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Immunogenicity of Therapeutic Proteins

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Causes of Immunogenicity

- Sequence differences between therapeutic protein and endogenous protein
- Structural alterations
  - Aggregation
  - Oxidation
  - Deamidation and degradation
  - Conformational changes
- Storage conditions
- Production/purification
- Formulation
- Route, dose and frequency of administration
- Immune status of patient
- Genetic background

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Immunogenicity Prediction

- May play a role in future drug development
- Could be useful in early drug development and in design of second-generation products
- Could significantly reduce development costs
- The story is still building

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Animal Models

- At this time, animal models cannot predict immunogenicity in humans
- Factors limiting predictive value
  - Immune system differences between humans, other primates, and other mammals
  - Lack of 100% homology between human therapeutic protein and non-human endogenous protein

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Animal Models for Differential Immunogenicity

- Animal models can be useful for comparing immunogenicity of 2 similar products
  - Parent and second generation product
  - Original therapeutic and product after process changes have been made

NOTE: This will still not necessarily reflect what happens in humans, but may provide advance warning if comparator has different immunogenicity profile from original

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Value of Preclinical Immunogenicity Assessment

- Interpret toxicology findings (was the animal exposed to the therapeutic protein or was the protein neutralized by the immune response)
- Provide insight into potential consequences of immunogenicity
- However, preclinical findings are not predictive for human immunogenicity

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How Do T-Cells Boost an Immune Response?

- Initial immune response is typically IgM, low affinity, and low concentration
- T-cell help is needed for class switching and affinity maturation that is required for a robust immune response
- High affinity mature antibodies of the IgG class are more likely to neutralize effects of therapeutic proteins

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Clinical Trials

- Immunogenicity is best determined through controlled clinical trials
  - Studies need to be powered to detect immunogenicity
  - Duration should be at least 6 months to 1 year
  - Ab samples taken at time when circulating drug has cleared or methods utilized to compensate for high levels of circulating drug
  - Assays should be robust, sensitive, specific, and validated
  - Binding and neutralizing Abs should both be measured

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Significance of Antibody Results

- Factors effecting interpretation of results
  - Magnitude of response (titer)
  - Duration of response (continuous or sporadic)
  - Correlation with AE
  - Correlation with change in PK (sustaining or clearing)
  - Biologically neutralizing antibodies

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Relevance of Antibody Response

- Examine relevance by patient
  - Determine effect of immune response on each patient
- Assess impact of immune response in patients on the project
  - Track rate of antigenicity
  - Track magnitude of immune response
  - Track rate of neutralizing antibody formation

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Antibody Significance

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 reaction
5) Cross-reacting with endogenous protein

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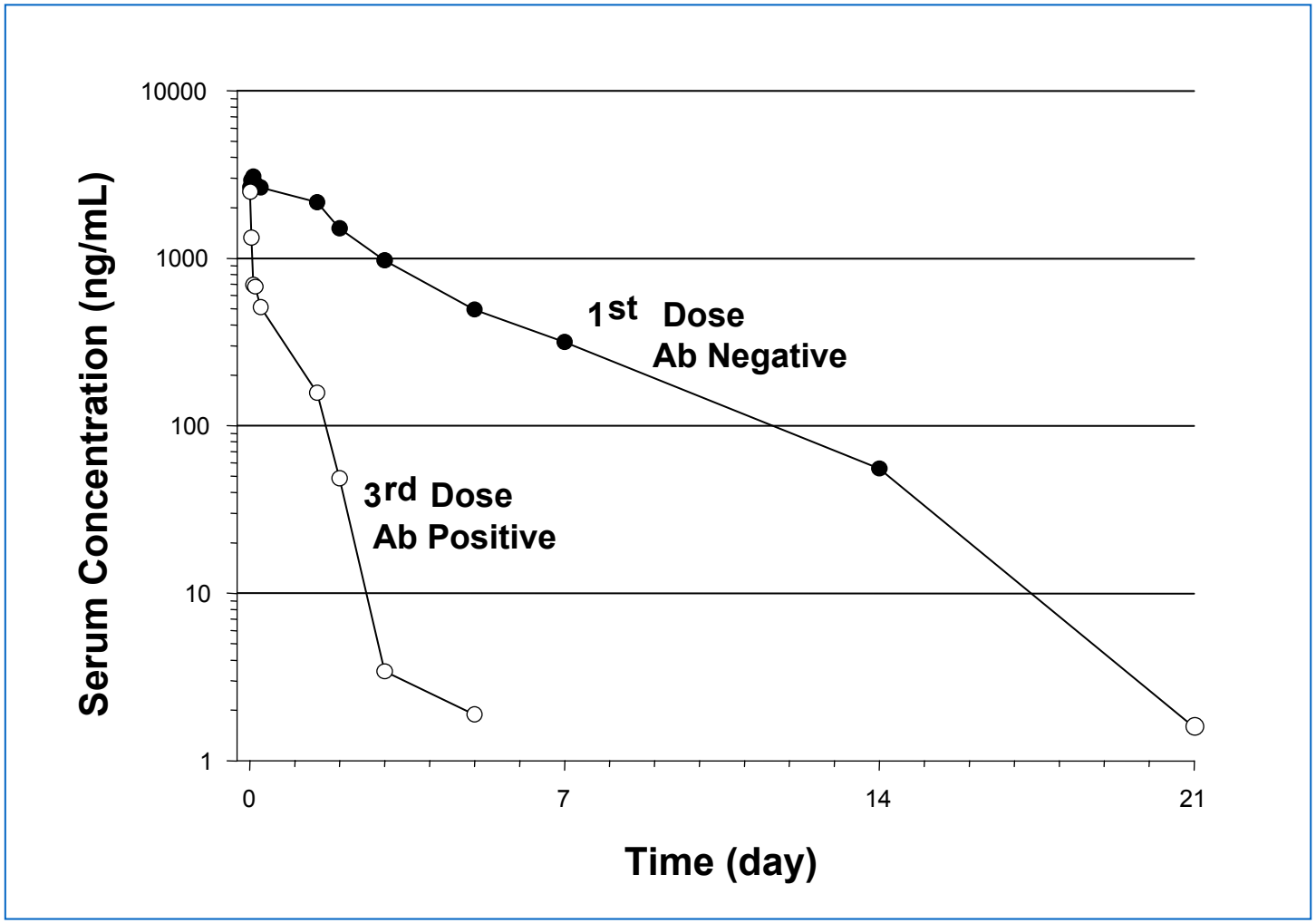
“Clearing” Antibody



