EUROPEAN BIOANALYSIS FORUM –
YOUNG SCIENTIST SYMPOSIUM

Evaluation of Multiple ADA Assay Formats for Achieving Highest Drug Tolerance

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Agenda

- Understanding the importance of drug tolerance testing in ADA assays
- Addressing the current challenges of immunoassays
- Comparison of multiple ADA methods to demonstrate best achievable drug tolerance vs assay sensitivity
  - Cost of reagents, assay time and complexity of analytical procedure
- Providing the method development team with a potential guide/solution in terms of drug tolerance discussions with clients during initial assay setup stages

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Considerations with immunogenicity testing

Overestimation and underestimation of ADA

Effect of acid and heat denaturation

Presence of free drug can result in decrease of assay sensitivity

Matrix interference

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Drug tolerance (DT)

What is it and why is it important?

- Drug tolerance of an assay is defined as the measure of the maximum concentration of drug that can be present in a sample and not prevent a positive sample from being detected
  - Assesses if an assay is adversely influenced by the presence of high levels of drug
- Increasingly important in the rise of biologics with longer half-lives as samples contain higher concentrations of drug at numerous time points
  - Within clinical dosing, the C(trough) is considered the minimum required DT level
  - Misinterpretation can have dangerous consequences
- FDA recommendation that antibodies should be evaluated down to 100 ng/mL for human samples and 250-500 ng/mL for nonclinical testing
  - For those that reach low DT, optimisation required to ensure false-negatives are not produced during screening

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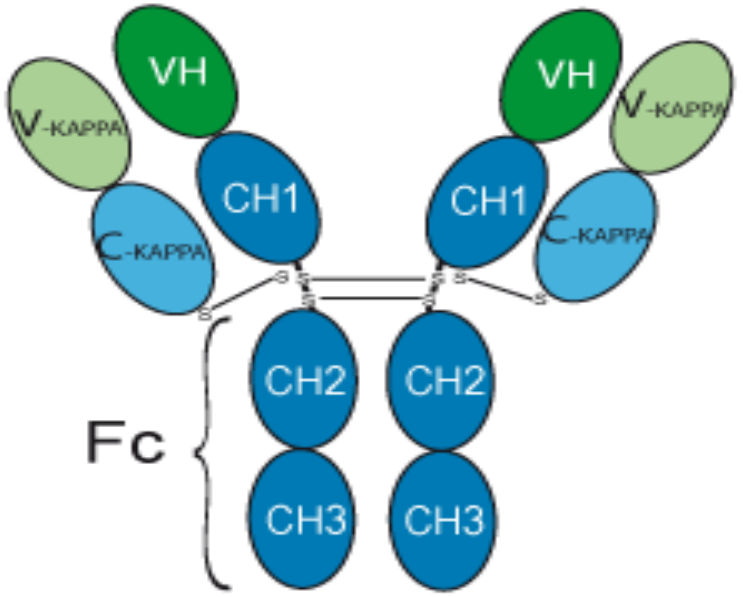
Trastuzumab – Herceptin®

- Humanised IgG1 kappa monoclonal antibody
- Targets and binds to the extracellular domain of the human epidermal growth factor (HER2)
- HER2 is responsible for the mediating of cell signalling, proliferation and differentiation
- Trastuzumab binds to HER2:
  - Inhibiting HER homo-dimerisation and cells undergo arrest in the G1 phase
  - Prevention of PI3K and MAPK pathways
  - Stimulates platelet-activating factors, resulting in antibody dependent cytotoxicity and leading to death of cells that express HER2
- Average C(trough) in clinical studies is 47 µg/mL



(*DrugBank, 2022*)

