Session 4

From	To	Name	Organization	Title
1600	1630	Kyra Cowan	Merck KGaA	Clinical Immunogenicity: Case Studies in the Life Cycle of ADA Assays
1630	1650	Daniel Kramer	Sanofi	Risk Based Approach
1650	1710	Jonas Blaes	Abbvie	Regulatory requirements translated into day-to-day practice
1710	1730	Michael Partridge	Regeneron	Are we over reporting ADA?
1730	1800	All	Close out discussion and Q&A	

Clinical Immunogenicity

Case Studies in the Life Cycle of ADA Assays

Kyra Cowan

March 24, 2020

MERCK

0 Introduction

Key Points

What you can count on:

Change.

What you can’t count on:

What exactly those changes will be.

- Colleagues
- Institutional expertise, strategies
- External partnerships (Pharma to CRO and vice-versa)
- Health authority recommendations
- Methodologies

Story Summary

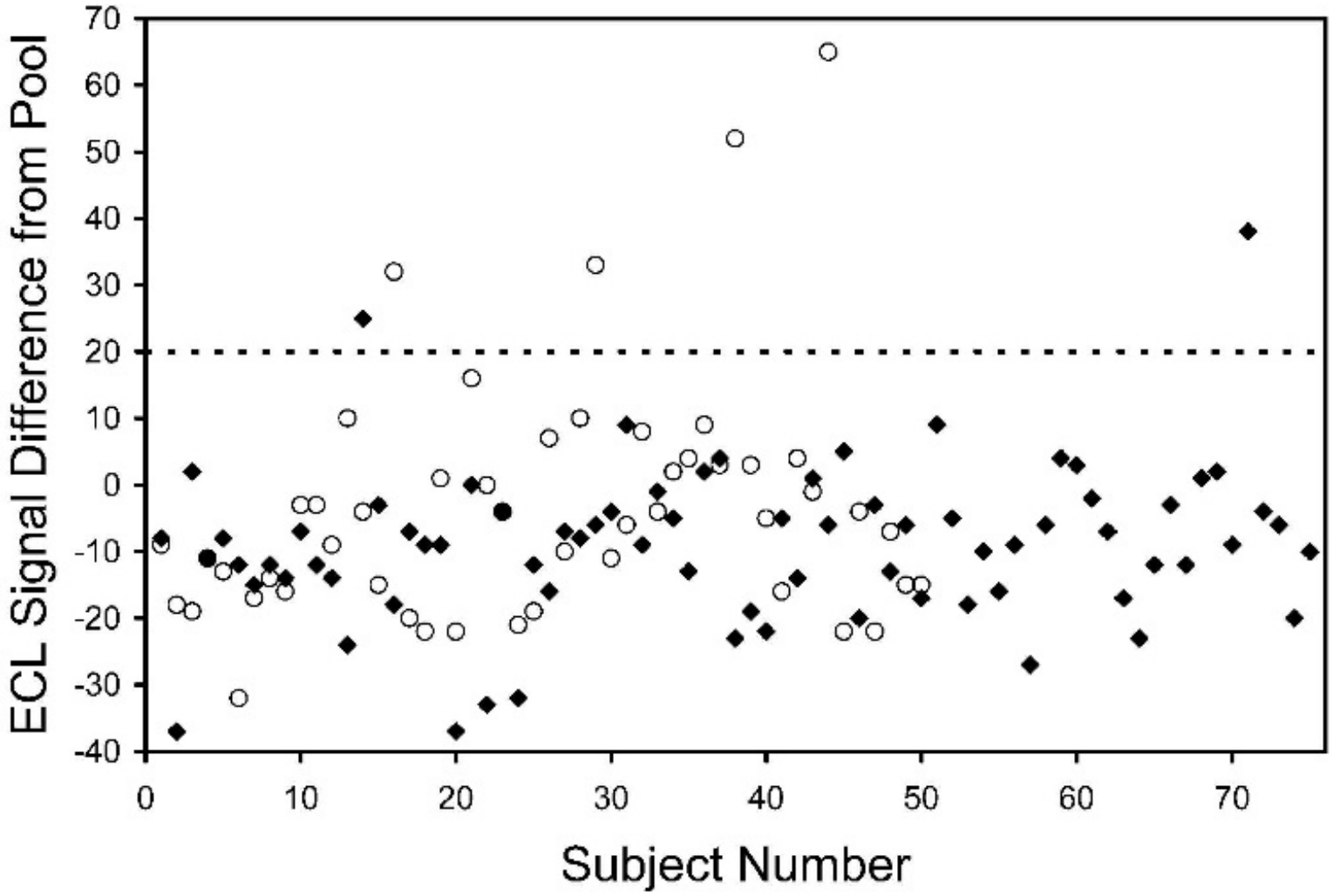
Changes can occur in ADA assays over time for long term, multi-site clinical trials:

- **Cut point**
  - Sample basis for establishment of cut point
  - In-study cut points for different disease states and studies in different populations
- **Change/addition of laboratories at CROs, or could be transferring in-house to CRO**
  - Additional Capacity
  - Lab Closures
  - China - samples need to be run in-country
- **Change of assay format**
  - Difficulties in validation of assay at second lab
  - Detection of different positives
  - New technologies

- **Take-home lessons**

1 Using Correct ADA Screening cut point factor

For example: Calculating a cut point



- Evaluate distribution of naïve samples
- Eliminate outliers
- Calculate a 95% confidence interval for a 5% false positive rate

Often performed for normal healthy (black diamonds) plus another, disease population (white circle) and if no meaningful difference, keep using normal healthy cut point factor for convenience

For the example on the left, the normal healthy sera were from the validation and the disease population was baseline samples from full clinical development of a rare indication.

Case Study 1 – Original Cut Point False Positive Rate <2%

Normal Human Serum cut point established-during initial validation and used for clinical trial sample analysis:

Trial	Samples	% Positive	% False Positive
1	299	0	0
2	9471	1.3	0.3

False Positive Rate (FPR) <2%

They used the wrong material - NHS

Case Study 1 –

Cut point re-established using study (disease specific) baseline samples

Trial	Samples	% Positive	% False Positive
1	301	2.0	7.4
2	9471	1.8	5.8

Lower cut point resulting in FPR between 2-11%

Result in overall percent positive subjects across 3 trials in ~2% increase in incidence of treatment-emergent immunogenicity.

Correct material from study – population serum

Case Study 2 – Original Cut Point False Positive Rate >11%

Commercial disease state sera used for initial validation cut point of ~1.06.

Several clinical studies had false positive rates significantly in excess of 11%

Case Study 2 – Study-Specific Cut Points

Cut points re-established using study baseline samples:

Trial	Samples	% Positive	% False Positive
1	3206	10.3	5.0
2	9471	10.2	6.9
3	661	13.8	8.8

Study specific cut point results in FPR between 2-11%

At least look at the study samples

Cutpoint Take Home

Assay validation with commercially available samples may not accurately emulate study samples.

Many factors can affect cut-point:

- Disease state
- Population/race
- Population/age

Test pre-dose samples from clinic.

Can assess FPR and adjust accordingly:

Viswanath Devanarayan, et. al., *Recommendations for Systematic Statistical Computation of Immunogenicity Cut Points*, AAPS Journal, Vol. 19:1487.

1. If false positive rate between 2% to 11% use pre-study validation SCP and CCP
2. If FPR is less than 2% or greater than 11%, new study-specific SCP and CCP should be determined using clinical study baseline samples.

2 Changing Anti-Drug Antibody Assay

Changes in ADA Assay

1. **Lab 1 – Original ADA assay**
    1. **Stepwise ECL Bridging**
2. **Lab 2 -**
    1. Concerns about Lab 1 capacity for phase 3 trial testing
    2. Attempt to transfer assay to Lab 2 – stepwise assay did not transfer well
    3. Modified homogeneous ECL bridging assay developed and validated
    4. Lab 1 announced shut down of lab

"Frank! Is it wise to change your horse mid-stream?"