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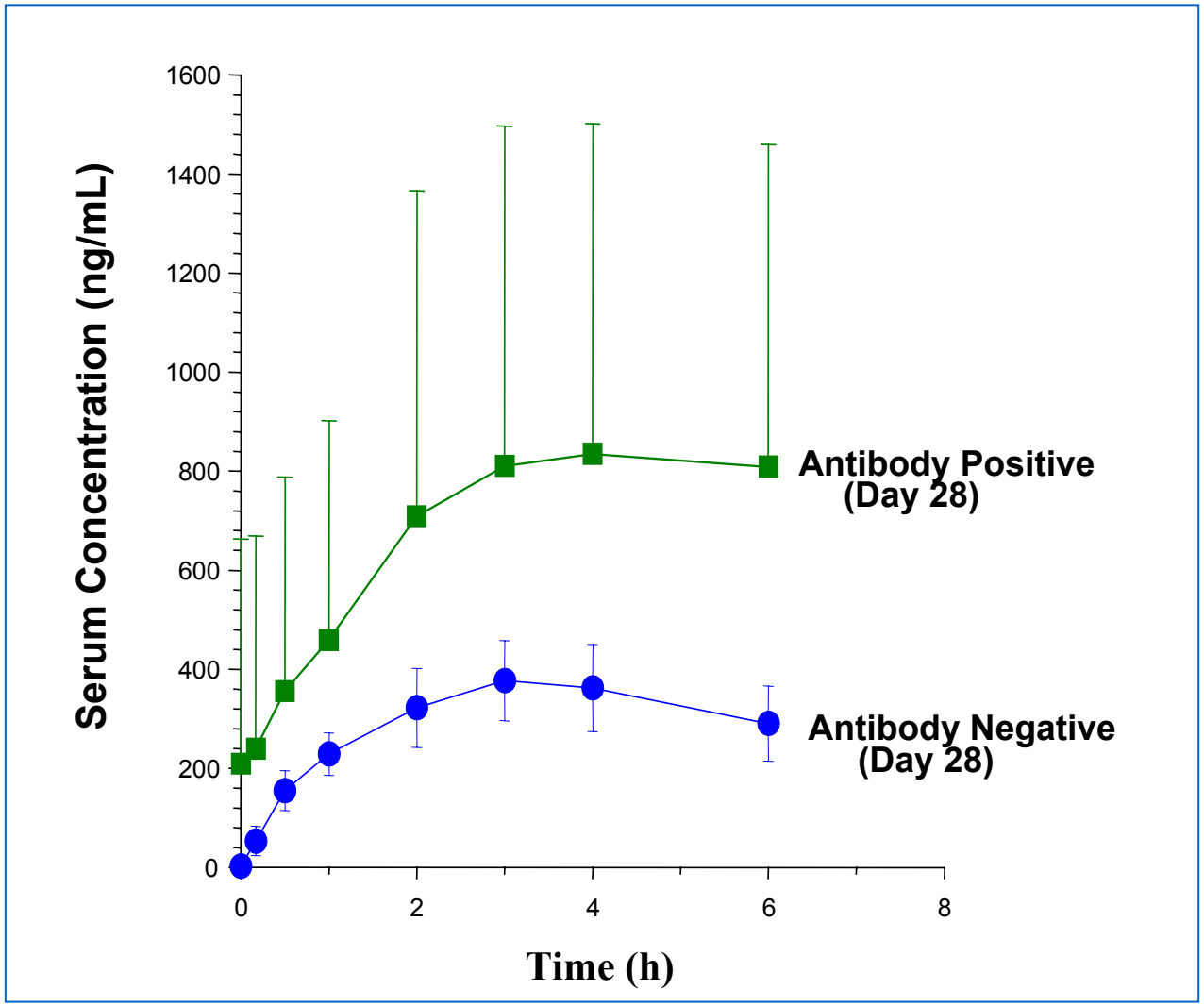
“Sustaining” Antibody



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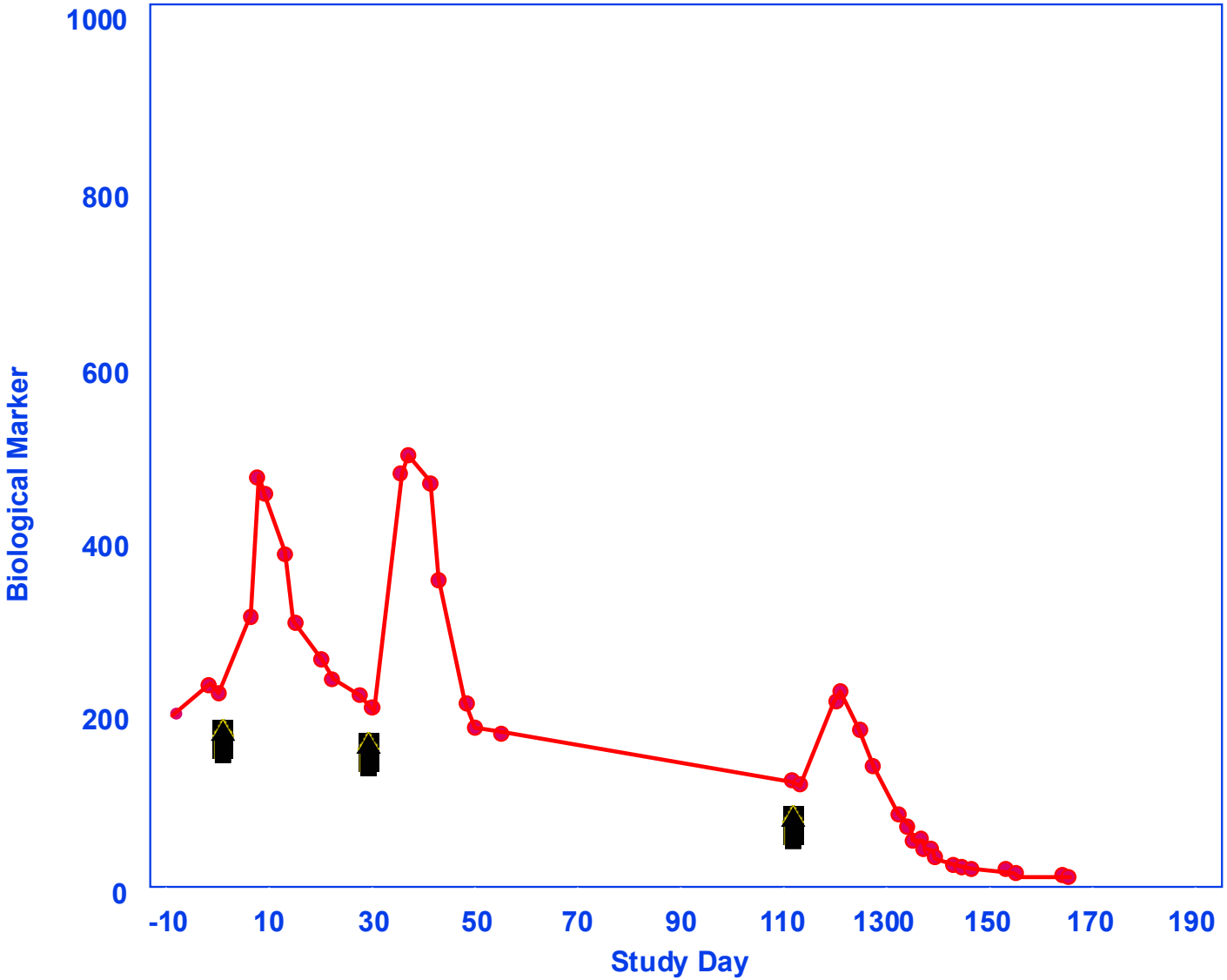
Drug Induces Neutralizing Antibody to Drug and to Endogenous Protein



