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Binding Antibodies: Assay Methodologies, Screening Confirmation, Characterization of Anti-Drug-Antibodies

EIP Open Symposium Kopenhagen 2012

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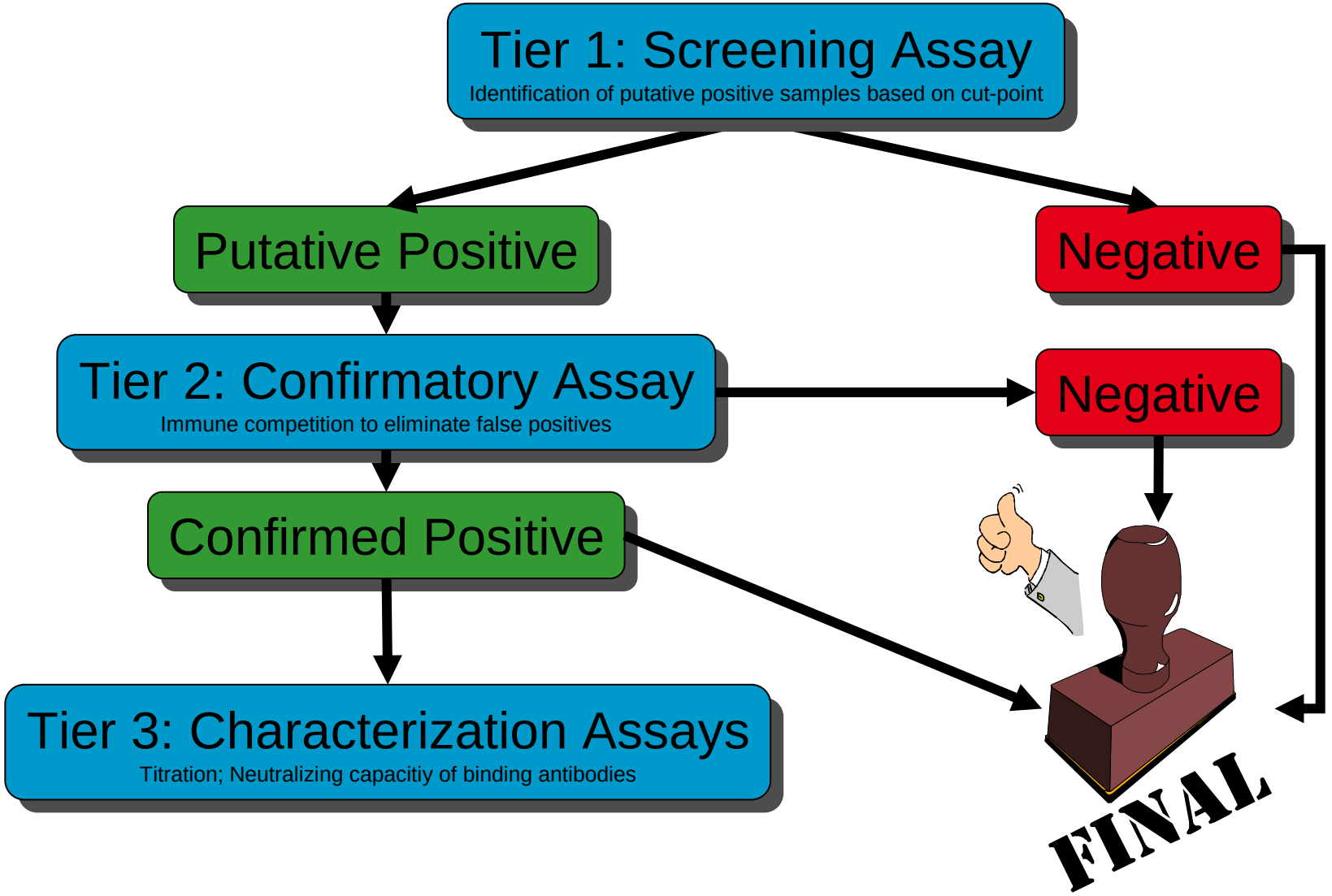
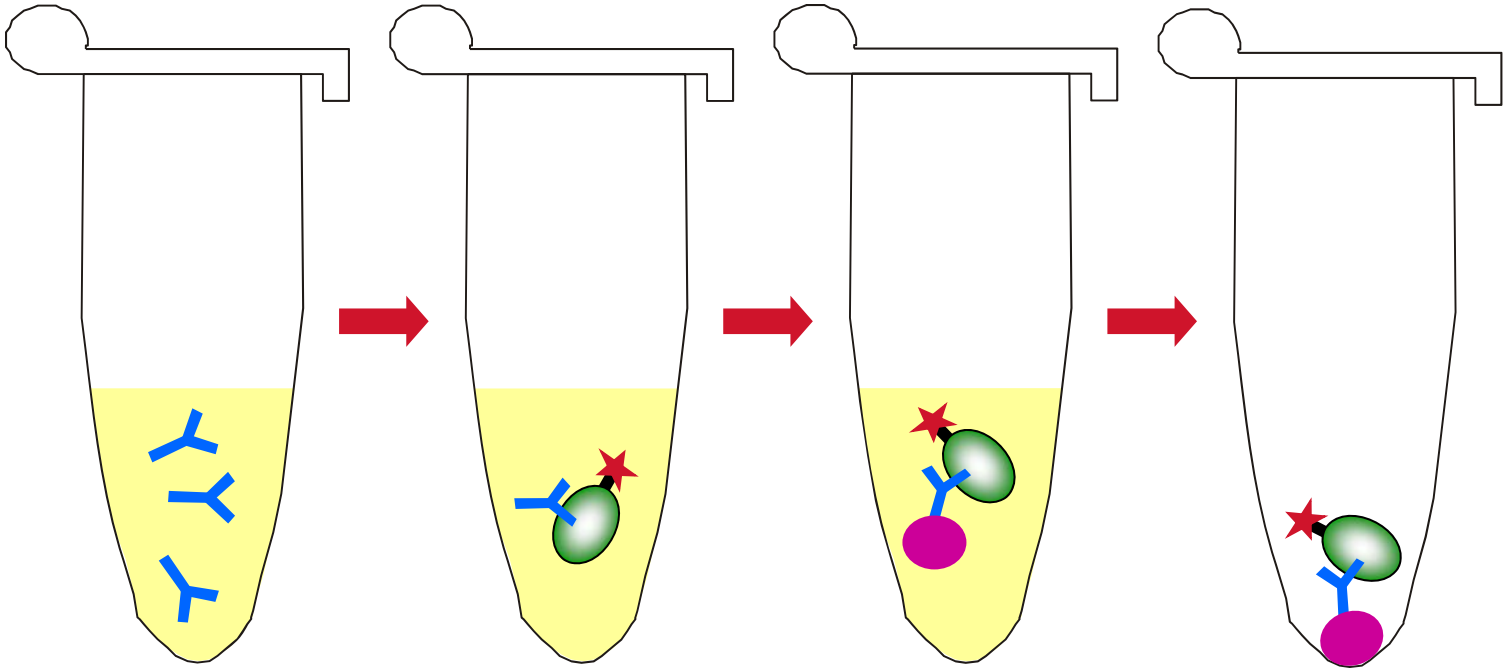
Immunogenicity Testing – How…?



1st Tier: Screening Assay
Radio-Immunoprecipitation (RIP)



Add iodine-labeled drug to serum containing anti-drug antibodies

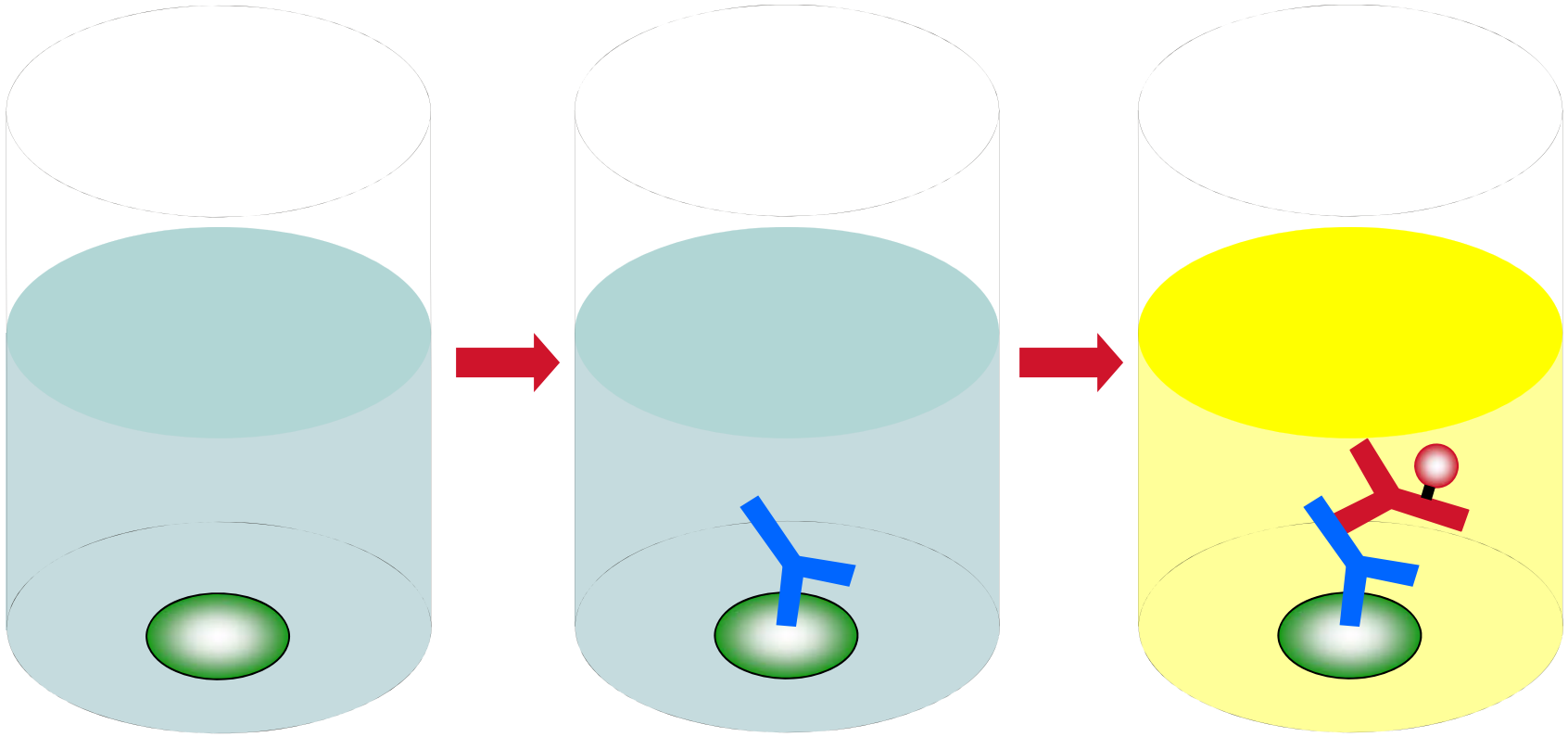
Add protein A coated beads

Centrifuge measure pellet

1st Tier: Screening Assay
Radio-Immunoprecipitation (RIP)

