

Assays and Strategies for Immunogenicity Assessment

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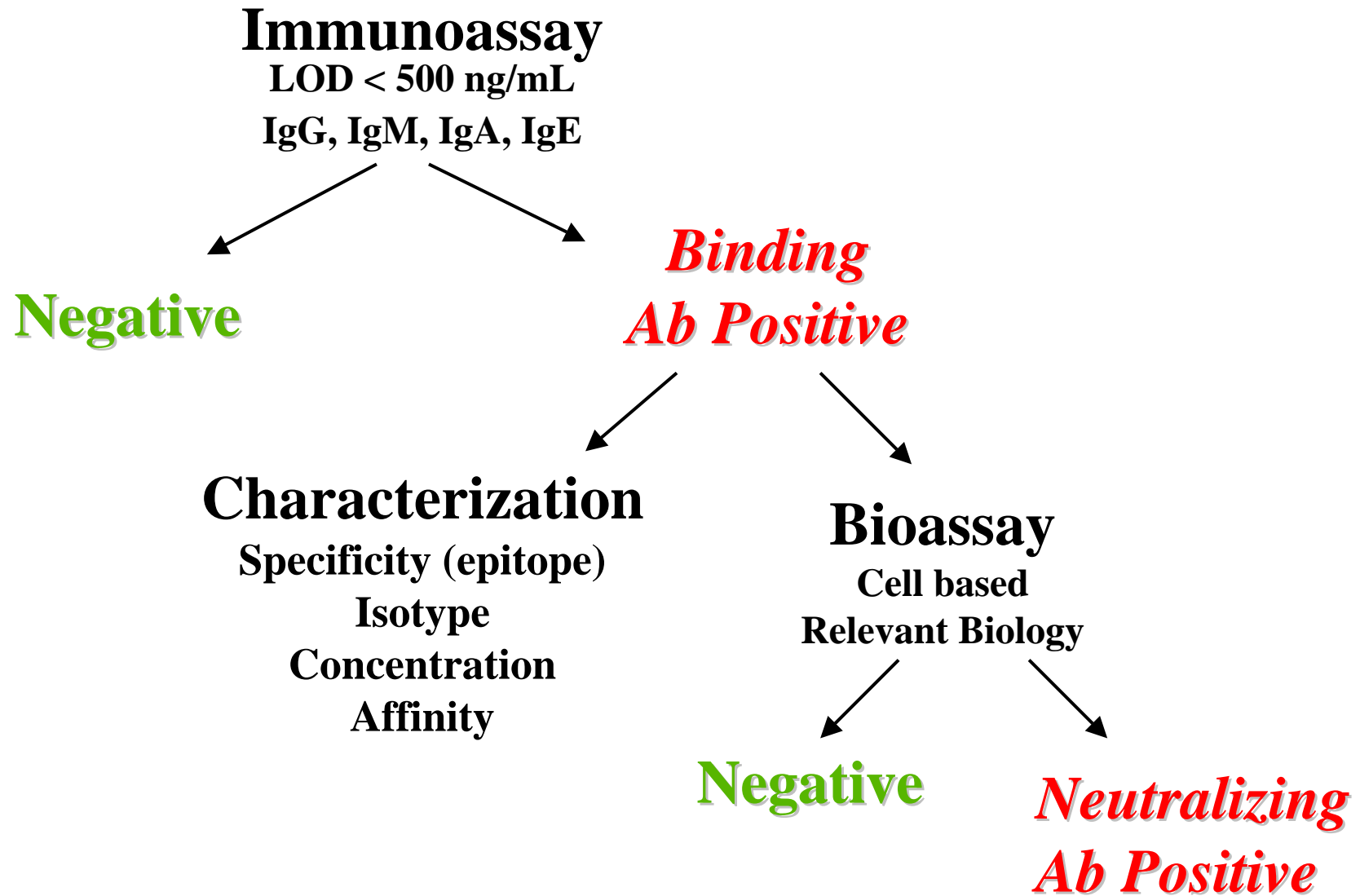
Executive Director, Medical Sciences

Clinical Immunology, Amgen

General Antibody Assay Strategy

- Correlation of clinical findings with presence of Ab provides the most significant information
- Screening immunoassay used to prioritize samples for bioassay determination
- Screening assay attributes: sensitive, able to detect all classes, able to detect low affinity Abs
- New immunoassay technologies allow thorough characterization of antibodies prior to bioassay
- Bioassay determines ability to neutralize effect of drug
- Assay tier
 - screening immunoassay
 - confirmatory immunoassay
 - bioassay

Immunogenicity Assay Strategy



Clinical Immunology Terms

Antibody Response = all antibodies generated
in a patient in response to a drug

Clinically Relevant Ab =

- 1) Clearing Ab
- 2) Sustaining Ab
- 3) Neutralizing Ab
- 4) Allergic rxn
- 5) Cross-reacting
w/ endogenous protein

Analytical Procedures for Detection of Binding Antibodies

- Radioimmune precipitation (RIP)
- ELISA/ECL
- Biosensor
- Bioassay

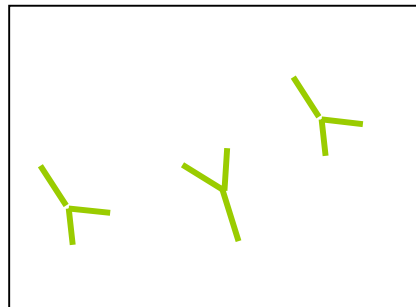
Strengths/Weaknesses of Analytical Procedures

- RIP
 - (+) Sensitive, inexpensive, equipment readily available
 - (-) May not detect early immune response, may be influenced by high levels of circulating drug
- ELISA
 - (+) Sensitive, inexpensive, equipment readily available
 - (-) May not detect early immune response (especially rapidly dissociating or low affinity Abs), may be influenced by high levels of circulating drug (especially bridging format)

