Quotient Bioresearch

Application of the Gyrolab to Pharmacokinetic, Pharmacodynamic and Immunogenicity Assays in a Regulated Laboratory

3rd EBF International Symposium

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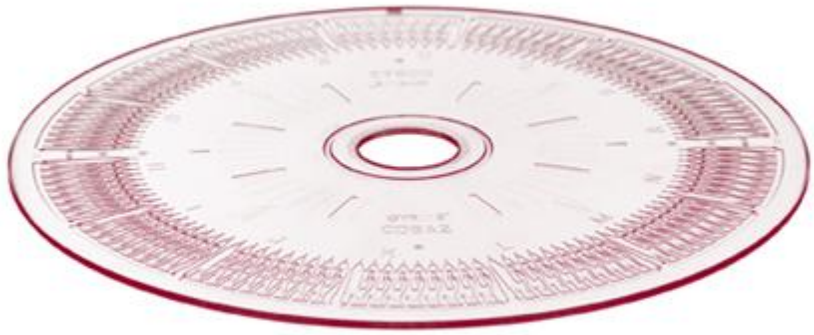
Quotient Bioresearch

Overview

- The Gyrolab Platform
- Integration of the Gyrolab to a regulated laboratory
- Why consider use of the Gyrolab?
- Method Validation
- Bioanalytical Method Examples
- Pharmacodynamic Method Example
- Immunogenicity Method Example
- Conclusions

The Gyrolab Platform

- Automated Immunoassay Analyser
- Bioaffy CD microlaboratory
- Nanofluidic column system
  - 1000 nL, 200 nL or 20 nL
  - 96 Columns per CD1000
  - 112 Columns per CD200
- 5 CD Capacity
- Streptavidin coated beads
- Alexa-647 detection label
- Open assay development platform
- Manual Instrument Loading



Regulated Laboratory

- Instrument Qualification
  - Installation Qualification
    - Engineers Report
  - Operational Qualification
    - Does the Instrument Perform according to the manufacturer's specifications in your laboratory?
  - Stress Testing
    - What happens when things go wrong?
  - Preventative Maintenance
    - Regular servicing and Maintenance to ensure correct operation
  - Performance Qualification
    - Demonstrating that the instrument operation is acceptable

Why Use the Gyrolab?

- The Gyrolab is a valuable addition to the regulated laboratory
  - Open ended platform allowing development of your own assays
  - Low Sample Volume
  - Fast Individual Batch Turnaround Time
  - Rapid Method Development
  - (Relatively) Simple Reagent Labelling
  - Low intra-assay drift
  - Good reproducibility between replicates
  - Reduced variability between operators
  - Gyrolab Viewer gives additional information on detection label capture

Gyrolab Method Validation

- Dependent on the assay type
- Pharmacokinetic Assays:
  - Follow the ligand-binding assay guidance documents
  - Validation process should model procedure intended for sample analysis.
- Pharmacodynamic Assays:
  - May be differing degrees of validation dependent on end assay use?
  - Follow a full PK validation for Primary Endpoints
- Immunogencity Assays:
  - Guidance Documents
  - Extent of validation can be tailored dependent on development phase
- Additional considerations
  - Characterisation of labelled reagents
  - On-board stability of samples and reagents

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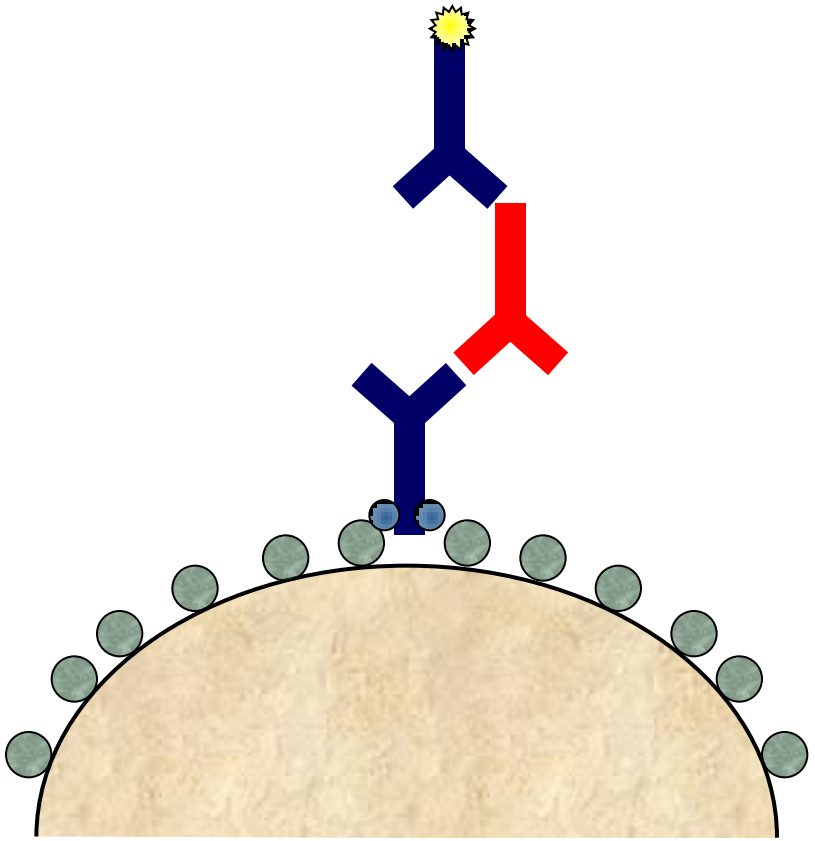
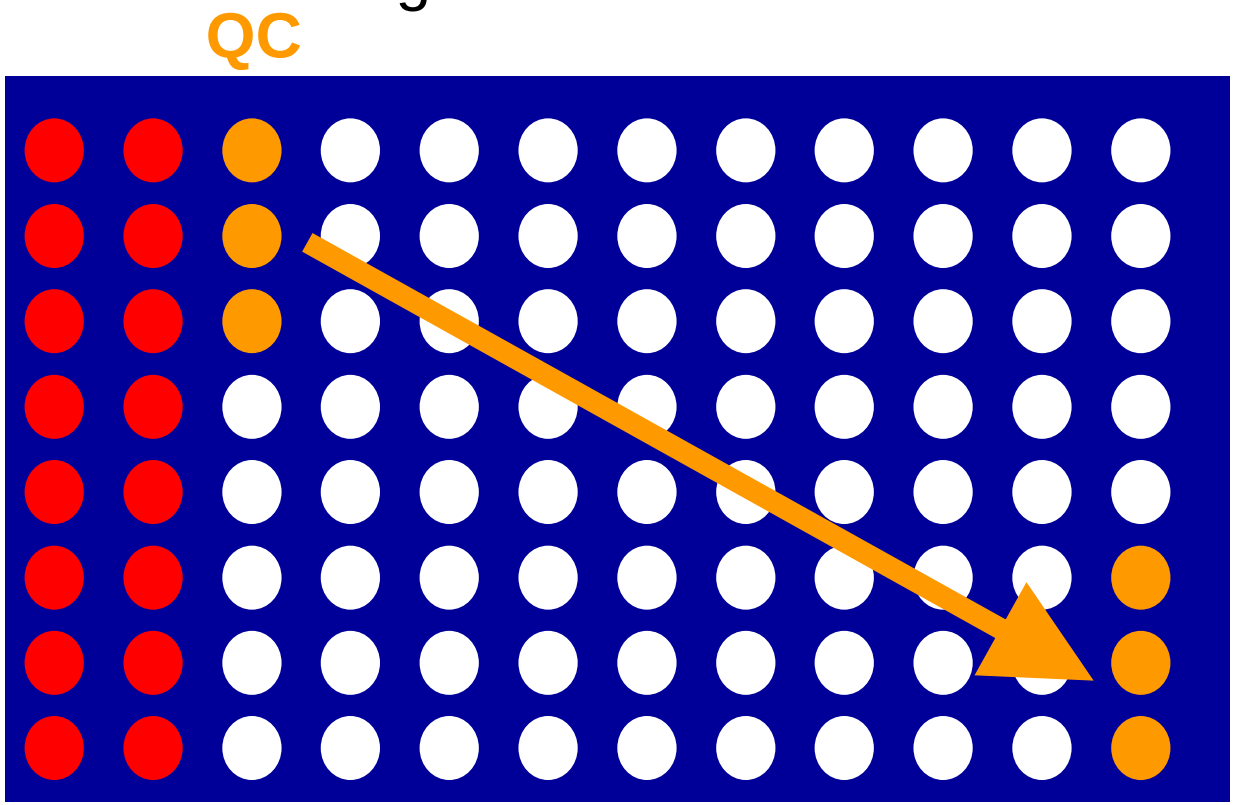
Pharmacokinetic Assay 1