Lab 1 Assay vs Lab 2 Assay Comparison

Parameter	Lab 1	Lab 2
Matrix	Serum	Serum
Analysis Method	ECL - Stepwise	ECL - Homogenous
Screening Cutpoint Factor	1.25	1.12
Confirmation Cutpoint	49.5%	22.5%
Sensitivity (ng/mL)	5.12	10.0
Drug Tolerance	250ng/ml PC can tolerate up to 125ug/ml	250 ng/mL PC can tolerate up to 100 µg/mL
MRD	1 (60)*	60
Titer	(2-fold) 1, 2, 4, 8, 16, 32, 64, 128	(3-fold) 60, 180, 540, 1620, 4860, 14580, 43740, 131220

*Before draft guidance – MRD should include all dilutions

(60): Lab used „1" for MRD but in reality it should have been 60 if done correctly

Case 1 - Data Comparison for Positive Samples Lab 1/2

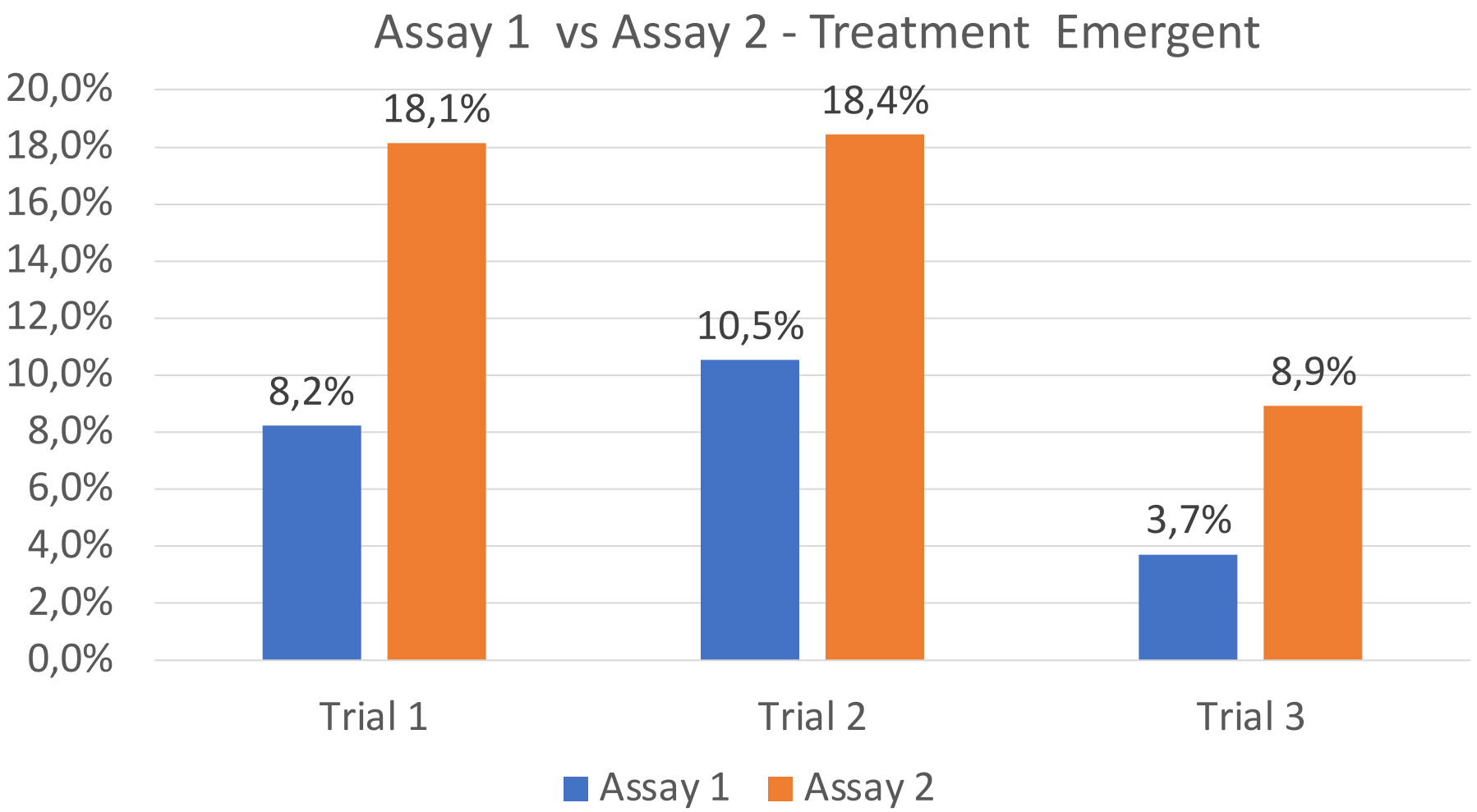
	Lab 1 - Stepwise			Lab 2 - Homogeneous		
Sample	SCREEN	CONFIRM	TITER	SCREEN	CONFIRM	TITER
1	Negative	n.a	n.a	Positive	Positive	180
2	Negative	n.a	n.a	Positive	Positive	180
3	Negative	n.a	n.a.	Positive	Positive	60
4	Positive	Negative	n.a.	Positive	Positive	180
5	Positive	Negative	n.a.	Positive	Positive	540
6	Positive	Negative	n.a.	Positive	Positive	540
7	Positive	Positive	2	Positive	Positive	180
8	Positive	Positive	4	Positive	Positive	540
9	Positive	Positive	8	Positive	Positive	1620
10	Positive	Positive	8	Positive	Positive	1620
11	Positive	Positive	8	Positive	Positive	43740
12	Positive	Positive	32	Positive	Positive	14580
False Positive	**3.0%**	**2.0%**	**1.0%**	**12.3%**	**4.0%**	**8.3%**