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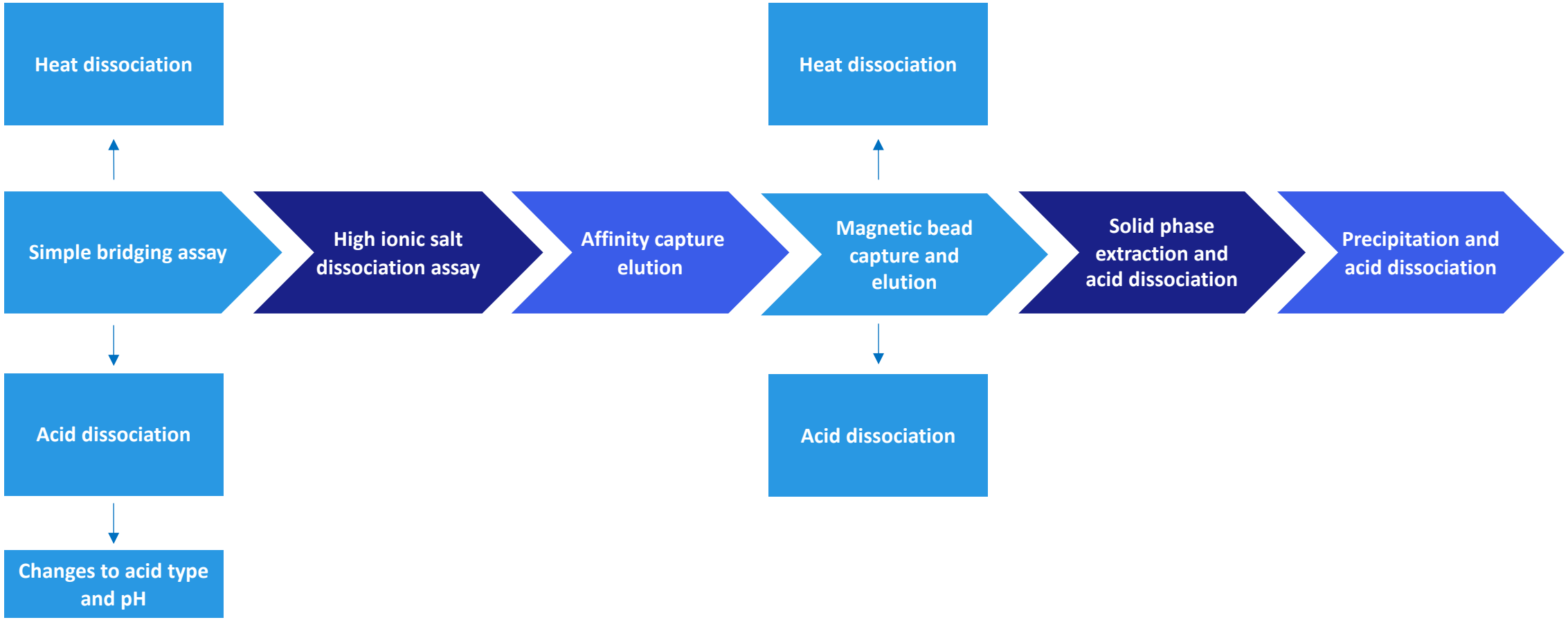
Method development process



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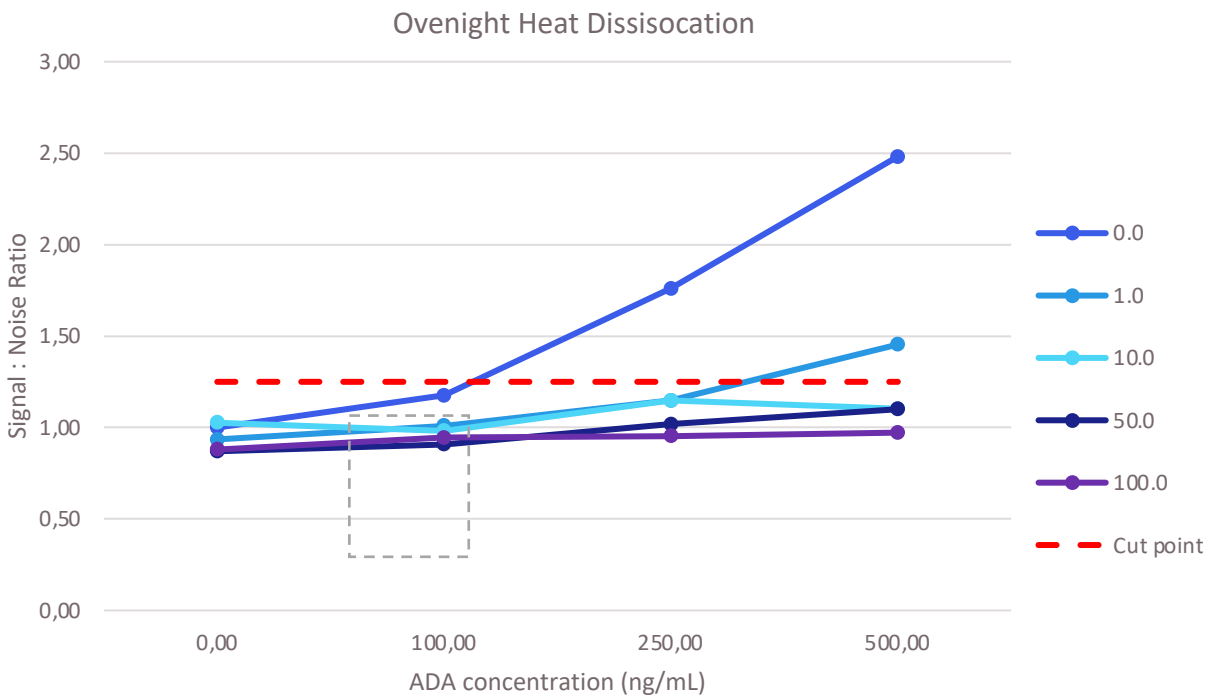
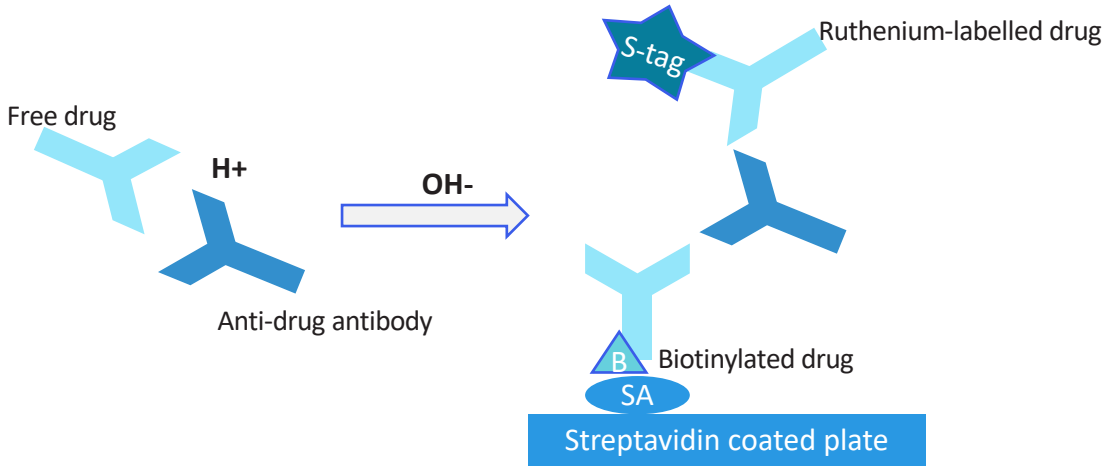
Heat dissociation





