Automated Singlicate Biomarker Assay: Enhancing Assay Performance by Surrogate Matrix Optimization

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EBF 21-Nov-2024

Surrogate Matrix: Essential for Endogenous Analytes

1. **Purpose**: used preparing calibration standards and/or quality control (QC) samples without the interference of endogenous analytes.

2. **Selection**: The surrogate matrix should closely mimic the biological matrix in terms of composition and behavior within the assay.

3. **Method Development/Validation**: demonstrate suitability of the use of surrogate matrix.

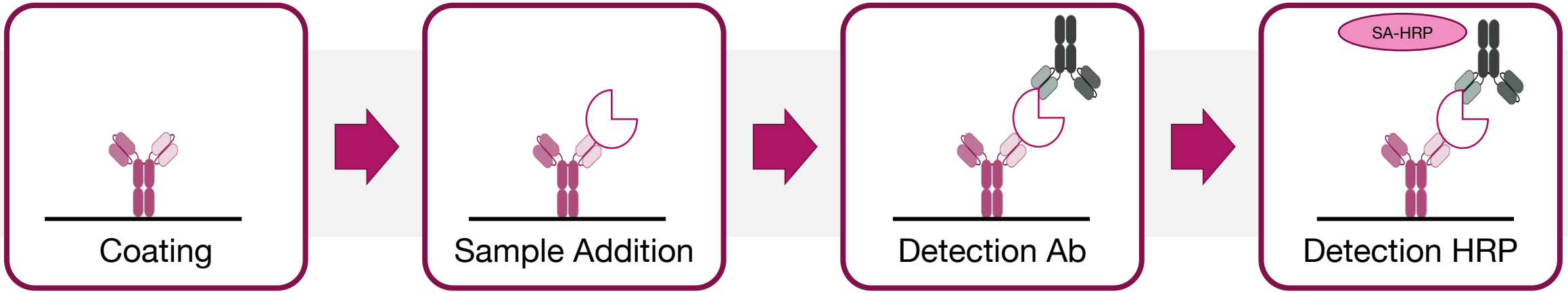
Challenge of Choosing the Appropriate Matrix for calibrators and QCs

Surrogate Matrix	Composition	Matrix effect	Lot-to-lot Variation	Adsorption of target	Considerations
Ideal surrogate matrix	Matrix prepared using an anti-target antibody		X		• Availability of anti-target antibody • Not suitable for large volumes
Simple buffer	Buffer containing protein (typically, BSA or Casein)	X		X	• Addition of detergents, protease inhibitors etc.
Complex surrogate matrix (CSM)	Commercially available matrix	X	X		• Assay life-cycle management
	Extracted matrix using charcoal	X	X	X	• Low extraction efficiency for large molecules • Does not resemble that of original matrix
	Matrix derived from other species	X	X		• No interfering endogenous counterpart present

Case Study: Development of an Exploratory Biomarker ELISA Assay for Detecting Changes in Endogenous Peptide Hormone after Treatment in Human Plasma

Detecting Endogenous Peptide Hormone in Human Plasma

- Format: Direct Sequential ELISA
- Target: 16kDa peptide hormone, low ng/ml concentration in plasma

