitivity

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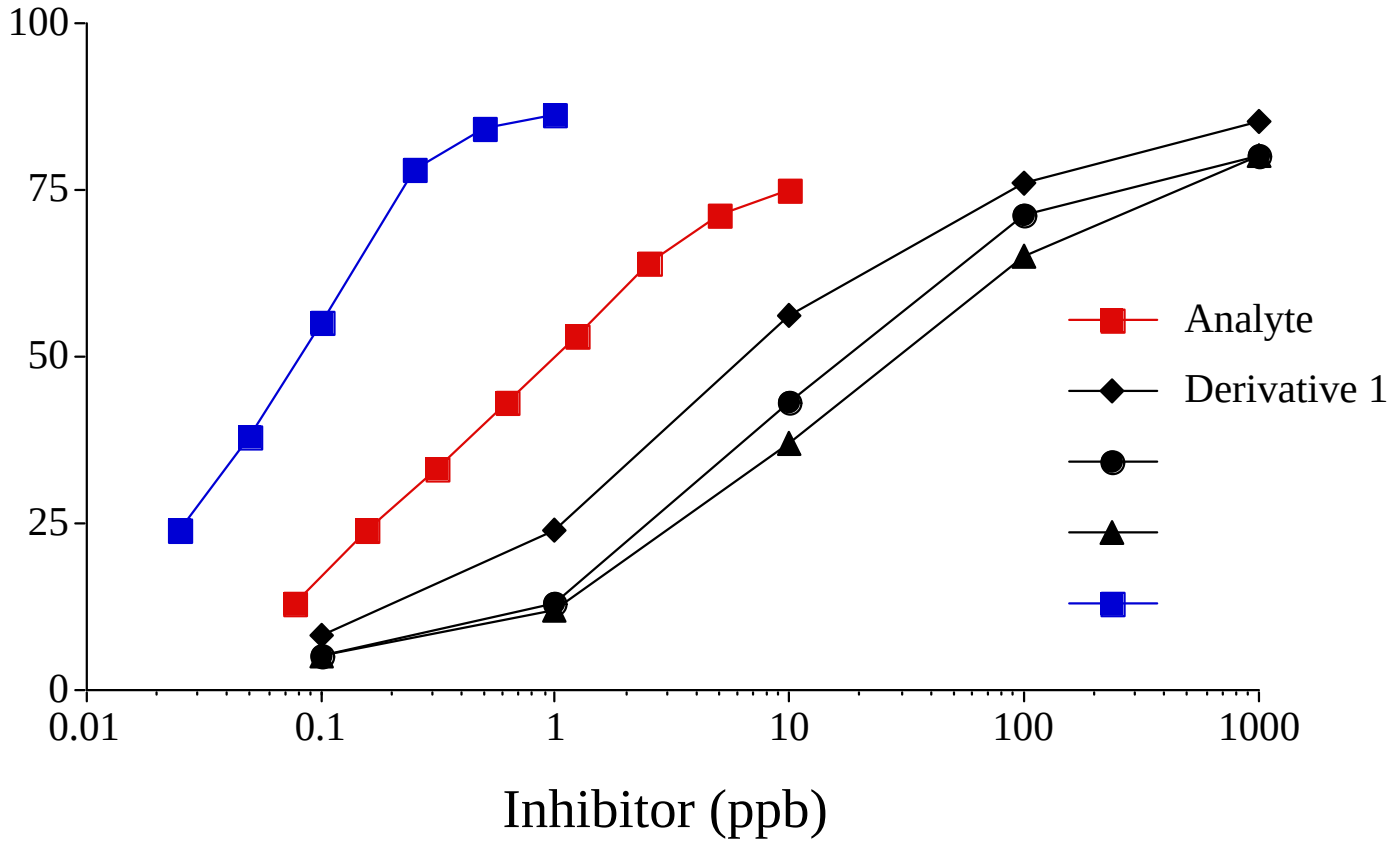

*AEIC, 1998

Designing Broad Reactivity to 16 Polyaromatic Hydrocarbons

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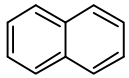
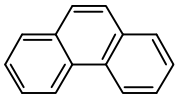
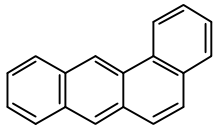
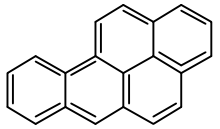