- Pre-clinical determination of MAb in serum
- Bioaffy 1000CD
- SIA mode
- Biotin-labelled drug-specifc capture reagent
- Sample containing MAb
- Alexa-647 labelled Anti-drug host species detection



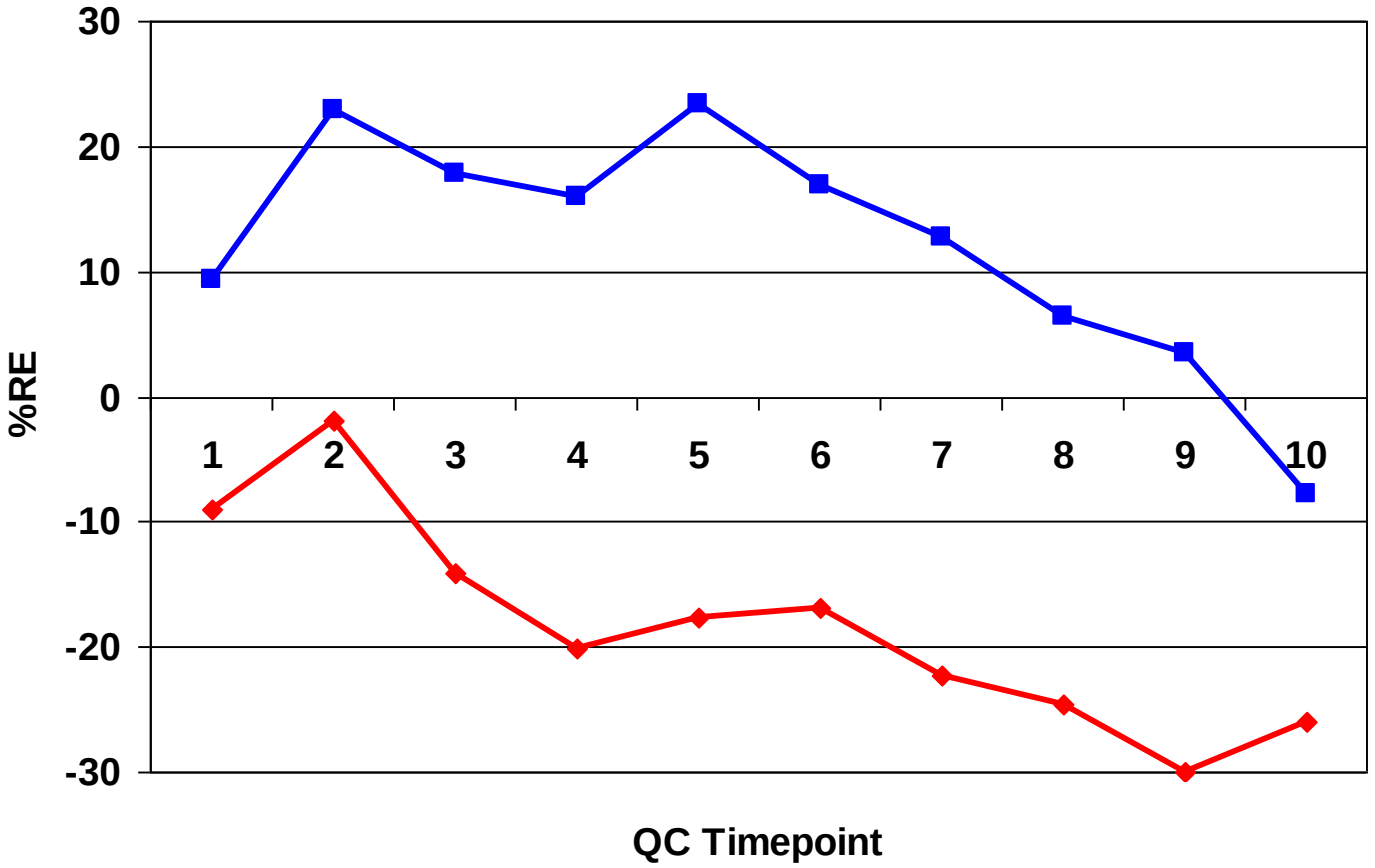
Assay Drift

- ELISA Method for Monoclonal Antibody Therapeutic
- Timed addition of reagents

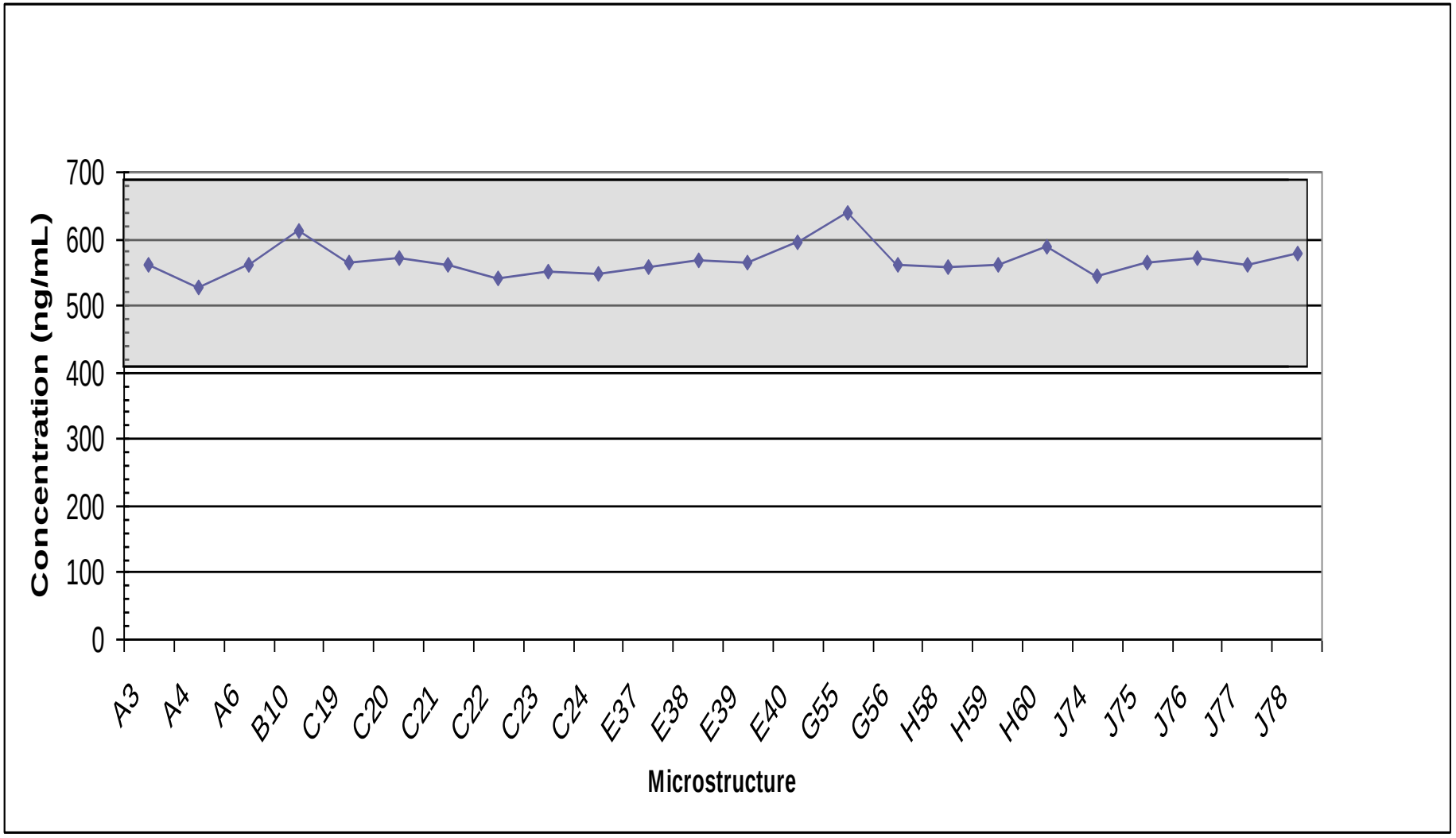


