



Drug Tolerance in ADA Analysis

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- **What is Drug Interference and Why an issue in ADA analysis?**
- **Methodologies to overcome Drug Interference**
 - **Acid Dissociation**
 - **Affinity Capture Elution (ACE)**
 - **Solid Phase Elution with Acid Dissociation (SPEAD)**
- **Case Studies**
 - **Herceptin**
 - **Avastin**
- **Summary**

- **The maximum amount of free drug in a study sample that allows detection of ADA at an acceptable sensitivity**
- **Why an issue?**
 - **the presence of high drug levels in study samples can interfere with detection of ADA, especially in bridging assay formats**
 - **Particularly relevant in multiple dose studies where drug concentrations may remain high for a significant period of the study**
 - **Can interfere with PK and PD evaluations**

DRUG TOLERANCE



Compound Type	MW (Kda)	Test Method	Drug Tolerance ($\mu\text{g/mL}$)	Drug Tolerance (nM)	Sensitivity (ng/mL)
Peptide		ELISA	8.1		566
PEG-peptide		RIPA	2.5		11
Peptide	3	RIPA	1.0	333	69
PEG-peptide		ACE ECL	0.002		69
Fusion protein		ECL	0.5		22
Fusion protein		ACE ECL	0.46		94
Enzyme		ECL	102		195
MAb	150	ACE elisa	500	3333	8
MAb	150	ECL	200	1333	63
PEG-peptide		ECL	0.08		127
Peptide		ECL	20		10

Addressing Drug Tolerance

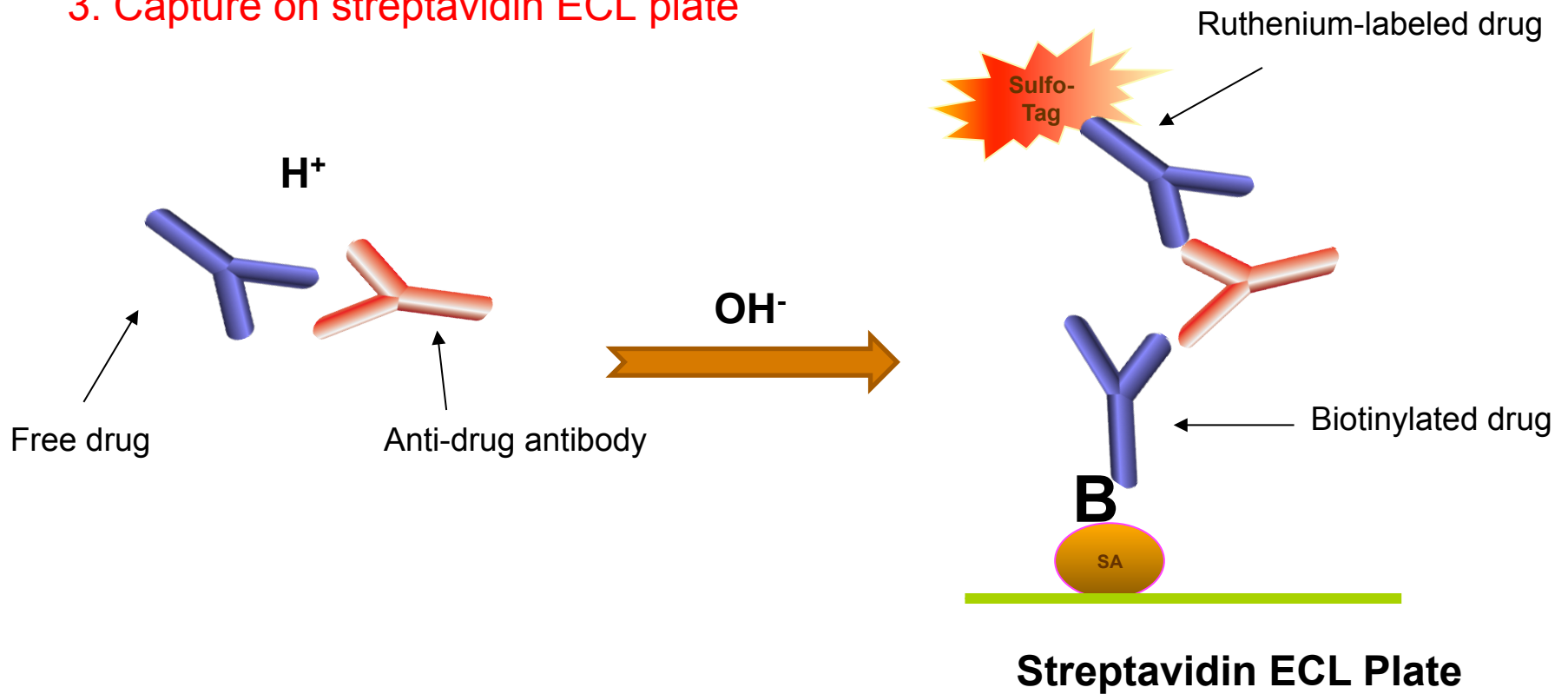
- **First line approach often taken :**
 - **Acid dissociation using Acetic Acid between 300 and 600mM**