- Advantages
  - Sensitivity
  - Rather high drug tolerance
- Disadvantages:
  - Low throughput
  - Restricted availability of CROs
  - Specificity (prone to artefacts)
  - Radiolabelling process can mask/denature epitopes recognized by anti-drug antibodies
  - Protein A/G are known of having different affinities to different isotypes

1st Tier: Screening Assay
Direct ELISA



Immobilize drug

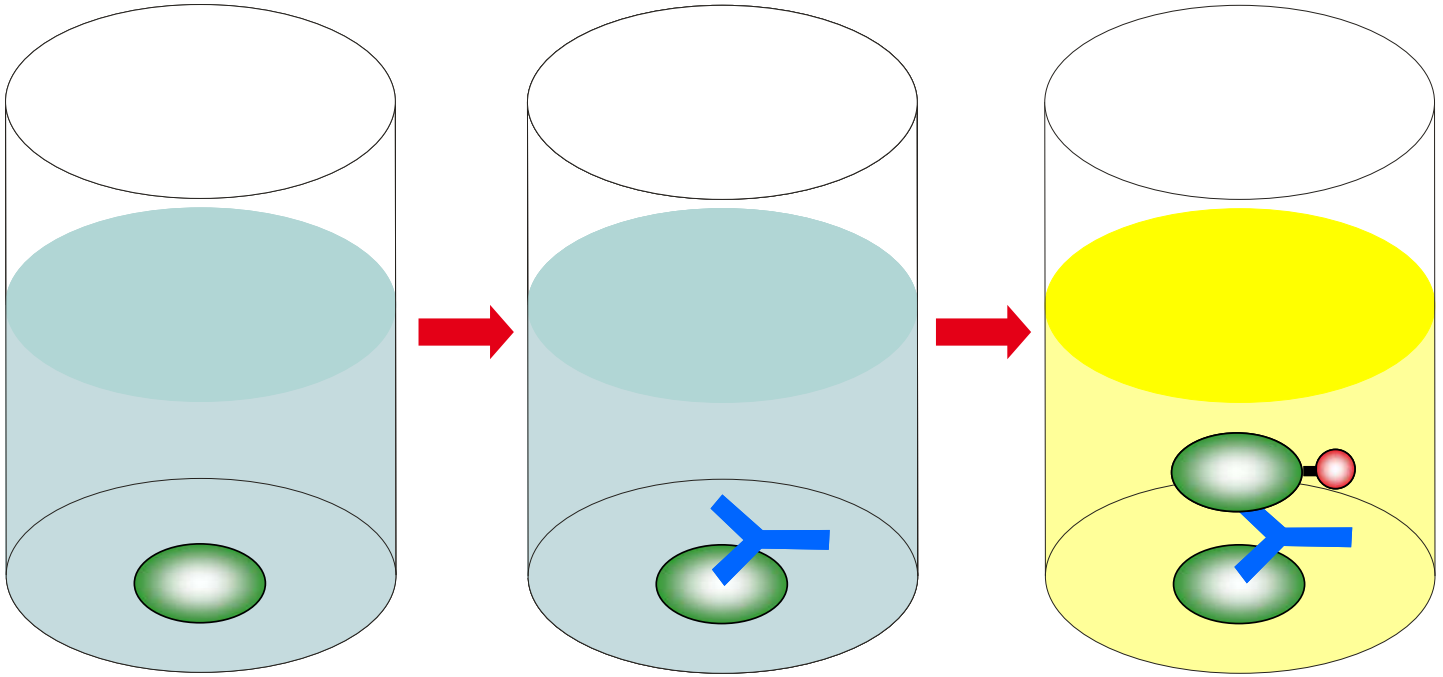
Add serum containing anti-drug antibodies

Detect with enzyme labeled polyclonal secondary antibody

1st Tier: Screening Assay
Direct ELISA

- Advantages:
  - Sensitivity
  - Commercial available secondary antibodies
- Disadvantages:
  - Source of the positive control has to be the same as that of the anti-drug antibodies
  - Specificity (unspecific binding to matrix components)
  - Restricted detection of low-affinity antibodies

1st Tier: Screening Assay
Bridging ELISA



Immobilize drug

Add serum containing anti-drug antibodies

Detect with labeled (e.g. biotinylated) drug

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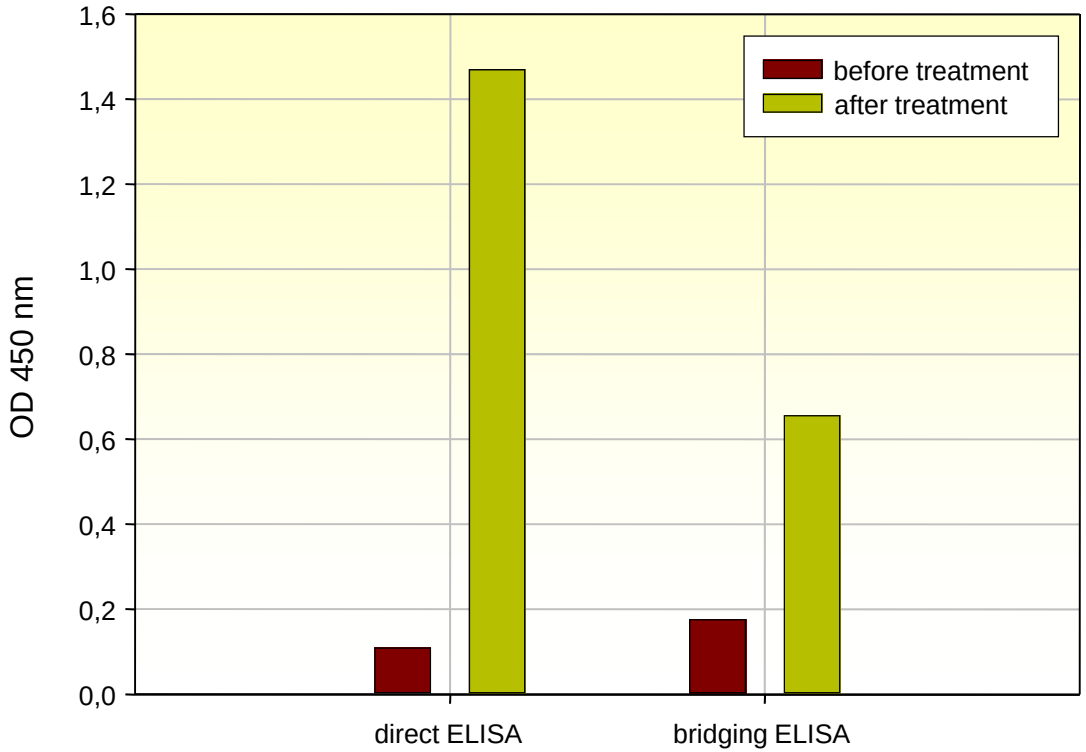
1st Tier: Screening Assay
Bridging ELISA

- Advantages
  - High throughput
  - Specificity (two-fold binding of drug required for signal)
  - Possibility to use any positive control binding to the drug (independent of species)
- Disadvantages
  - Sensitivity (special orientation of immobilized drug required)
  - Restricted detection of low-affinity antibodies
  - Biotinylation might mask/denature epitopes recognized by anti-drug antibodies

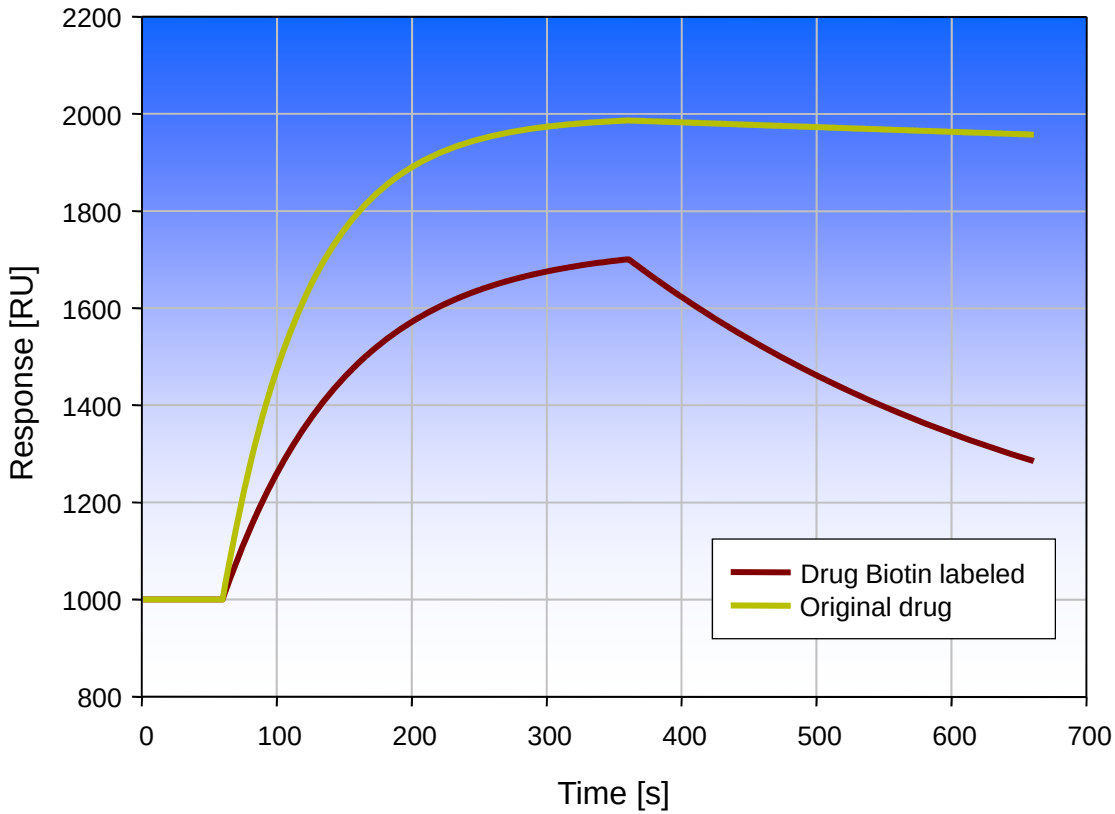
1st Tier: Screening Assay
Bridging ELISA