ELISA

- Significant decrease in recovery across assay plate

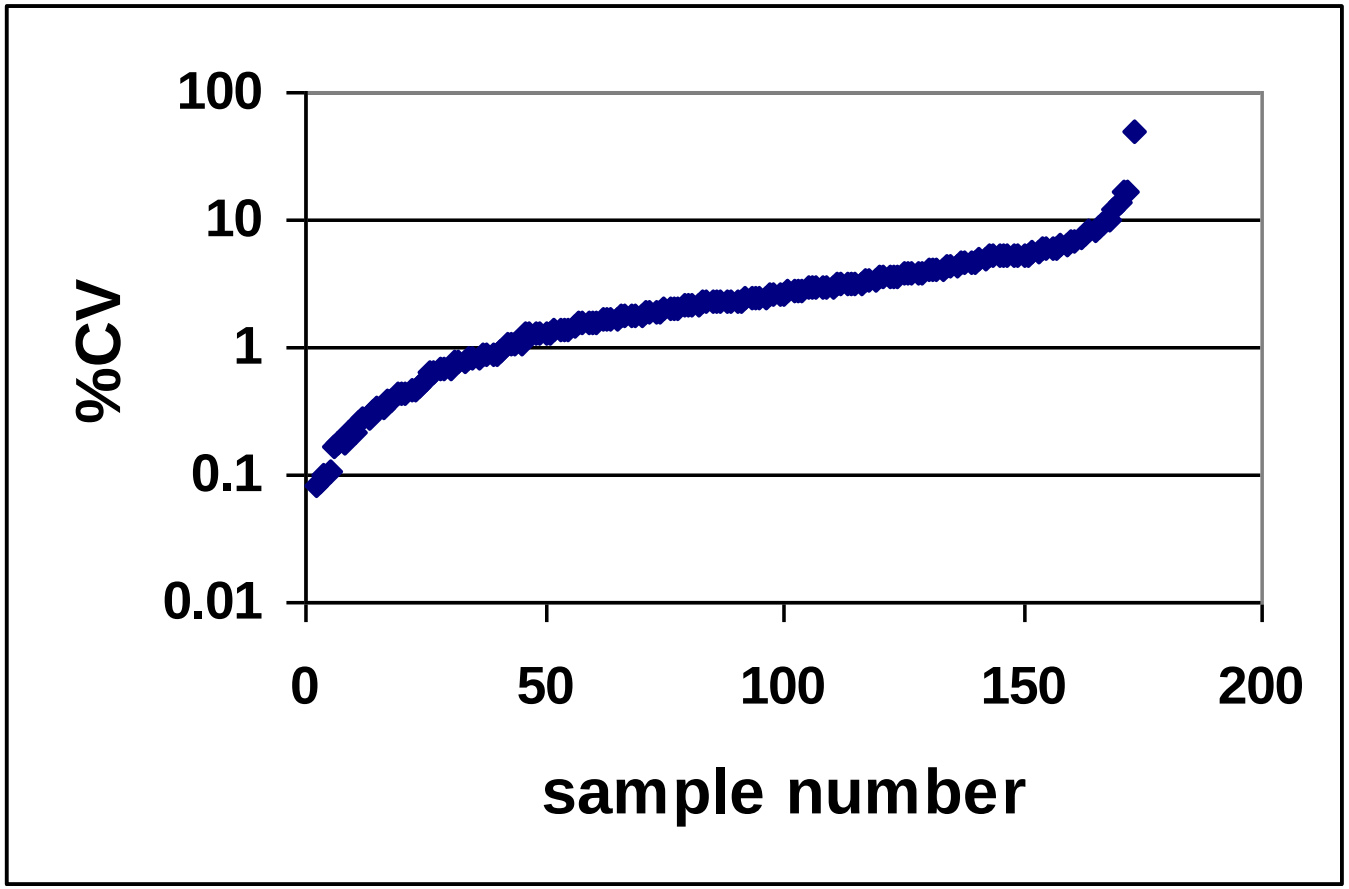


Gyrolab Assay Drift



Gyrolab Precision

- Precision between measured concentrations for replicates



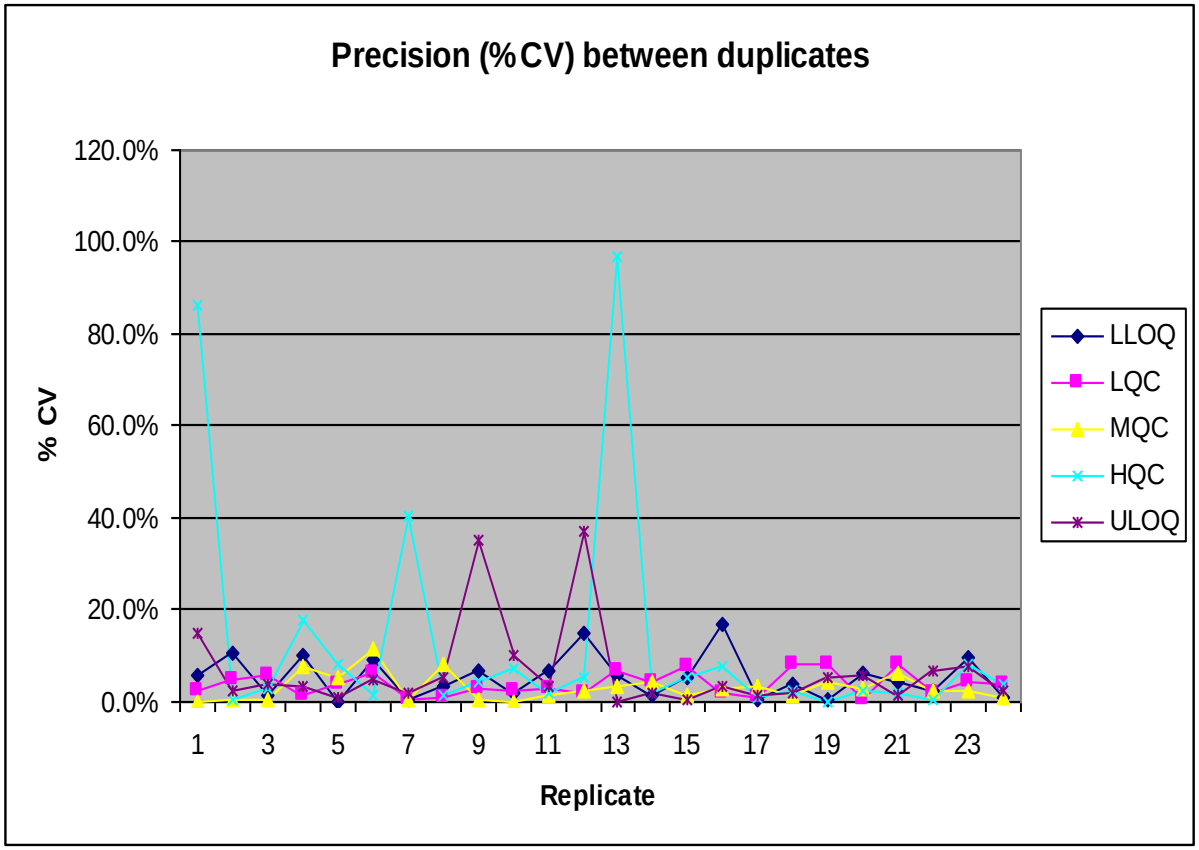
Pharmacokinetic Assay 2

- 2-fold Dilution
- LLOQ 5 ng/mL serum
- ULOQ 1250 ng/mL
- Linearity of Dilution >10,000-fold