Ind. Samples – could come from the same pt

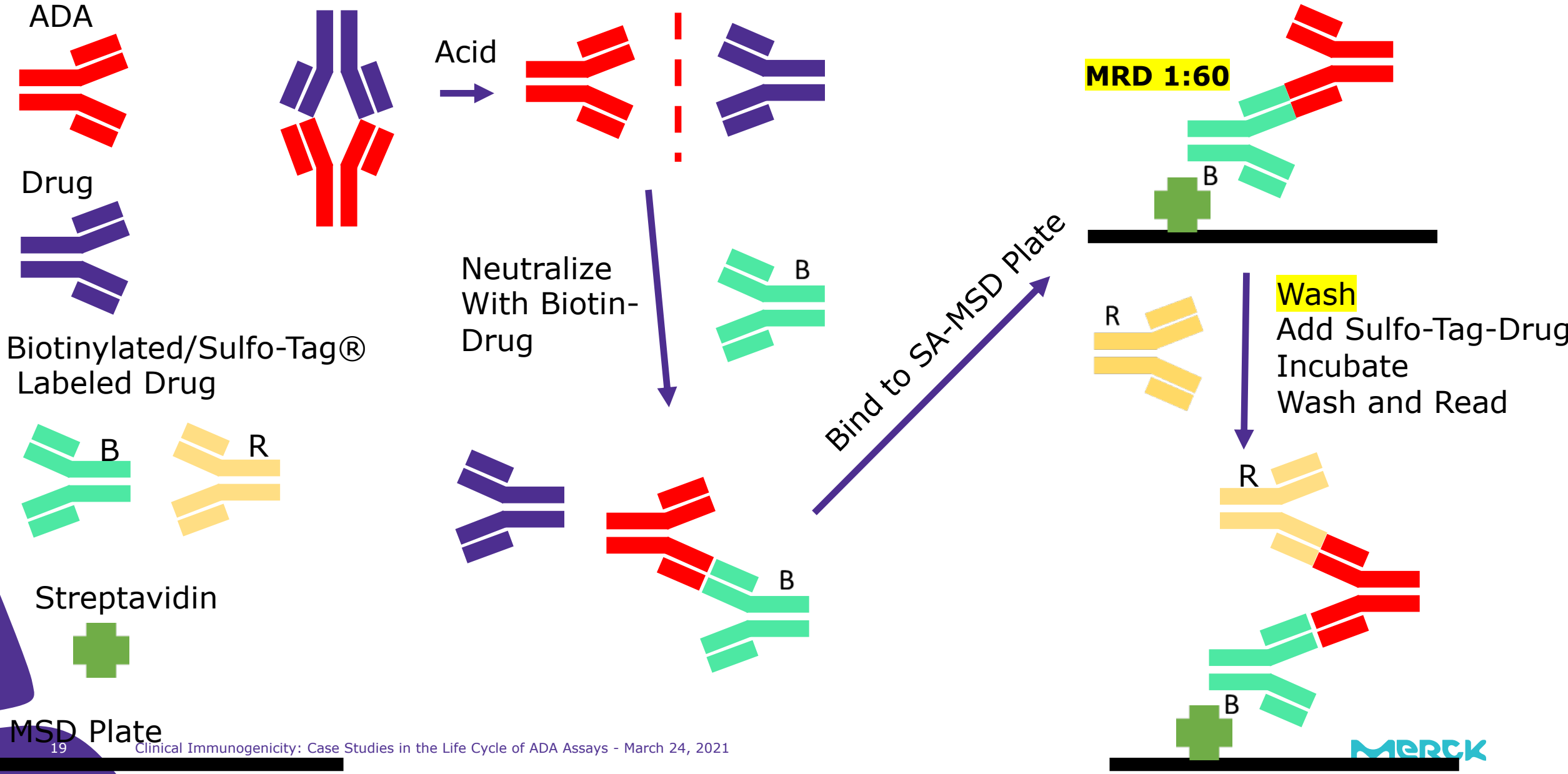
Case 2 - Data Comparison for Positive Samples Lab 1/2

