EBF YOUNG SCIENTIST SYMPOSIUM 2022

Development of an Immunogenicity Method Using Volumetric Absorptive Microsampling (VAMS® Technology), Whole Human Blood and the Meso Scale Discovery® (MSD®) platform

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neoteryx™ The promise of microsampling, delivered

Session description and objectives

This session will focus on ADA method development using the Mitra® microsampling devices with VAMS® technology for sample collection.

By the end of the session we will have covered:

- The ADA bridging assay developed
- The development assessments performed
- Results obtained so far
- What are our next steps?

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Background

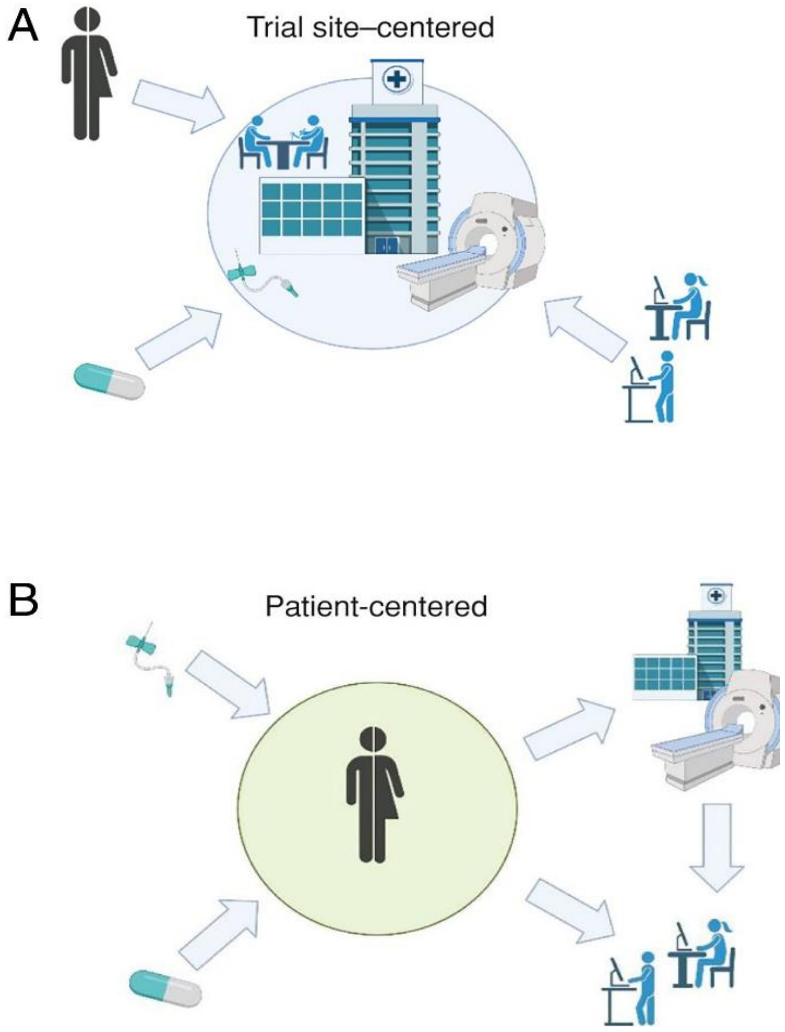
Venipuncture → Microsampling

Preclinical

- Supports 3Rs commitments (replacement, reduction, refinement)
- Lower-volume draws, less animals, increases data reliability

Clinical

- Improved patient access→ Decentralized clinical trials
- Real-time therapeutic drug monitoring
- Facilitates long-term follow-ups
- Less invasive → Patient comfort
- Overall reduced cost



Banks, 2021

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Why Mitra® Devices with VAMS® Technology?

- Fixed volumes: 10, 20 or 30 µL
- Different collection formats
- User-friendly → less invasive/more convenient
- Classed as CE-IVD devices
- No need for temperature controlled transport

Possible disadvantages

- Haematocrit effect unclear
- Number of repeats
- Impact of anti-coagulant presence in lab-generated samples
- Low blood volumes can be hard to elute

Mitra® devices
(Neoteryx, 2022)