- **One Size Does not fit all**
 - **Optimisation**
 - **Acid Concentration?**
 - **Dissociation / Neutralisation Time?**
 - **Temperature?**
 - **Acid type – e.g. Acetic, Glycine?**
 - **Alternative formats**
 - **ACE – Acid – Capture – Elution: Several variations**
 - **SPEAD Solid phase extraction with acid dissociation**

- **Case Study: Anti-Avastin**
 - **Three different formats developed for Avastin**
 - **Bridging ECL with Acetic Acid Dissociation**
 - **ACE**
 - **SPEAD**
 - **Early in development, the impact of added drug was evaluated on these formats**
 - **Most promising format was to be chosen for further development**

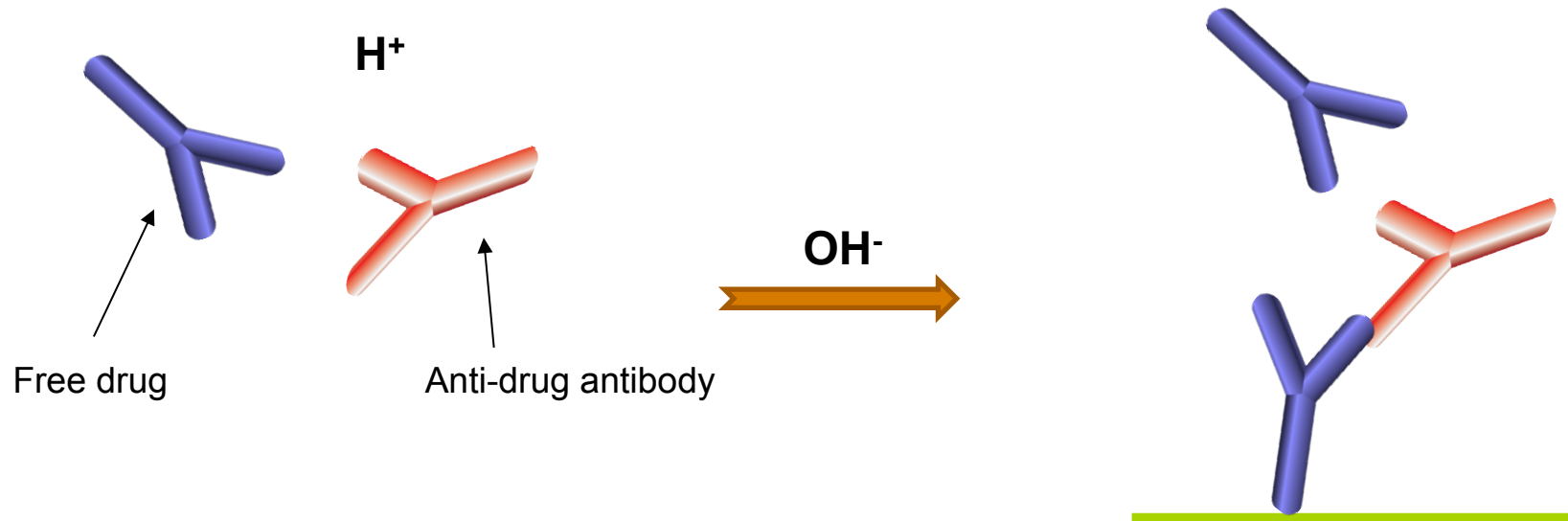
Bridging ECL format

1. Acid dissociate and neutralize
2. Incubate with biotin- and ruthenium-labeled drug
3. Capture on streptavidin ECL plate