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Strategy for Immunogenicity Testing

- Tiered approach
  - Screening immunoassay
  - Confirmatory immunoassay
  - Bioassay for neutralizing antibodies
- Sensitive, specific, and robust methods required
- Validation of assays to allow interpretation of results
- Incorporate “risk-based” approach

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How Should Antibodies be Tested?

- Many different formats available
- No “perfect” assay currently exists

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Immunoassay Platforms for Detecting Antibodies

- ELISA
  - Bridging format
  - Direct format
  - Indirect format
- Radioimmune precipitation
- Surface plasmon resonance
- Electrochemiluminescence

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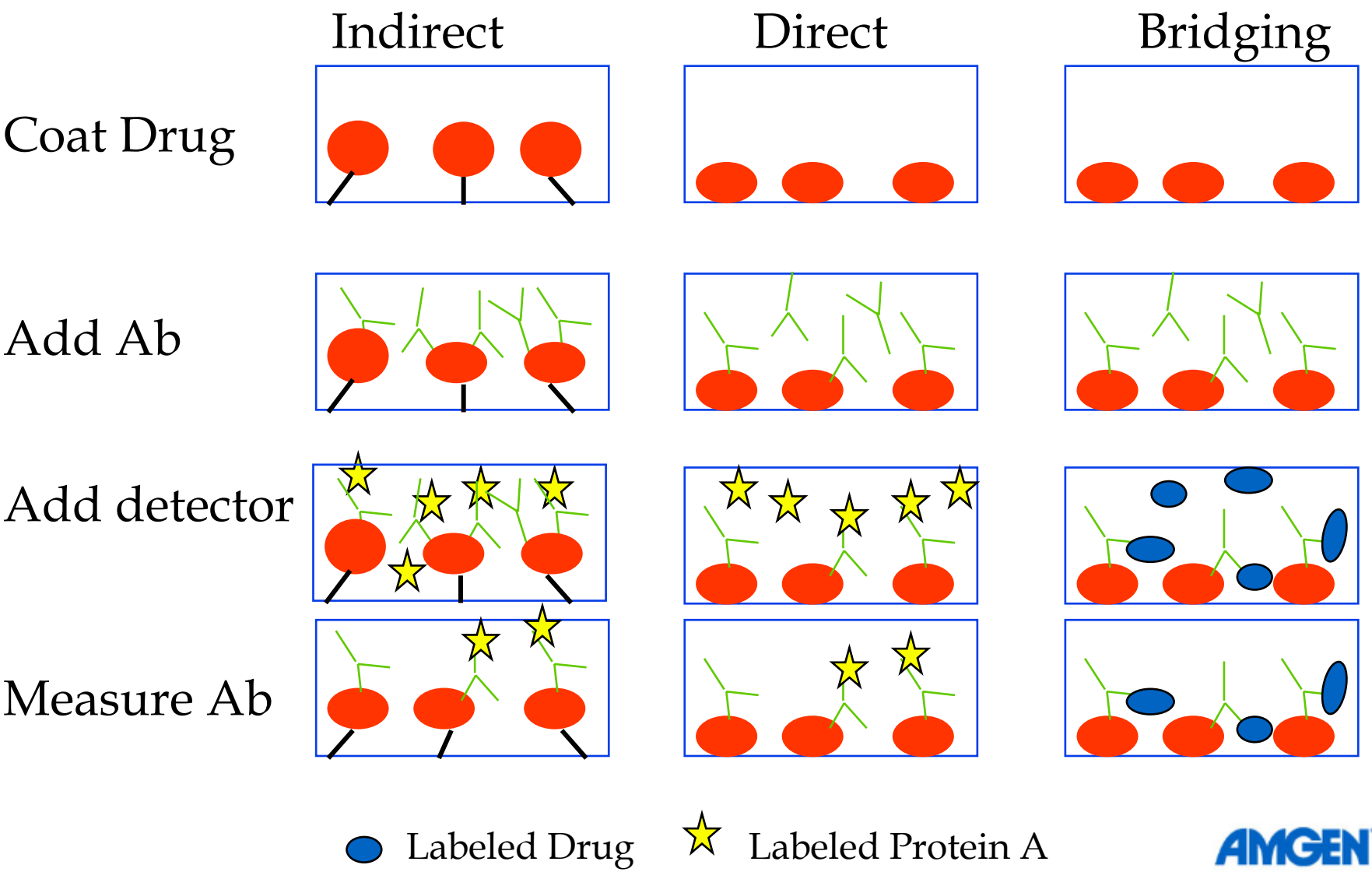
ELISA Platforms