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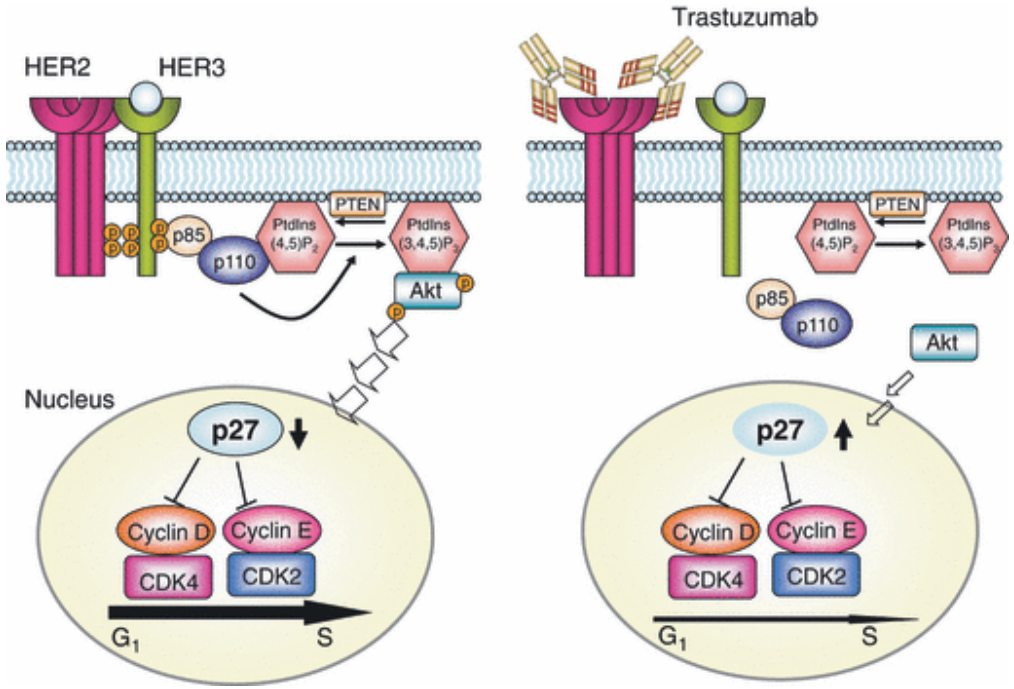
Why Trastuzumab?

- Humanised monoclonal IgG1κ
- Binds to the extracellular domain of HER2*
- Approved in 1998 → Well-known and studied
- Suppresses cancer cells growth, proliferation and survival
- Indications:
  - HER2+ breast cancer
  - HER2+ metastatic gastric/gastroesophageal junction adenocarcinoma