Independent of the incubation times, the achieved Drug tolerance was 0 µg/mL at the recommended sensitivity for clinical studies of 100 ng/mL.

Assay type		*Level of Trastuzumab in matrix (µg/mL)*				
		0	1	10	50	100
Positive control level (100 ng/mL)	Heat Dissociation (1 hour)	+	-	-	-	-
	Heat Dissociation (4 hours)	+	-	-	-	-
	Heat Dissociation (24 hours)	+	-	-	-	-

The highlighted boxes represent signals above the positive cut point.

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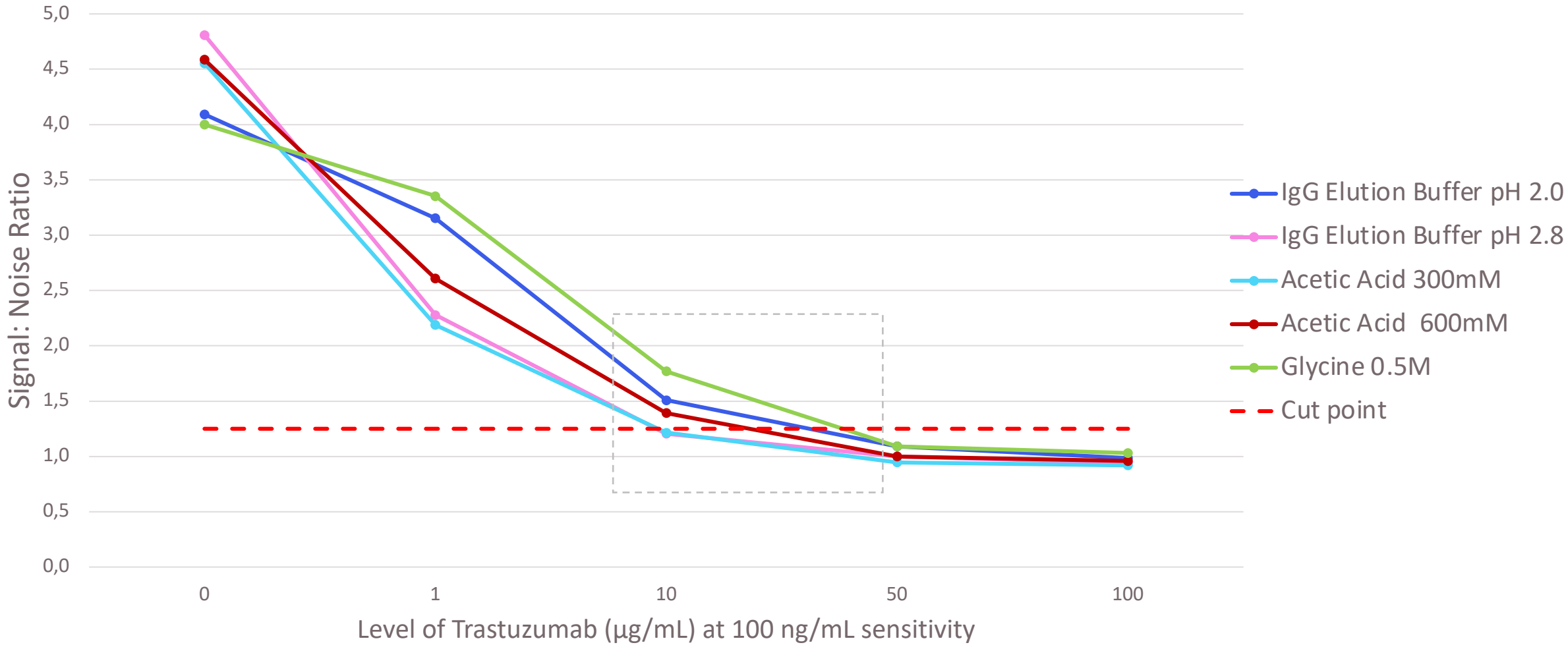
Acid dissociation