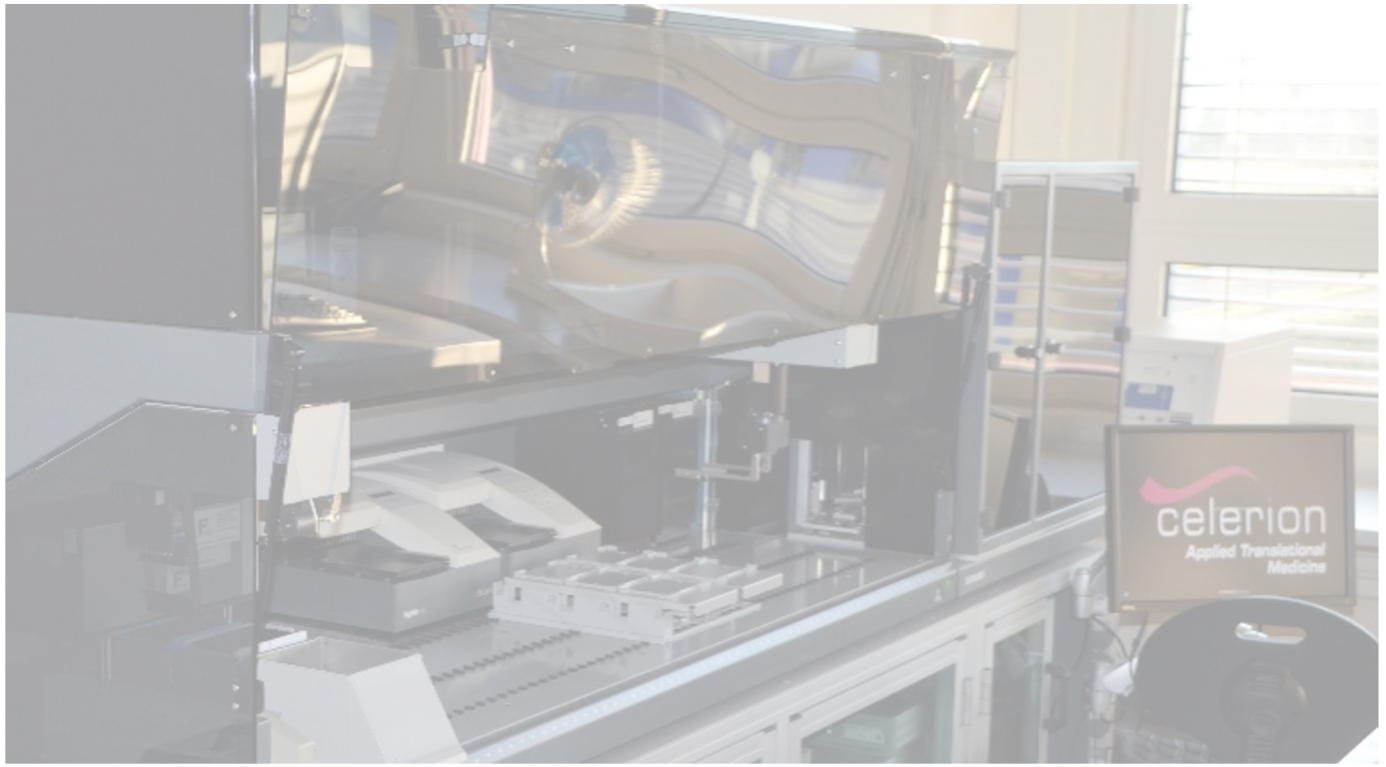


- Aim: develop an assay based on validated kit that allows for high throughput
  - Automatable
  - Singlicate