Mukohara, 2010

*HER2: **H**uman **E**pidermal growth factor **R**eceptor protein

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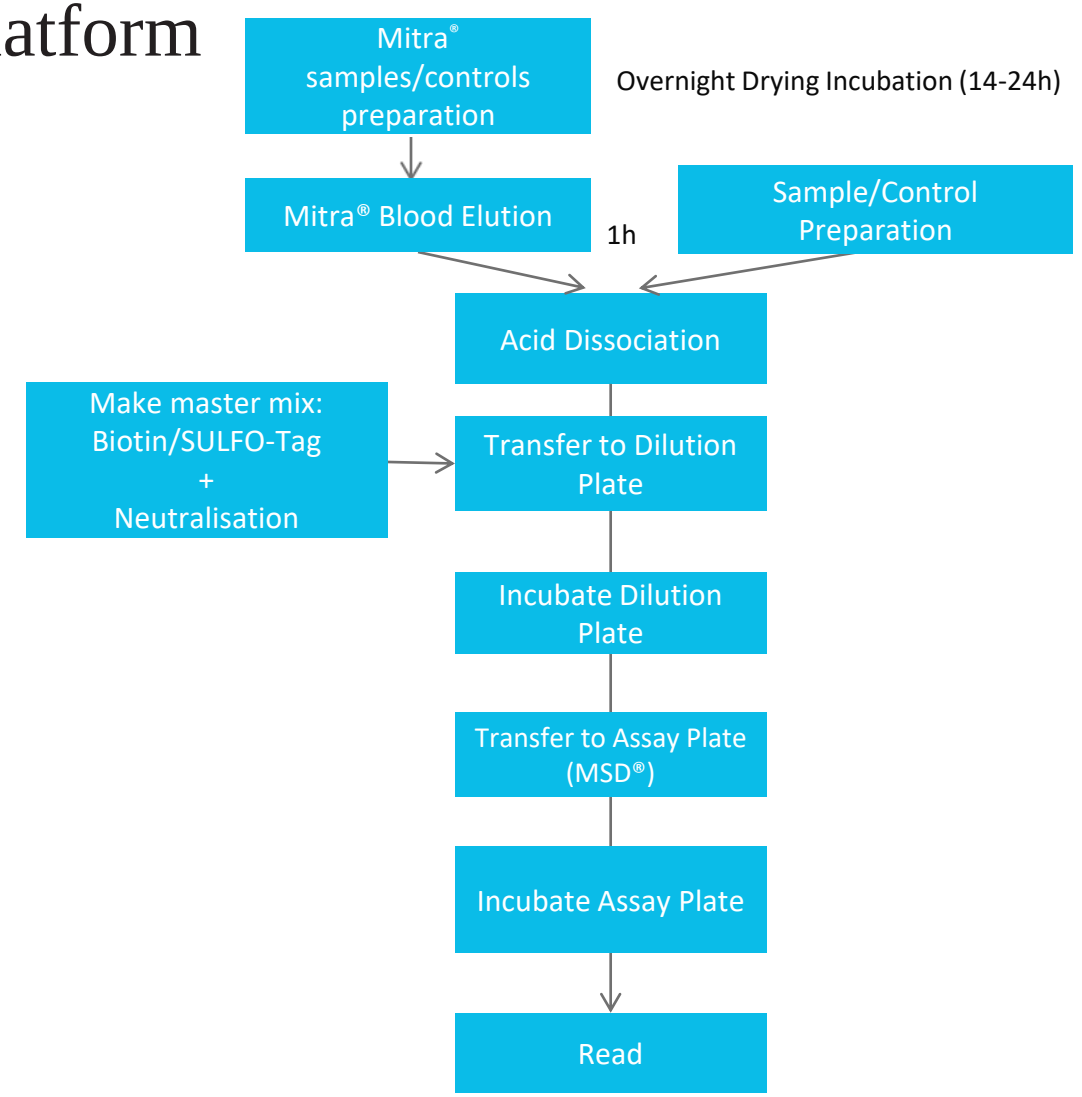
The ADA Bridging Assay on the MSD® Platform

Advantages

- Bivalent binding capability – high specificity, sensitivity, low background
- Often better drug tolerance as increases sensitivity
- MSD®: faster, lower volumes and better sensitivity

Risks

- IgG4 may not be detected, due to *in vivo* exchange of half molecules
- Conformation changes
- Epitope masking during labelling



MRD: 1 in 20 (1 in 5 in LowCross Buffer, 1 in 2 Acid Dissociation and 1:1 neutralisation)

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Development Assessments

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Sample Extraction Techniques

- Samples loaded into 96-well dilution plate
- Tips take up sample and placed into polypropylene tube for drying
- Tips dried for 14-24 hours (overnight) at +25°C
- Elution buffer added to tubes holding dried tips for 60 minutes with agitation (450 rpm) at +25°C
- Multiple elution buffers evaluated
  - EB1: LowCross Buffer (LCB)
  - EB2: 1x PBS
  - EB3: 1x PBS with 0.1% Tween-20 (PBST1)
  - EB4: 1x PBS with 0.05% Tween-20 (PBST2)
  - EB5: 1x PBS with 0.05% Tween-20, NaN3 (BSC Class I)

Mitra® Clamshell
(Neoteryx, 2022)

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Feasibility

Duplicate	NC (0 ng/mL)			LPC (50 ng/mL)			HPC (25,000 ng/mL)			PSCP
	Observed Response (ECL)	S/N Ratio	Pos/Neg	Observed Response (ECL)	S/N Ratio	Pos/Neg	Observed Response (ECL)	S/N Ratio	Pos/Neg	
1	88	1.0	Negative	134	1.6	Positive	26,026	304.1	Positive	107
2	86	1.0	Negative	136	1.6	Positive	28,123	328.6	Positive	
3	85	1.0	Negative	134	1.6	Positive	27,682	323.5	Positive	
4	83	1.0	Negative	134	1.6	Positive	30,861	360.6	Positive	
5	88	1.0	Negative	128	1.5	Positive	27,886	325.8	Positive	
6	85	1.0	Negative	136	1.6	Positive	27,346	319.5	Positive	