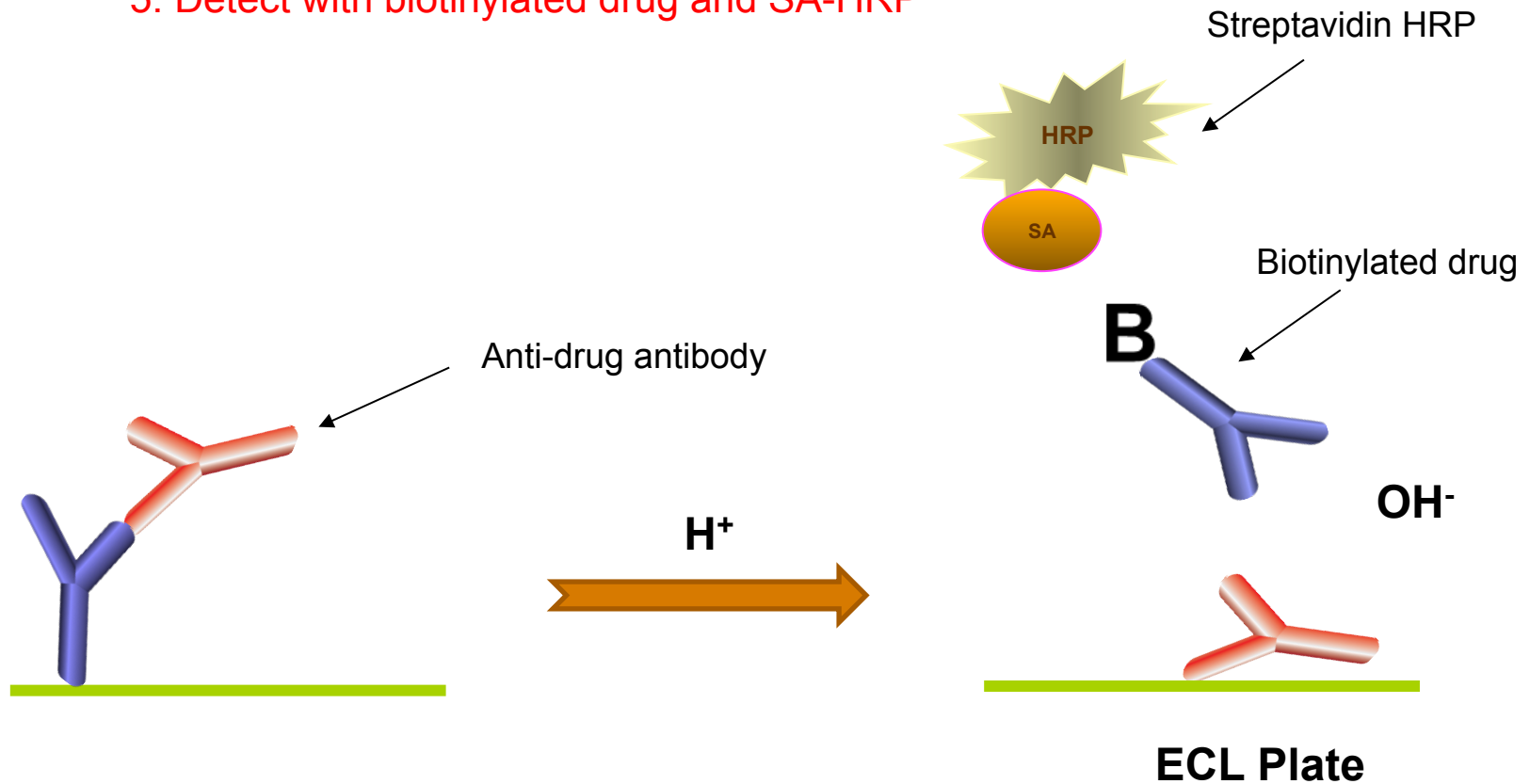
“ACE” assay format (1)