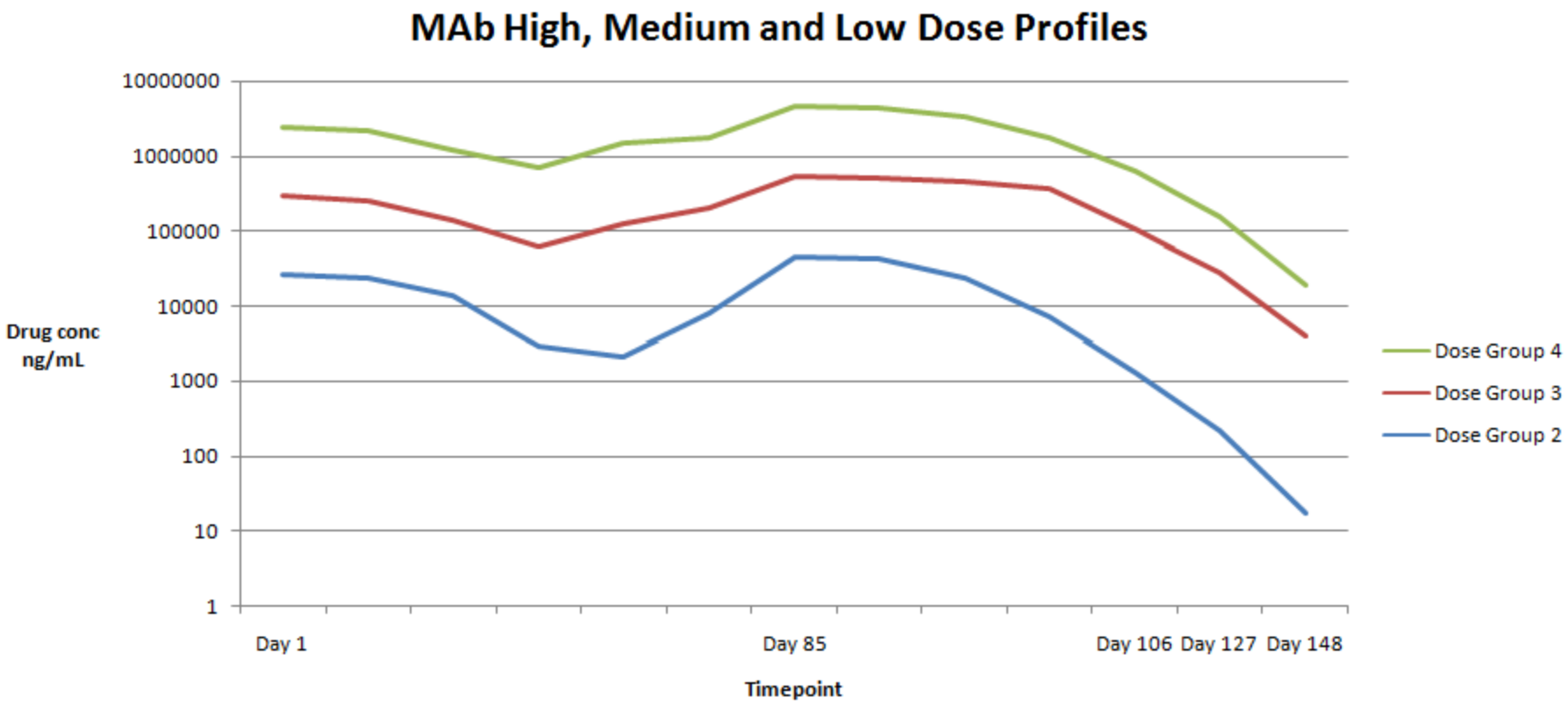
Intra-Batch Accuracy (%RE)	-17% to 13%
Intra-Batch Precision (%CV)	2.4% to **37.0**%
Inter-batch Accuracy (%RE)	6% to 13%
Inter-Batch Precision (%CV)	12.5% to **22.3**%

Validation Precision

% CV > 20%	
Sample ID	Measured Concentration (ng/mL)
HQC	763
	185
HQC	**517**
	935
ULOQ	1620
	975
ULOQ	1690
	988
HQC	860
	160

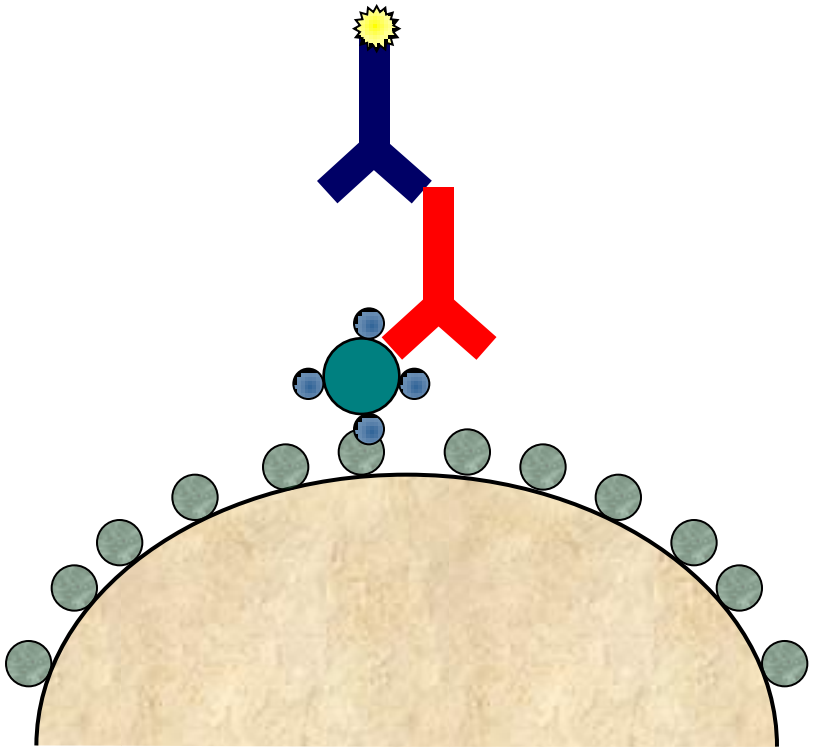


13 Week GLP Tox Study

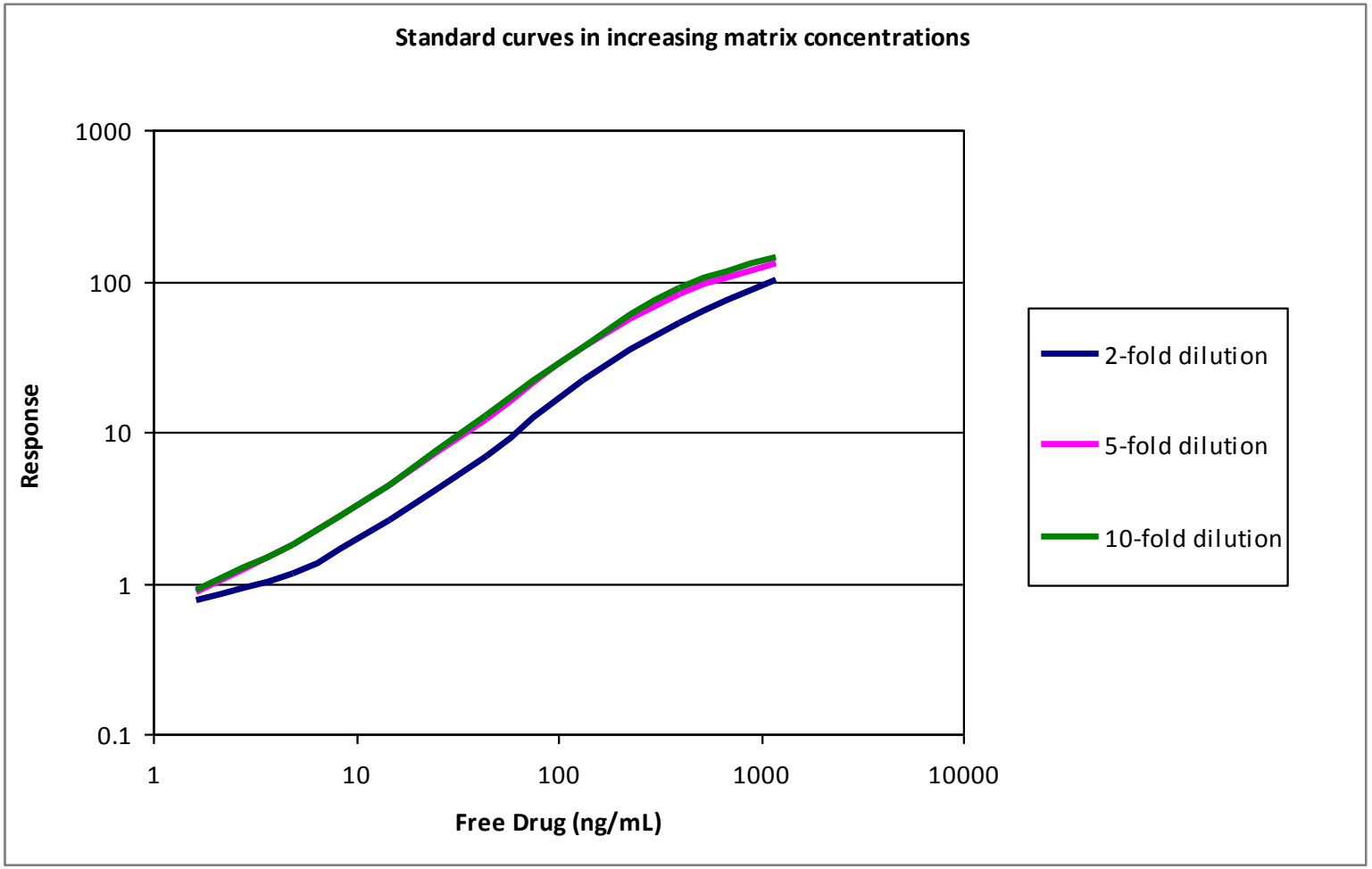


Pharmacokinetic Assay 3

- Determination of Free Drug
- Bioaffy 1000CD
- Capture: Biotin-labelled drug target
- Sample: Matrix containing Drug and Drug+Target complex
- Detection: Alexa 647-labelled anti-drug host species detection reagent
- Flow through method gives short time where sample is in contact with capture reagent
- Lower interference from dissociation of drug + target complexes