HUMAN SERUM

MITRA® BLOOD

Condition	NC (0 ng/mL)			LPC (50 ng/mL)			HPC (25,000 ng/mL)			PSCP
	Observed Response (ECL)	S/N Ratio	Screen Pos/Neg	Observed Response (ECL)	S/N Ratio	Screen Pos/Neg	Observed Response (ECL)	S/N Ratio	Screen Pos/Neg	
Elution Buffer 1	105	1.0	Negative	127	1.2	Negative	19,764	188.2	Positive	131
Elution Buffer 2	88	1.0	Negative	117	1.3	Positive	19,952	228.0	Positive	109
Elution Buffer 3	91	1.0	Negative	117	1.3	Positive	16,340	180.6	Positive	113
Elution Buffer 4	90	1.0	Negative	118	1.3	Positive	19,090	213.3	Positive	112
Elution Buffer 5	90	1.0	Negative	121	1.4	Positive	17,517	195.7	Positive	112

NC: Negative Control

LPC: Low Positive Control

HPC: High Positive Control

PSCP: Plate-Specific Cut Point

S/N: Signal/Noise

ECL: Electrochemiluminescence Units

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Sensitivity

- Positive controls spiked in blood at different levels and loaded on Mitra® tips. After overnight storage, samples eluted with different buffers

Anti-Trastuzumab Antibody Diluted Concentration (ng/mL)	Blood + LCB		Blood + PBS		Blood + PBST2	
	S/N Ratio	Pos/Neg	S/N Ratio	Pos/Neg	S/N Ratio	Pos/Neg
1,024,000	744.3	Positive	1,073.1	Positive	1,207.3	Positive
51,200	598.3	Positive	420.6	Positive	374.6	Positive
25,600	151.7	Positive	157.2	Positive	148.1	Positive
12,800	122.9	Positive	70.7	Positive	71.0	Positive
6,400	60.8	Positive	34.5	Positive	31.7	Positive
3,200	26.7	Positive	17.8	Positive	18.7	Positive
1,600	14.3	Positive	8.4	Positive	9.0	Positive
800	8.0	Positive	4.6	Positive	5.1	Positive
400	3.9	Positive	2.7	Positive	3.1	Positive
200	2.5	Positive	1.8	Positive	2.0	Positive
100	1.8	Positive	1.3	Positive	1.4	Positive
50	1.4	Positive	1.0	Negative	1.1	Negative
25	1.1	Negative	0.9	Negative	0.9	Negative
12.5	1.1	Negative	0.9	Negative	0.9	Negative
6.25	1.1	Negative	0.9	Negative	0.9	Negative

Target sensitivity for clinical ADA assays

- Target sensitivity achieved

For reference: Sensitivity obtained in human serum: 50 ng/mL

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Selectivity

- 10 individuals, healthy matrix, analysed blank and spiked to LPC and HPC

Individual	Blank				LPC (100 ng/mL)				HPC (25,000 ng/mL)			
	Screening		**Confirmation**		**Screening**		**Confirmation**		**Screening**		**Confirmation**	
	Observed Response (ECL)	Pos/Neg	Observed Response (ECL)	Inhibition (%)	Observed Response (ECL)	Pos/Neg	Observed Response (ECL)	Inhibition (%)	Observed Response (ECL)	Pos/Neg	Observed Response (ECL)	Inhibition (%)
1	65	Negative	64	1.5	162	Positive	64	60.5	23,323	Positive	82	99.6
2	68	Negative	66	2.9	130	Positive	65	50.0	22,954	Positive	76	99.7
3	66	Negative	65	1.5	141	Positive	70	50.4	19,854	Positive	72	99.6
4	71	Negative	69	2.8	130	Positive	73	43.8	24,651	Positive	72	99.7
5	69	Negative	64	7.2	128	Positive	67	47.7	27,352	Positive	72	99.7
6	73	Negative	63	13.7	127	Positive	66	48.0	23,557	Positive	72	99.7
7	72	Negative	61	15.3	100	Positive	65	35.0	27,713	Positive	79	99.7
8	64	Negative	68	-6.3	138	Positive	67	51.4	27,025	Positive	72	99.7
9	65	Negative	69	-6.2	126	Positive	67	46.8	12,917	Positive	76	99.4
10	69	Negative	63	8.7	133	Positive	62	53.4	12,981	Positive	74	99.4

- Selectivity passes in all individuals assessed

Screening Cut Point = NC*1.25

Confirmation Cut Point = 30%

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Drug Tolerance (DT)

- Positive controls spiked in blood containing drug levels in range expected in clinical samples

Level of TRASTUZUMAB in Matrix (µg/mL)	Observed Response (ECL)					Approximate Cut-Point (ECL)
	Positive Control Level (ng/mL)					
	0	50	100	250	25,000	
0.0	96	120	155	250	20,380	113
0.5	91	105	136	209	15,880	
1.0	94	117	129	217	15,813	
2.0	86	108	114	208	14,445	
2.5	93	110	118	199	11,974	
5.0	86	114	137	173	9,803	
7.5	86	99	109	166	9,133	

Normalisation factor: 1.25; Mean NC= 90.3

- Drug tolerance meets study requirements

The ***drug tolerance*** and ***sensitivity*** are based on a ***surrogate positive control*** and they may not be representative of ADA in the study population.