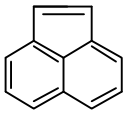
Naphthalene



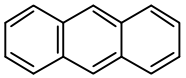


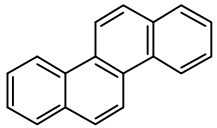
Benzo[a]anthracene



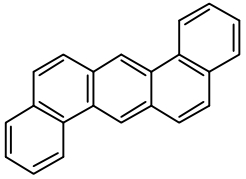


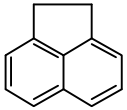
Acenaphthylene

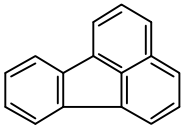


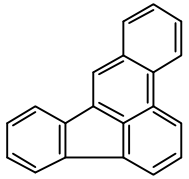


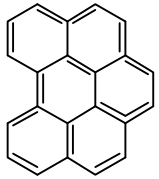
Chrysene

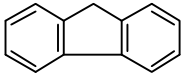


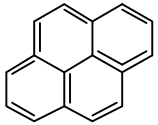


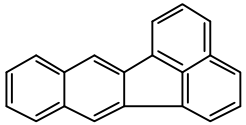


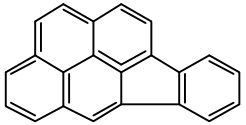




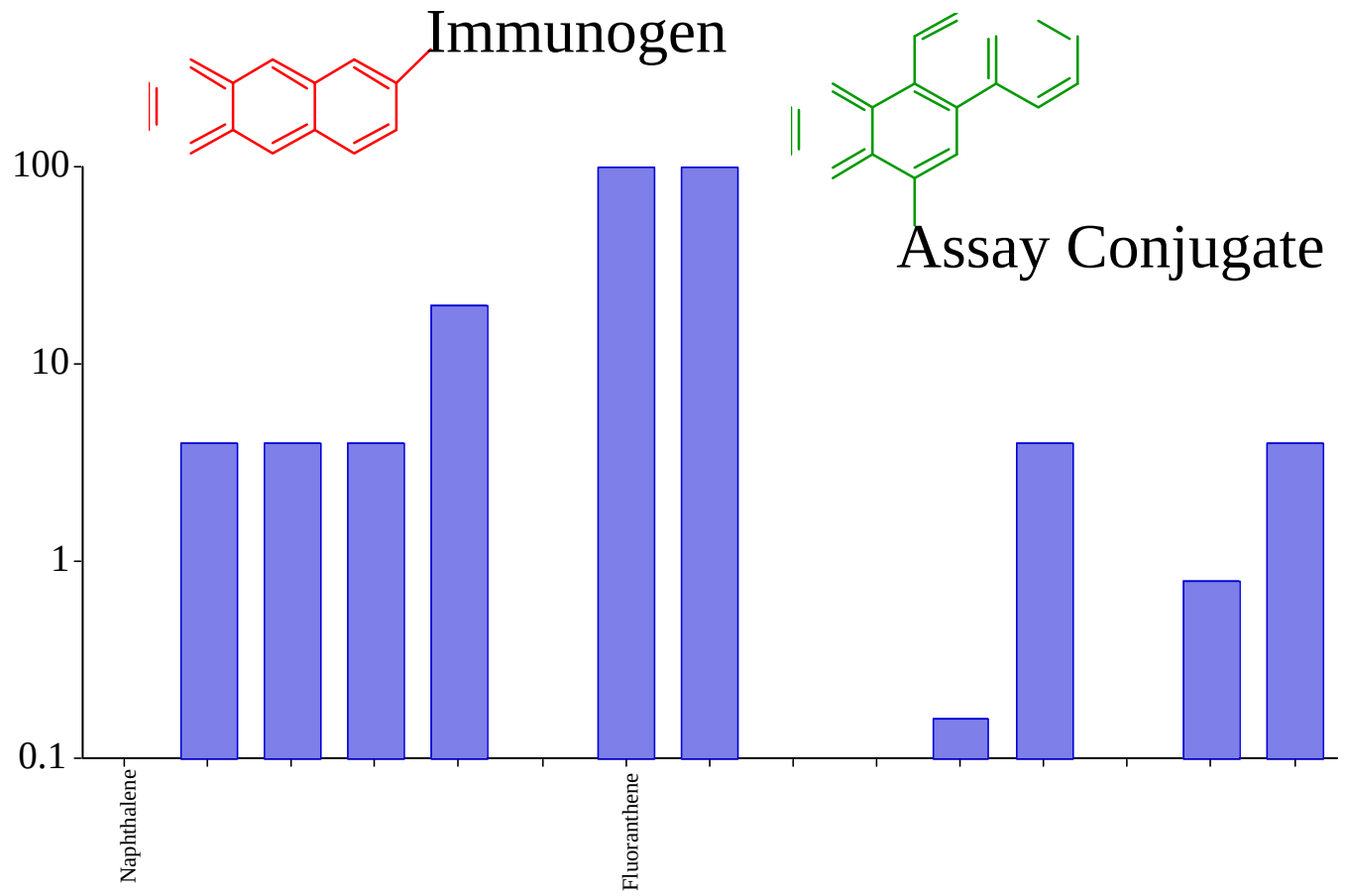








The Effect of the Antibody and Assay Conjugate Pair on Specificity



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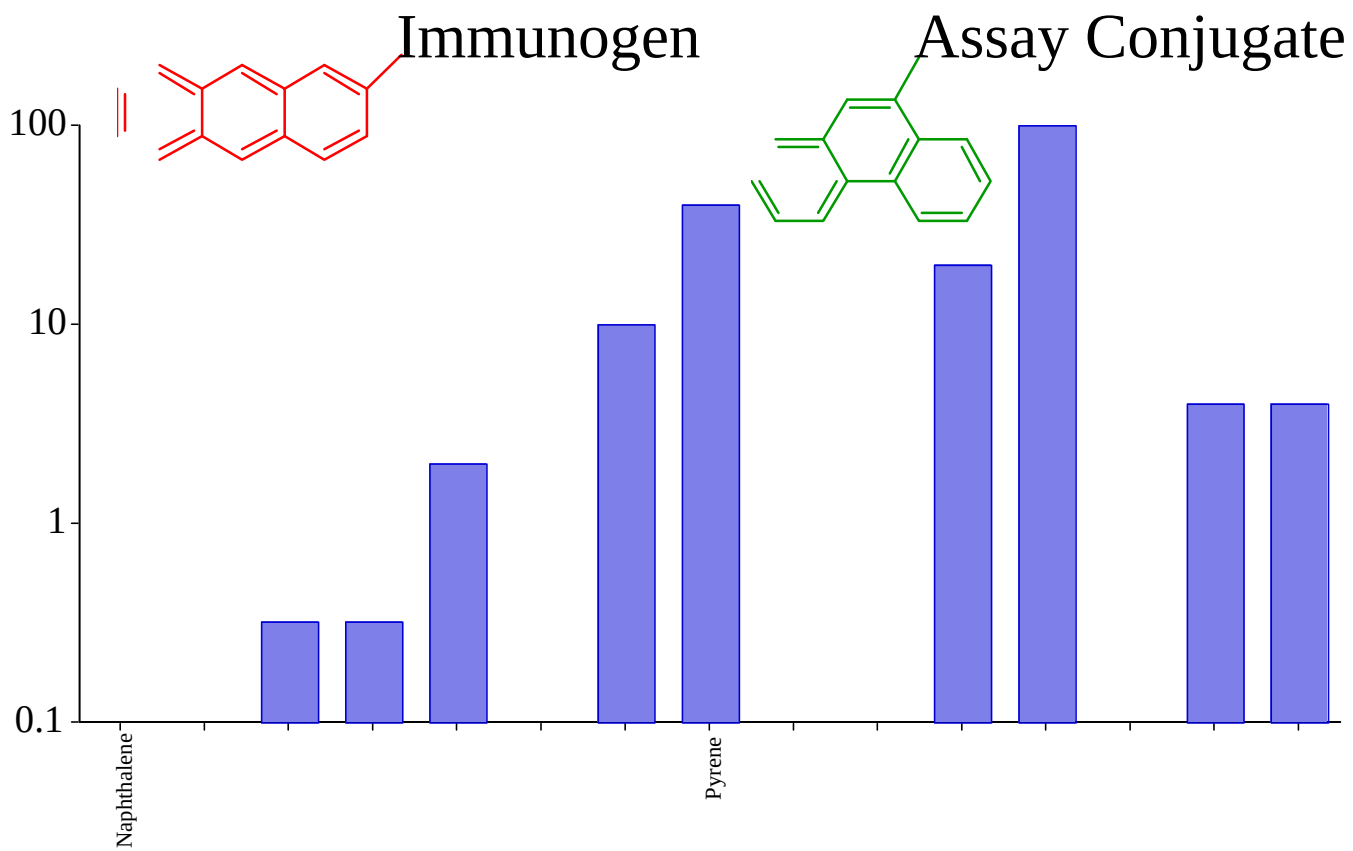