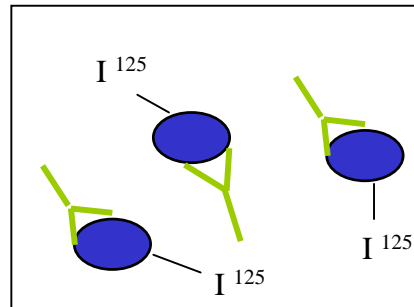
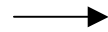
Strengths/Weaknesses of Analytical Procedures

- ECL
 - (+) Sensitive, can be modified to respond in the presence of high levels of circulating drug
 - (-) Equipment can be expensive, may not easily detect rapidly dissociating Abs
- Biosensor
 - (+) Method of choice for detecting early immune response, Ab characterization capabilities
 - (-) Expensive equipment, generally less sensitive than RIP or ELISA/ECL (although is more sensitive for rapidly dissociating Abs)

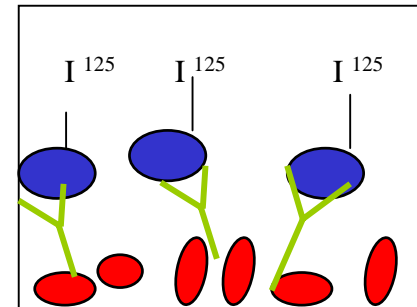
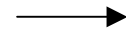
Radioimmune Precipitation Platform



Dilute sample



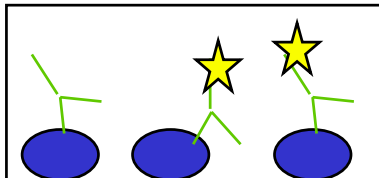
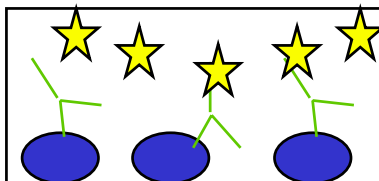
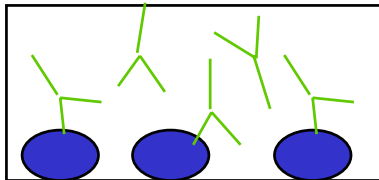
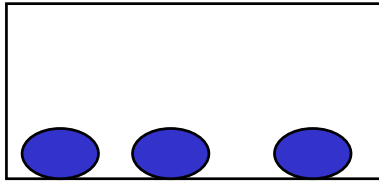
Add radioactive-labeled drug



Add Protein A,
precipitate Ab,
and measure
labeled drug

ELISA Platform

Direct



Coat Drug

Add Ab

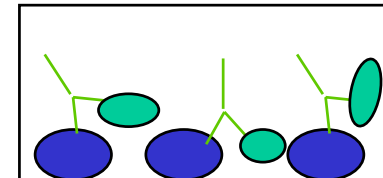
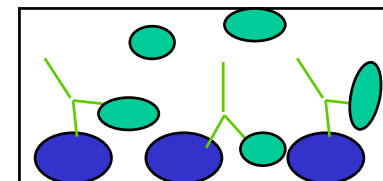
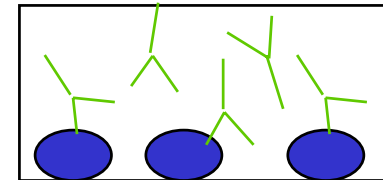
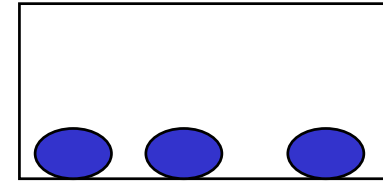
Add detector

★ Labeled Protein A

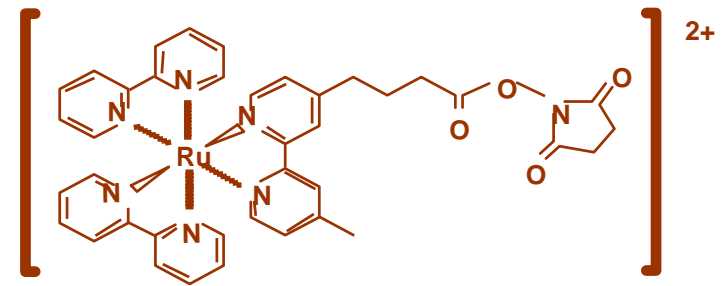
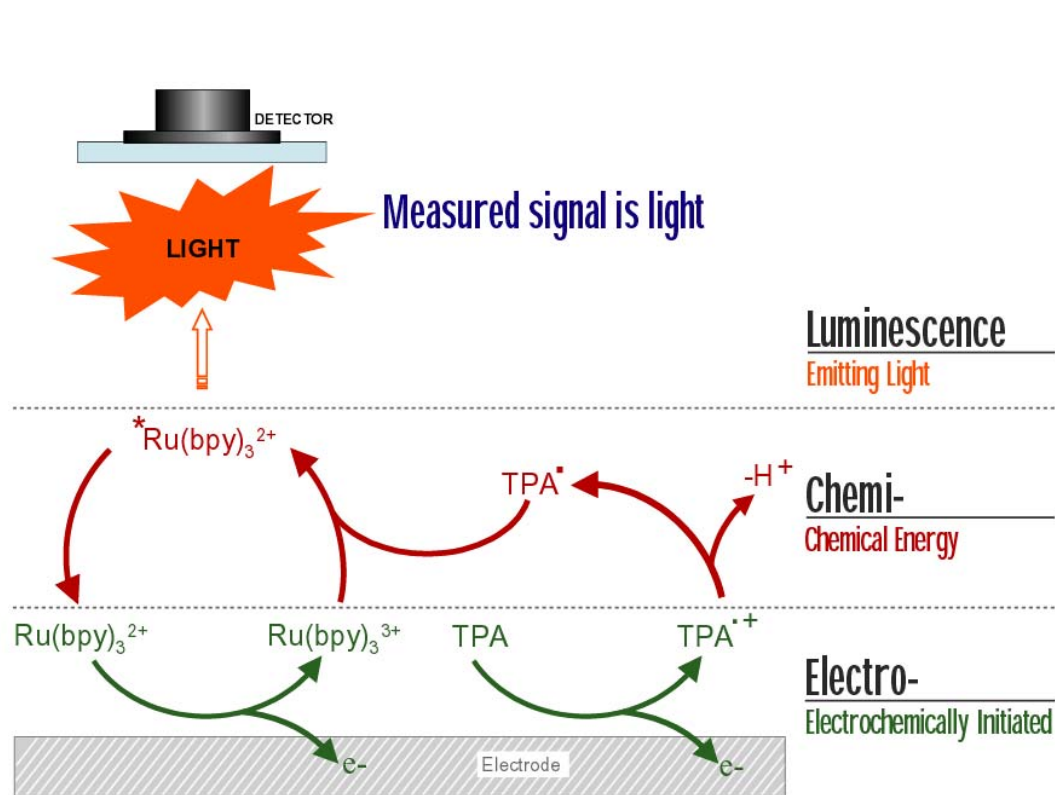
● Labeled Drug