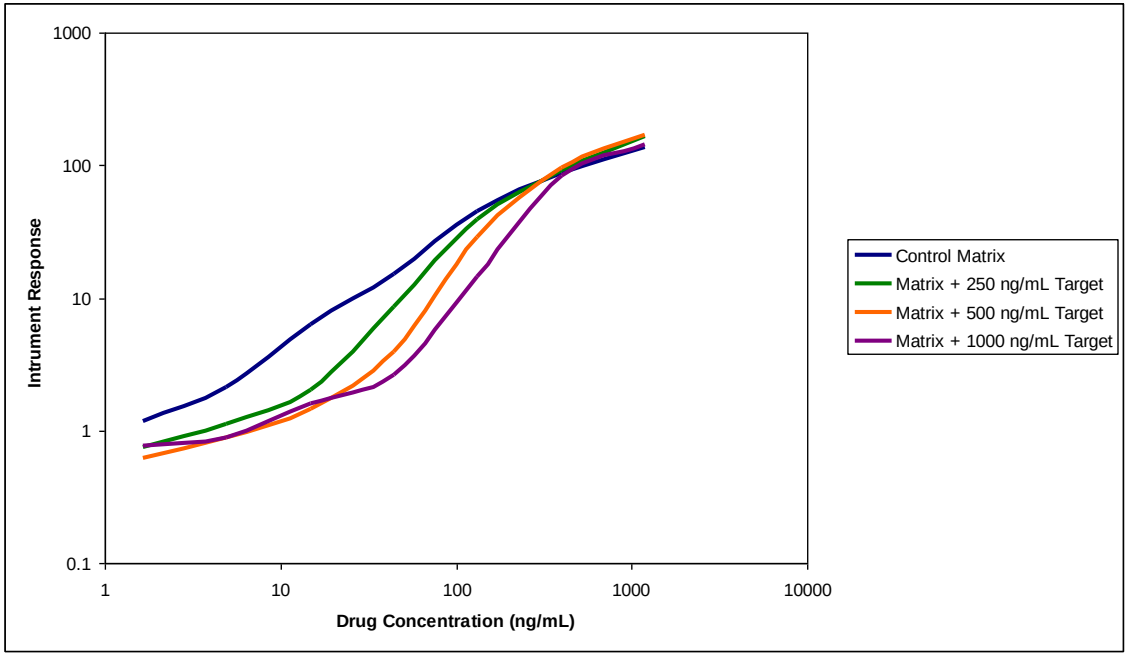


Matrix effects



Pharmacokinetic Assay 2

- Very early pre-clinical development
- Low sample volume
- Minimal dilution
- No custom reagents available
- Demonstrated target interference



Sample ID	LLQ	LQC	MQC	HQC	ULQ
Target Concentration (ng/mL)	15	45	250	4500	6000
Mean Concentration (ng/mL)	14.8	46.4	256	3810	4730
%RE	-1.3	3.1	2.4	-15.3	-21.2
SD	3.12	6.15	21.9	836	1200
%CV	21.1	13.3	8.6	21.9	25.4

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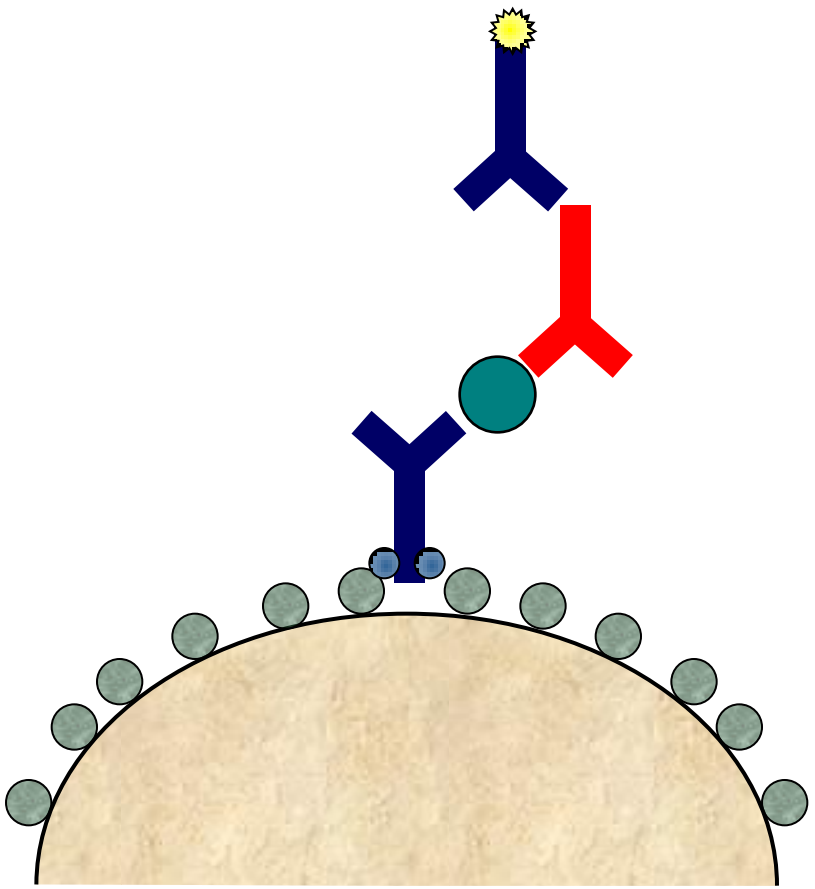
Total Drug Target Assay

- Total Drug Target Assay
- Pre-incubate samples with excess of drug
- Bioaffy SIA application
- Capture: Biotin-anti-target monoclonal Ab
- Sample: Target + drug complex
- Detection: Alexa 647-labelled anti-drug Ab
- 1% PMT



Total Drug Target Assay

- High abundance drug target
- Poor-recovery for spiked samples

ID	QC1	QC2	QC3	QC4	QC5
Target concentration (ng/mL)	**E + 20,000**	**E + 10,000**	**E + 5,000**	**E + 1,500**	**E**
	24540	**14540**	**9539**	**6039**	**-**
Batch 1 (n=4)	17480	11430	7395	5611	4540
Batch 2 (n=4)	16690	12300	8479	6159	5285
Batch 3 (n=4)	13730	10890	8544	5589	5015
Batch 4 (n=4)	15110	11610	7645	5551	4581
Mean Concentration (n=16) (ng/mL)	**15900**	**11600**	**8020**	**5730**	**4860**
Mean %RE	**-35.1**	**-20.0**	**-15.9**	**-5.1**	**7.1**
Mean %CV	**14.9**	**10.2**	**14.2**	**7.0**	**11.7**