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Pros and Cons - ELISA

- Pros
  - Established technology, equipment and expertise readily available
  - Sensitive
  - Inexpensive and high throughput
  - Bridging format is highly specific
- Cons
  - Can suffer from high background
  - Limited in ability to detect low-affinity antibodies
  - Difficult to confirm IgM antibodies in competition experiments

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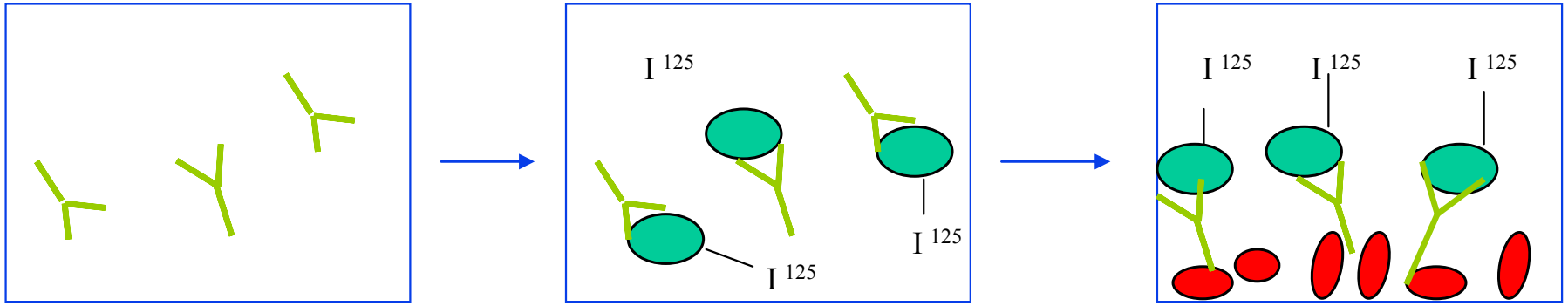
Radioimmune Precipitation Assay



Dilute sample

Add radioactive-labeled drug

Add Protein A, precipitate Ab, and measure labeled drug

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Pros and Cons - RIA

- Pros
  - Highly sensitive
  - Established method
- Cons
  - Use of radioactivity
  - Not ideal at detecting low-affinity antibodies
  - May not detect early immune response

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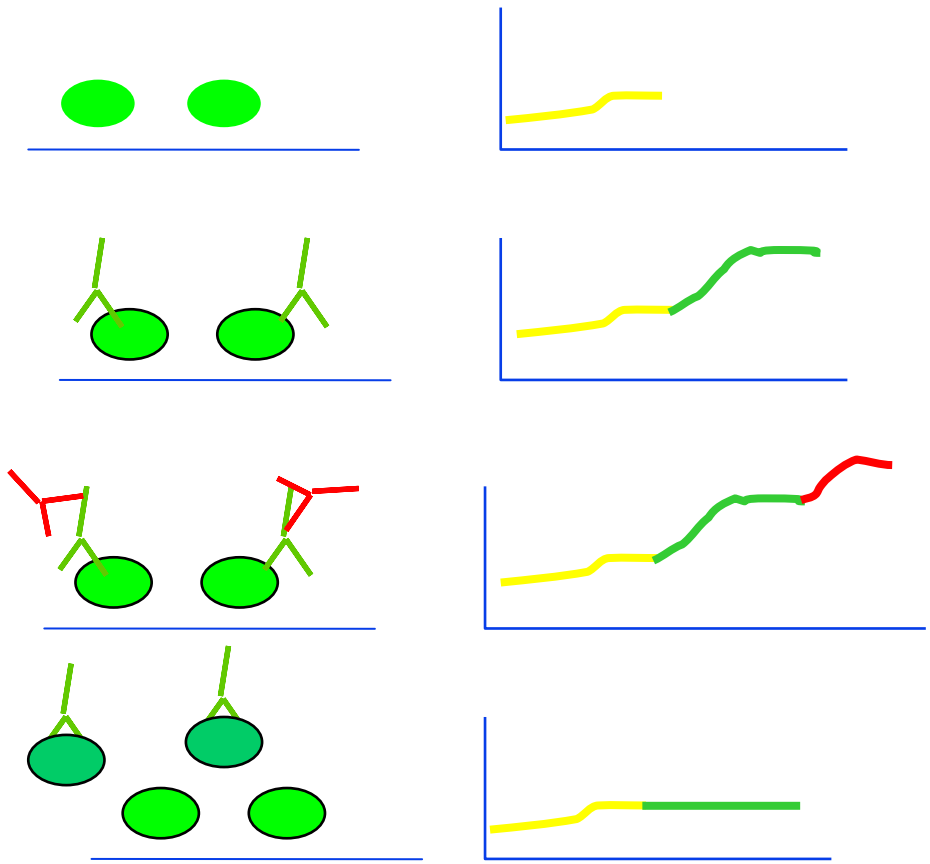
Surface Plasmon Resonance (Biacore)

Event	Sensorgram
Immobilize Drug	
Add Sample	
Confirm binding is antibody	
Inhibit binding w/drug	