Benefits of Assay Automation – High Sample Throughput

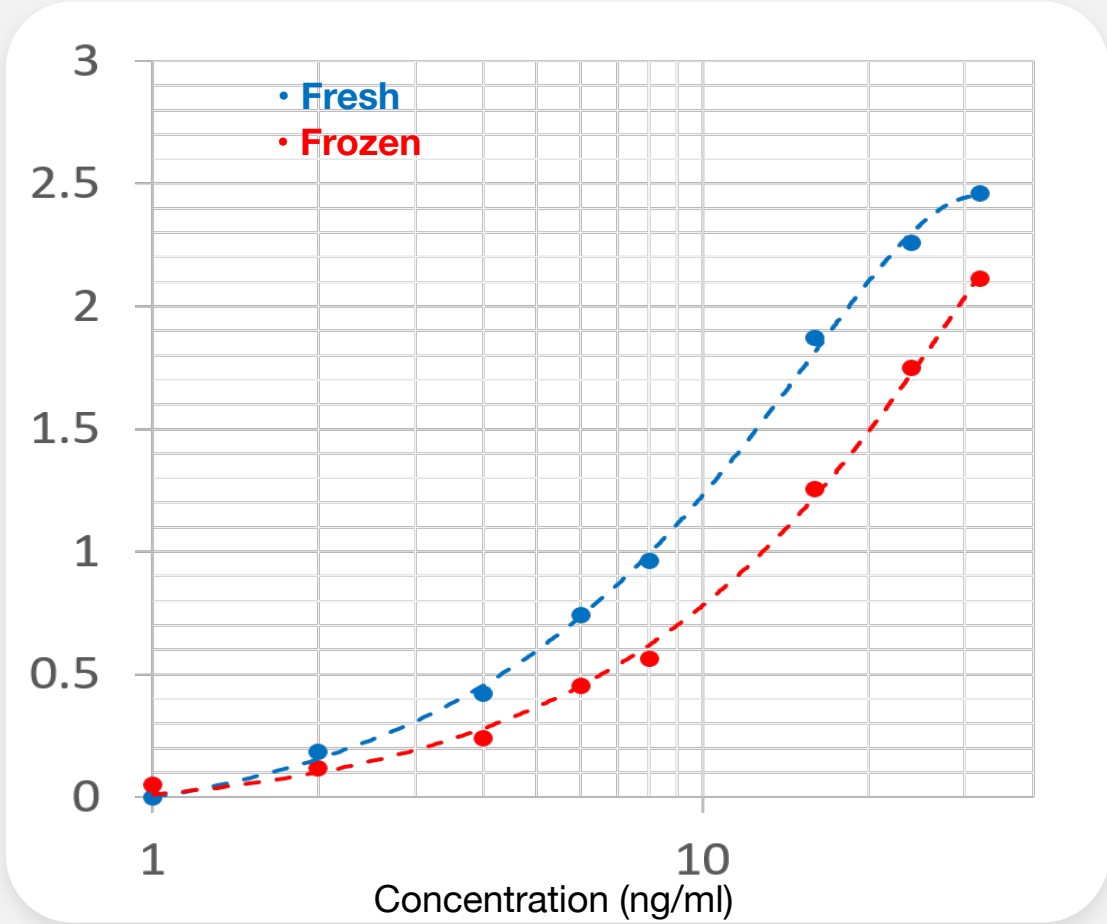
Advancements in Fully Automated Sample Analysis Systems

- Increased Throughput
- Enhanced Traceability
- Greater Reliability
- Improved Reproducibility
- Increased Robustness



Initial Buffer Testing for Standard Curve Preparation

- **Reagent Diluent**
  - Freshly prepared and frozen standard curves are not superimposable
  - Stability of standards is questionable after freezing at -20°C



Reagent Diluent = PBS +1% BSA

Mitigation Strategies for Observed Differences

1. Addition of Additives	▪ Increase BSA concentration ▪ Addition of Tween-20 ▪ Addition of glycerol ▪ Addition of EDTA 
2. Changing buffer system	▪ HEPES ▪ PBS
3. Factors influencing curve	▪ Storage temperature ▪ Heat inactivation
4. Matrix derived from other species	▪ Test different complex surrogate matrices