Evaluation of acid



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Acid dissociation

Level of Trastuzumab in Matrix (µg/mL)	*Signal : Noise Ratio at Positive Control Level (100 ng/mL)*				
	Elution buffer pH 2.0	Elution buffer pH 2.8	Acetic Acid 300M	Acetic Acid 600M	Glycine 0.5M
0.00	4.092	4.809	4.554	4.586	4.000
1.00	3.154	2.279	2.189	2.608	3.354
10.00	1.508	1.206	1.216	1.392	1.769
50.00	1.092	1.000	0.946	1.000	1.092
100.00	0.985	0.941	0.919	0.959	1.031

The highlighted boxes represent signals above the positive cut point.

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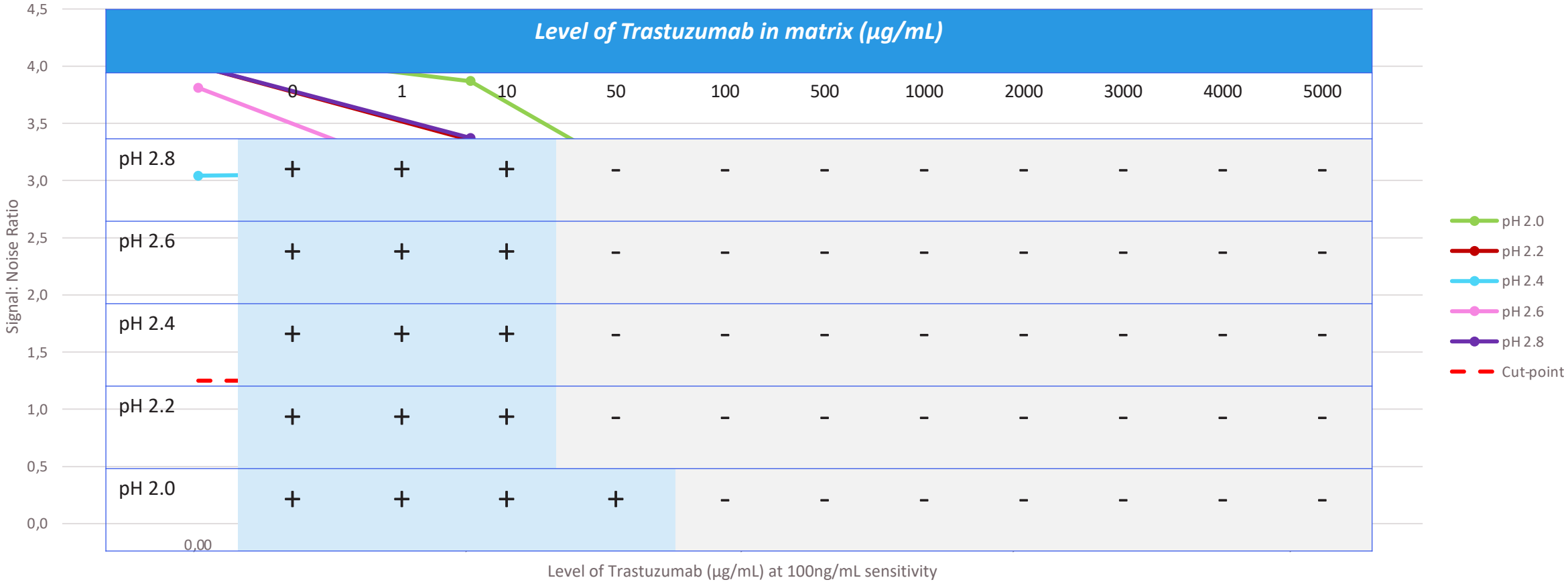
Acid dissociation