Sensitivity

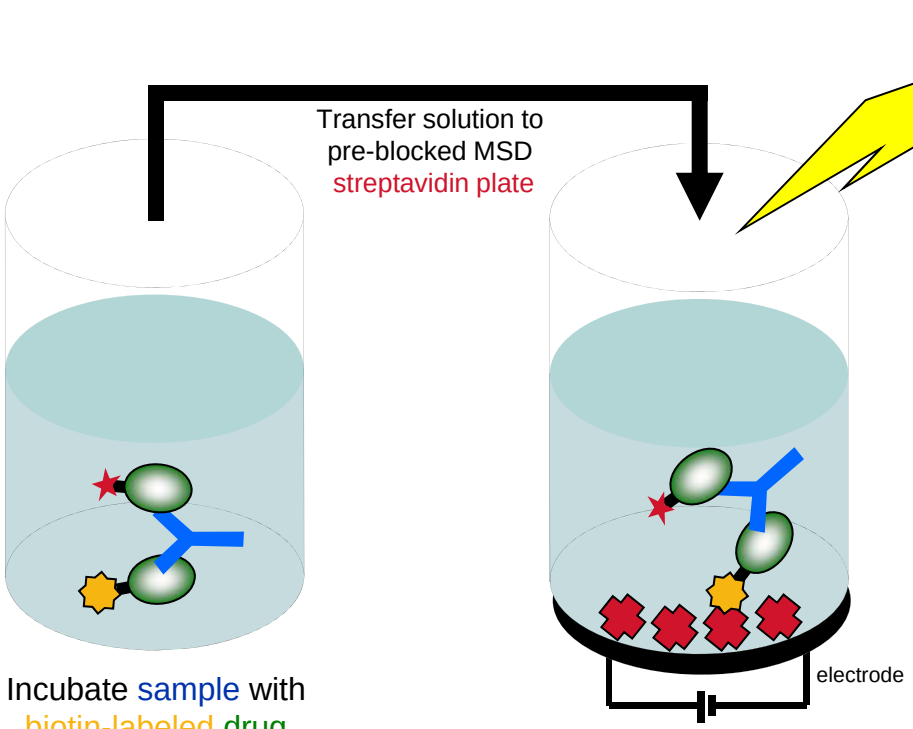


1st Tier: Screening Assay
Bridging ELISA

Masking of binding epitopes by biotin



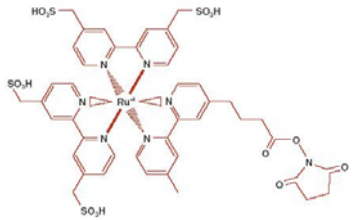
1st Tier: Screening Assay
Electrochemiluminescence (ECL)



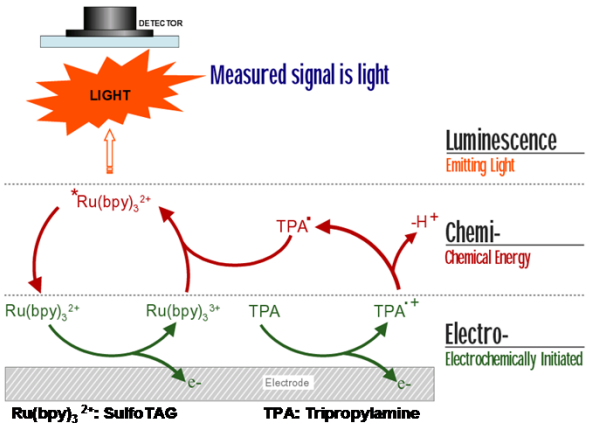
Incubate sample with biotin-labeled drug and sulfoTAG labeled drug

Wash assay plate; add Read Buffer; Read plate

sulfoTag emits light when electrochemically stimulated



Ruthenium (II) Sulfo-tris-bipyridine NHS ester = sulfoTAG



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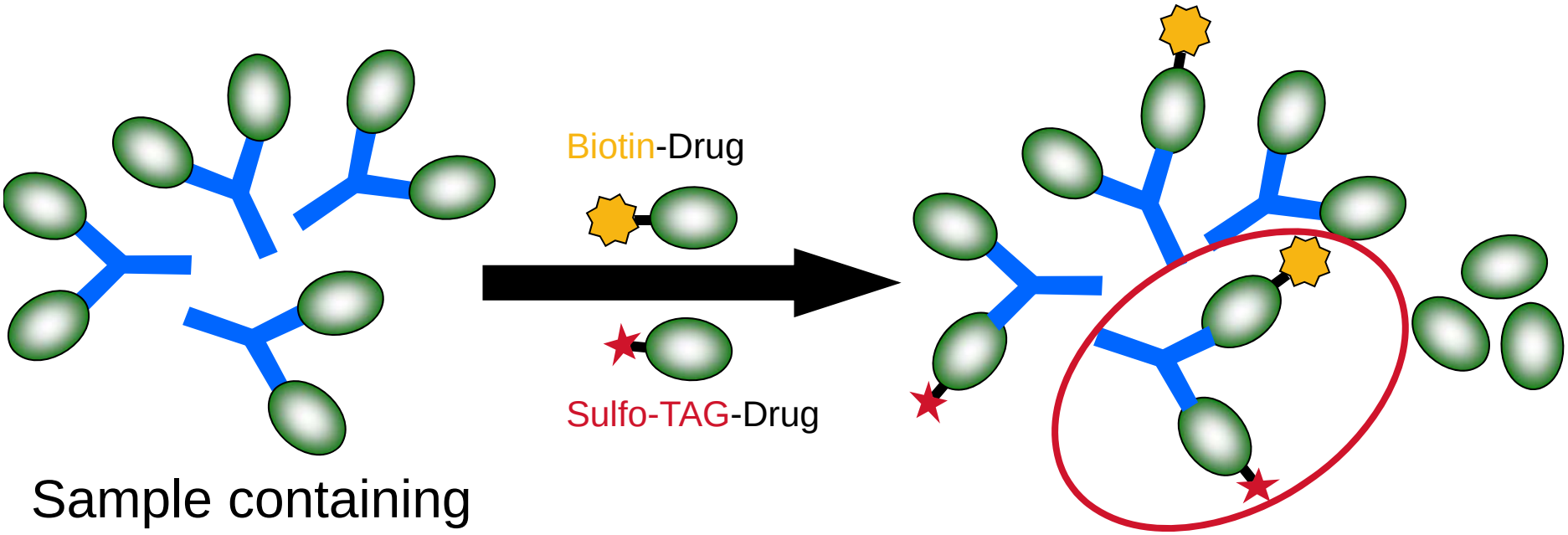
1st Tier: Screening Assay
Electrochemiluminescence (ECL)

- Advantages
  - Electrochemiluminescence technology offers sensitivity and large dynamic range
  - Less washing steps allow the detection of low affinity anti-drug antibodies
  - Better tolerance for drug than ELISA
  - Possibility for multiplexing (epitope mapping)
- Disadvantages
  - The use of two conjugated reagents increases the risk of masking of binding epitopes

1st Tier: Screening Assay
Electrochemiluminescence (ECL)



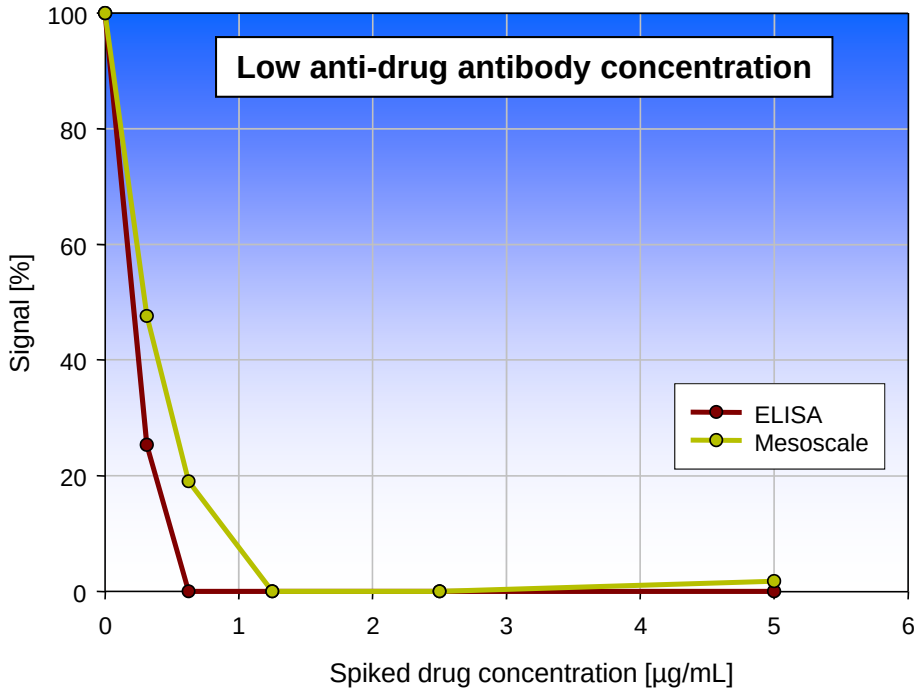
Sample containing excess of drug

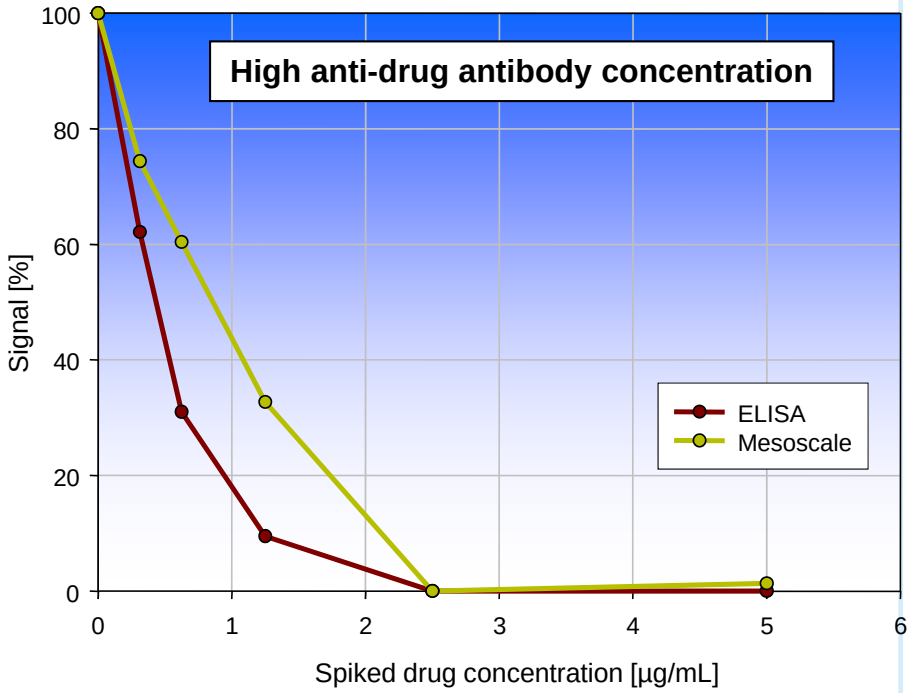
Detectable in MesoScale

1st Tier: Screening Assay
Electrochemiluminescence (ECL)