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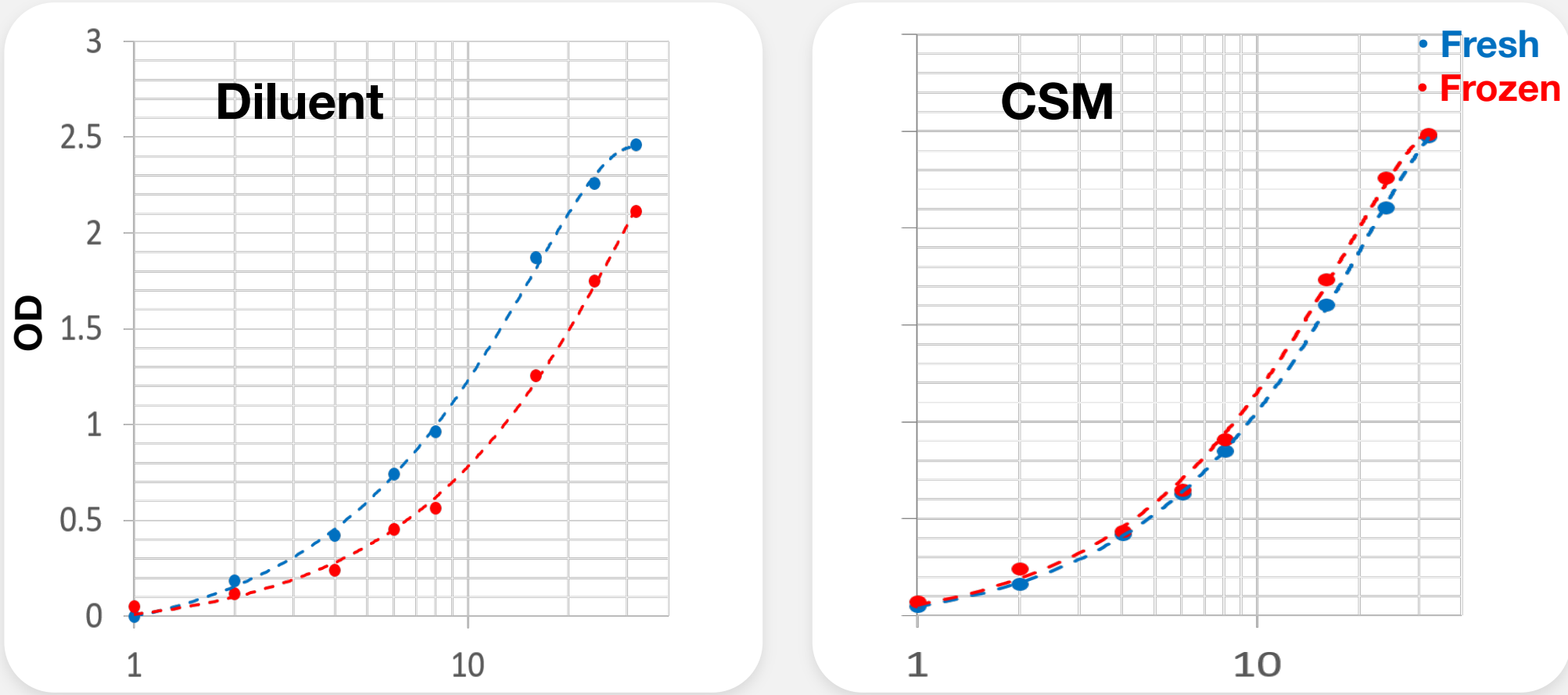
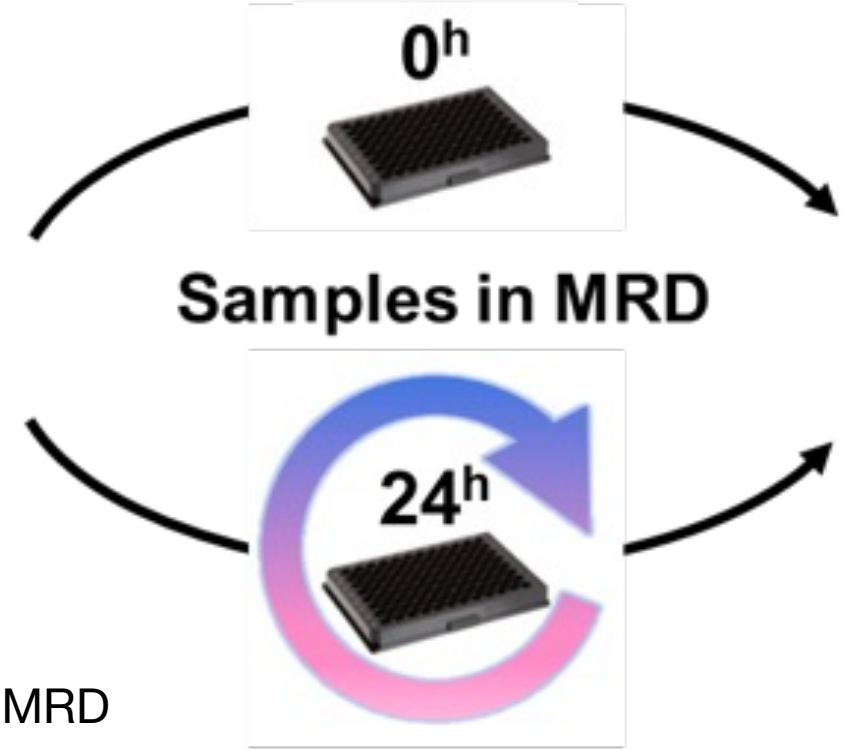
Resolving Differences by Using a Complex Surrogate Matrix