1. Acid dissociate and neutralize
2. Capture on drug-coated plate

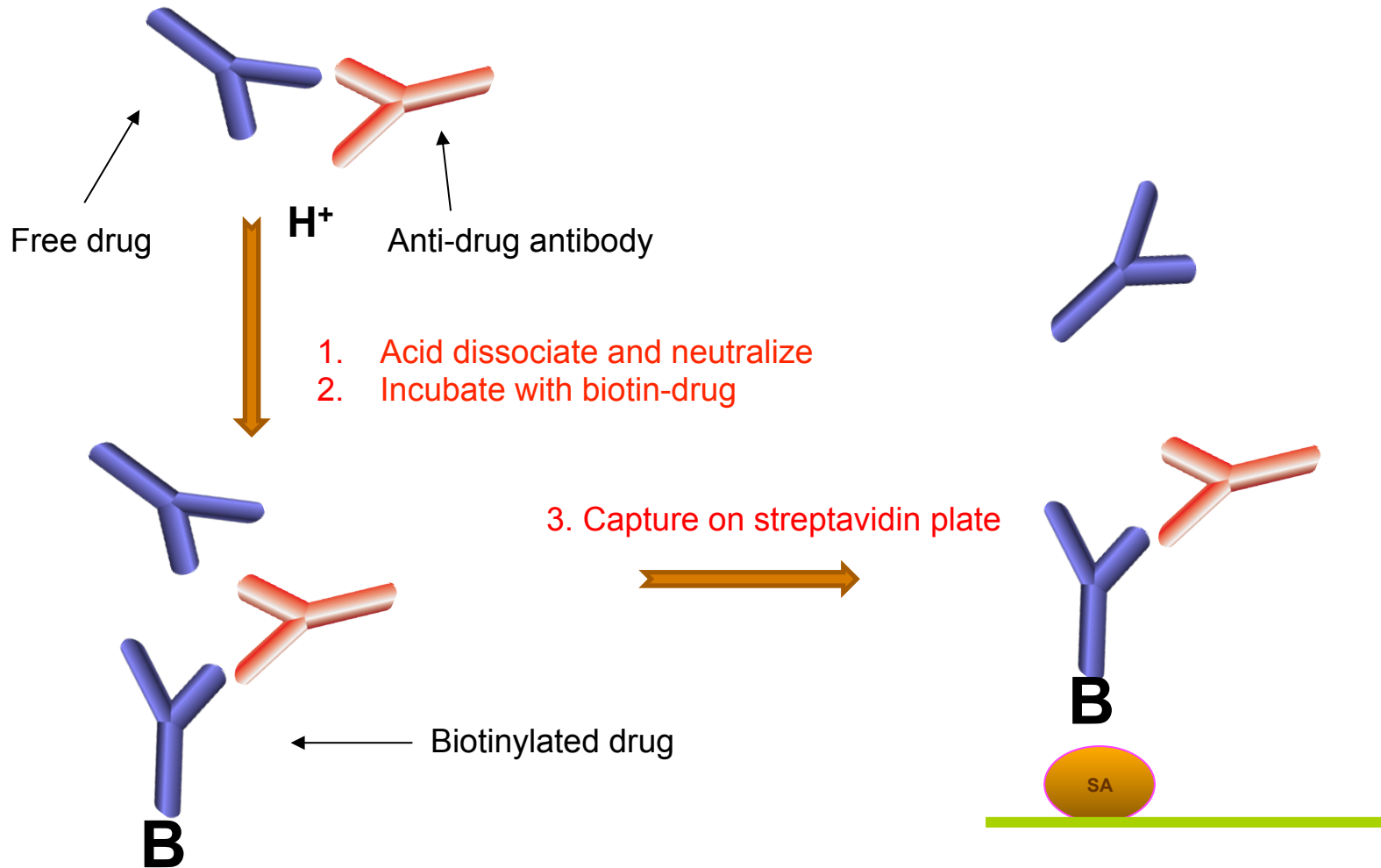


“ACE” assay format (2)

- 3. Elute using acid
- 4. Neutralize and coat on second plate
- 5. Detect with biotinylated drug and SA-HRP