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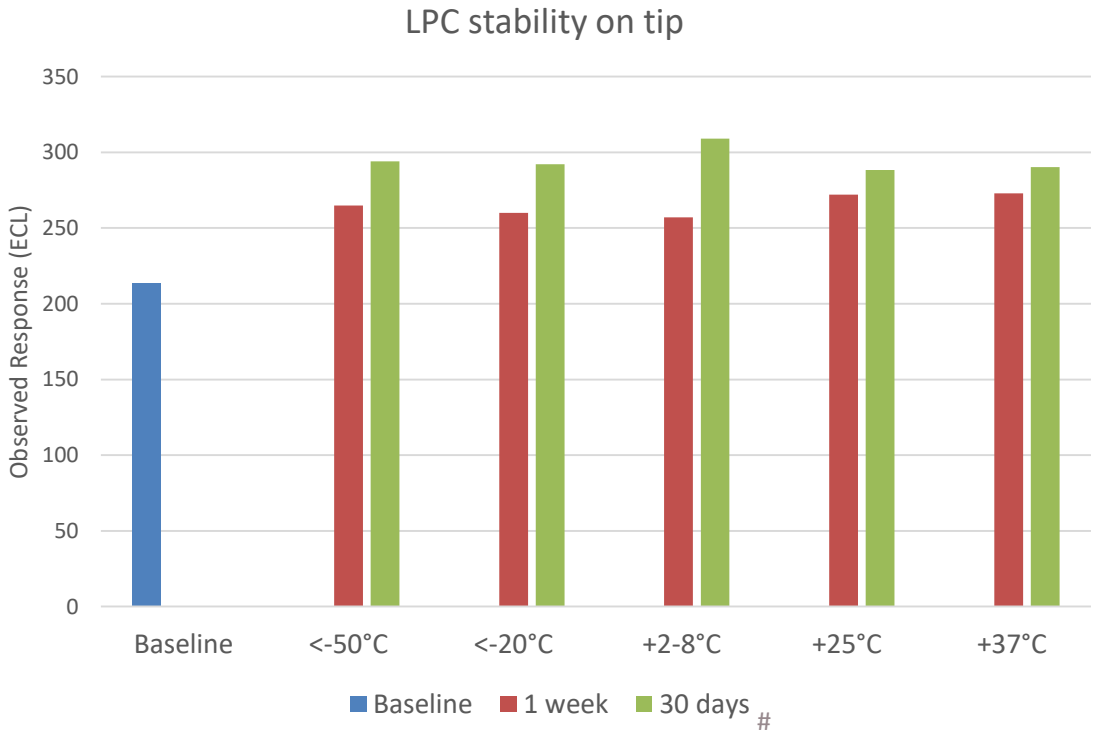
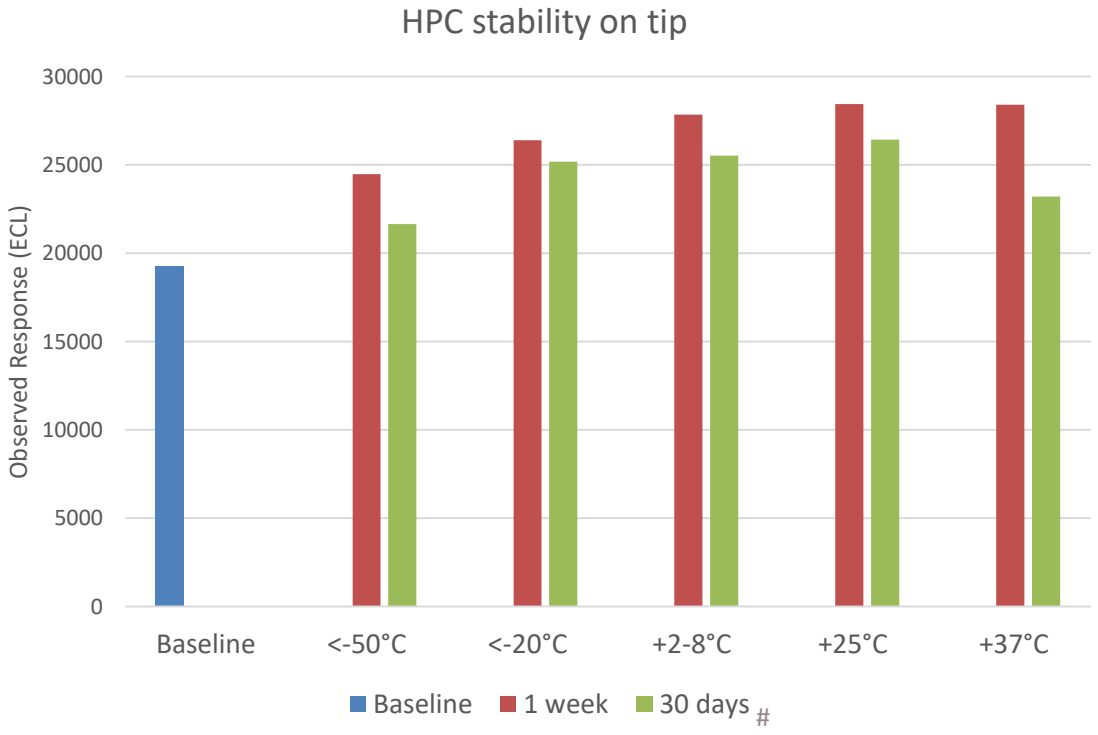
Stability (Dried on Mitra® Tip)