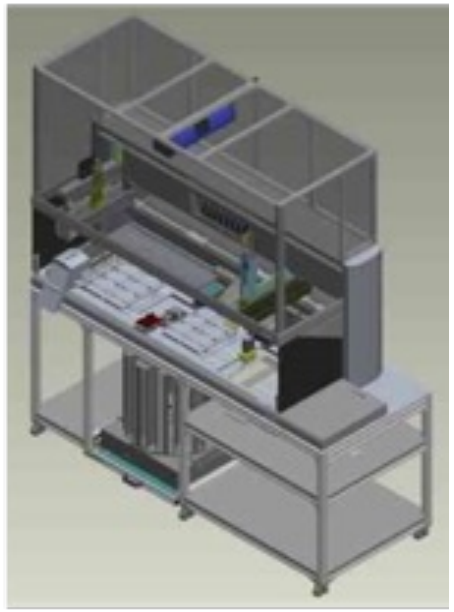
- Behavior of the standard curve before and after optimization of the surrogate matrix



Automated Assay: Eliminating Manual Pipetting

Sample Preparation up to MRD

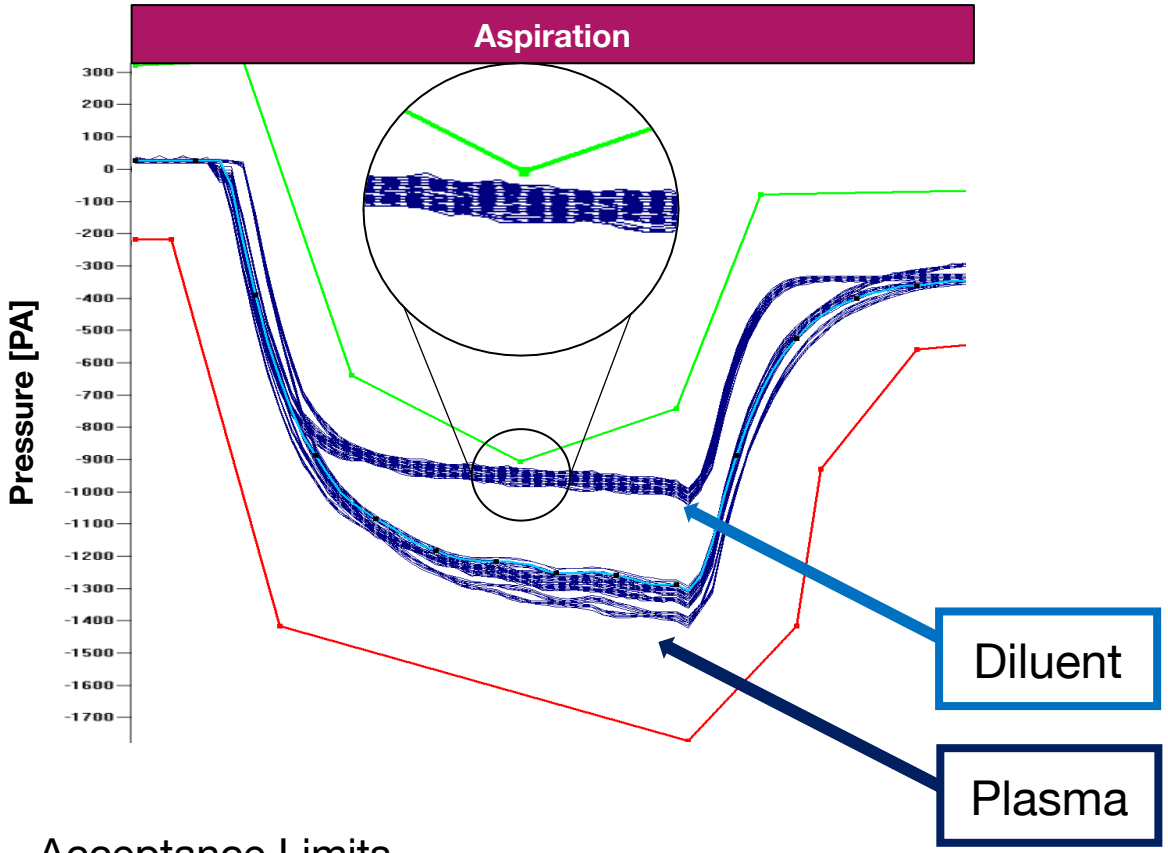