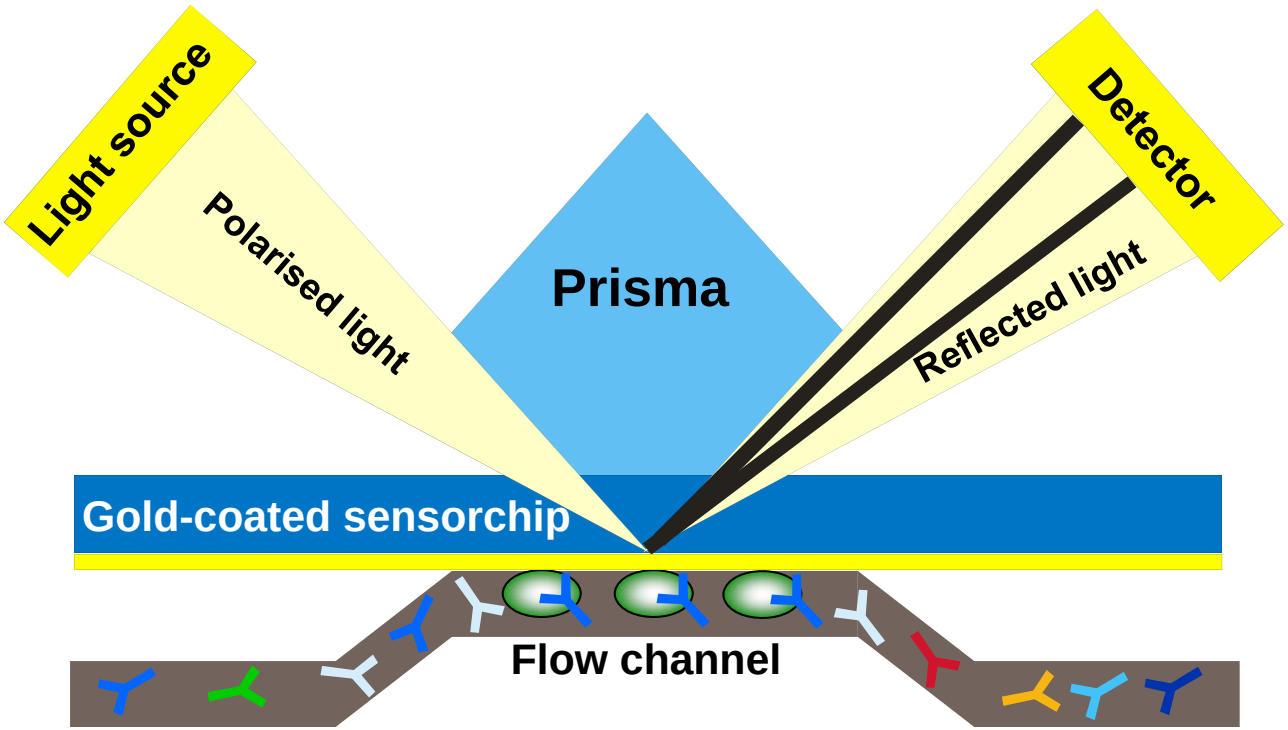
Drug Interference

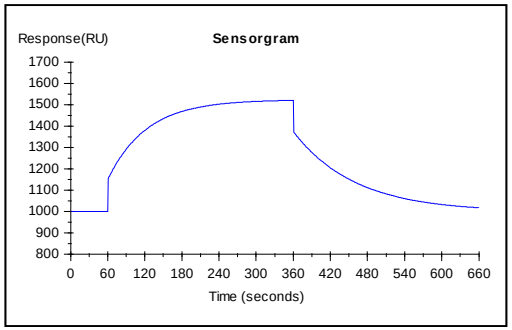
Positive samples were spiked with increasing amounts of drug and analyzed in Mesoscale and (bridging) ELISA