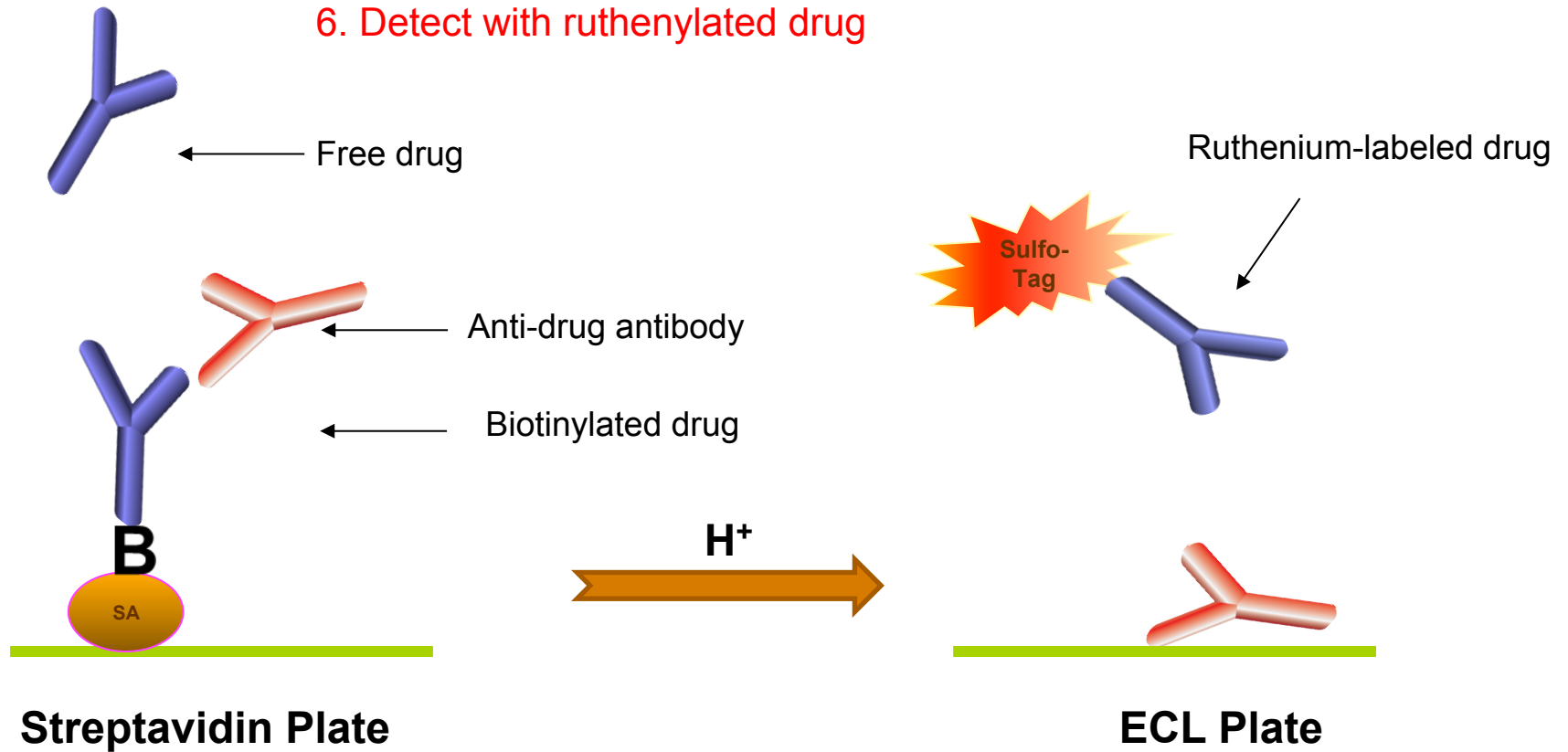


“SPEAD” assay format (1)



“SPEAD” assay format (2)

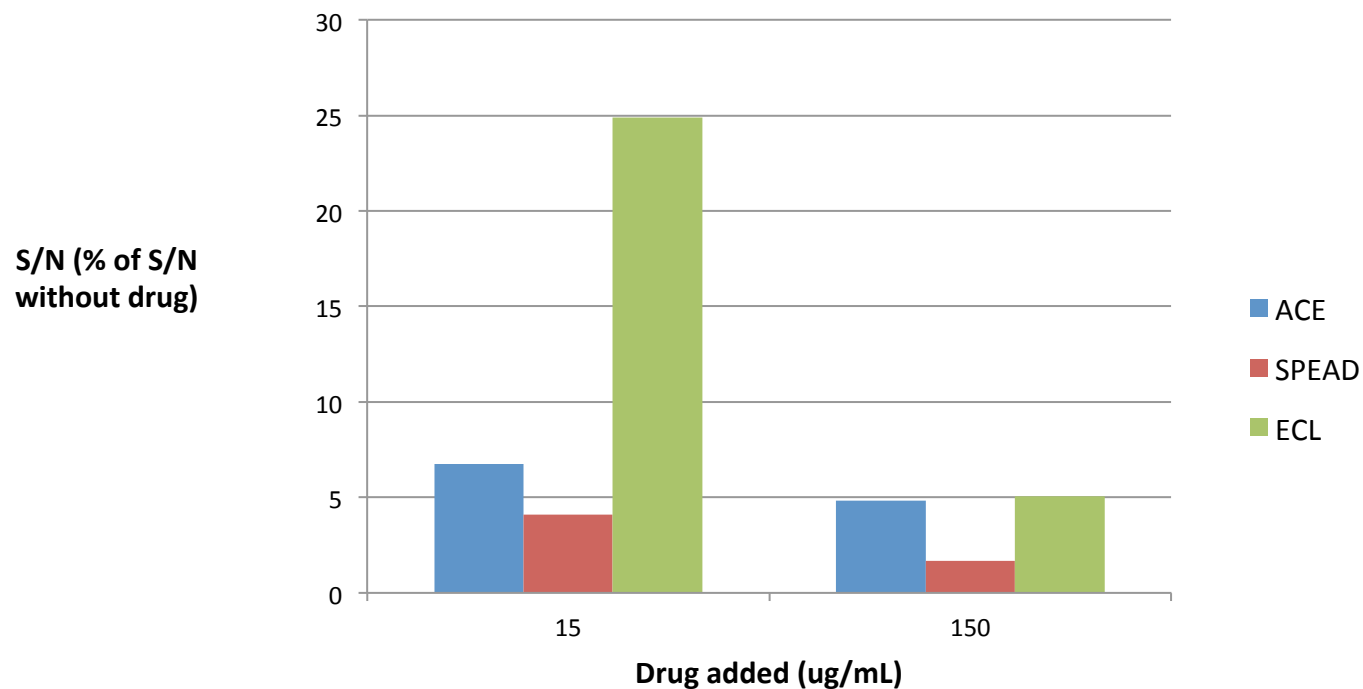
4. Elute using acid
5. Neutralize and coat on ECL plate
6. Detect with ruthenylated drug