	Lab 1 - Stepwise			Lab 2 - Homogeneous		
Sample	Screen	Confirm	Titer	Screen	Confirm	TITER
1	Negative	n.a	n.a	Positive	Positive	60
2	Negative	n.a	n.a	Positive	Positive	60
3	Negative	n.a	n.a	Positive	Positive	60
4	Negative	n.a	n.a	Positive	Positive	60
5	Negative	n.a	n.a	Positive	Positive	180
6	Negative	n.a	n.a	Positive	Positive	1620
7	Negative	n.a	n.a	Positive	Positive	1620
8	Positive	Negative	n.a	Positive	Positive	60
9	Positive	Negative	n.a	Positive	Positive	180
10	Positive	Negative	n.a	Positive	Positive	540
11	Positive	Positive	1	Positive	Positive	60
12	Positive	Positive	4	Positive	Positive	540
13	Positive	Positive	4	Positive	Positive	1620
14	Positive	Positive	16	Positive	Positive	1620
15	Positive	Positive	8	Positive	Positive	1620
16	Positive	Positive	8	Positive	Positive	1620
17	Positive	Positive	64	Positive	Positive	4860
18	Positive	Positive	8	Positive	Positive	4860
19	Positive	Positive	16	Positive	Positive	4860
20	Positive	Positive	4	Positive	Positive	14580
False Positive	6.2%	1.4%	4.8%	7.8%	2.8%	4.9%