Measure Ab

Bridging

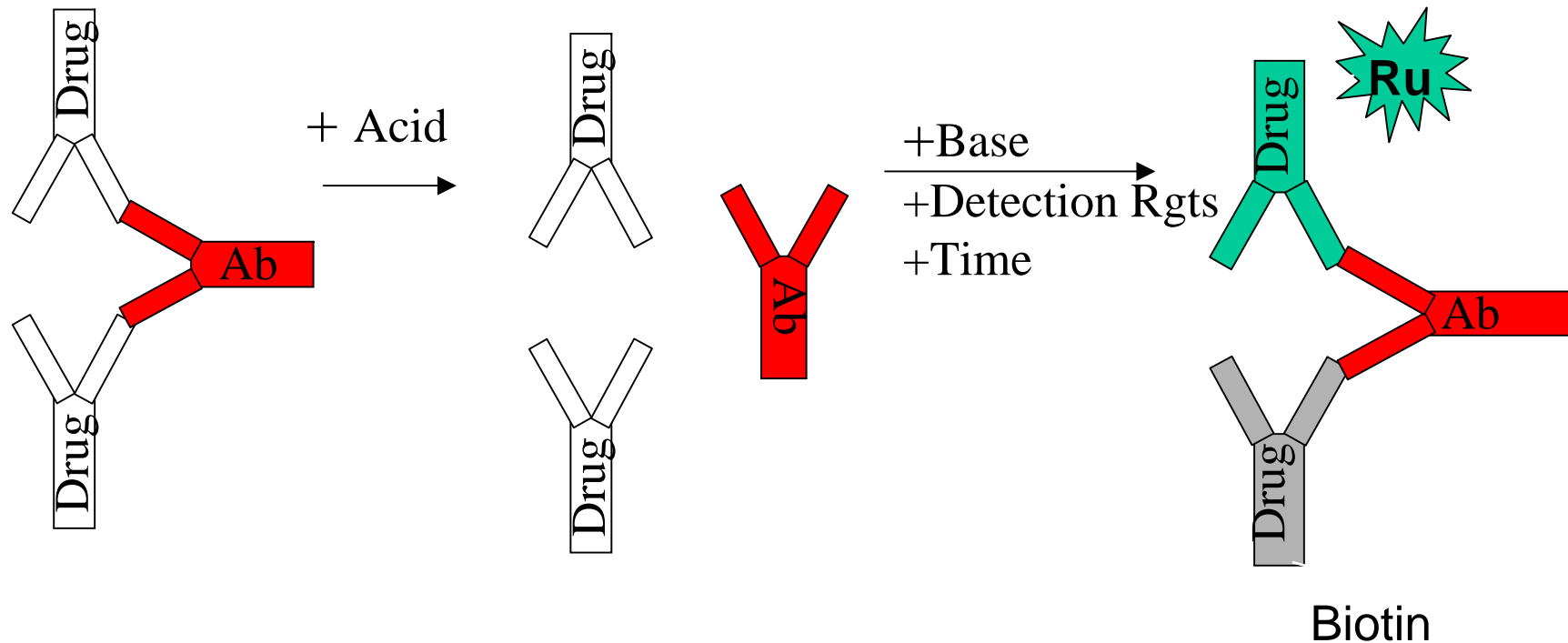


Electrochemiluminescence Detection (ECL)