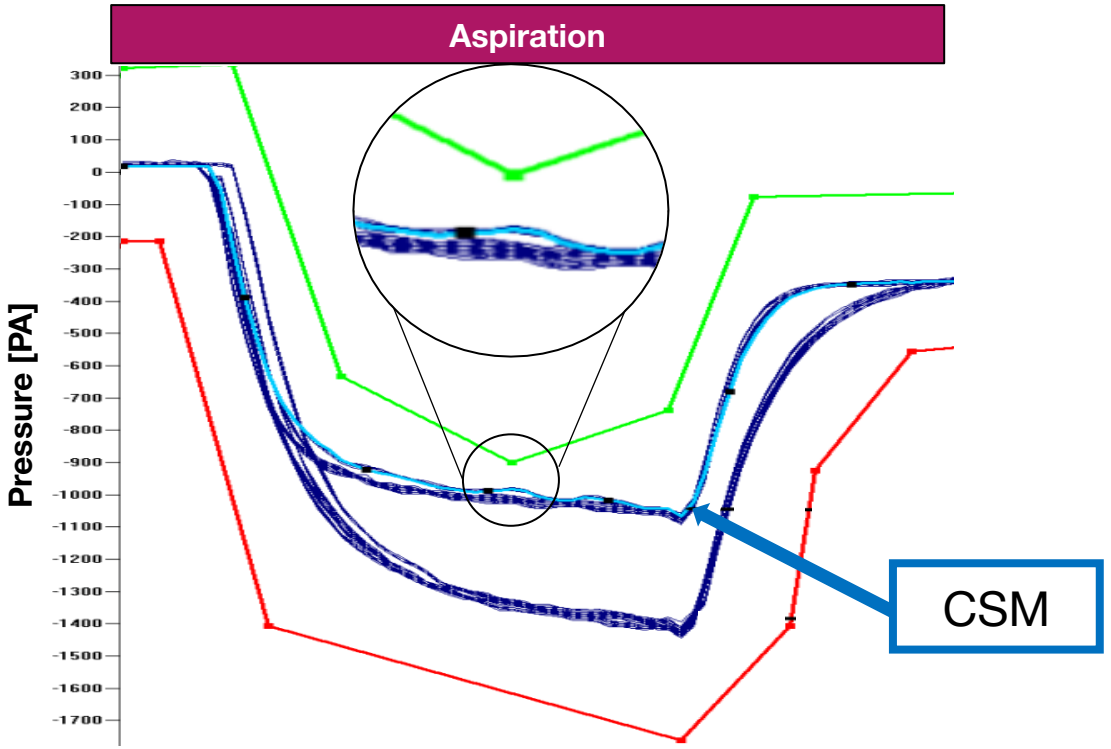
Sample Analysis

Impact of Varying Viscosity on Robotic Sample Pipetting

- **Total Aspiration and Dispense Monitoring (TADM)**: pipetting steps managed by the Hamilton robot