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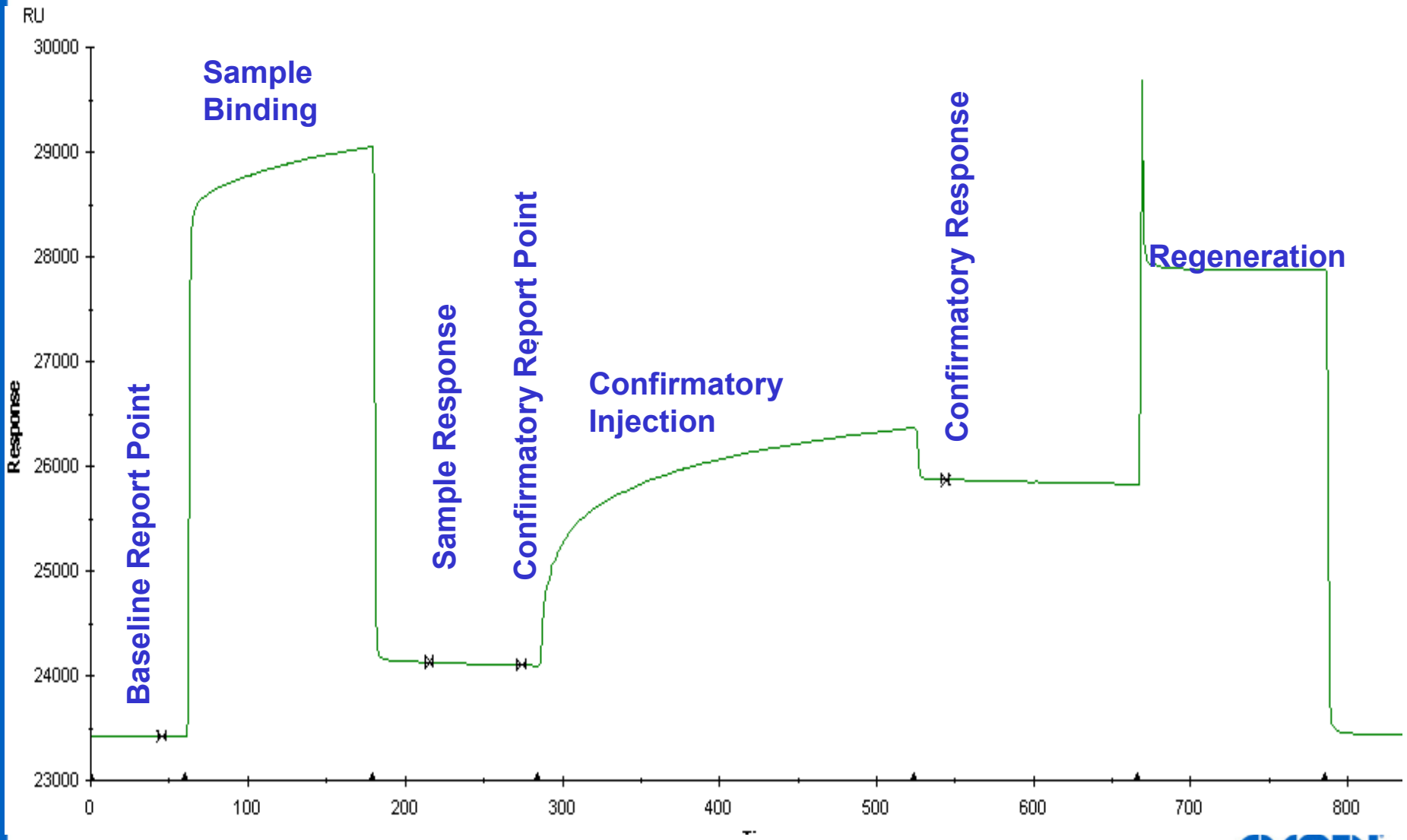
BIAcore Sample Analysis Sensorgram



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Pros and Cons - SPR

- Pros
  - Real-time detection is ideal for detection of low affinity Abs
  - Very good method for detecting early immune response
  - Ability to characterize detected Abs
- Cons
  - Equipment expense
  - Not as sensitive as other methods
  - Not as established as other methods
  - Throughput is moderate to low

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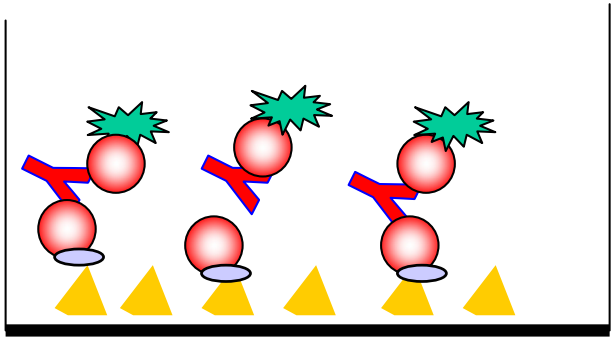
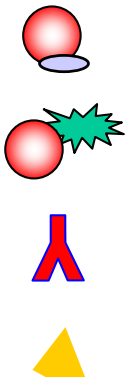
Electrochemilluminescence Assay (ECL)



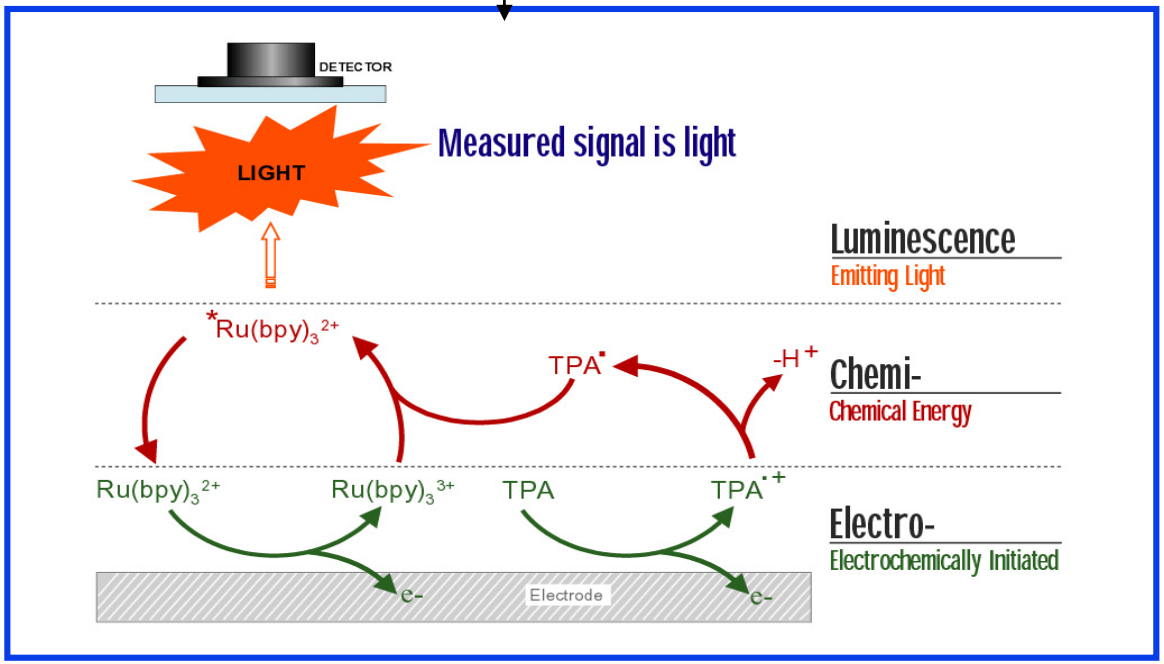


Biotinylated TP

Ruthenylated TP

Anti-TP antibody

Streptavidin



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Pros and Cons - ECL

- Pros
  - Highly sensitive
  - Broad dynamic range
  - Generally better than ELISA for detecting early immune response
  - Can be automated for high throughput
- Cons
  - Limited availability, not yet well-established
  - Can be subject to pronounced “hook effect”
  - May not detect all low affinity Abs