1st Tier: Screening Assay
Biacore



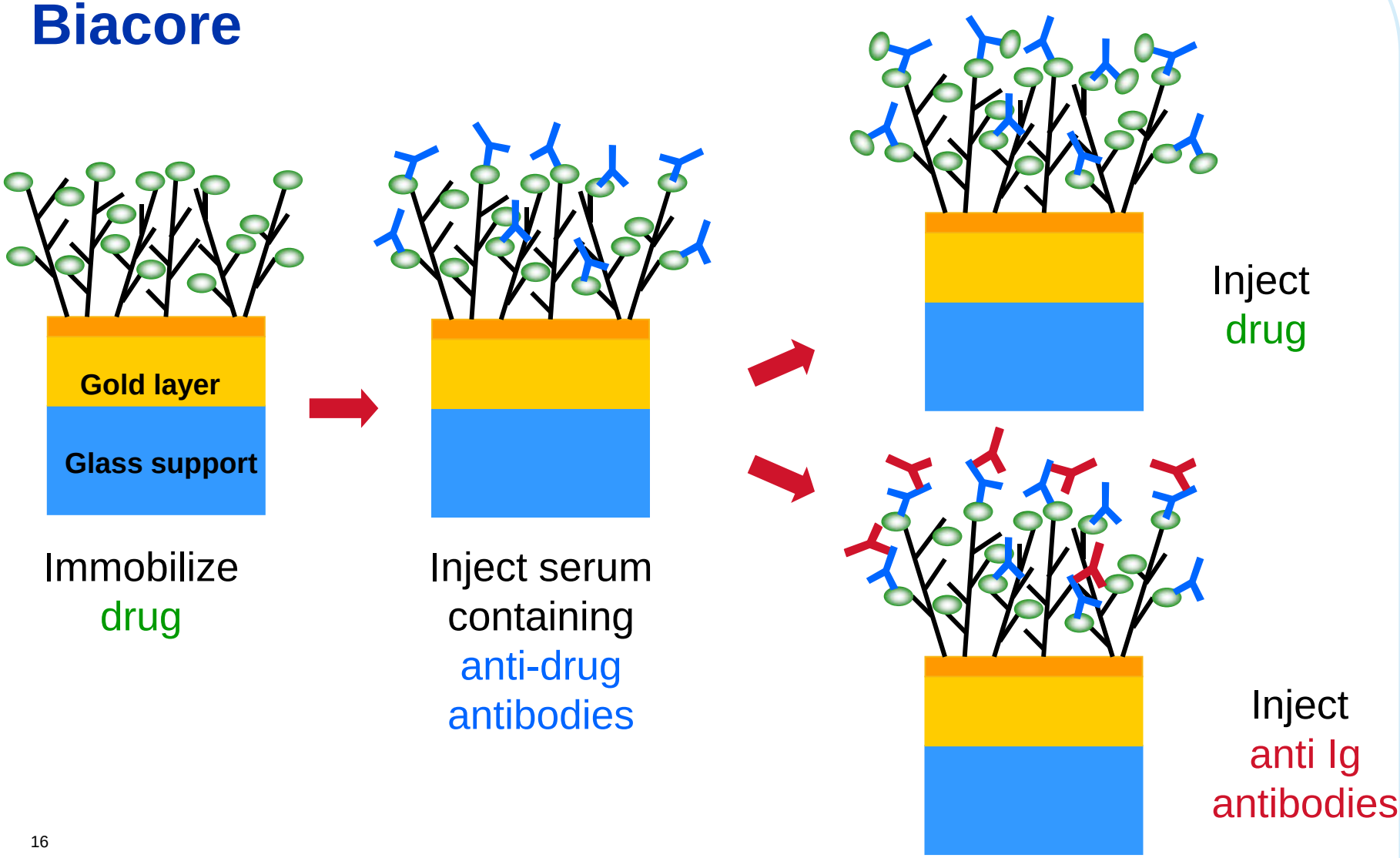


Drug immobilized on sensorchip

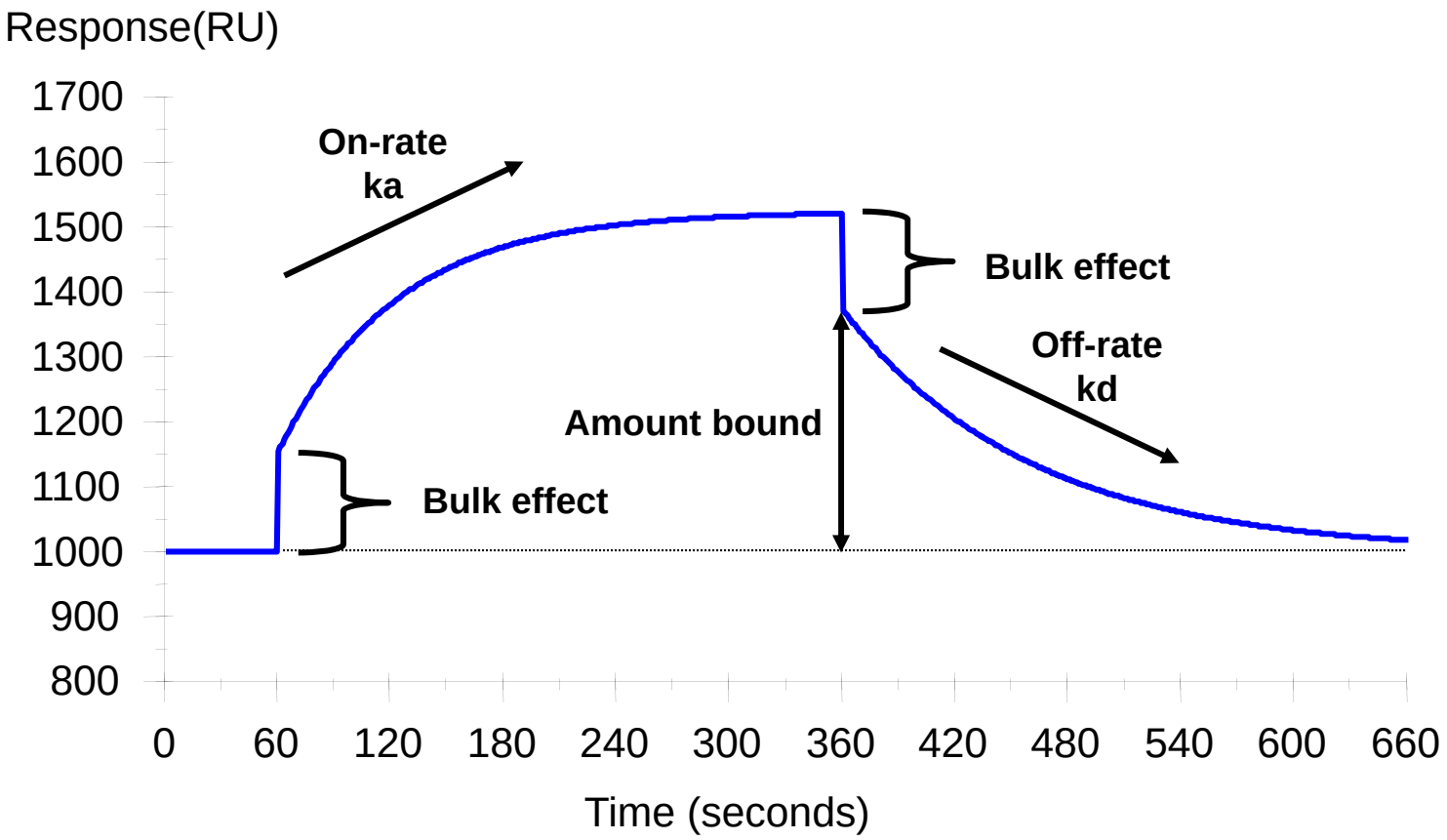
⬇

Injection of serum containing anti-drug antibodies

1st Tier: Screening Assay
Biacore



1st Tier: Screening Assay
Biacore

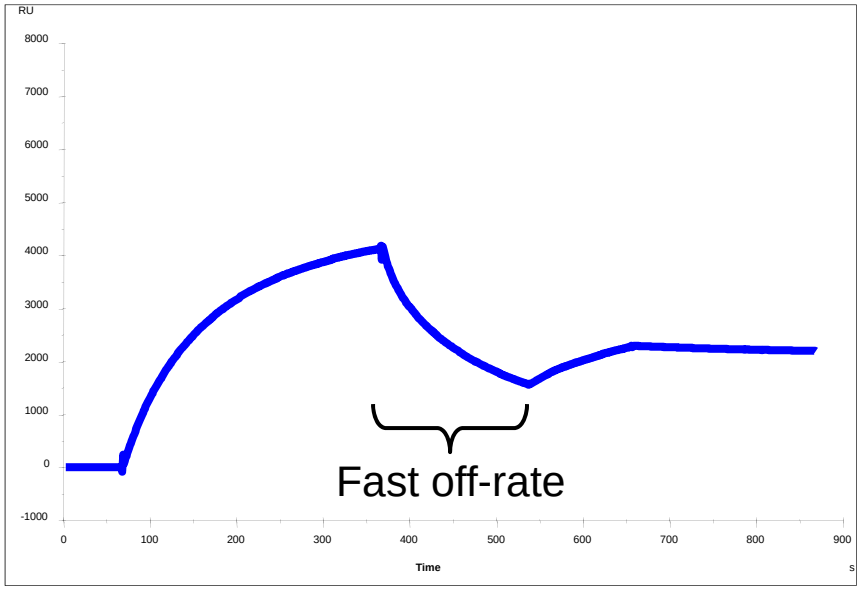


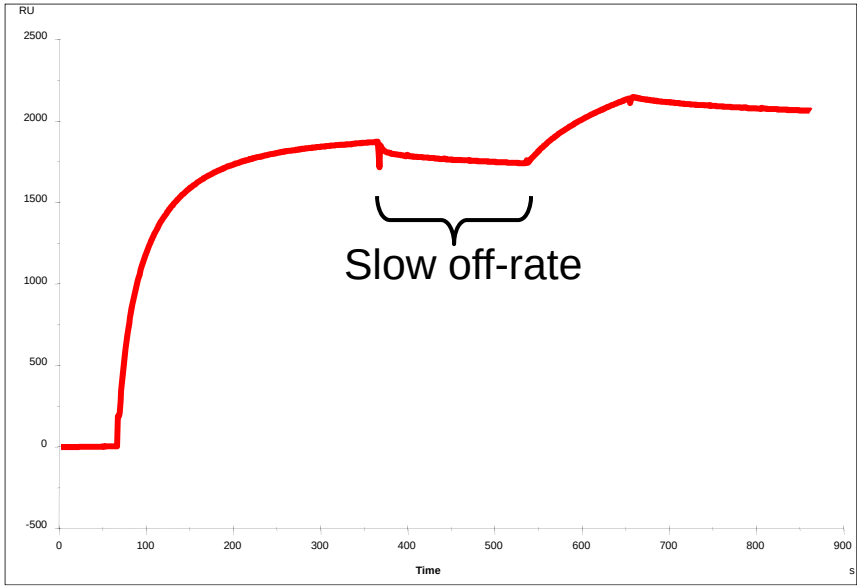
1st Tier: Screening Assay
Biacore

- Advantages:
  - Large dynamic range
  - No secondary reagents required
  - Detection of low affinity antibodies
  - Sensograms include information about affinity of anti-drug antibodies
  - Easy procedure for isotyping
  - Easy procedure for epitope mapping
- Disadvantages:
  - Structure of drug might be influenced by chemical coupling
  - Less sensitive than ELISA
  - Time consuming
  - Costs

1st Tier: Screening Assay
Biacore

- Sensograms contain information about affinity of anti-drug antibodies





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1st Tier: Screening Assay
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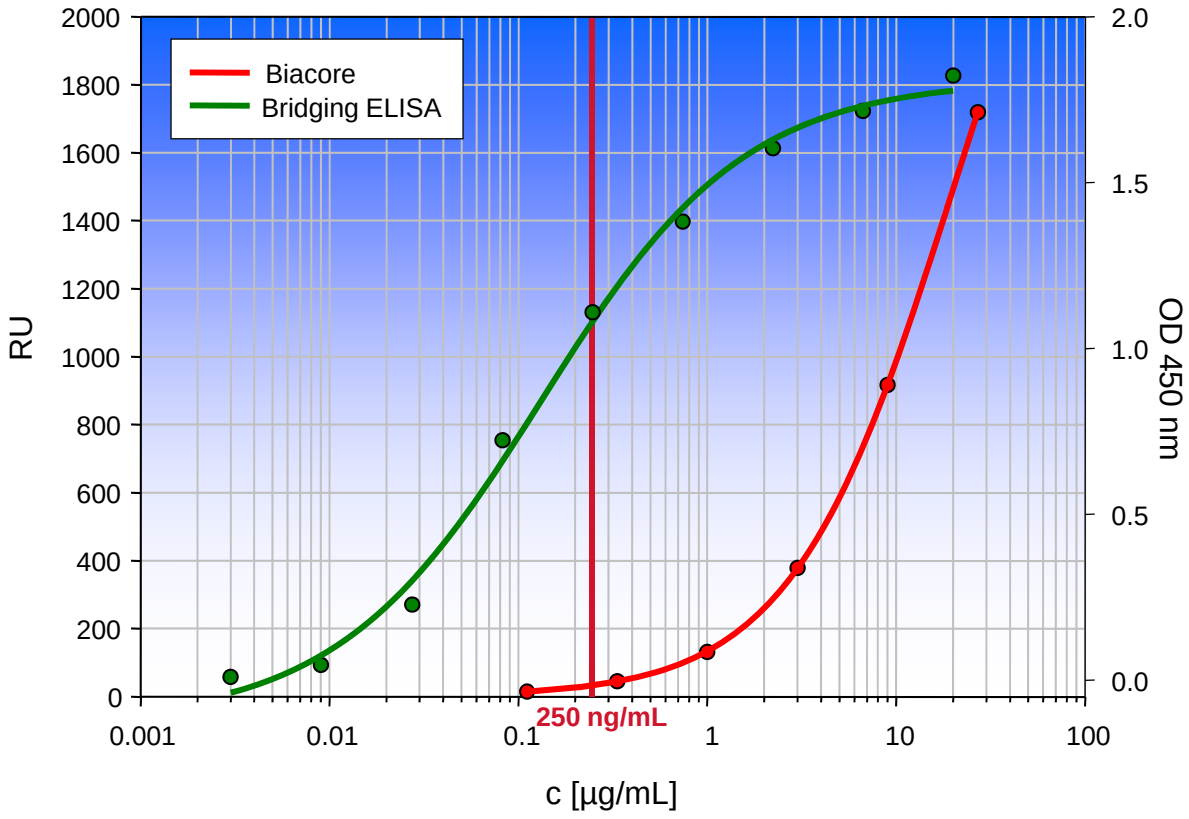
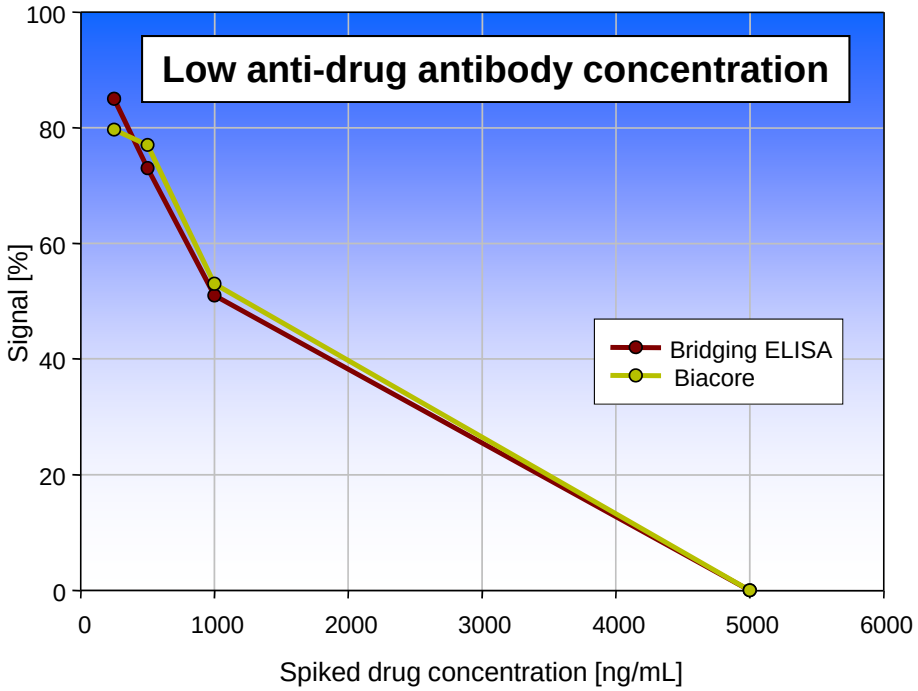
Sensitivity / Dynamic range