Acceptance Limits



- 5-fold Pre-diluted samples exhibit similar behavior to calibrator in a complex surrogate matrix (CSM)

Proven Suitability of Complex Surrogate Matrix in A&P Runs

	Precision (%CV)	Bias (%)	Total Error (%)
LLOQ	2.3	-7.6	9.9
LQC	1.7	-6.6	8.3
MQC	2.5	-7.9	10.4
HQC	3.1	-5.1	8.1
ULQC	4.4	-8.8	13.2

- Calibrators prepared in complex surrogate matrix
- Endogenous QCs: LQC, MQC and HQC
- Recombinat QCs: LLOQ & ULOQ (surrogate matrix)

Comparable A&P: Automated vs Manual Processing

	Automated Processed Run			Manually Processed Run		
	Precision (%CV)	Bias (%)	Total Error (%)	Precision (%CV)	Bias (%)	Total Error (%)
LLOQ	2.3	-7.6	9.9	5.8	-2.9	8.7
LQC	1.7	-6.6	8.3	4.4	8.5	13.0
MQC	2.5	-7.9	10.4	2.8	19.4	22.2
HQC	3.1	-5.1	8.1	2.0	16.9	18.8
ULOQ	4.4	-8.8	13.2	5.0	4.1	9.1

- Acceptance Criteria met in both, automated and manual runs
- ~30% reduction in total error was achieved using the automated system

Validation A&P Data Supportes Singlicate Analysis

- Assessment of validation data conducted in duplicate analysis
- Singlicate results were derived by using the first replicate

	Duplicate Analysis			Singlicate Evaluation		
	Precision (%CV)	Bias (%)	Total Error (%)	Precision (%CV)	Bias (%)	Total Error (%)
LLOQ	7.8	-3.5	11.3	8.6	-3.4	12.0
LQC	6.8	-0.9	7.6	8.7	-1.2	9.9
MQC	10.1	4.0	14.1	12.0	3.2	15.2
HQC	11.6	0.8	12.5	15.3	0.5	15.8
ULQC	11.2	-7.8	19.0	22.9	-5.7	28.6

Selectivity Validation Data Supports Singlicate Analysis

Individual	Duplicate Analysis					Singlicate Evaluation				
	-	Low Spike		High Spike		-	Low Spike		High Spike	
	ng/mL	ng/mL	%Bias	ng/mL	%Bias	ng/mL	ng/mL	%Bias	ng/mL	%Bias
1	1.22	4.39	4.0	21.7	-6.5	1.2	4.37	4.0	21.6	-6.9
2	2.11	5.15	0.8	24.3	0.8	2.12	5.16	0.8	24.1	0.0
3	1.95	4.53	-8.5	19.6	-18.3	1.92	4.47	-9.1	19.4	-18.8
4	1.95	4.58	-7.5	20.1	-16.3	1.92	4.52	-8.7	20.6	-14.2
5	2.14	4.46	-13.2	**18.7**	**-22.4**	2.08	4.54	-10.6	**18.1**	**-24.9**
6	2.38	5.37	-0.2	23.1	-5.3	2.35	5.27	-1.5	22.6	-7.4
7	2.09	4.72	-7.3	19.8	-17.8	2.02	4.62	-8.0	19.4	-19.2
8	2.23	4.98	-4.8	22.5	-7.0	2.15	4.93	-4.3	21.9	-9.5
Lipemic	6.67	9.2	-4.9	26.4	-8.0	6.73	9.05	-7.0	27.2	-5.2
Hemolyzed	2.75	5.43	-5.6	21.7	-12.5	2.61	5.25	-6.4	20.6	-16.3
Pool control	3.34	6.01	-5.2	21.7	-14.2	3.31	5.83	-7.6	20.8	-17.8