Total Drug Target Assay

- Reproducibility for individual serum samples
- % CV <15% across

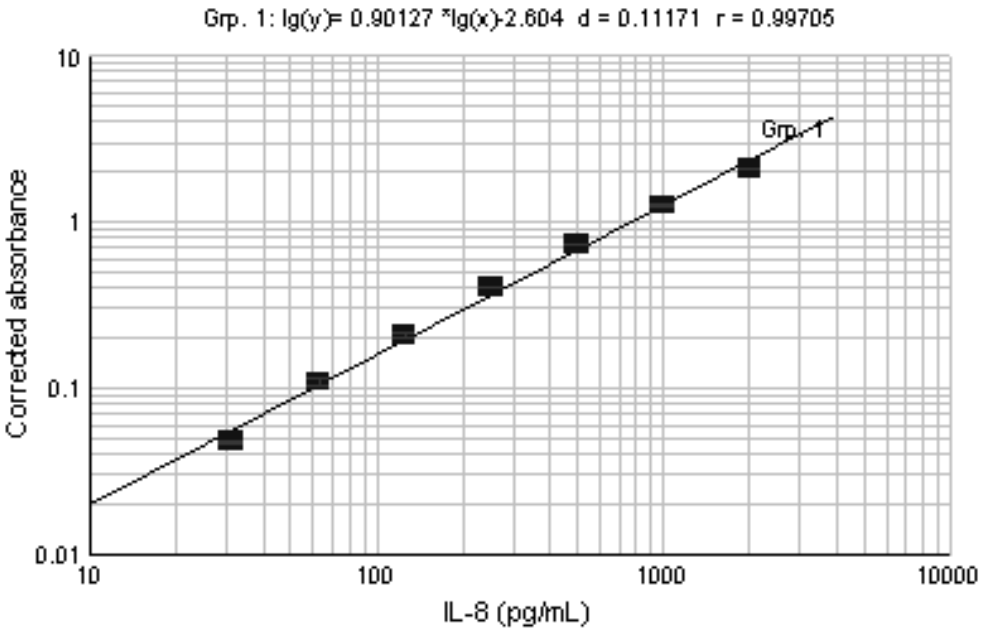
ID	S1	S2	S3	S4	S5	S6
Mean Concentration (n = 4x3 duplicates)	4650	5630	4220	3370	5300	5160
Mean Precision (%CV)	12.2	7.7	11.0	9.3	11.5	12.9

Parallelism

- Samples diluted 5, 10 or 20-fold prior to 2-fold MRD
- Precision calculated across 3 dilutions
- Obvious under-recovery for undiluted samples
- All samples <LLOQ at 50-fold dilution

ID	Mean Concentration (ng/mL)	% CV
S1	4160	2.8
S2	7010	7.0
S3	5960	8.2
S4	5683	7.4
S5	7797	14.9
S6	5613	8.1
S7	6107	9.3
S8	5417	4.2

Biomarker Example – IL-8

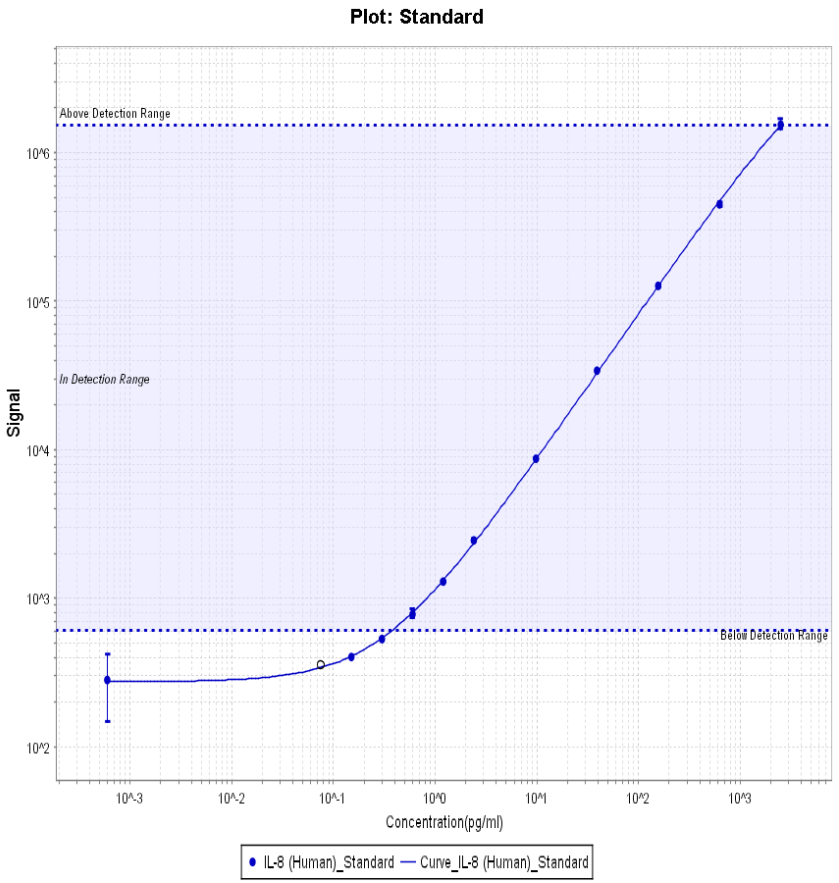


Range	31.2 – 2000 pg/mL
Intra-assay Precision (% CV)	<7.2 %
Intra-assay Accuracy (% RE)	< ±15 %
Inter-assay Precision (% CV)	< 15.0 %
Inter-assay Accuracy (% RE)	< ±18 %

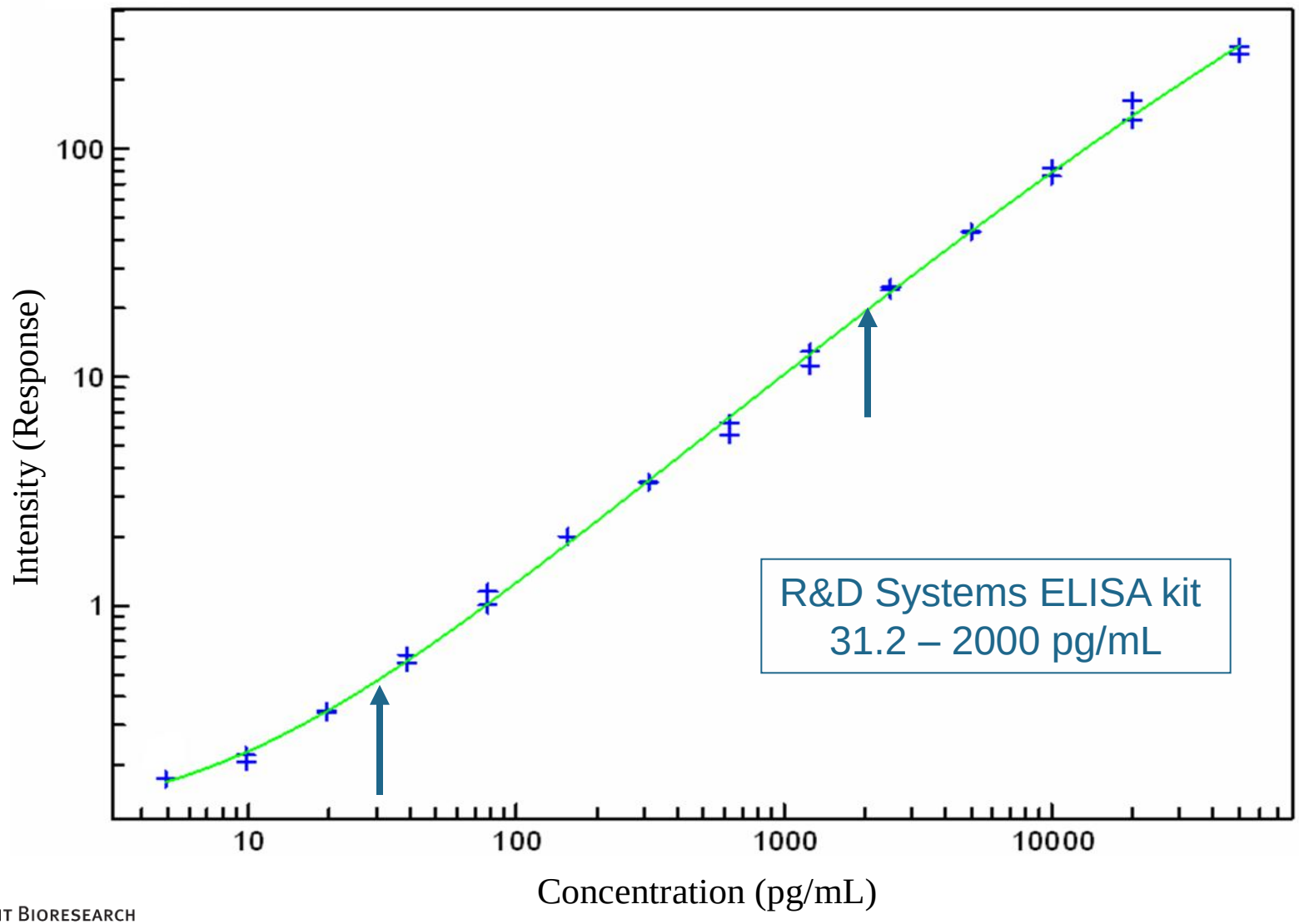
Interleukin-8 ECL

- 25 µL/well sample volume
- 4 hour assay

Sample	Target Conc. (pg/mL)	Mean Conc. (pg/mL) n=4	% RE	%CV
Serum	-	7.65	-	5.3
Serum	500	443	-11.5	2.9
Serum	1000	915	-8.5	4.3
Serum	2500	2735	9.4	8.4
Serum	4000	4277	6.9	6.3
Serum	8000	9637	20.5	8.6
Serum	-	8.43	-	8.0