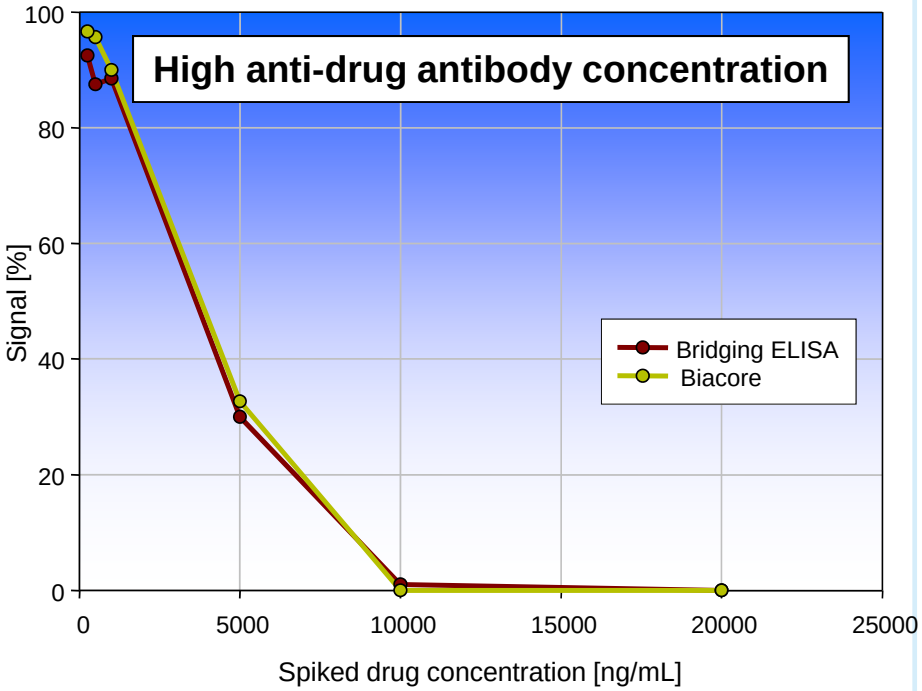


1st Tier: Screening Assay
Biacore

Drug Interference

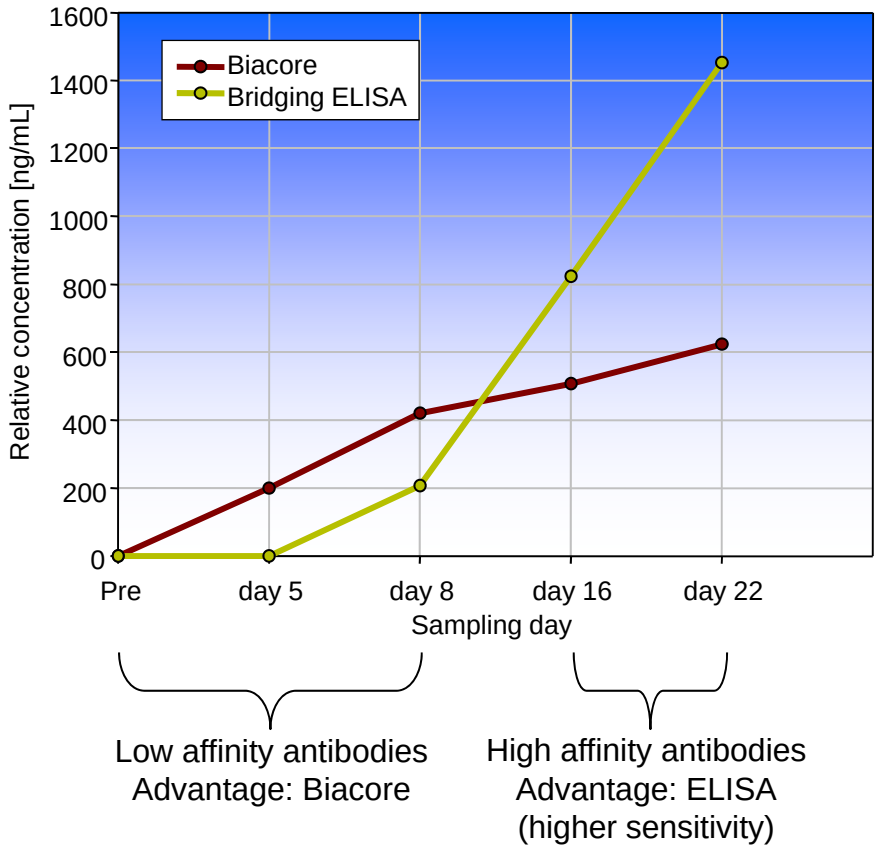
- Positive samples were spiked with increasing amounts of drug and analyzed in Biacore and (bridging) ELISA