A&P Assessment Confirm Assay Performance in Full-Throughput Singlicate Runs

	Singlicate Analysis		
	Precision (%CV)	Bias (%)	Total Error (%)
LLOQ	2.6	-3.4	6.0
LQC	2.3	3.4	5.7
MQC	2.0	4.1	6.1
HQC	4.3	-1.7	5.9
ULQC	6.8	-11.9	18.6

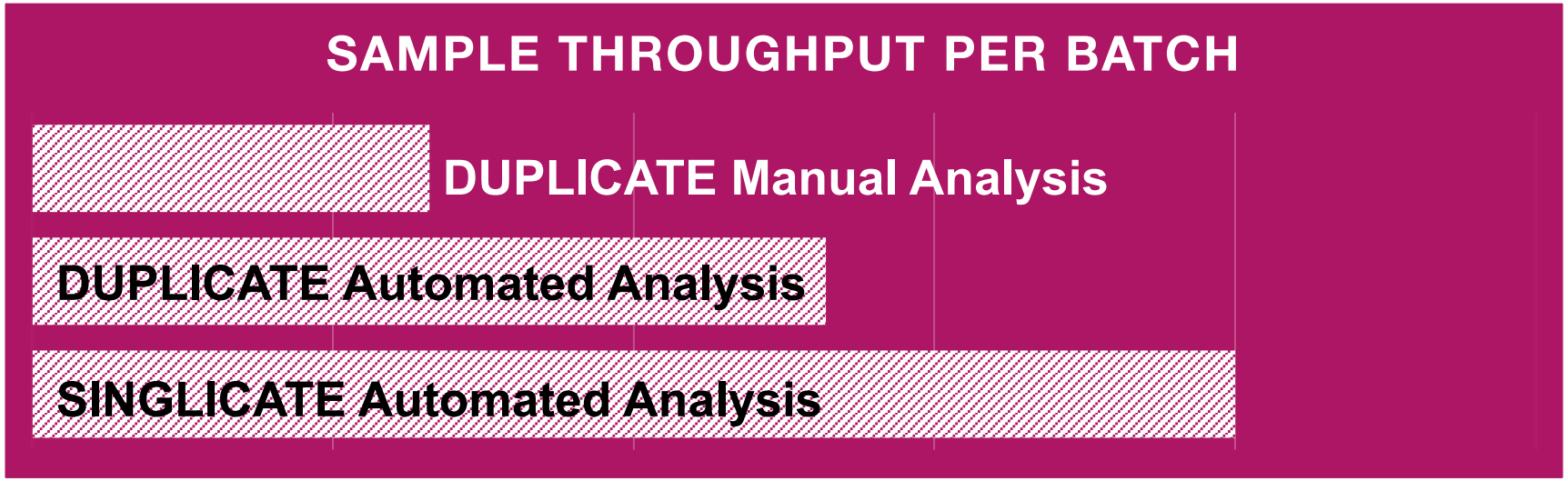
Batch Size:
5 plates (~400 samples)

Sample Processing Time: Increased compared to manual

✓ **Method Suitability Confirmed and Successfully Validated**

Summary

- An automated singlicate biomarker ELISA assay has been successfully validated:
  - matrix optimization significantly enhanced assay performance
  - Automation and singlicate analysis offer an opportunity to increase throughput without compromising data quality



Thanks to:
Marleen Lutz
Rebeca Schibli
Elisabeth Friedhoff
Lysie Champion
Wibke Lembke
Petra Struwe

THANK YOU