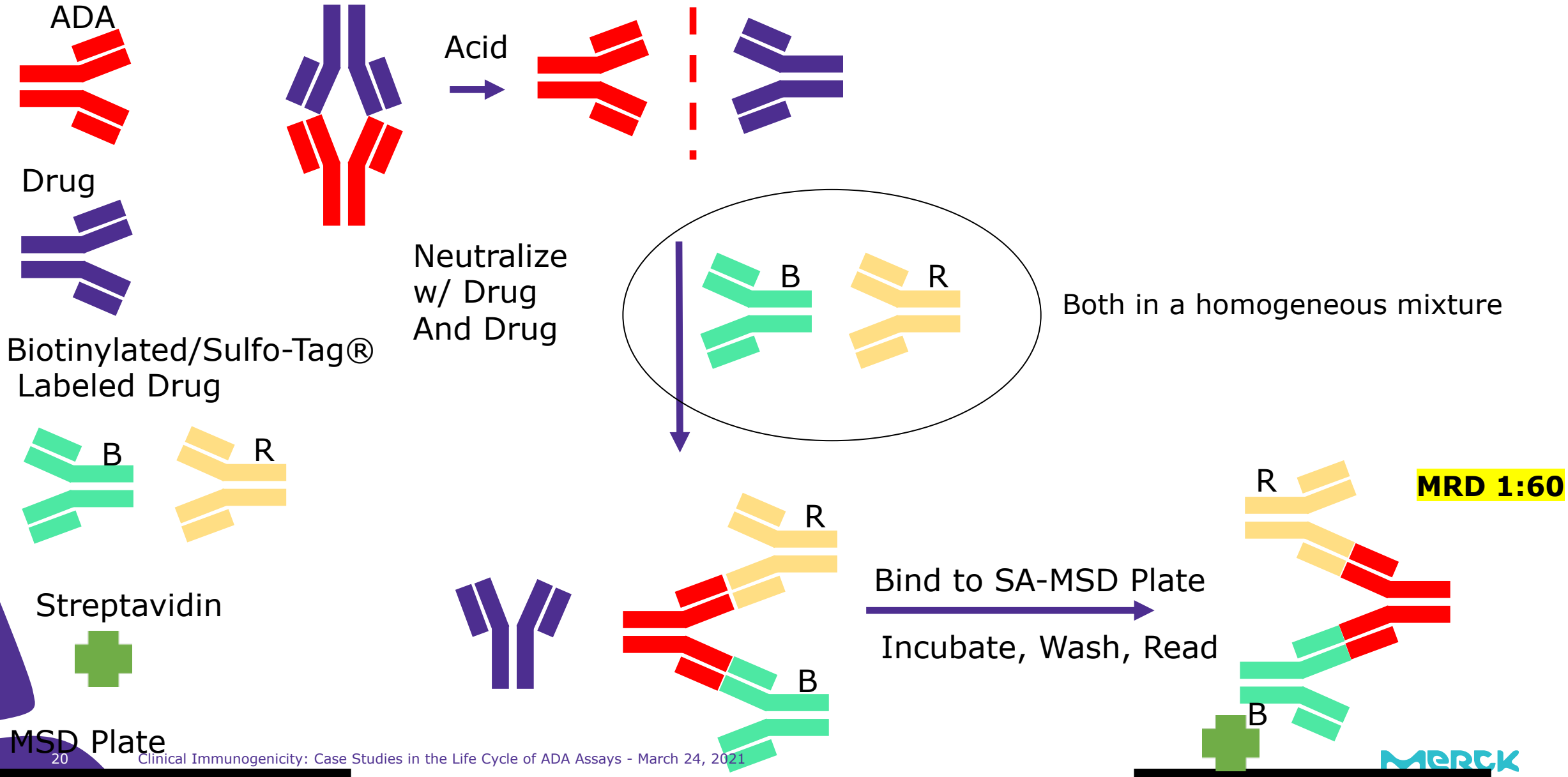
ADA Patient Positives Across Multiple Studies



ADA Lab 1 – Step-Wise Electrochemiluminescence Format



ADA Lab 2 – Homogeneous Electrochemiluminescence Format



Assay Format Changes

- Lab changes happen (for a variety of reasons) and are not necessarily avoidable
- Transferring assays between labs does not always go smoothly