- Positive controls spiked in blood and loaded on Mitra® tips
- Baseline analysed after overnight incubation
- Mitra® tips stored for 1 week or 30 days at different temperature to mimic real world (including extremes)





- No evidence of instability

Correction factor applied to 30 day stability data due to dilution error during analysis

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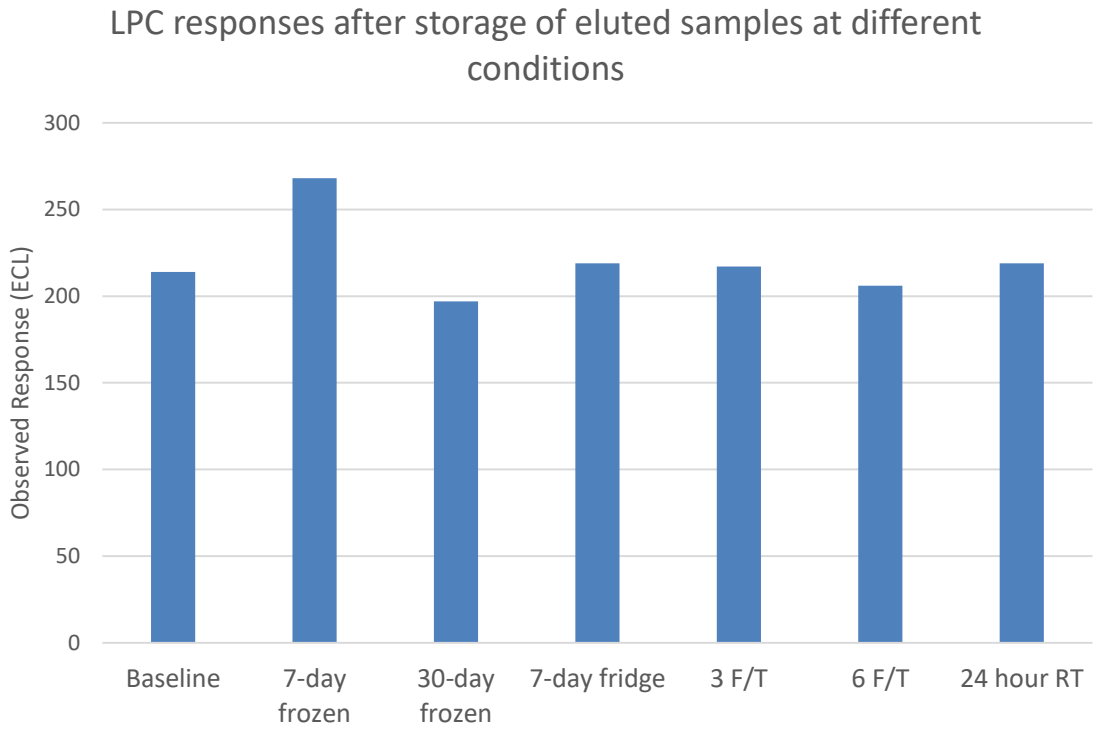
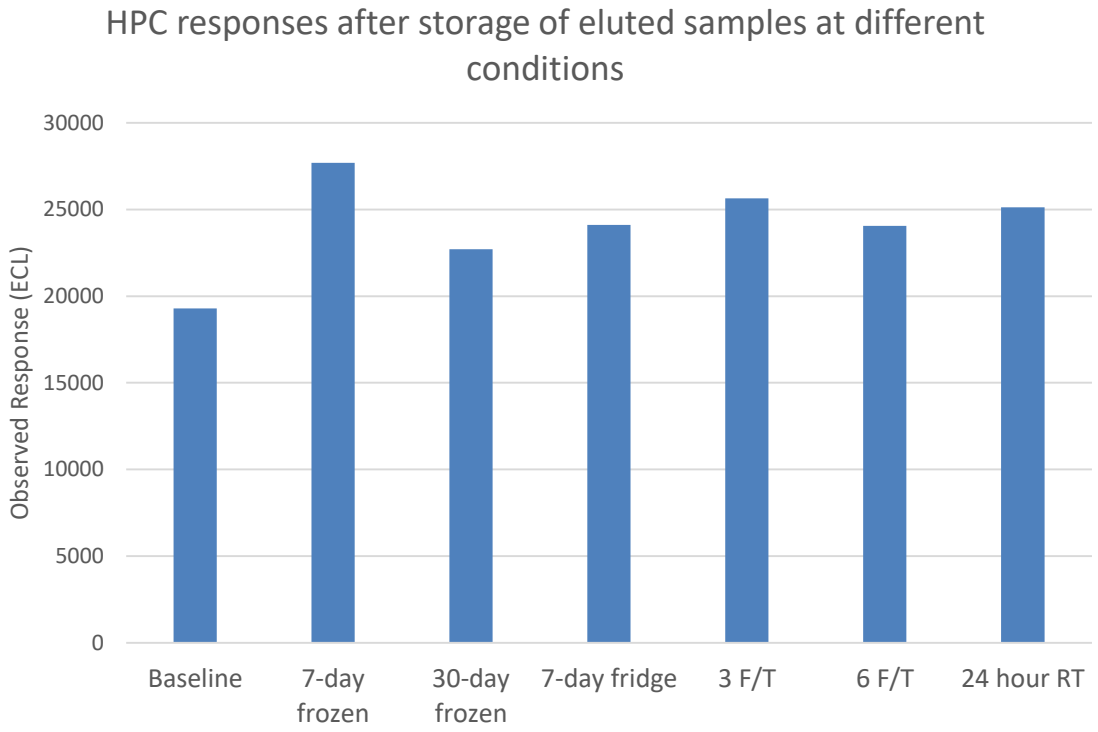
Stability (Eluted Sample)

- Positive controls spiked in blood and loaded on Mitra® tips, then eluted after overnight incubation
- Baseline analysed after elution
- For stability assessments, eluted samples stored at different conditions





- No evidence of instability

PSCP for the Baseline assessment was 151, the PSCP for the 7-day assessment was 212, and the PSCP for the other stability assessments was 156.

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Intra-Assay Precision