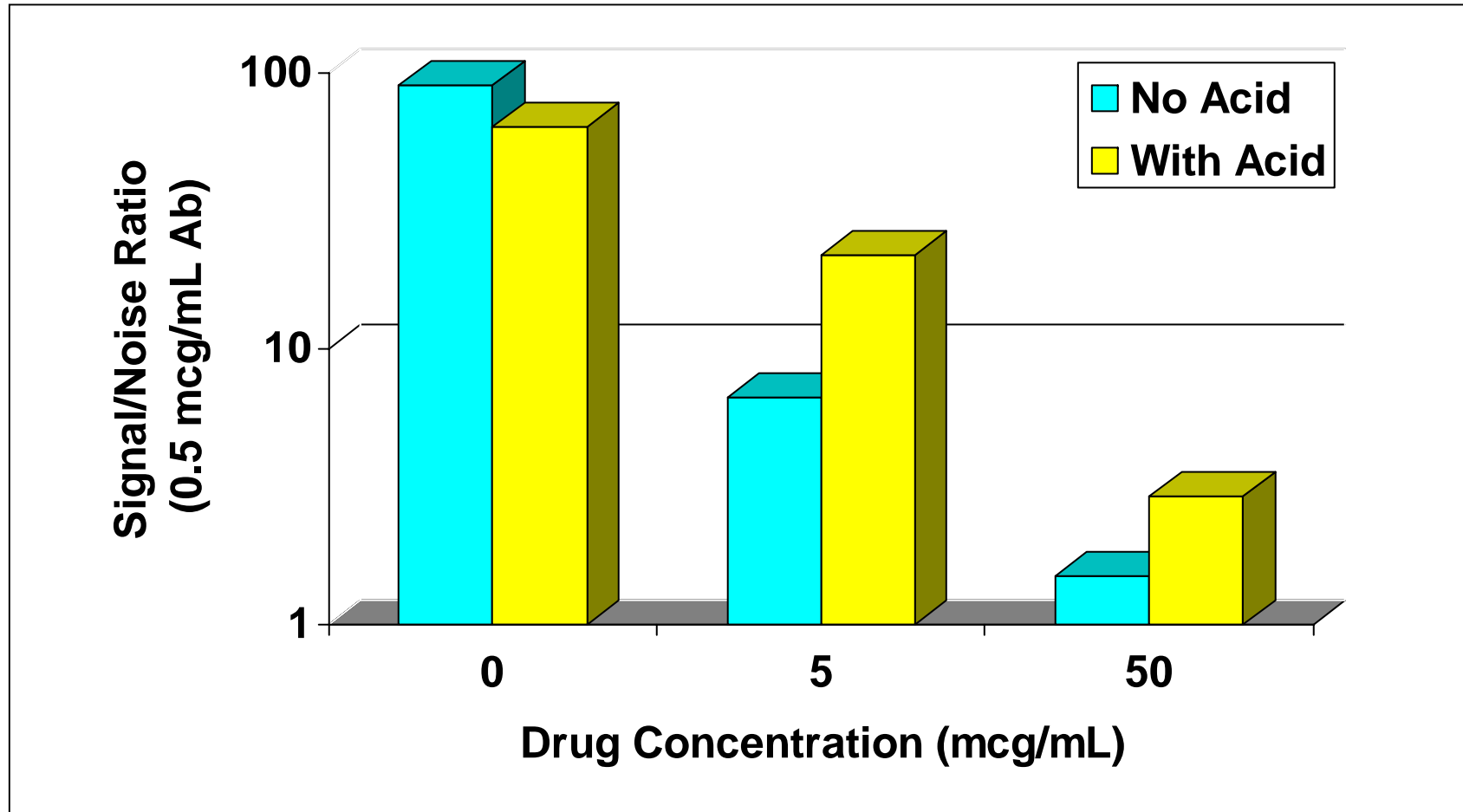
- Selective
- Convenient immobilization chemistry
- Robust, stable
- Few interferences

Acid Dissociation Reduces Drug Interference



Ref: Moxness et al. *Clin Chem* 2005; 51:1983-1985.

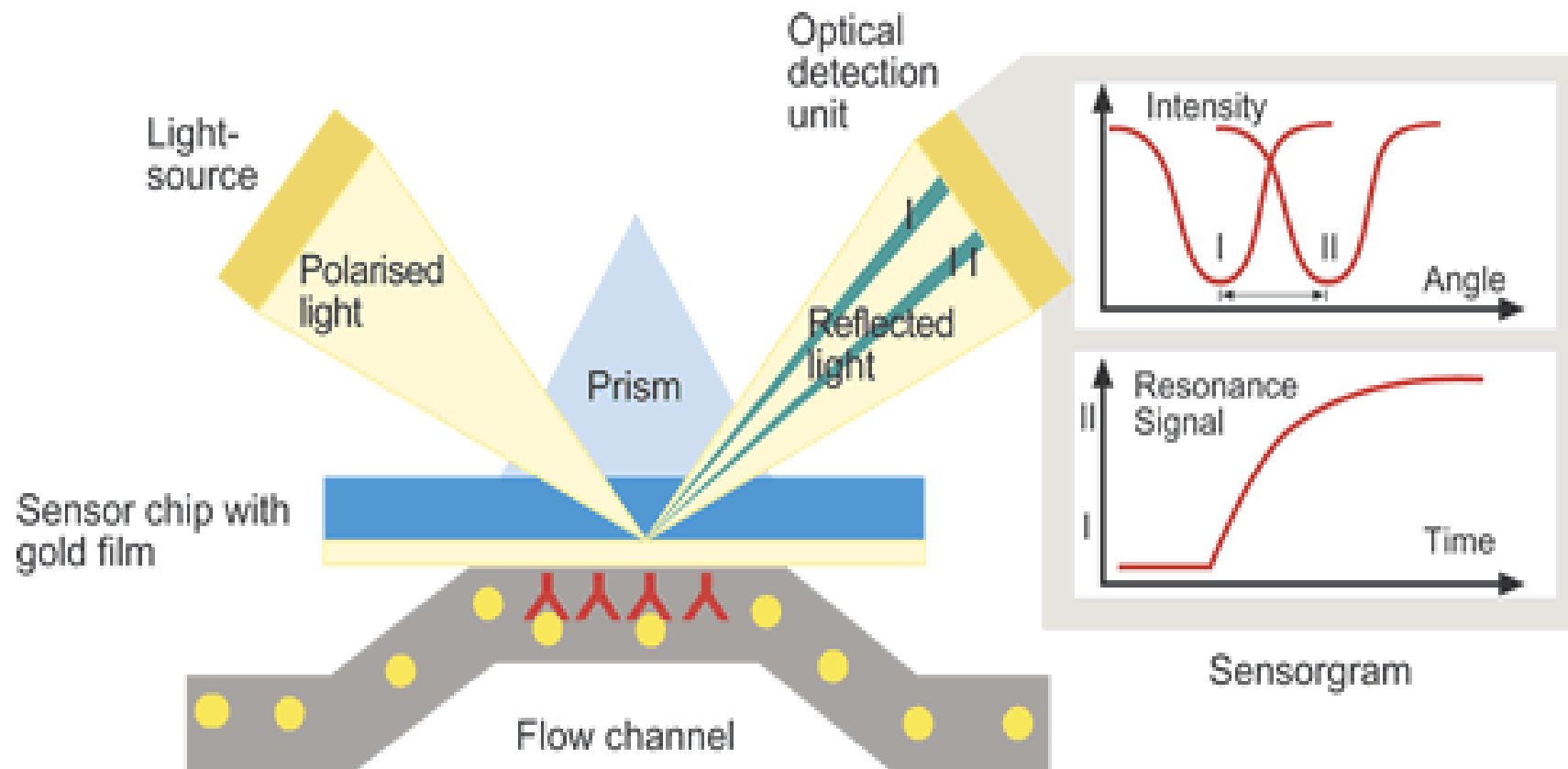
Acid Dissociation Enhances Signal in Presence of Drug