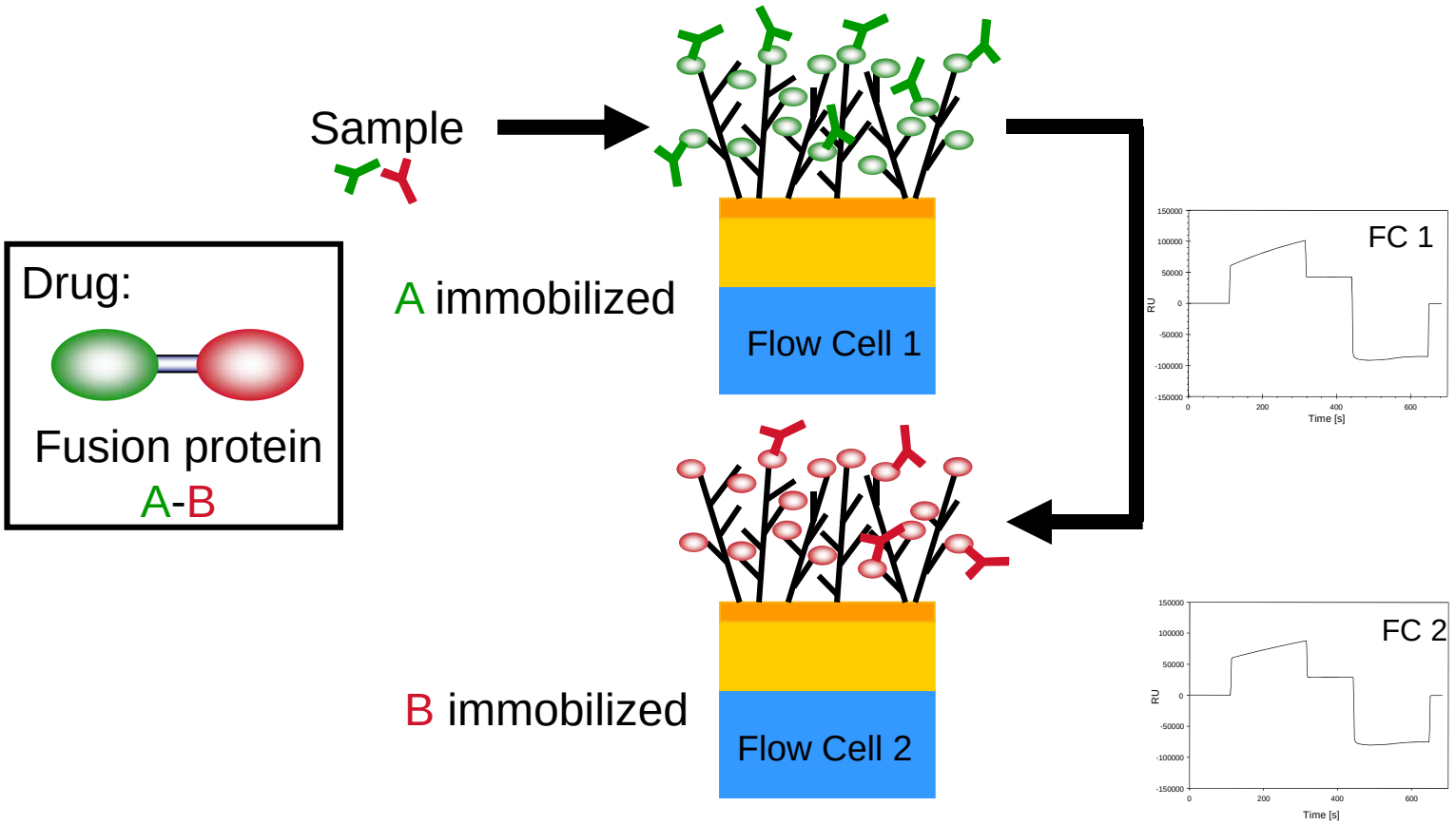
1st Tier: Screening Assay
Biacore

Low Affinity Antibodies

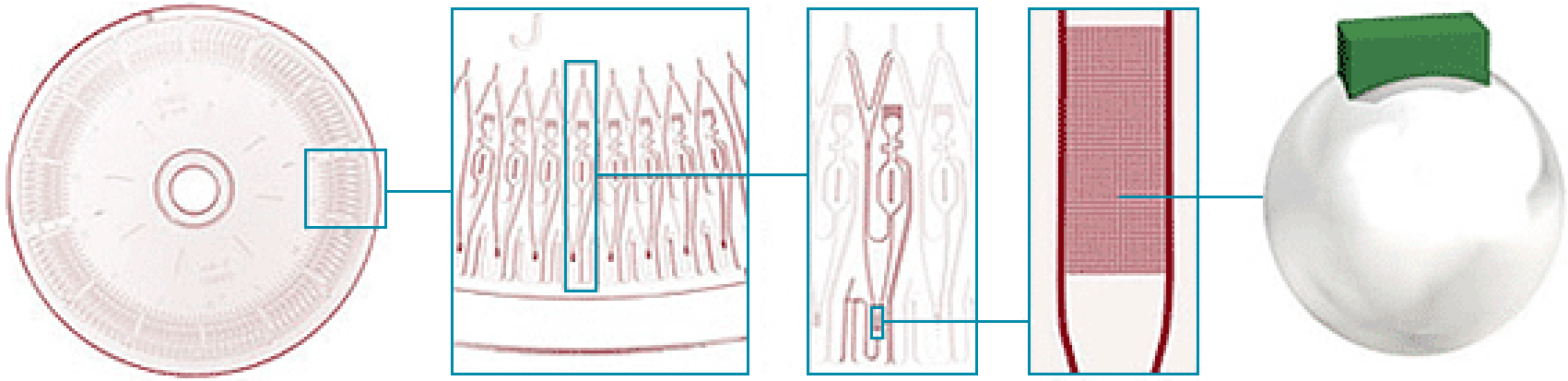


1st Tier: Screening Assay
Biacore

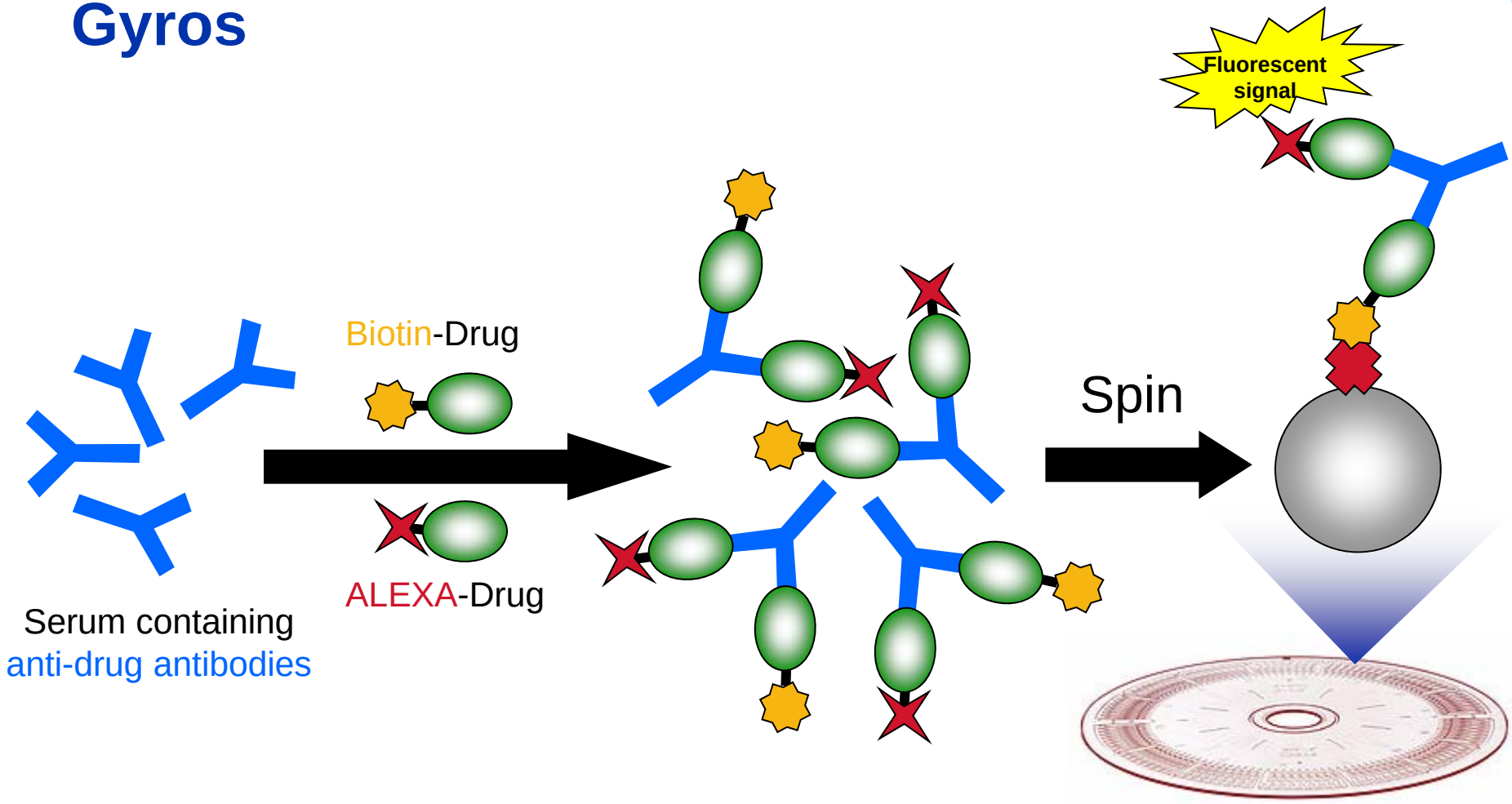
- Epitope Mapping



1st Tier: Screening Assay
Gyros



1st Tier: Screening Assay
Gyros



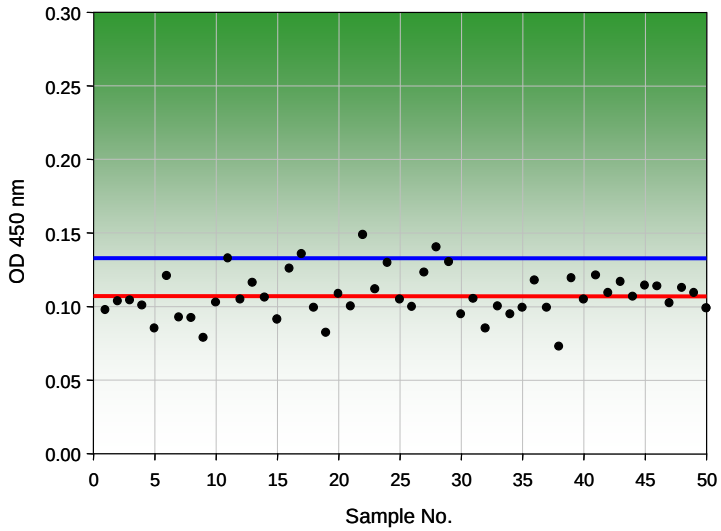
1st Tier: Screening Assay
Gyros

- Advantages:
  - The Gyros technology offers sensitivity and large dynamic range
  - Detection of low affinity antibodies (homogenous format)
  - Rather high drug tolerance (homogenous format)
  - Requires only small sample volumes
  - Epitope mapping possible
  - High throughput
  - Automatization reduces variability (less manual pipetting steps)
- Disadvantages:
  - The use of two conjugated reagents increases the risk of masking of binding epitopes
  - Costs
  - Carry over

1st Tier: Screening Assay
Cut-Point

Problem: Immunogenicity is a relative thing => criteria for positive samples needed

- Determination of the „non specific background“ (NSB) by testing of 50 serum samples of untreated animals or patients on three different days
- Cut-Point: NSB + 1.645 x Standard Deviation (=> 5 % false positives)
- Positive: Response ≥ Cut-Point



Cut Point = NSB + 1.645 x SD
Mean Response (=NSB)

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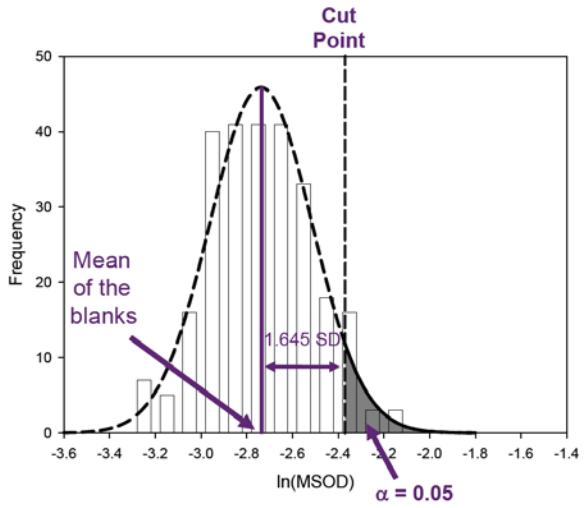
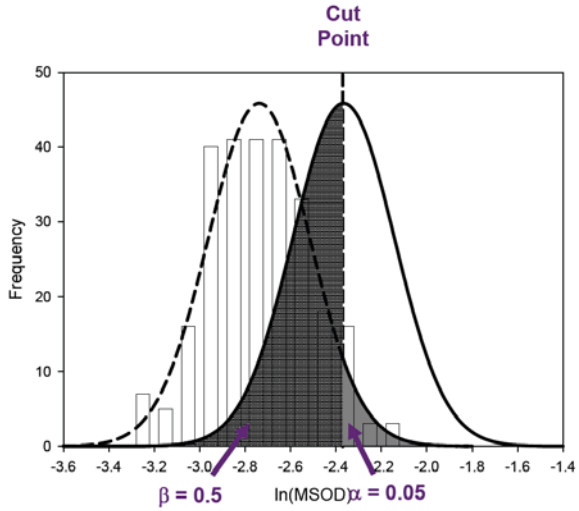
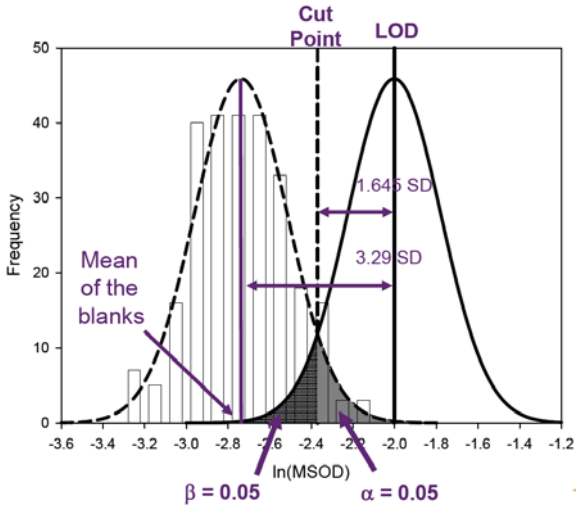
1st Tier: Screening Assay
Some Statistics

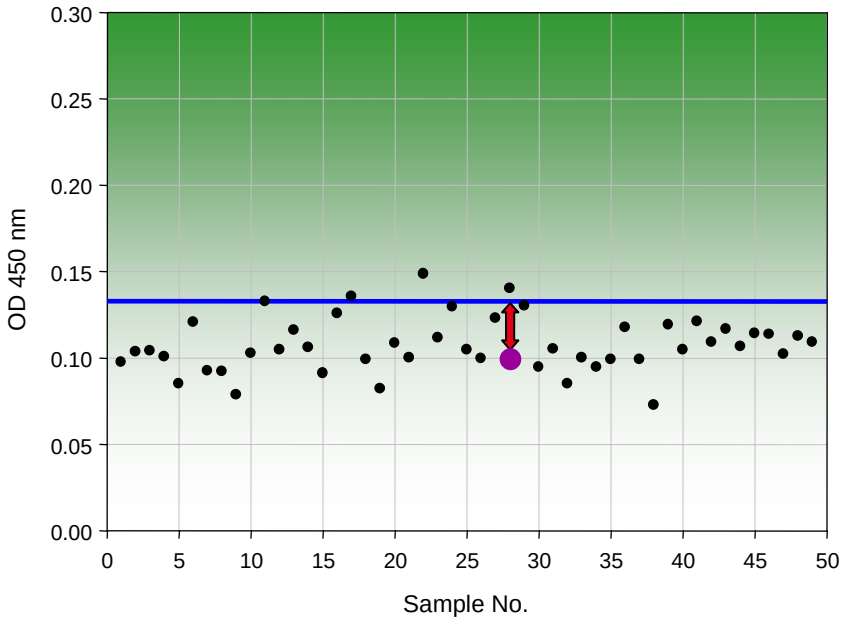






1st Tier: Screening Assay
Normalization of Cut-Point

Problem: **Usually assay signal will vary between runs => Cut-Point normalization necessary**

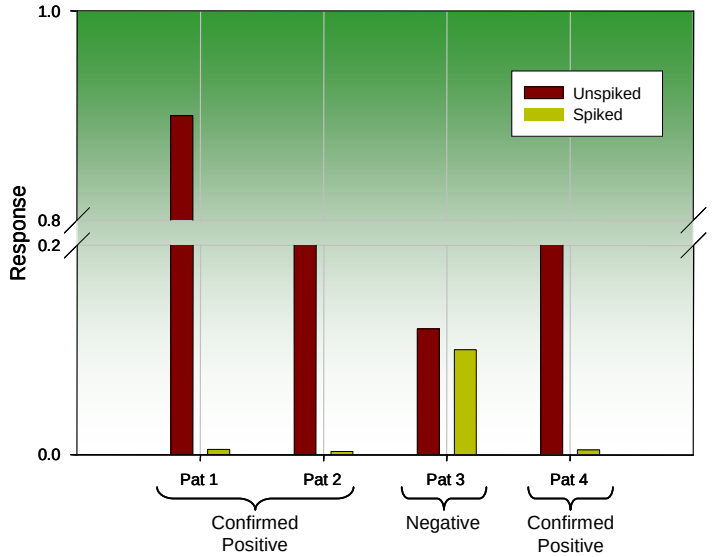


Normalization factor = relative response Cut-Point – relative response negative control

For each batch the normalization factor is added to the relative response of the negative control to set the cutpoint

2nd Tier: Confirmatory Assay

- Due to the 5% false-positive rate built into the screening cut point, samples showing a response at or above the assay cut-point can just be considered “putative positive” for the presence of BAbs.
- The confirmation of true positives among the putative positive samples requires the demonstration of specific binding to the drug:
  - A putative positive sample is re-tested in the presence and absence of an excess of drug.
  - The specificity cut point is defined as the percent inhibition at or above which a sample is considered as “confirmed positive”.