Evaluation into effect of pH change

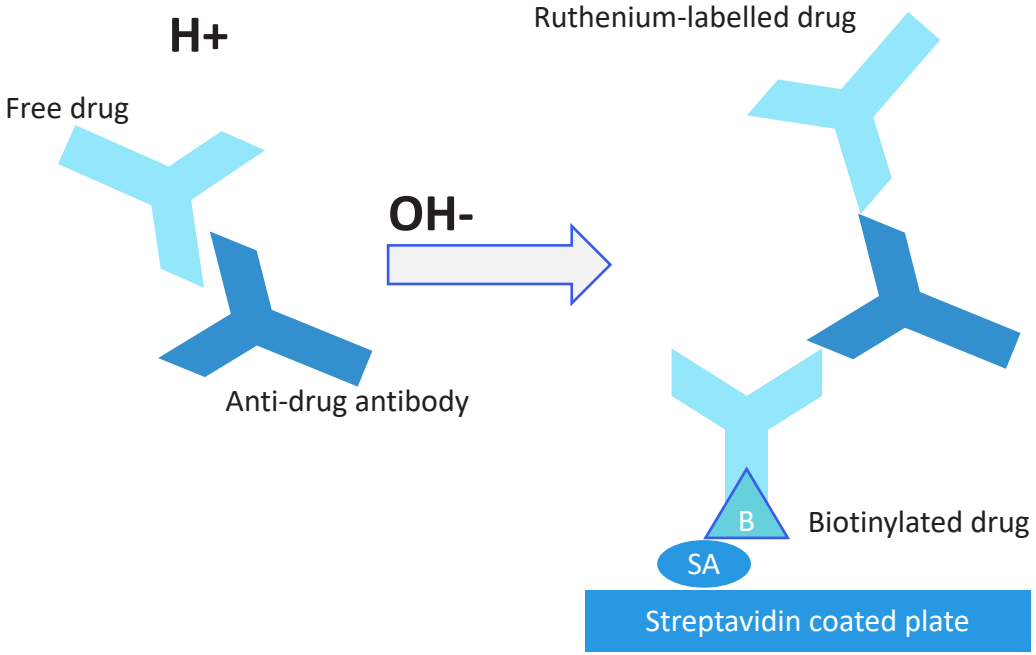


The highlighted boxes represent signals above the positive cut point.

HISDA High ionic salt dissociation assay

Level of Trastuzumab in Matrix (µg/mL)	*P/N Ratio at Positive Control Level (ng/mL)*		
	100.00	250.00	500.00
0.00	1.549	2.305	3.793
1.00	1.280	1.744	2.037
10.00	1.110	1.329	1.500
50.00	1.134	1.171	1.073

The highlighted boxes represent signals above the positive cut point.



Drug Tolerance
Not optimal reaching only 1 µg/mL at 100 ng/mL with 62°C heat

Assay Time & Complexity
Utilises the simple standard bridging assay with MgCl
Total procedure can take around 2 hours

Cost of Reagents
Assay only uses MgCl which is £62 for a 1kg so is inexpensive

Optimisation Rationale
Checkerboard approach with changes to dilutions of samples in MgCl and Mastermix

Future Optimisation Work
Trialling different temperatures for the dissociation of ADA as the initial incubation was not effectively dissociating

Increasing agitation speed

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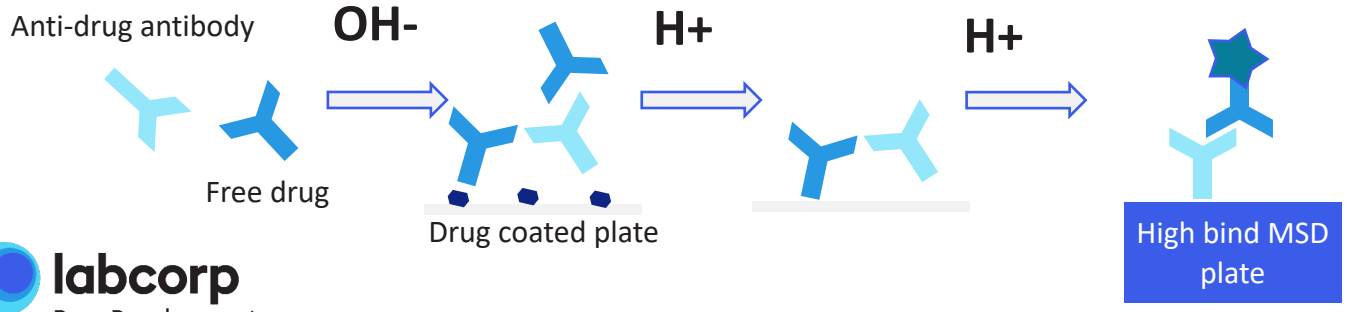
ACE Affinity capture elution

Level of Trastuzumab in Matrix (µg/mL)	*P/N Ratio at Positive Control Level (100 ng/mL)*		
	Format 1	Format 2	Format 3
0.00	8.000	6.476	1.947
1.00	1.604	2.679	1.213
10.00	1.743	1.155	1.067
50.00	1.109	0.810	1.000
100.00	0.782	0.774	0.973

The highlighted boxes represent signals above the positive cut point of 1.25.



Format 1: 2-day assay with overnight neutralisation on high bind MSD

Format 2: 1 day assay on a high bind MSD

Format 3: 2-day assay with master mix concentration on Strep MSD plate

Drug Tolerance
Format 1 achieved highest

Assay Time and Complexity
Dependent on format
For highest DT, 2 day assay required
More technically complex due to the use of two acid dissociation steps

Cost of Reagents
Difference within MSD plates with formats
High bind plates retail slightly more than Streptiviidin MSD plates
Format 3 has the additional use of Biotin