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Characterization of Antibodies

- Isotype determination
- Binding inhibition with soluble drug
- Determination of relative binding affinity
- Relative antibody concentration
- Specificity to native and second generation product
- Ability to neutralize in a cell-based system

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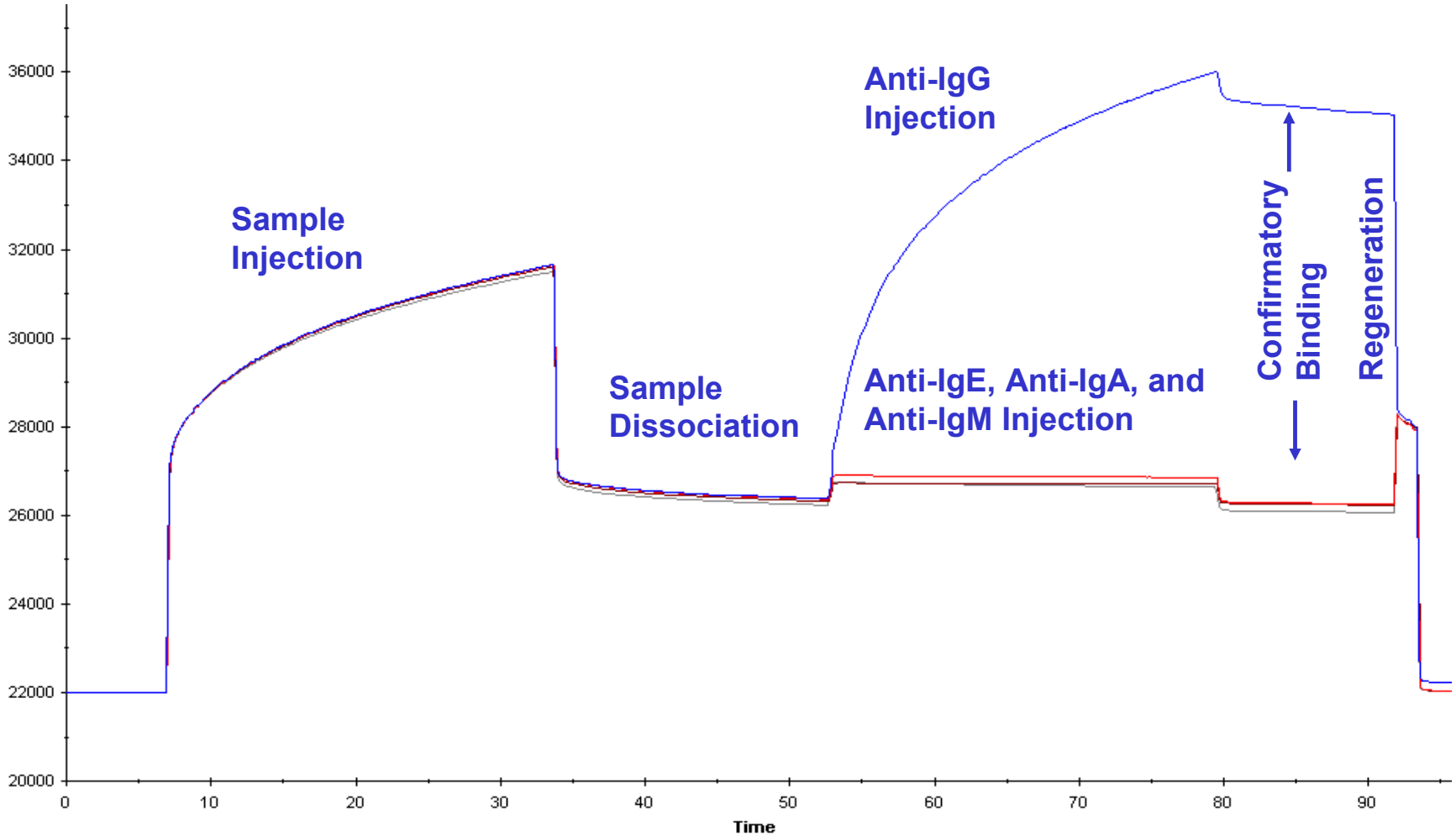
Biacore: Determination of Antibody Isotype