IL-8 Gyrolab assay: 9.8 – 20000 pg/mL



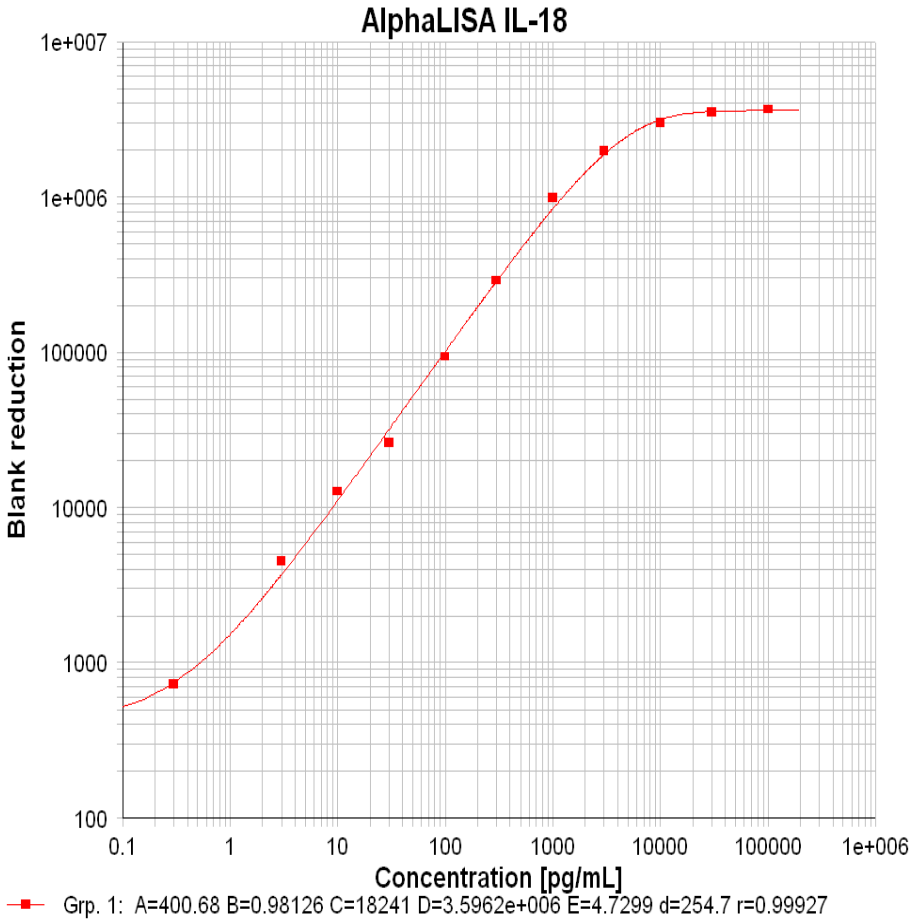
Gyrolab IL-8

ID	Conc. pg/mL	INTRA-ASSAY				INTER-ASSAY	
		Batch 1		Batch 2			
		%CV	%RE	%CV	%RE	%CV	%RE
QC1	30	2.3	-10	4.6	-12	3.8	-11
QC2	250	0.6	-5	5.6	8	7.8	2
QC3	1000	3.3	3	1.8	-3	4.1	0
QC4	3000	2.3	9	1.2	-2	5.8	3
QC5	8000	3.7	46	5.3	24	9.8	33

Interleukin-8 - AlphaLISA

- 5 μL Sample volume
- No Dilution required
- 1.5 hours incubation

Spike Conc. (pg/mL)	Target Conc. (pg/mL)	Mean Conc. (pg/mL) n=4	% RE	% CV
0	N/A	9.0155	-	10.6%
15	24.0155	20.805	-13.4%	16.6%
150	159.0155	129.24	-18.7%	8.3%
1500	1509.0155	1226.5	-18.7%	10.6%



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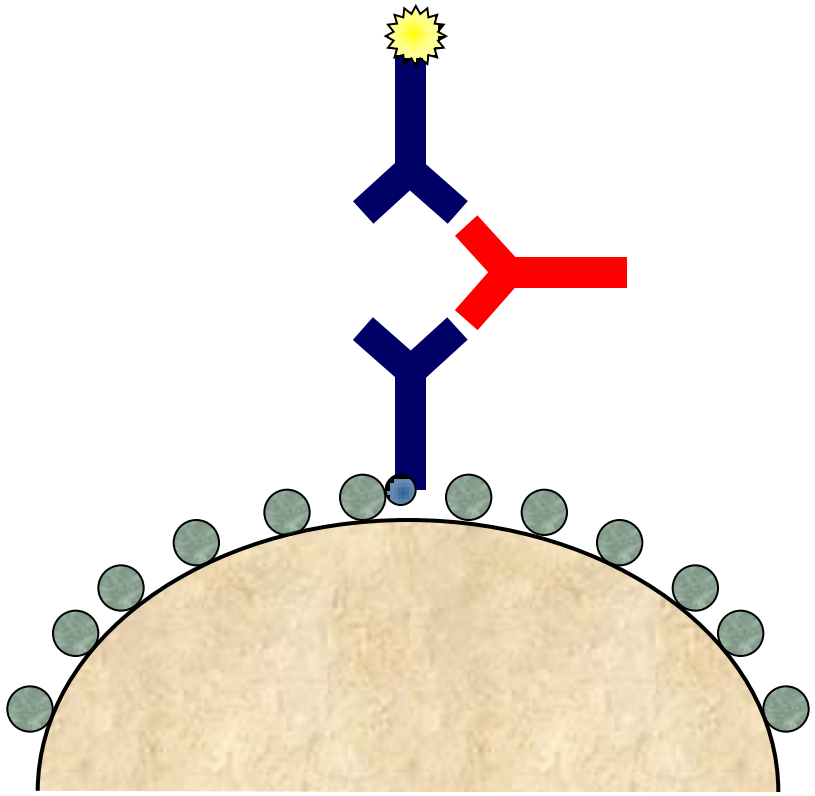
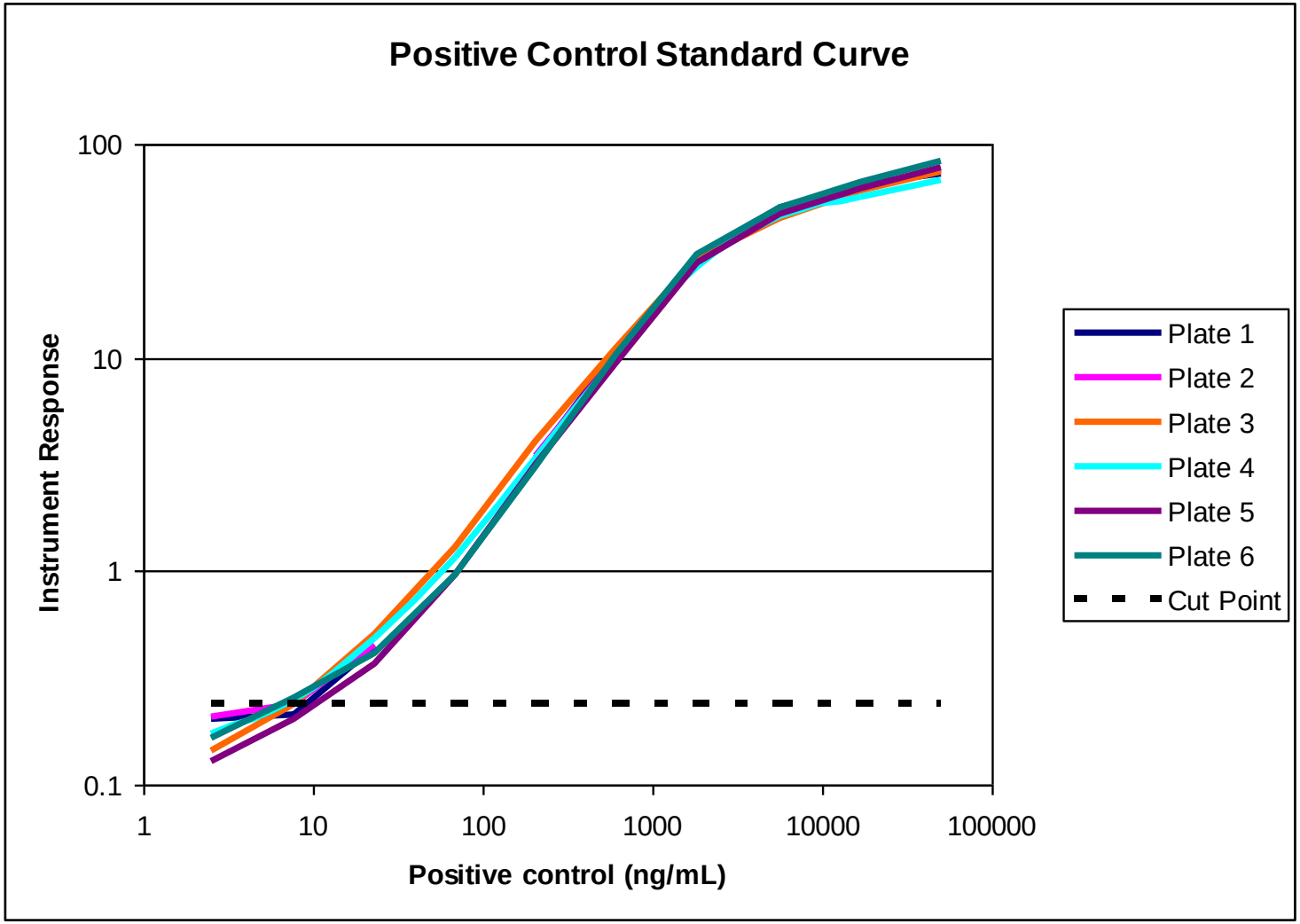
Immunogencity Assay 1