- NC and positive controls prepared in blood and loaded on Mitra® tips, then eluted after overnight incubation

Sample	NC (0 ng/mL)				LPC (100 ng/mL)				HPC (25,000 ng/mL)			
	Screening		Confirmation		Screening		Confirmation		Screening		Confirmation	
	Observed Response (ECL)	Screen Pos/Neg	Observed Response (ECL)	Inhibition (%)	Observed Response (ECL)	Screen Pos/Neg	Observed Response (ECL)	Inhibition (%)	Observed Response (ECL)	Screen Pos/Neg	Observed Response (ECL)	Inhibition (%)
1	73	Negative	70	4.1	141	Positive	72	48.9	26,111	Positive	81	99.7
2	70	Negative	67	4.3	143	Positive	68	52.4	23,598	Positive	79	99.7
3	69	Negative	68	1.4	142	Positive	68	52.1	22,965	Positive	80	99.7
4	73	Negative	67	8.2	145	Positive	70	51.7	24,355	Positive	78	99.7
5	71	Negative	64	9.9	143	Positive	70	51.0	22,381	Positive	78	99.7
6	73	Negative	66	9.6	146	Positive	69	52.7	21,037	Positive	77	99.6
Mean (ECL)	72		67		143		70		23408		79	
Mean (% Inhibition)	-			6.3				51.5	-			99.7
Precision (%)	**2.5**		**3.0**	**55.6**	**1.3**		**2.1**	**2.7**	**7.4**		**1.9**	**0.0**