Biacore 3000



Surface Plasmon Resonance



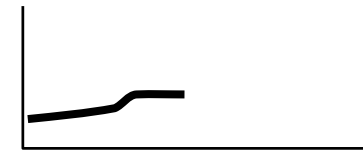
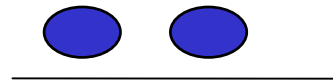
Biosensor Assay Platform



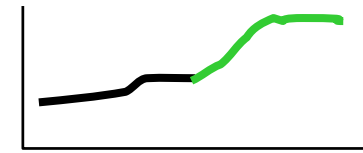
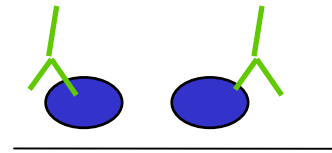
Event

Sensorgram

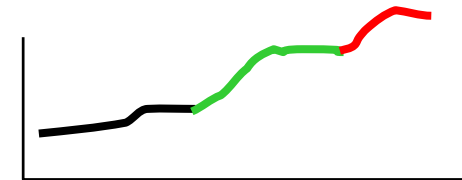
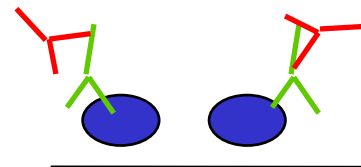
Immobilize Drug



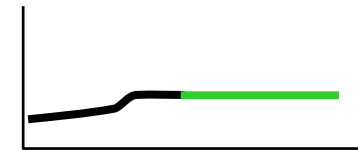
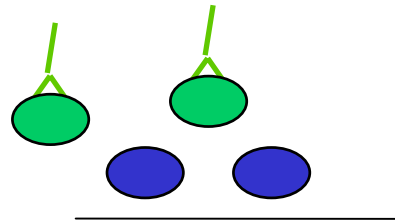
Add Sample