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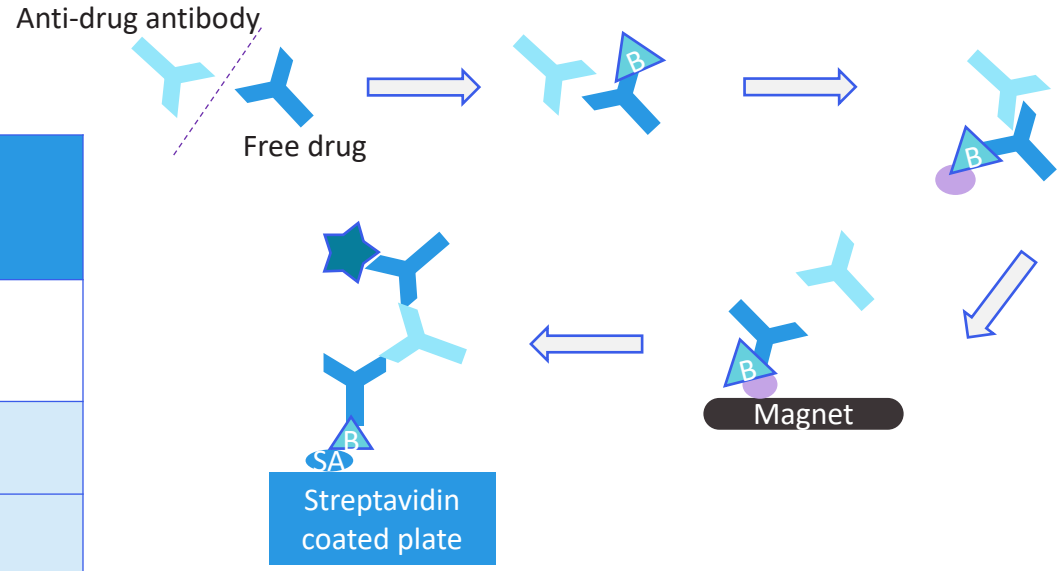
BEADs Biotin-extraction and acid dissociation

Level of Trastuzumab in Matrix (µg/mL)	*P/N Ratio at Positive Control Level (ng/mL)*		
	100	250	500
0.00	4.811	11.774	20.151
1.00	2.377	4.283	8.283
10.00	1.604	2.151	3.717
50.00	1.113	1.528	1.811
100.00	1.075	1.302	1.547
500.00	1.038	1.000	1.113
1000.00	0.943	1.038	1.094

The highlighted boxes represent signals above the positive cut point.



Drug Tolerance

The recommended sensitivity by the FDA is 100 ng/mL and at that level, the achieved DT was 10 µg/mL

Assay Time and Complexity

1 day assay

Requires training as it is a complex assay that requires use of magnetic wash programmes

Cost of Reagents

High

Use of NanoLink Streptavidin Magnetic Beads which retail £2400 for 10mL

Around £100 for one plate

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BEHDs Biotin-extraction and heat dissociation

Level of Trastuzumab in Matrix (µg/mL)	*P/N Ratio at Positive Control Level (ng/mL)*		
	100	250	500
0.00	7.361	14.213	35.738
1.00	2.344	4.066	7.689
10.00	1.672	3.016	4.475
50.00	1.295	1.738	3.210
100.00	1.180	1.656	2.279
500.00	0.984	1.016	1.295
1000.00	0.934	1.000	1.016

The highlighted boxes represent signals above the positive cut point.

Drug tolerance

DT reached 50µg/mL at the required 100 ng/mL Above the C(trough) level, which is comparable to Glycine at pH 2.0

Assay Time and Complexity

1 day assay

Requires 62°C heat dissociation step as an alternate for acid for the dissociation of ADA: drug complexes

Cost of Reagents

High

Use of NanoLink Streptavidin Magnetic Beads

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SPEAD Solid phase extraction and acid dissociation

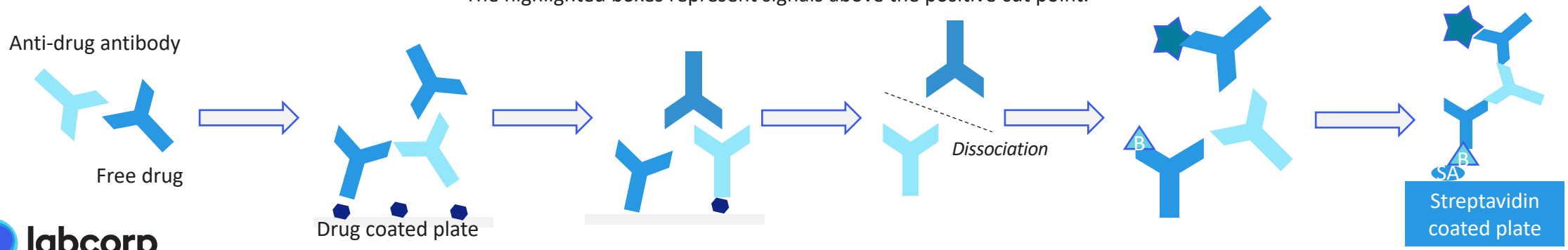
Level of Trastuzumab in Matrix (µg/mL)	P/N Ratio at Positive Control Level (ng/mL)		
	100	250	500
0.00	1.317	3.756	2.817
1.00	1.390	2.659	4.732
10.00	1.598	3.622	4.890
50.00	1.841	3.122	3.317
100.00	1.427	2.085	3.524
500.00	1.146	1.220	1.768
1000.00	1.061	1.244	1.598
2000.00	0.976	1.110	1.256

The highlighted boxes represent signals above the positive cut point.