- Excellent reproducibility seen

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Inter-Assay Precision

- NC and positive controls from across different assay runs

Run	NC (0 ng/mL)	LPC (100 ng/mL)				HPC (25,000 ng/mL)			
		Screening		Confirmation		Screening		Confirmation	
	Observed Response (ECL)	Observed Response (ECL)	Pos/ Neg	Observed Response (ECL)	Inhibition (%)	Observed Response (ECL)	Pos/ Neg	Observed Response (ECL)	Inhibition (%)
1	84	142	Positive	-	-	15,920	Positive	-	-
2	83	145	Positive	-	-	14,838	Positive	-	-
3	67	165	Positive	72	56.4	30,678	Positive	84	99.7
	71	167	Positive	69	58.7	31,847	Positive	83	99.7
4	78	178	Positive	74	58.4	27,625	Positive	80	99.7
	74	159	Positive	69	56.6	27,679	Positive	76	99.7
5	73	141	Positive	72	48.9	26,111	Positive	81	99.7
	70	143	Positive	68	52.4	23,598	Positive	79	99.7
	69	142	Positive	68	52.1	22,965	Positive	80	99.7
	73	145	Positive	70	51.7	24,355	Positive	78	99.7
	71	143	Positive	70	51.0	22,381	Positive	78	99.7
	73	146	Positive	69	52.7	21,037	Positive	77	99.6
6	88	172	Positive	-	-	19,827	Positive	-	-
	97	158	Positive	-	-	21,109	Positive	-	-
	85	167	Positive	-	-	21,699	Positive	-	-
7	132	172	Positive	-	-	14,825	Positive	-	-
	134	213	Positive	-	-	14,551	Positive	-	-
8	164	259	Positive	-	-	26,853	Positive	-	-
	175	274	Positive	-	-	22,796	Positive	-	-
9	113	159	Positive	-	-	11,488	Positive	-	-
	125	198	Positive	-	-	19,954	Positive	-	-
Mean (ECL)	95	171	-	70	53.9	22,006	-	80	99.7
Precision (%)	**34.2**	**21.7**	**-**	**2.9**	**6.1**	**24.9**	**-**	**3.1**	**0.0**

- Some drift in assay signal over time contributes to the higher imprecision observed – troubleshooting required

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Conclusions

VAMS® can help with more patient-focused sampling in clinical trials

VAMS® works for ADA assessments

Other subclasses of protein therapeutic need to be included in the assessments to increase confidence in the approach

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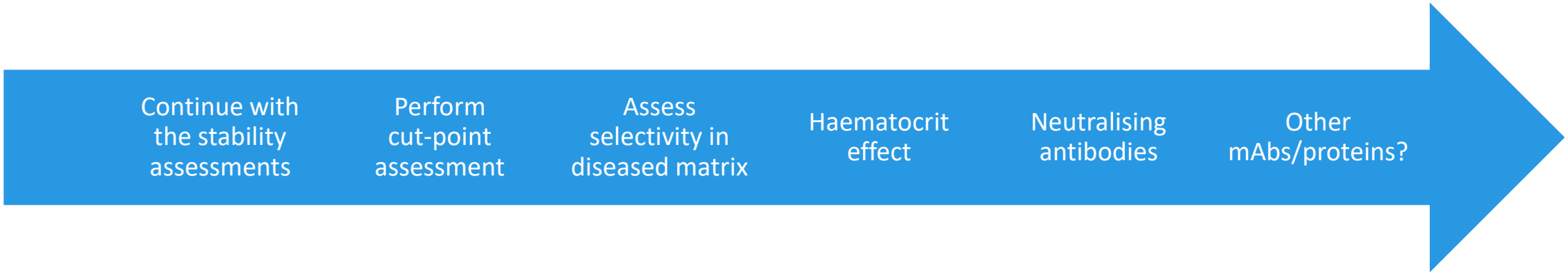
Next Steps…



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I would like to sincerely thank Traci Mizuno and James Rudge from Neoteryx®, Ranbir Mannu and Robert Nelson from Labcorp, and especially Sarah Malpas, without whose continual guidance, knowledge and help this project would not have gone ahead.

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Thank You for Listening!