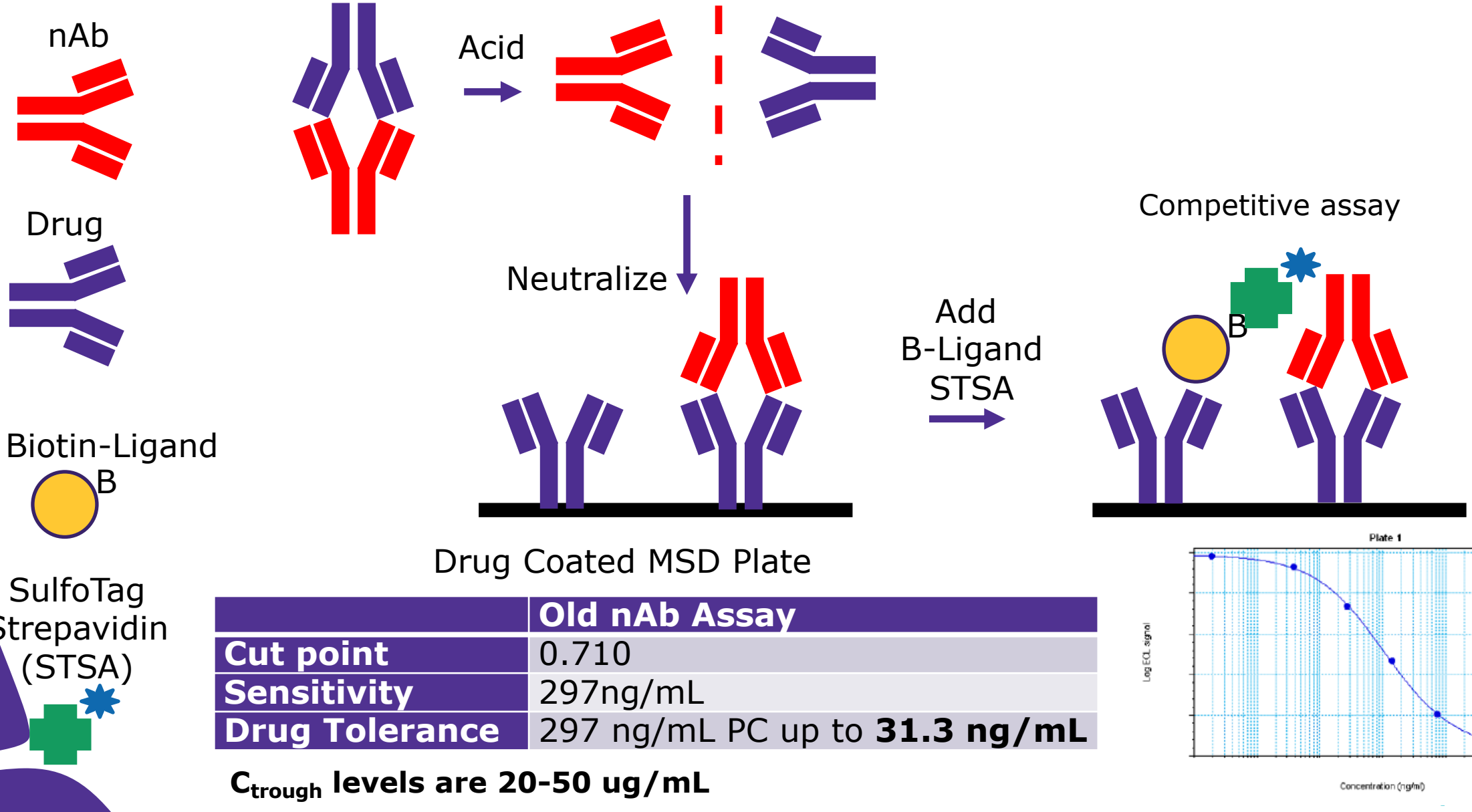
- To address this…
  - Clear communication in 2.7, integrated summary of immunogenicity (ISI)
  - Case-by-case basis with health authorities

3 Neutralizng Antibody Assay

Competitive Ligand Binding NAb assay

First generation neutralizing ADA assay had poor drug tolerance

First Generation Neutralizing Antibody Competitive Ligand Binding ECL Assay



	Old nAb Assay
Cut point	0.710
Sensitivity	297ng/mL
Drug Tolerance	297 ng/mL PC up to **31.3 ng/mL**

C_{trough} levels are 20-50 ug/mL

Concern over poor drug tolerance

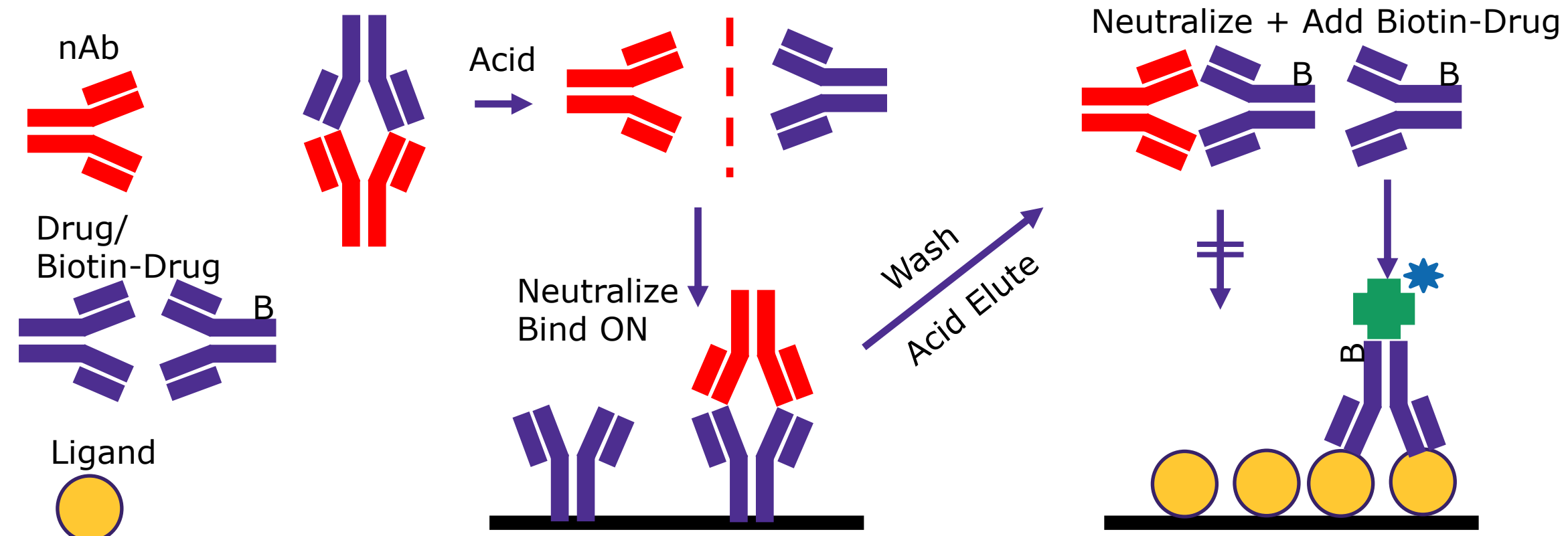
- By us, by HAs
- nAb antibodies detected multiple clinical trials at drug levels well in excess of drug tolerance of assay including at multiple c-trough time points
- the pAb PC in this assay may have underestimated the drug tolerance of treatment-emergent nAbs