Drug Tolerance
Achieving 100 µg/mL at 100 ng/mL
Above C(trough) level for Herceptin

Assay Time and Complexity
Similar design to ACE with 2 day assay and overnight neutralisation

Cost of Reagents
Low due to use of standard reagents



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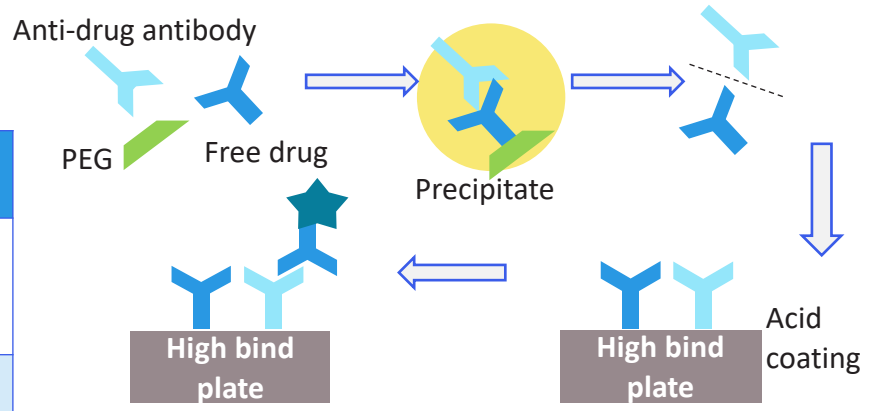
PANDA Precipitation and Acid Dissociation

Level of Trastuzumab in Matrix (µg/mL)	P/N Ratio at Positive Control Level (ng/mL)			
	0	100	250	500
0.00	1.000	1.364	2.333	4.515
1.00	1.015	1.455	2.288	3.773
10.00	0.970	1.409	2.061	3.076
50.00	0.955	1.379	2.091	3.403
100.00	1.045	1.591	2.333	3.621
500.00	0.970	1.652	2.682	3.697
1000.00	1.030	1.682	2.606	4.303
2000.00	0.985	1.788	2.652	4.348
3000.00	0.985	1.742	3.030	5.242
4000.00	0.985	2.015	2.985	5.909
5000.00	0.985	2.061	3.448	5.955

The highlighted boxes represent signals above the positive cut point.

This assay was able to produce the highest DT level of 5 mg/mL



Assay time

Assay is split over two days with an overnight incubation with PEG

Cost of Reagents

Polyethylene glycol is quoted at £105 per kg
- Around £1 for PEG buffers

Assay requires use of excess drug during coating of plate so higher cost of drug

Assay Complexity

Requires multiple optimisations

Concentrations of PEG have to be optimal for drug to ensure both sensitivity and specificity are balanced