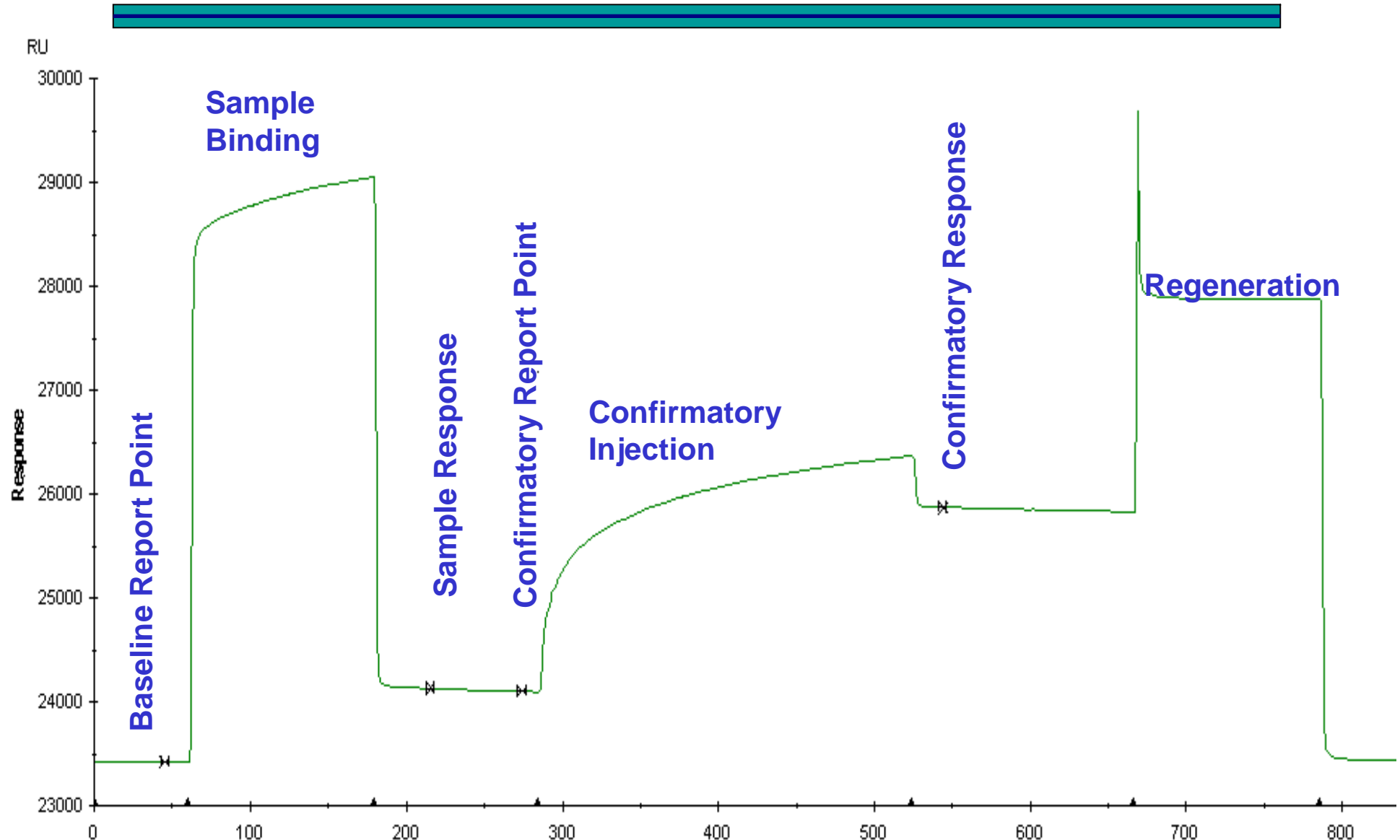
Confirm binding is antibody



Inhibit binding w/ drug



Biacore Sample Analysis Sensorgram



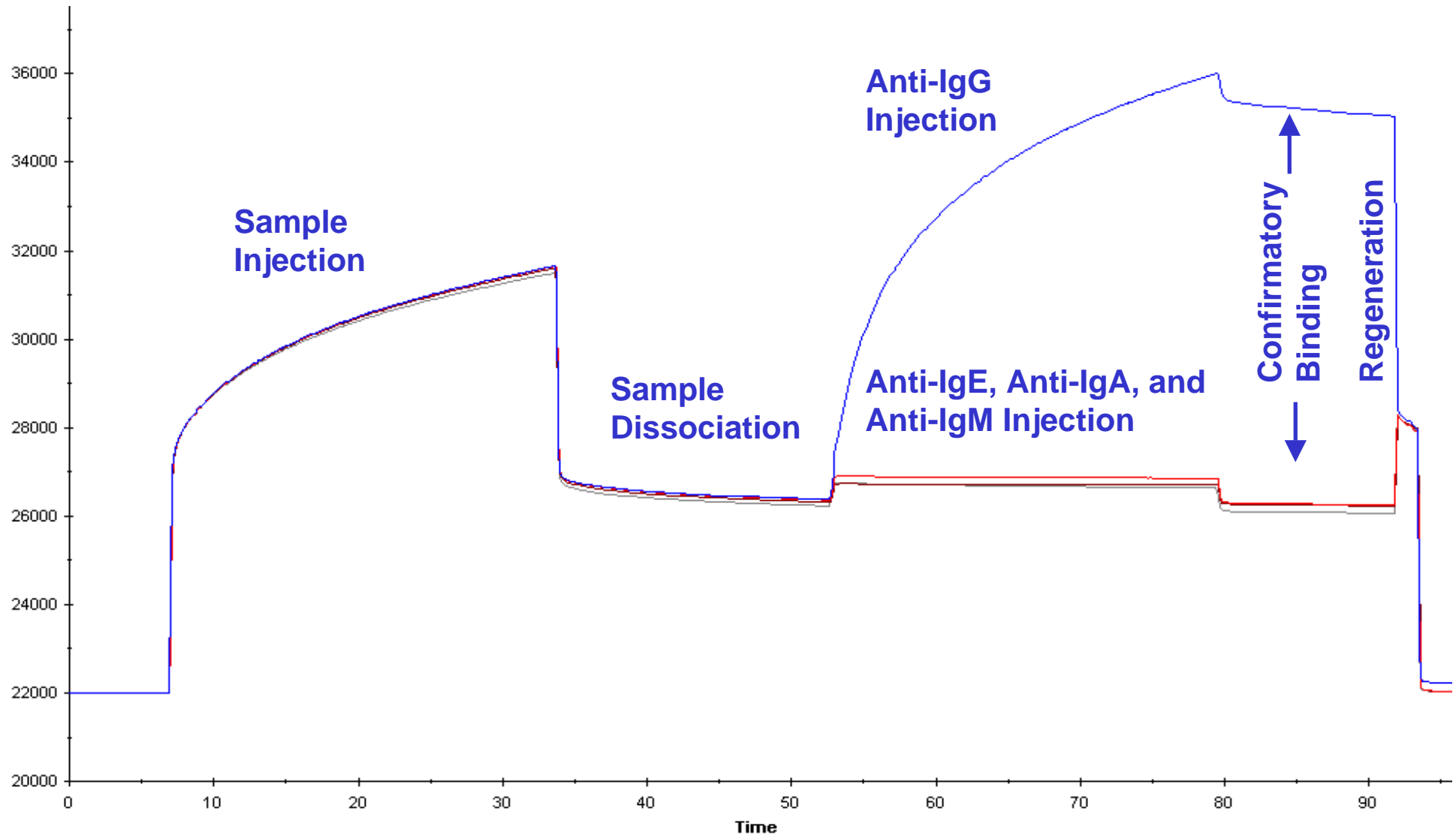
Interpretation of Biacore Results for Anti-Drug Antibodies

- The BIAcore is able to detect the presence of antibodies capable of binding to the immobilized drug.
- The BIAcore cannot determine if the detected antibodies are capable of neutralizing a biological effect of the drug.
- A bioassay is required to fully understand the significance of those antibodies.

Characterization of Antibodies

- Isotype determination
- Binding inhibition with soluble drug
- Determination of relative binding affinity
- Relative antibody concentration
- Specificity to native and derivatized product

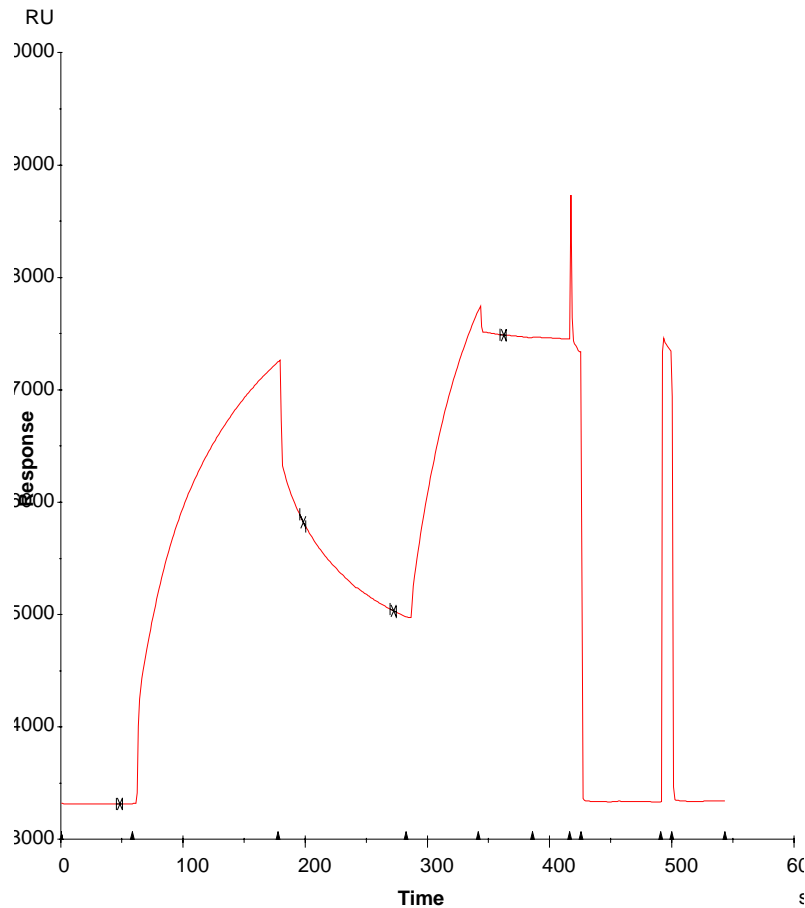
Biacore: Determination of Antibody Isotype