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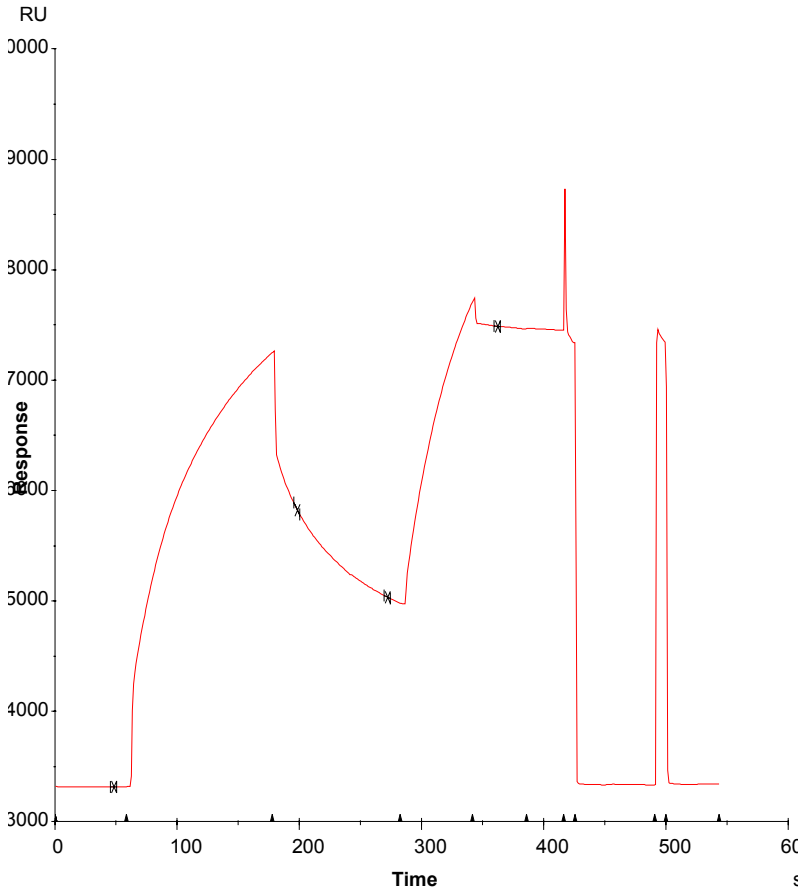
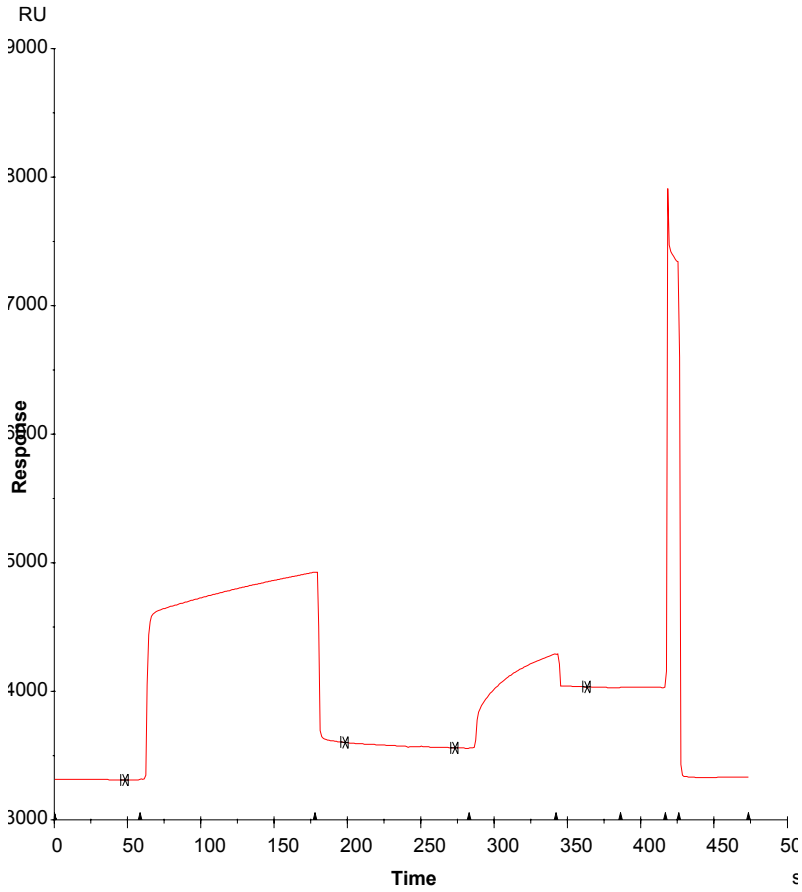
“High” and “Low” Affinity Antibodies



Low Affinity Antibody
(rapidly dissociating)



High Affinity Antibody

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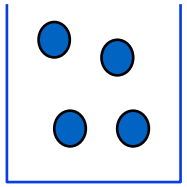
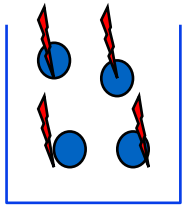
Bioassays for Neutralizing Antibodies

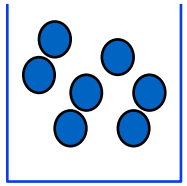
Culture cells



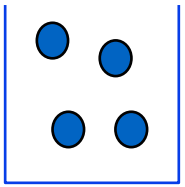
Add drug



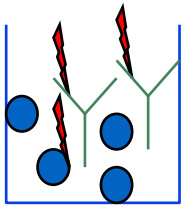
Measure biological response (proliferation)



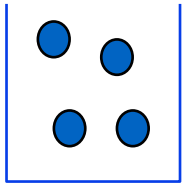
Culture cells



Add drug and Ab sample



Measure biological response (proliferation)



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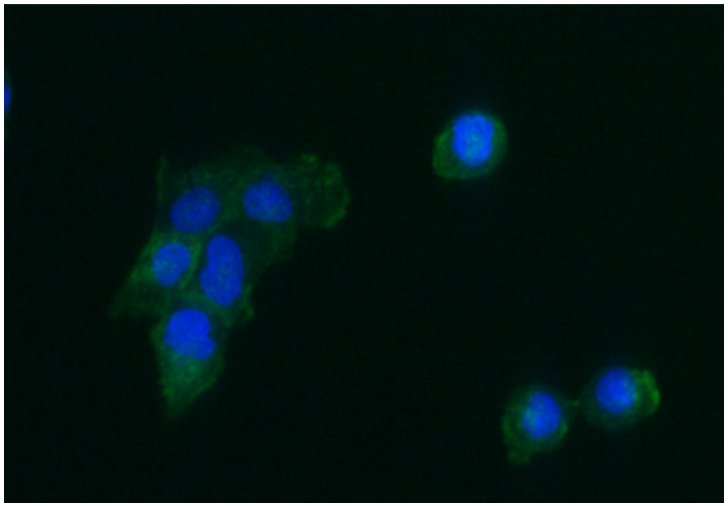
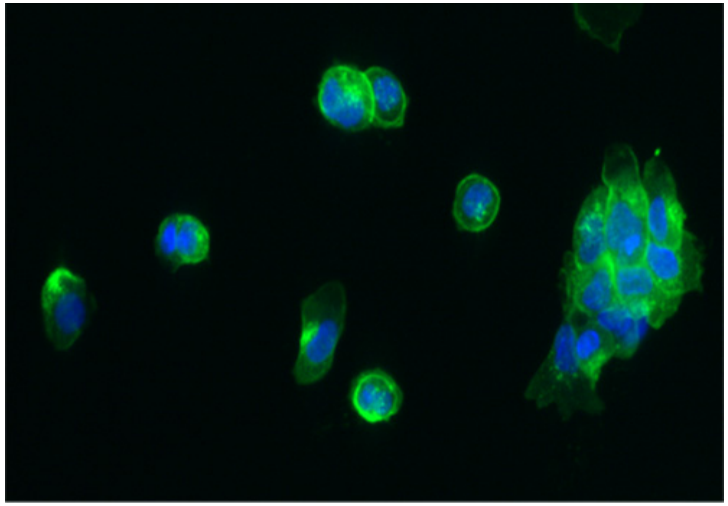
Receptor Tyrosine Kinase Activation

- Cell line untreated and treated with a growth factor for 20 minutes.
- Blue is Hoechst nuclear stain and green represents phosphorylated receptor antibody.