nAb Assay History

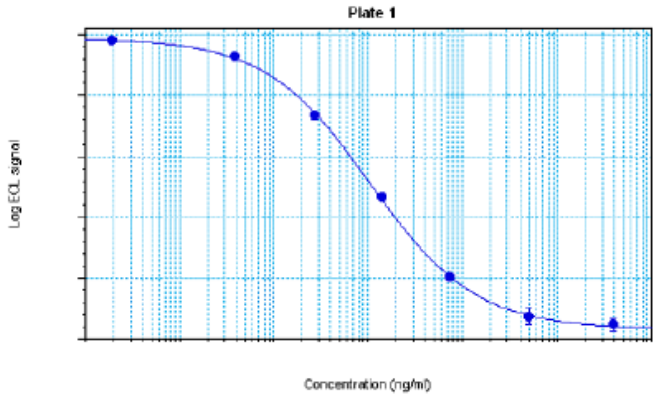
1. **Original NAb Assay**
   1. Poor drug tolerance
   2. Few positive samples

2. **New NAb Assay**
   1. Modified assay format
   2. New monoclonal PC
   3. More sensitive
   4. Better drug tolerance
   5. Significantly higher % positives

Second Generation Neutralizing Antibody Competitive Ligand Binding ECL Assay



	Old nAb Assay	New nAb Assay
Cut point	0.710	0.919
Sensitivity	297ng/mL	39.7ng/mL
Drug Tolerance	297 ng/mL PC up to **31.3 ng/mL**	39.7ng/mL PC at least **100ug/mL**



Case 1 - Comparison Old vs New Assay nAb Data
38 ADA Positive Samples

Old/New	Number	% ADA Pos Samples	GeoMean Titer
Neg/Neg	8	21.1%	69
Neg/Pos	**24**	**63.2%**	**1289**
Pos/Neg	**0**	**0.0%**	
Pos/Pos	6	15.8%	1946

Case 2 - Comparison Old vs New Assay nAb Data 200 ADA Positive Samples

Old/New	Number	% ADA Pos Samples	GeoMean Titer
Neg/Neg	65	32.5	177
Neg/Pos	**99**	**49.5**	**951**
Pos/Neg	**7**	**3.5**	**288**
Pos/Pos	29	14.5	3872

nAb Assay

New more drug tolerant assay detects significantly more NAb positive samples

What did we do to address this…

- Detecting more Nab positive samples, but no clinical sequelae correlated to this
- Case-by-case basis.

4 Take Home

Take Home Lessons

Changes in can occur in ADA assays over time for long term, multi-site clinical trials

Changes can occur for a lot of reasons:

New technologies, regulations, white papers, changing perceptions, changing labs…

So:

Think long-term as much as possible.

How does this help the clinician, the patient?

5 Thank you!

Kudos:
Jim Bourdage (B2S)