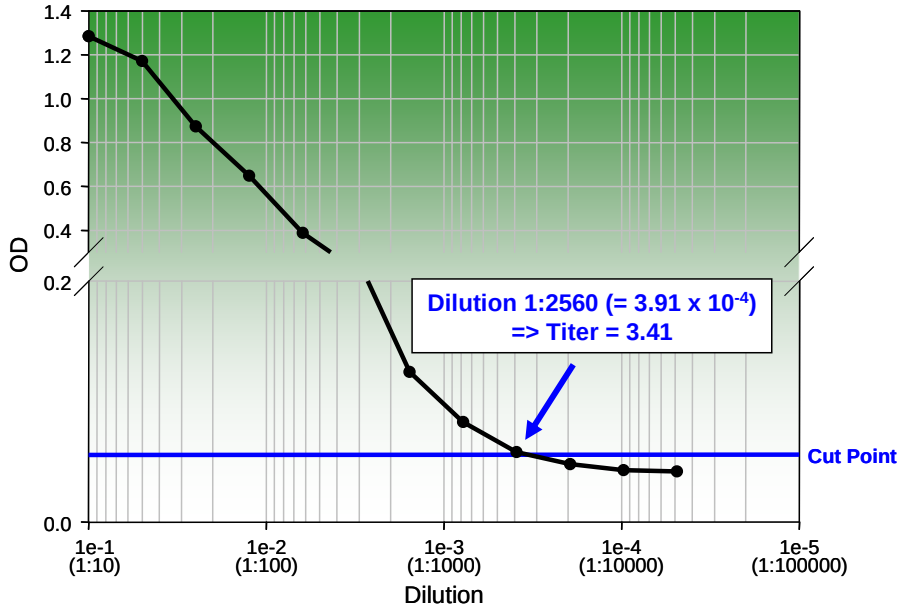


2nd Tier: Confirmatory Assay
Specificity Cut-Point

- Spike all individual samples from the cut-point determination (preferable in the same experiment) with an excess amount of drug and calculate the percent inhibition per sample: 100 x [1-(spiked/unspiked)]
- Calculate the specificity cut-point from the percent inhibition of all samples:
  Upper bound of a one-sided 99.9 % prediction interval (parametric: mean + 3.09 x SD or non-parametric: 99.9th percentile)
- A real sample in study showing a higher % inhibition after spiking of drug than the specificity cut-point is defined as „confirmed positive“

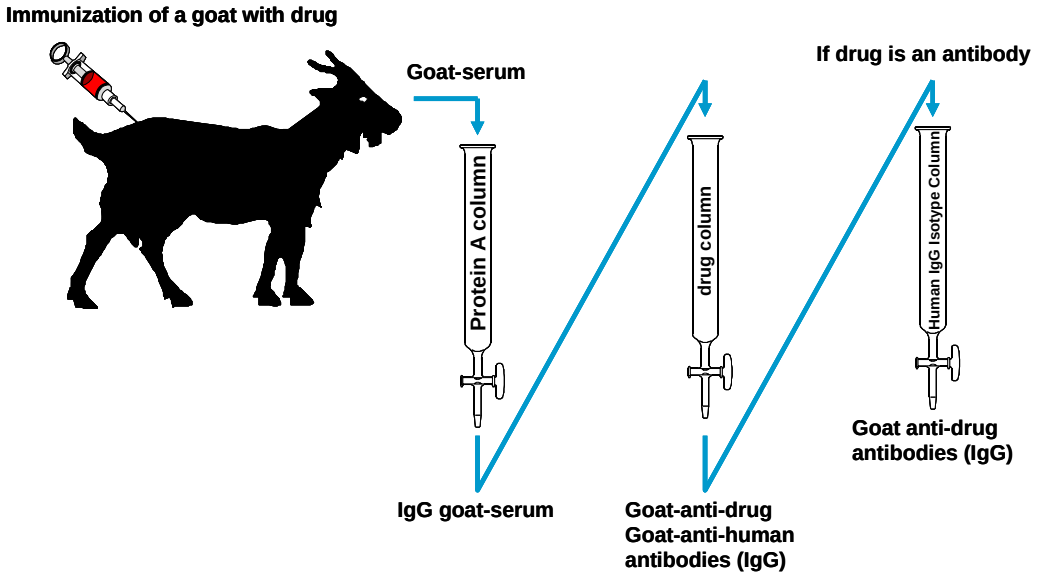
3rd Tier: Titration

- Aim:
  - Retrieve quasi-quantitative information for confirmed positive samples
- Procedure:
  - Serial dilution of confirmed positive samples
  - Titer = -log dilution factor of the last dilution that tests positive



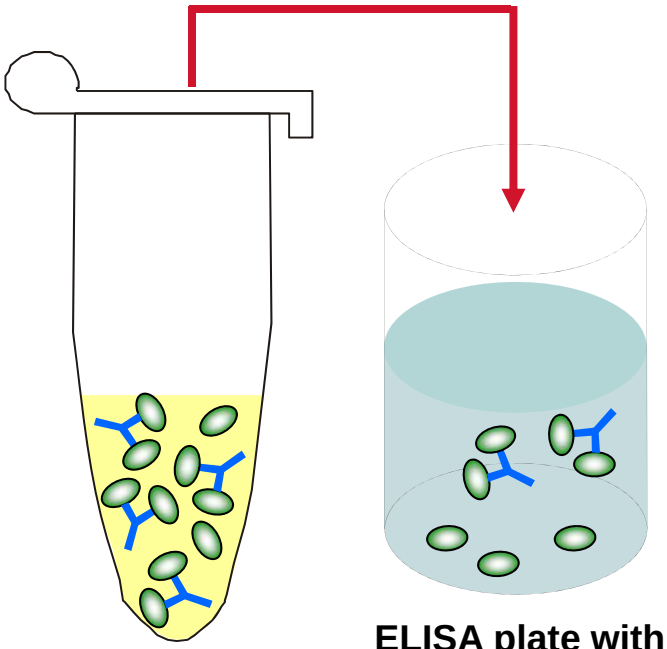
Challenges In Immunogenicity Testing
Positive Control

- In contrast to PK assays the analyte is not available in purified form
- Serum from animals (e.g. goats) hyperimmunized with the drug is used as control instead
- This surrogate control substantially differs from the measured human anti-drug antibodies in respect to affinity and avidity
- Consequently no exact numbers (e.g. for sensitivity) can be reported for Immunogenicity assays (but numbers relative to the positive control)



Challenges in Immunogenicity Testing
Drug Interference

- The presence of major amounts of drug interferes with the detection of anti-drug antibodies and leads to “false negatives”



Serum sample with excess of drug

ELISA plate with immobilized drug

No signal

- Solutions:
  - Wash-Out Samples
    - Draw blood samples for the detection of anti-drug antibodies several days/weeks after the last treatment (5-6 x t1/2)
    - Acid dissociation of the immunecomplexes

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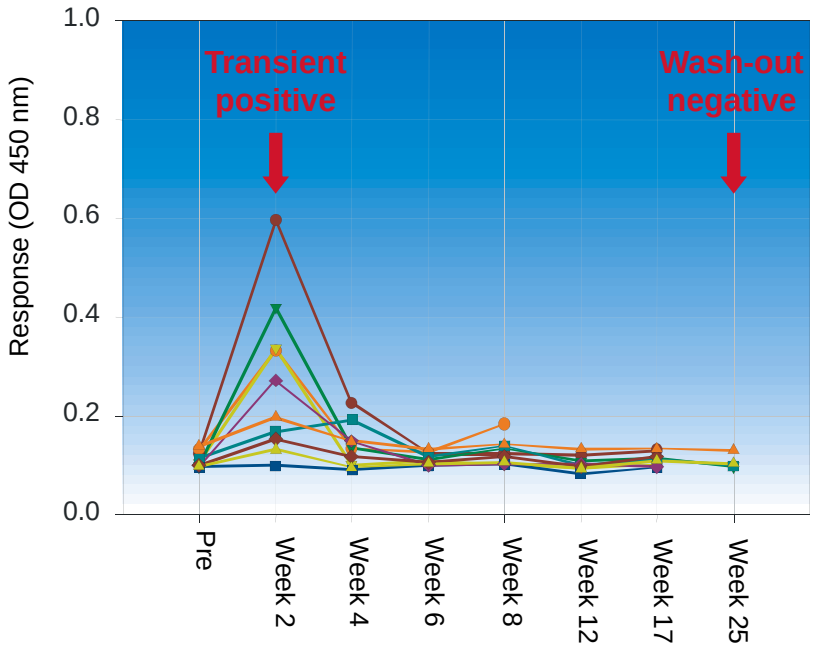
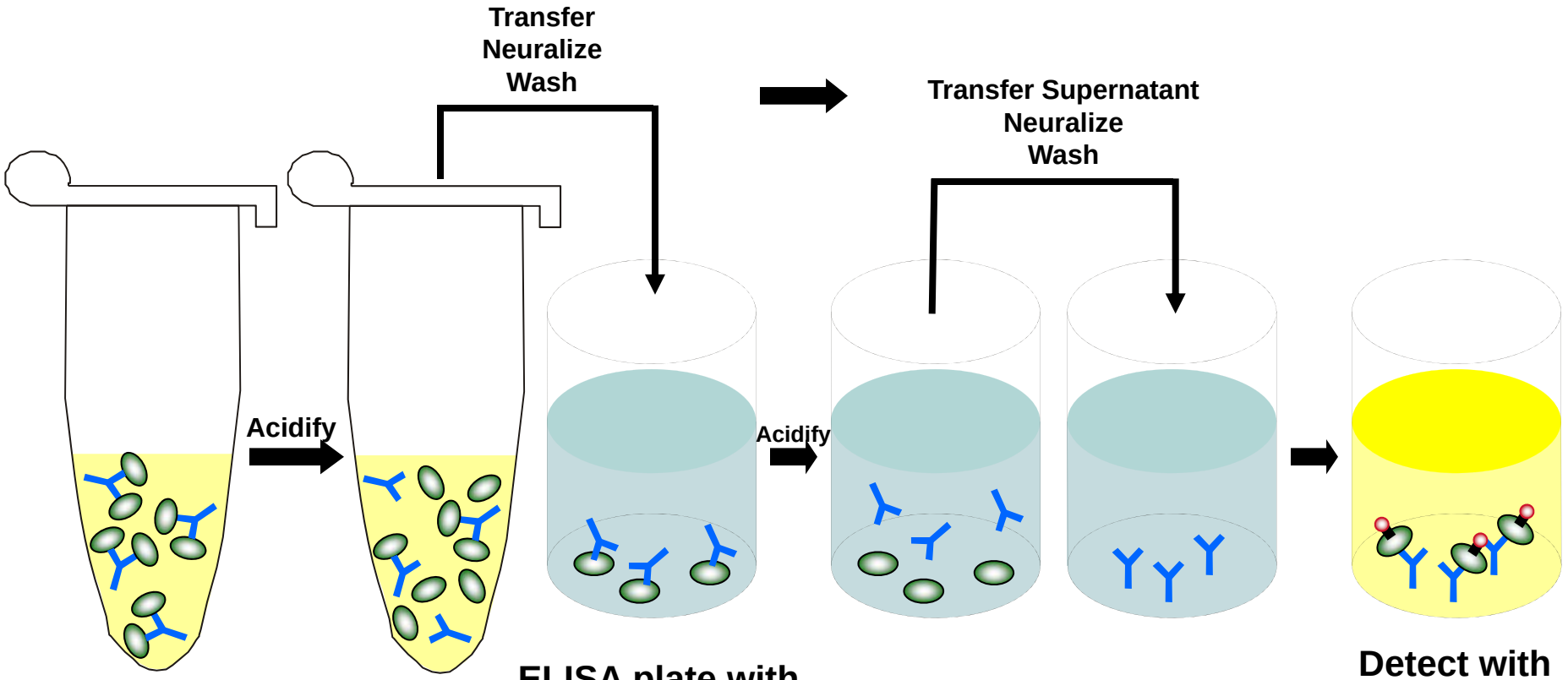
Wash-Out Samples

- Draw blood samples for the detection of anti-drug antibodies several days/weeks after the last treatment (5-6 x $t_{1/2}$)
- Problem: A transient immune response might not be detected in wash-out samples => acid dissociation assays might be needed



ACE Acid Dissociation Assay



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