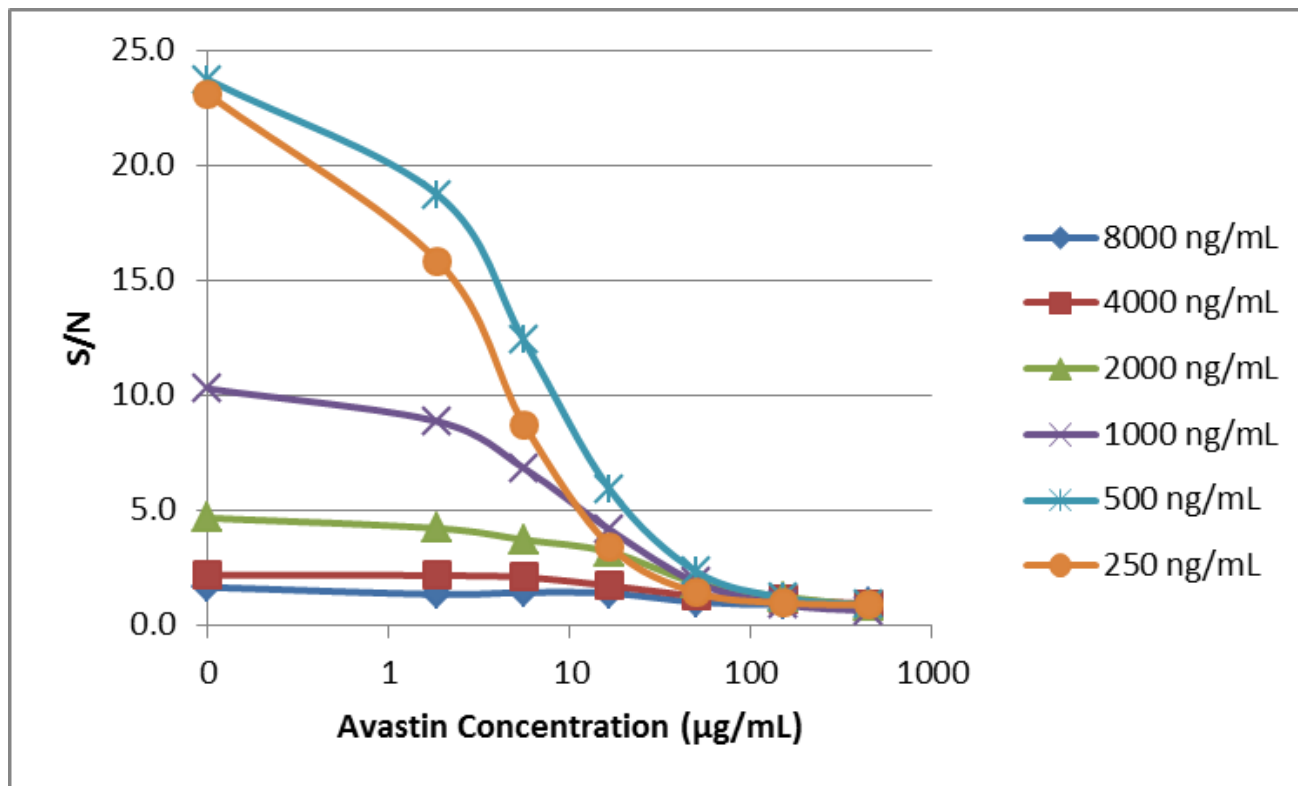


- **Three different formats developed for Avastin**
- **Signal-to-noise ration (S/N) was used to compare methods.**
- **15 or 150 $\mu\text{g}/\text{mL}$ of unlabeled drug with an ADA level of 500 ng/mL (required sensitivity level) was examined**
- **All three formats had S/N close to 1.0 at 150 $\mu\text{g}/\text{mL}$ drug = not tolerant to this level of drug**
- **Since bridging ECL assay had the highest S/N at 15 $\mu\text{g}/\text{mL}$ drug, this was deemed the most promising format and chosen for further optimization**