Not a one size fits all method

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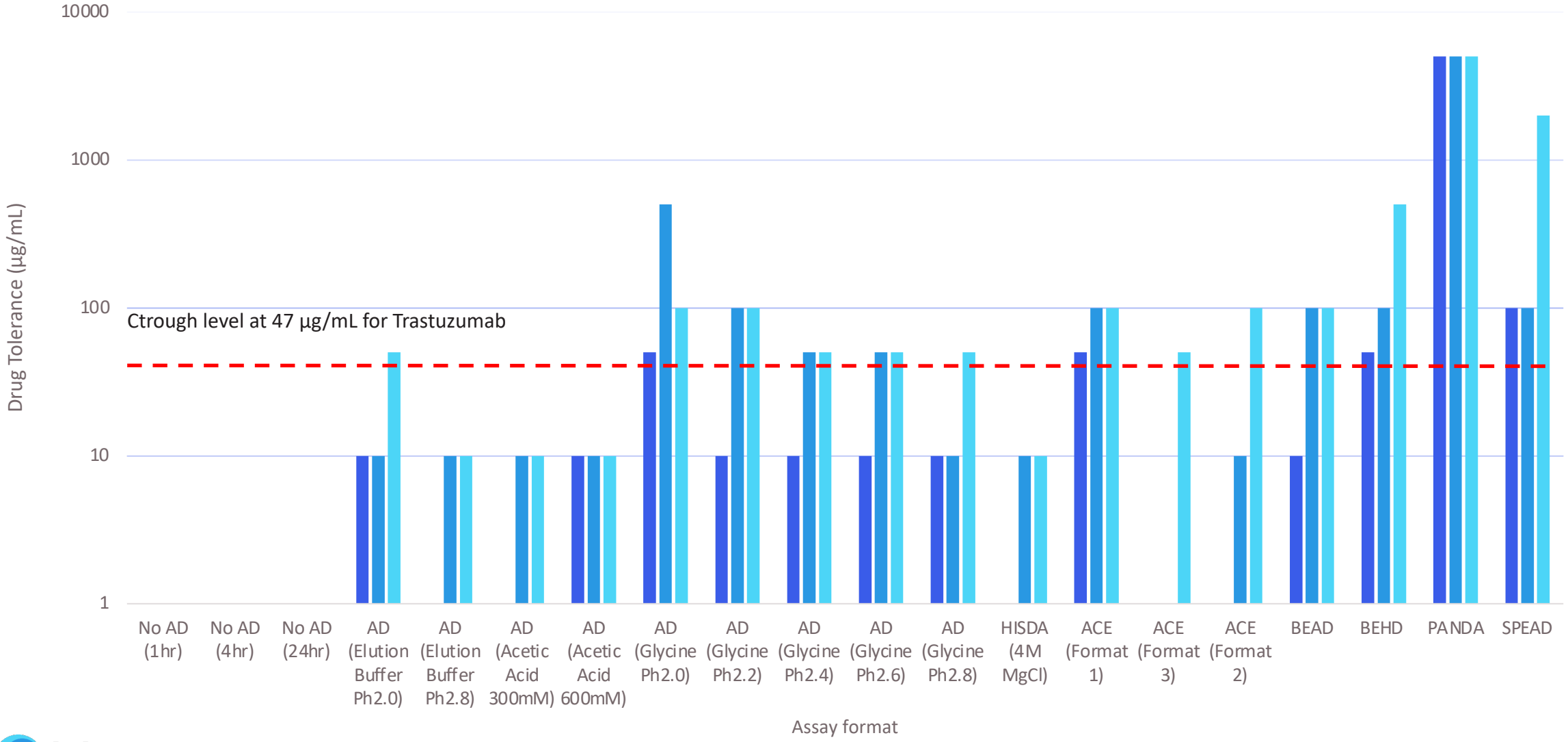
Format comparison



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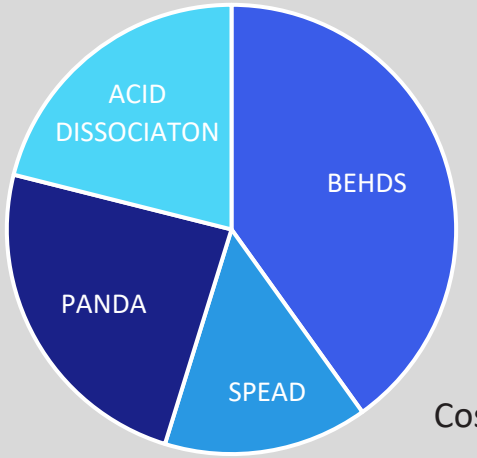
Conclusions

Methods that achieved above the clinical C(trough) level at 100 ng/mL sensitivity

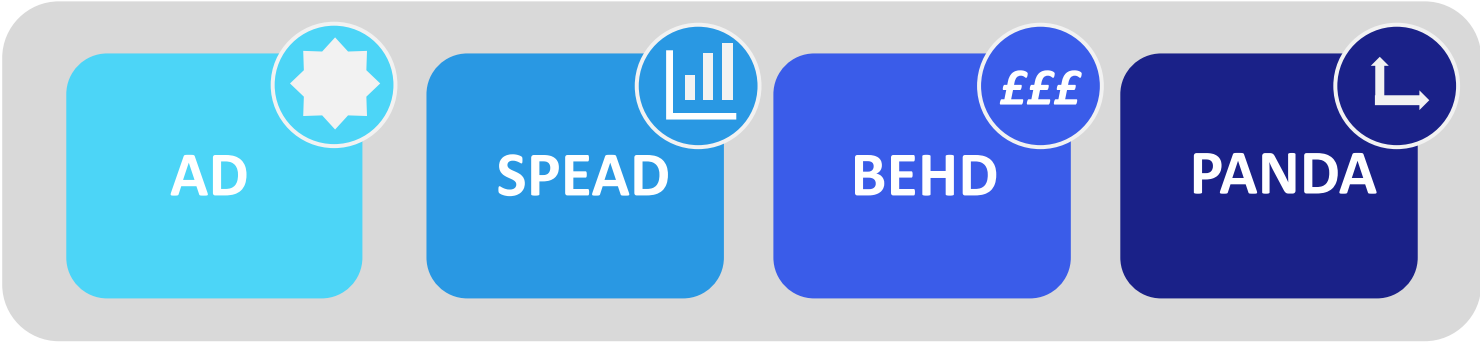
- Acid dissociation with glycine (pH2.0)
- Biotin-extraction and heat dissociation
- Precipitation and acid dissociation
- Solid-phase extraction and acid dissociation

Need for a simple, robust and not overly complex method to allow for high through-put when scaling up



Costing

Additional research with other monoclonal antibodies would aid in the development of a guide that can be used for initial assay development



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Thank you for listening!

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