- Sequential Addition Bridging Assay
- Preclinical Species
- Bioaffy 1000CD
- Capture: Biotin-labelled Drug
- Sample: Positive Control Ab
- Detection: Alexa-647 labelled Drug

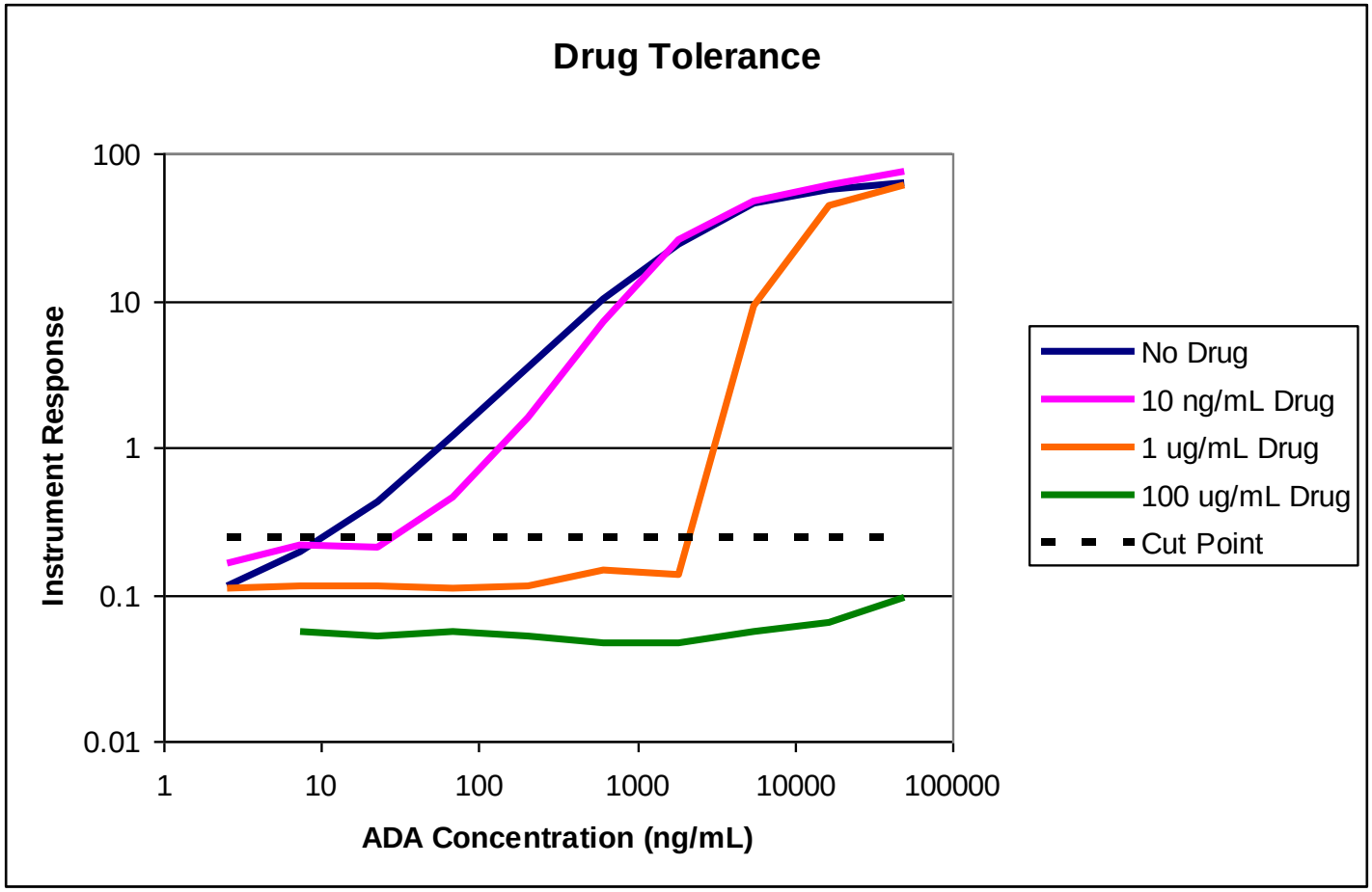


Sensitivity



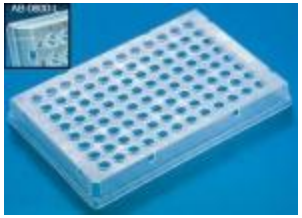
Drug Tolerance

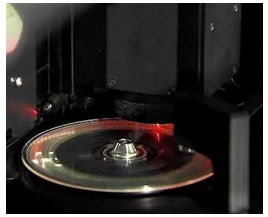
Method is not tolerant of 1 ug/mL drug in serum



Immunogenicity Learning Points

- If you have a low affinity positive control you will have poor assay sensitivity
- Sequential addition assays are vulnerable to drug interference
- Difficult to couple to acid dissociation
  - Can be achieved using a 1 step sample addition





Conclusions

- Is the Gyrolab a valuable addition to the regulated laboratory?
  - Gyrolab is just as susceptible to assay problems as other systems
  - Sample usage is not necessarily lower than other systems once minimum required dilutions are applied
  - Sample preparation time is roughly the same as ELISA/MSD
  - Reagents have to be labelled
  - Potential for laser signal change over time
  - Vulnerable to sample quality – aggregates and particulate matter can cause very heavy interference or blocked columns and needles.

Conclusions

- The Gyrolab is a valuable addition to the regulated laboratory
  - Open ended platform allowing development of your own assays
  - Low Sample Volume
  - Fast Individual Batch Turnaround Time
  - Rapid Method Development
  - (Relatively) Simple Reagent Labelling
  - Low intra-assay drift
  - Good reproducibility between replicates
  - Reduced variability between operators
  - Gyrolab Viewer gives additional information on detection label capture

Summary

- Gyrolab offers a robust open platform for rapid method development
- Fast single batch run-time makes the Gyrolab ideal for fast-turnaround studies
- Low sample volume can be advantageous in pre-clinical studies or rare matrices