Comparison of three assay formats

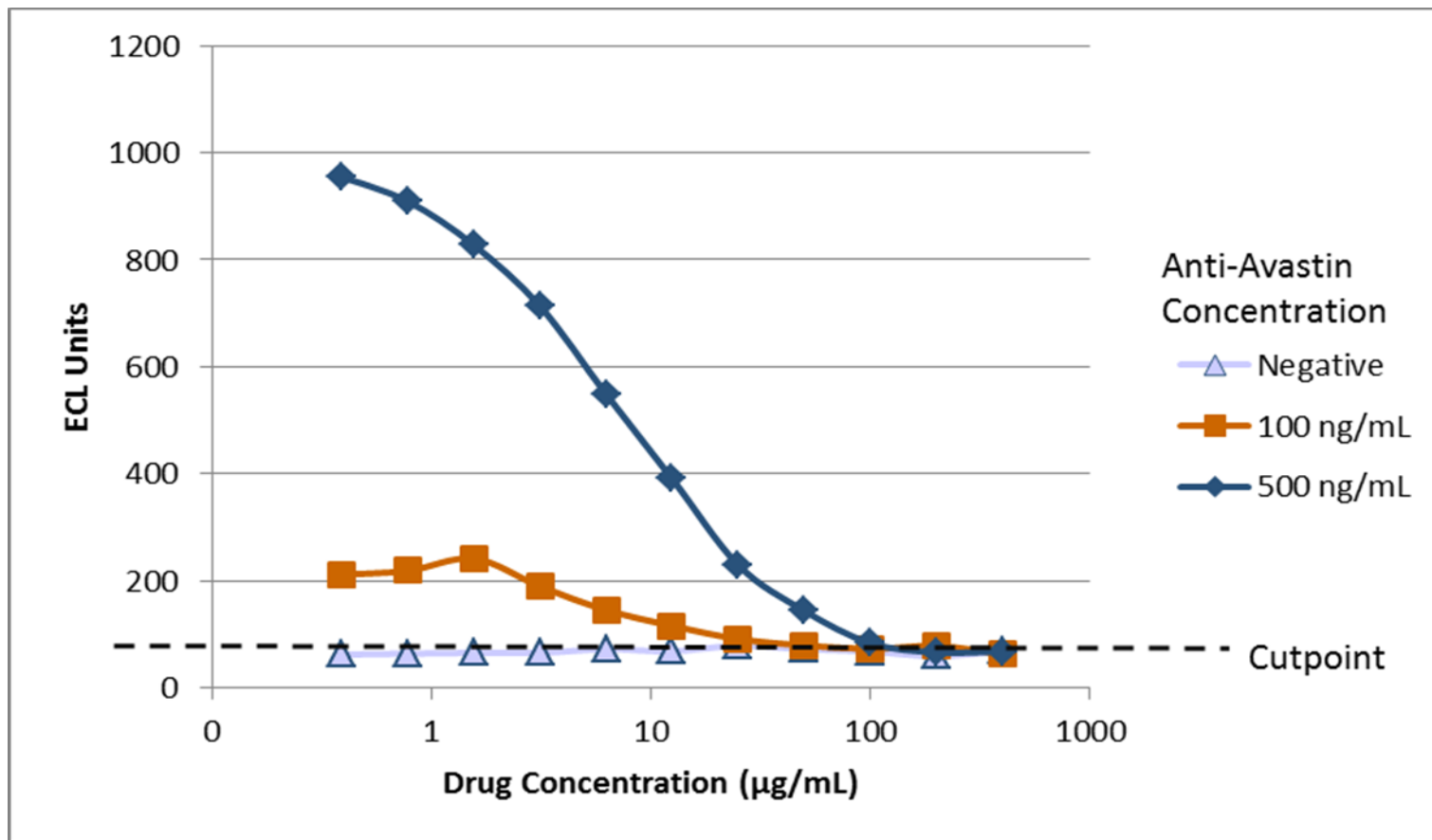
S/N at 500ng/mL ADA	Method		
[Drug] ($\mu\text{g/mL}$)	ACE	SPEAD	ECL
0	20.7	78.4	23.7
15	1.4	3.2	5.9
150	1	1.3	1.2





500 ng/mL of biotin- and ruthenium-labeled drug gave best combination of sensitivity and drug tolerance