The Effect of the Antibody and Assay Conjugate Pair on Specificity

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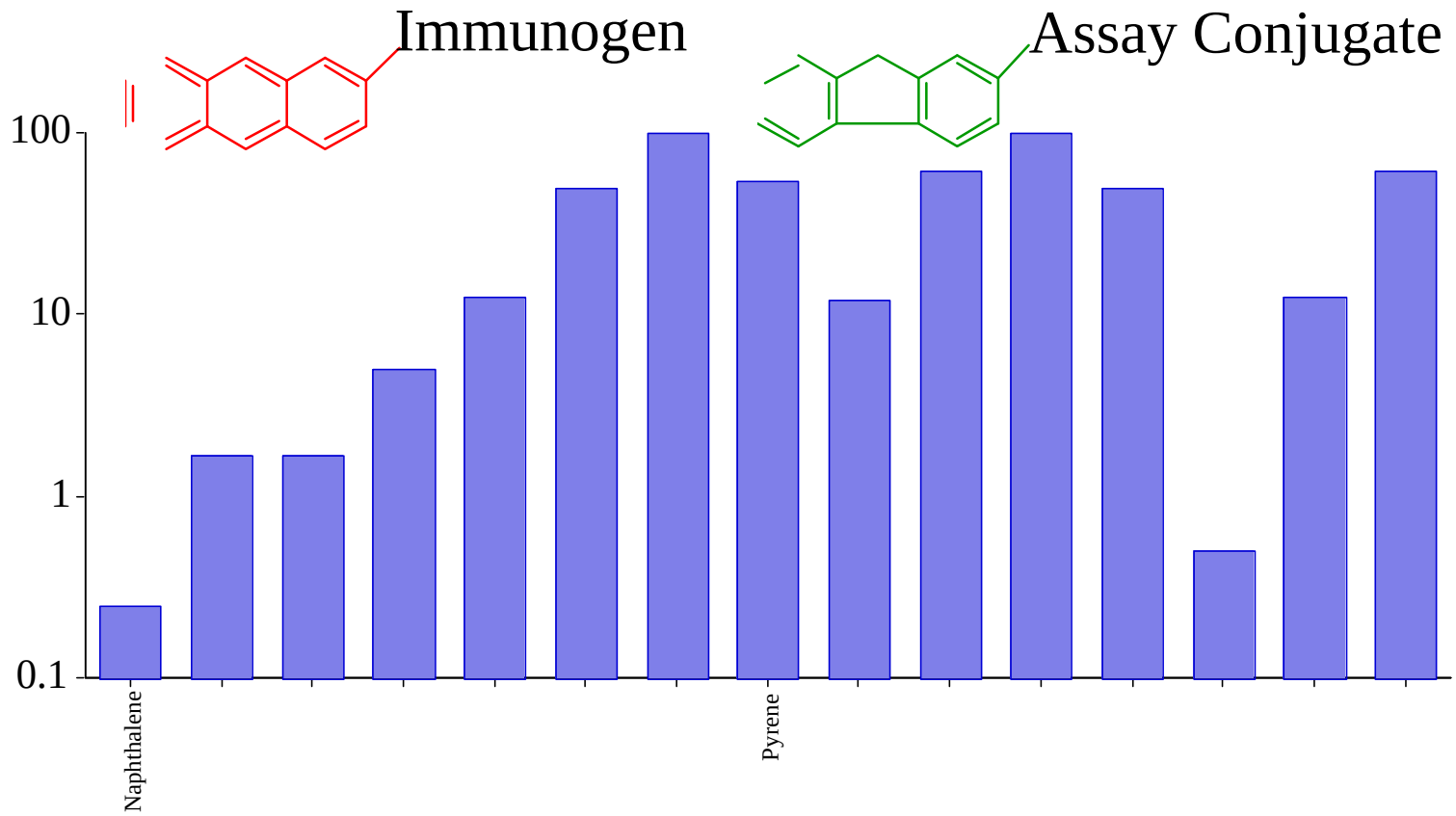

Principles of Immunochemistry

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- **Immunoassays are quantitative analytical methods**
- **Antibodies physically bind target analytes**
- **Strength of binding determines sensitivity**
- **Specificity**
  - Broad or specific (screening or quantitative)
  - Allows detection in complex matrix
    - Minimum sample preparation
    - Field-portable tests
- **Sensitivity and specificity determined by antibody and assay conjugate pair**
- **Flexible format provides for diverse applications**