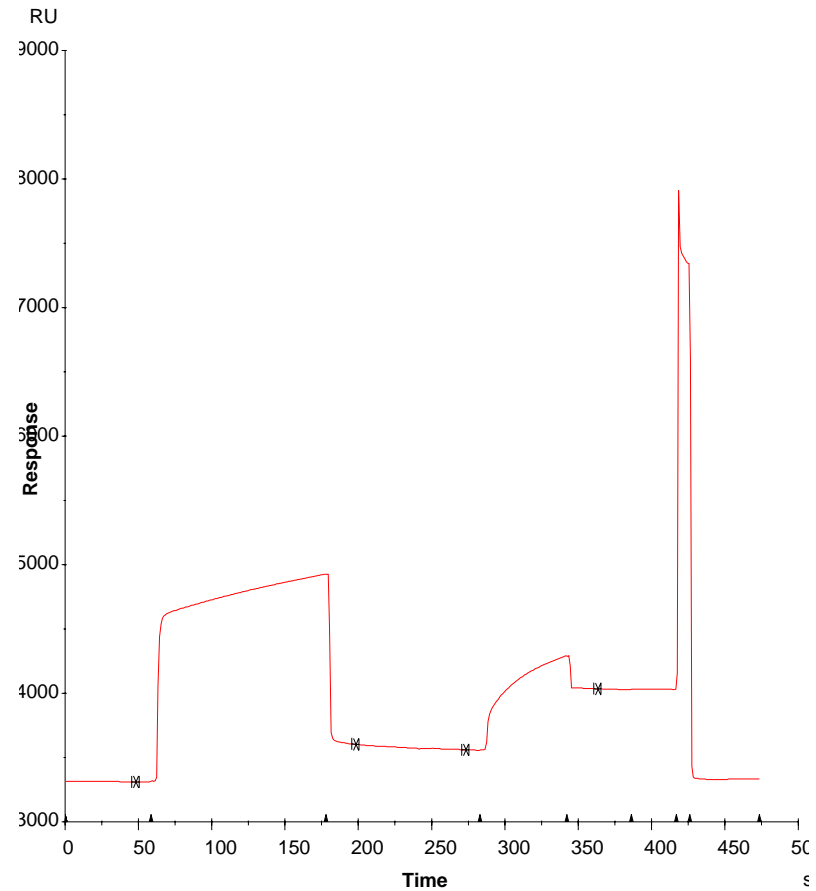
Determination of Relative Binding Affinity

- Procedure
 - Monitor dissociation rate as evidenced on sensorgram
 - Compare with positive control (high affinity)
- Interpretation
 - Comparisons can be made between the dissociation of samples and positive control