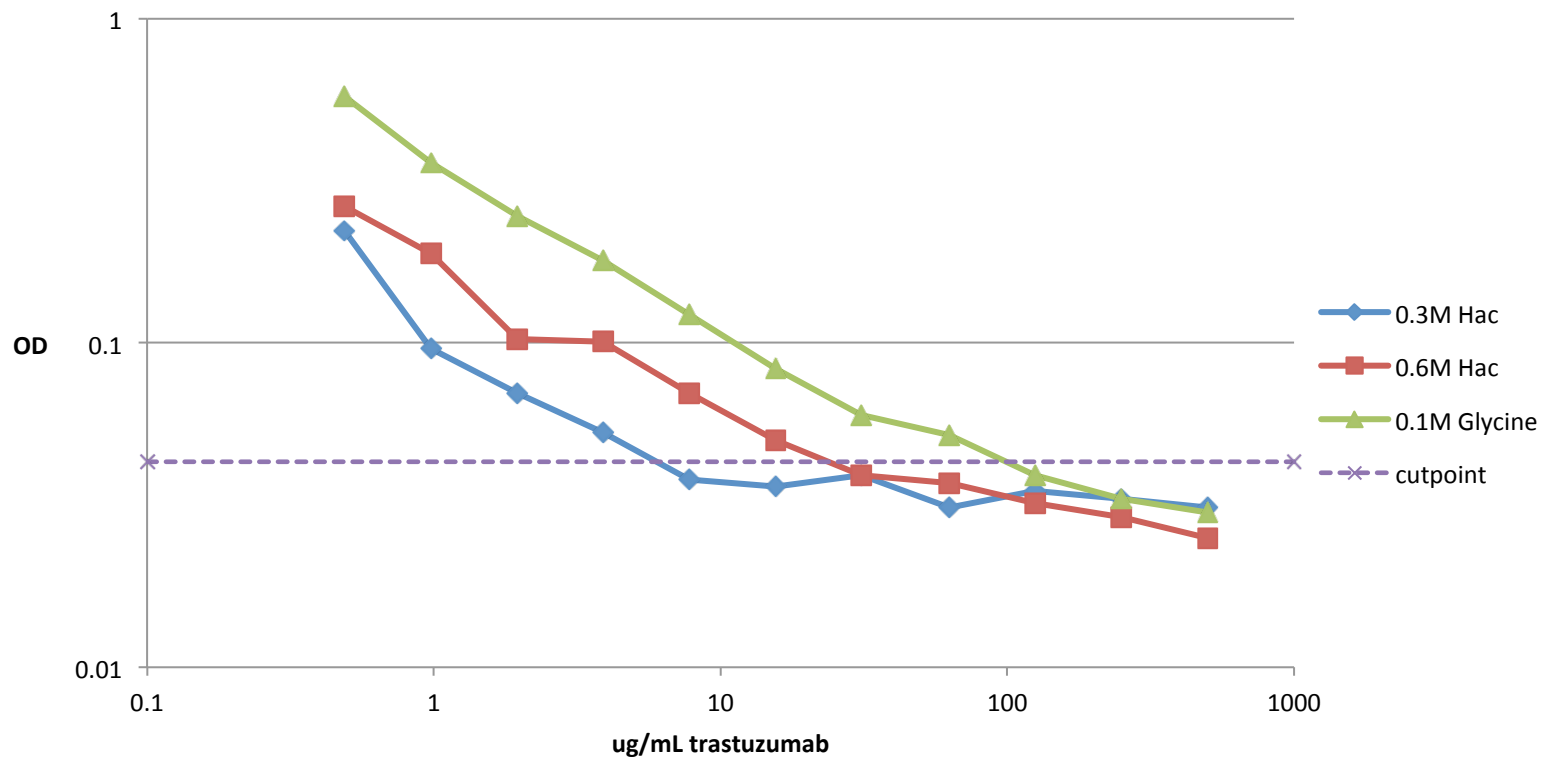
After establishment of cut point, drug tolerance determined to be 150 $\mu\text{g/mL}$ at 500 ng/mL ADA



- **Case Study:– Herceptin ADA - ACE**
 - **Format comparison**
 - **ACE selected**
 - **Optimisation of Acid conditions**
 - **Different concentrations and acids compared**

- **Acids Compared:**
 - **Acetic Acid (300 mM) → pH: 3.4**
 - **Acetic Acid (600 mM) → pH: 3.24**
 - **Glycine-HCl (100 mM) → pH: 3.02**

HERCEPTIN ADA ACE



	0.3M Acetic Acid	0.6M Acetic Acid	0.1M Glycine-HCl
Drug Tolerance	9 $\mu\text{g/mL}$	30 $\mu\text{g/mL}$	150 $\mu\text{g/mL}$

- **Summary**
 - **One size does not fit all**
 - **Optimisation for method and compound of interest essential**
 - **Method format:**
 - **Conventional Acid dissociation**
 - **ACE**
 - **SPEAD**
 - **pH, Concentration, Acid**