No treatment



+ Growth Factor

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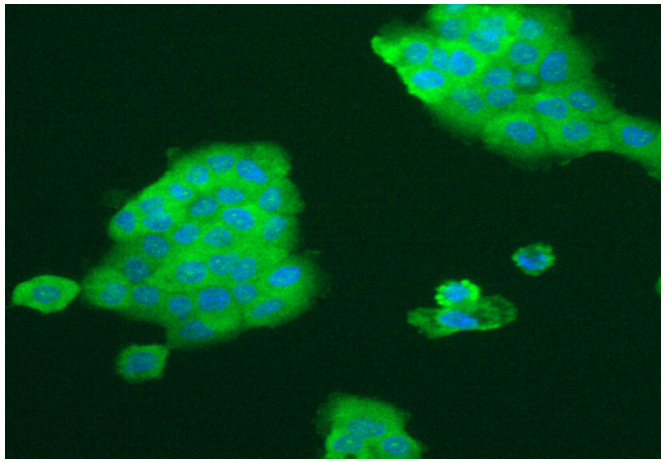
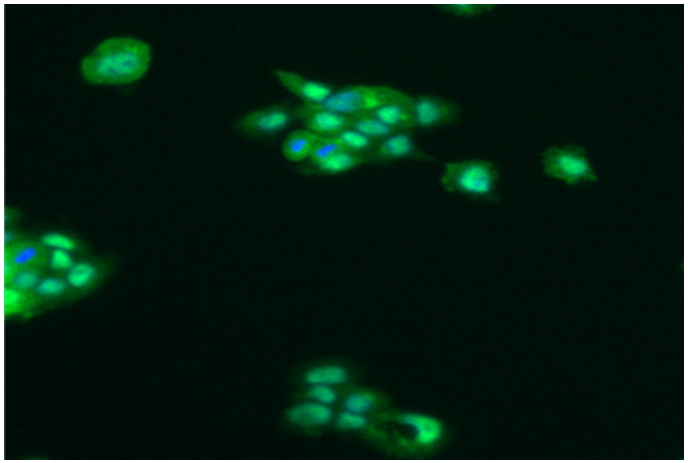
Transcription Factor Activation

- Cell line untreated and treated with a growth factor for 30 minutes.
- Blue is Hoechst nuclear stain and green represents a transcription factor.
- In untreated cells, is inactive and remains primarily in the cytoplasm. Upon activation, a transcription factor translocates to the nucleus to mediate gene expression.



No treatment



+ Growth Factor

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Neutralizing Antibodies

- Bioassay used to determine ability of the antibody to neutralize a biological effect of the drug in a cell-based system
  - Proliferation assay
  - Cytokine release assay
  - m-RNA measurement
- Bioassays typically more variable and less sensitive than immunoassays
- When a biological assay cannot be developed, can substitute a functional immunoassay

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Unique Features of Bioassays

- Determines effect of an antibody in a cell-based system
- Only assay that determines if an antibody can neutralize the biological effect of the drug
- Results must be coupled with a specific immunoassay to verify neutralization is due to antibody

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Antibody Response to Therapeutic Proteins

- Historically, many therapeutic proteins have induced antibody formation, effects of which range from minimal to significant
- Some of these antibodies are associated with serious adverse effects
- Both biopharmaceutical industry and regulatory agencies continue to partner for a better approach to antibody testing

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Antibody-Mediated PRCA

- Professor Nicole Casadevall reported in NEJM in 2002 on cases of antibody-mediated pure red cell aplasia in patients treated with ESAs
- These patients had neutralizing antibodies against erythropoietin
- Very few reports in the literature of this phenomenon prior to the Casadevall manuscript
- Focused attention on the analytical procedures used for detecting and characterizing antibodies against ESAs

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Summary of Anti-EPO Results

Subject	Immunoassays			Bioassay
	RIP*	ELISA	BIACORE	
001	+	+	+	+
002	+	+	+	+
003	+	+	+	+
004	+	–	+	+
005	+	–	+	+
006	+	+	+	+
007	+	+	+	+
008	+	+	+	+

* RIP = Radioimmune precipitation assay
+ = Positive for anti-rHuEPO antibodies
– = Negative for anti-rHuEPO antibodies

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Characterization of PRCA Antibodies

Relative Ab Concentration (mcg/ml)	Ab Dissociation Rate (RU/min)	Predominant Isotype
43.46	10.7	IgG4 (IgG1, 2)
15.86	3.9	IgG4 (IgG1, 3, 2)
14.57	6.2	IgG4 (IgG1, 2, 3)
5.96	1.9	IgG1 (IgG4)
6.57	2.1	IgG4 (IgG1, 2)
8.68	2.1	IgG1 (IgG4, 2)
4.78	1.7	IgG4 (IgG2, 1, 3)
4.10	1.6	IgG4 (IgG1)

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Antibody Related Issues in Clinical Studies and Post-Approval Stage

Patient safety

- Cross reactivity with endogenous proteins
- Allergic reactions
- Immune complexes-complement activation

Reduced efficacy

- Neutralizing and/or clearing antibodies

Altered PK

- Enhanced drug clearance
- Drug accumulation
- Interference with PK assay

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Conclusions

- Recombinant therapeutic proteins can be immunogenic
- Antibodies to therapeutic proteins can cause difficulties in preclinical animal studies and occasionally, serious side effects in humans
- Antibody testing method can greatly impact the results
- Careful antibody monitoring with appropriate assays is necessary throughout preclinical and clinical development in order to ensure safety and efficacy of therapeutic proteins

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Acknowledgements

Amgen's Clinical Immunology Department

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