“High” and “Low” Affinity Antibodies



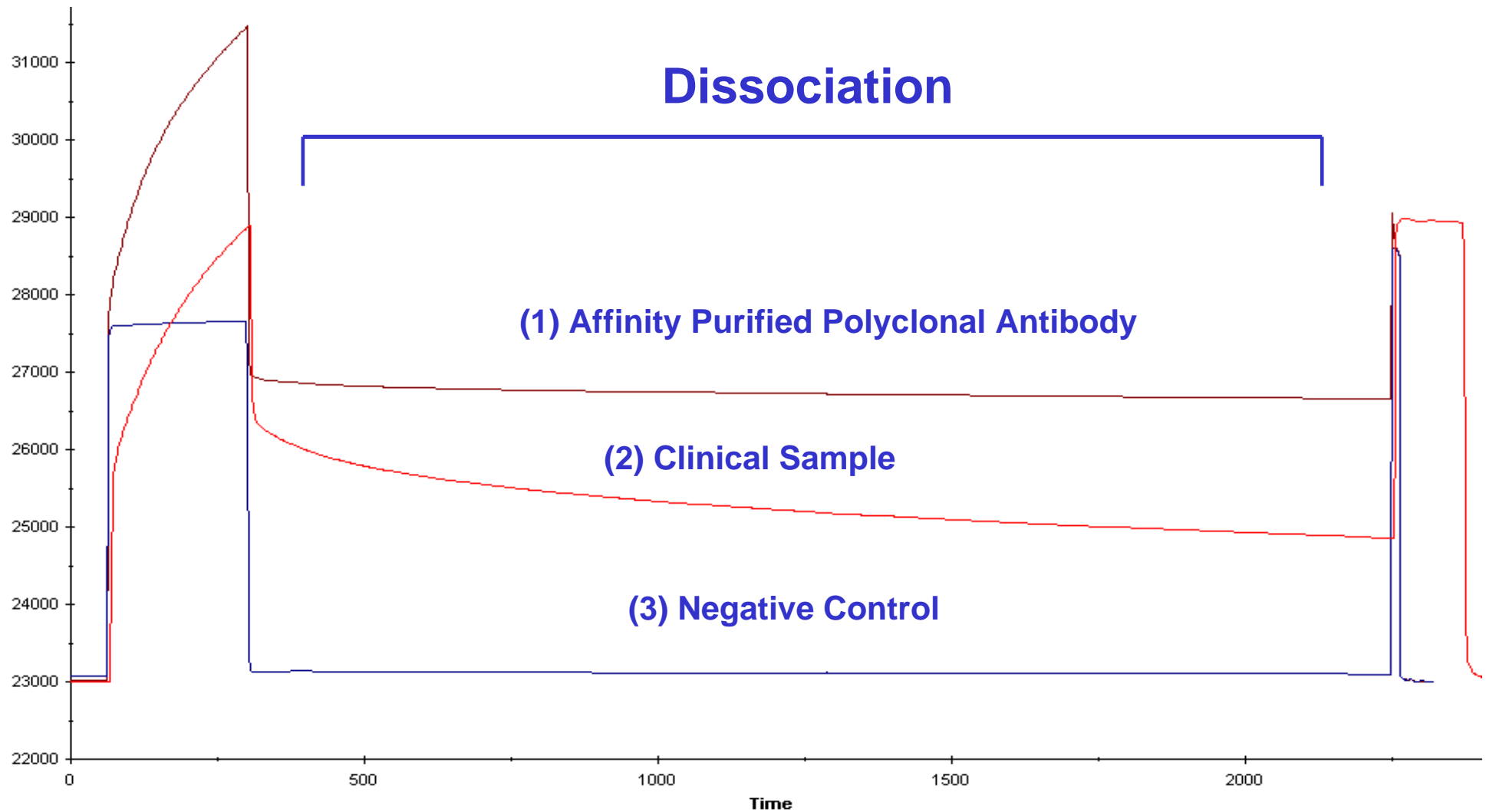
Low Affinity Antibody



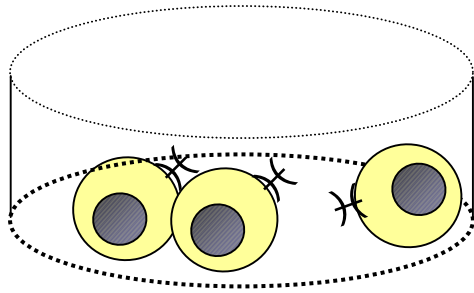
High Affinity Antibody


Note: Low affinity Abs that can be detected by Biacore are often not detected
By other methods, especially bridging ELISAs

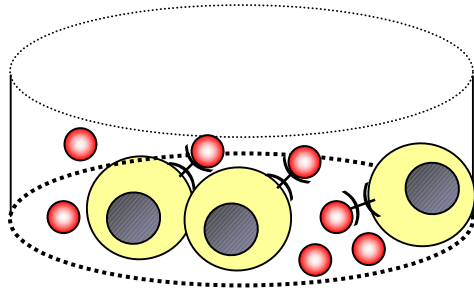
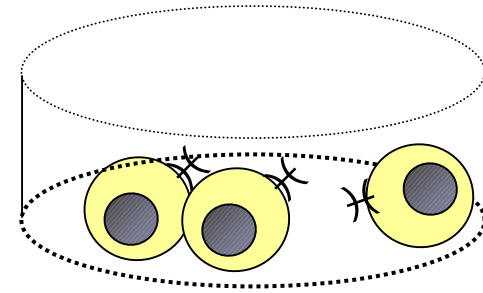
BIAcore: Determination of Antibody Dissociation Rates



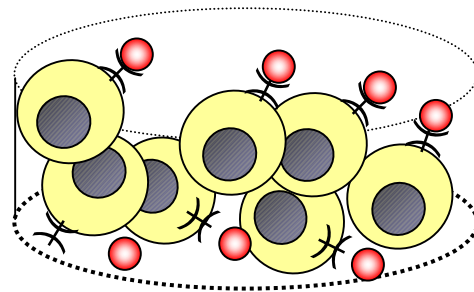
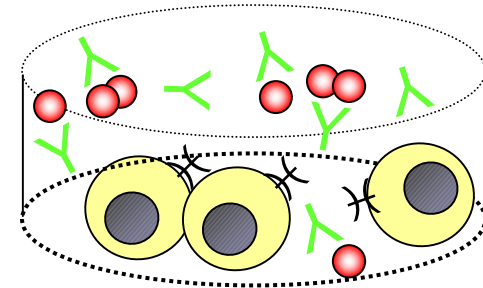
Bioassay Platform: Cell Proliferation



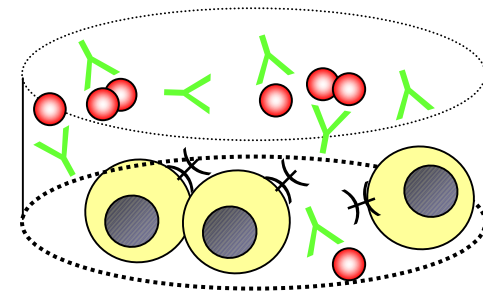
Cell line that is dependent on factor for growth 



Add drug (●) +/- patient serum sample (Y)



Measure proliferative response.
Inhibition of proliferation indicates presence of neutralizing antibodies.



Additional potential parameters: cytokine release, mRNA production, apoptosis

Challenges in Interpretation of Immunogenicity

- Ab detection hindered by soluble drug and is difficult with low affinity antibodies
 - Acid dissociation procedures have been developed to help in detecting Ab in presence of high drug levels
- As antibody assays improve in sensitivity we encounter detection of low level endogenous antibodies capable of binding to drug
- Important to have assays sensitive to detect earliest indication of an immune response
- Must be able to discriminate clinically relevant antibodies

Summary

- Many assay platforms available
 - Each has strengths and weaknesses
- Must be certain the assay detects all clinically relevant antibodies
 - Confirmatory and biological assays are critical
- Understanding the assay performance is critical to correct interpretation of results
 - Assays produce numerical readouts, important to consider that readout in the context of positive control
- Bioassays often correlate with clinical effect

References

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