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Singlicate Analysis in Ligand Binding Assays From Discovery to Regulated Clinical Studies: Implementation Strategies and Benefits

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INTRODUCTION

Singlicate analysis is a valuable tool that offers similar sensitivity and specificity to replicate analysis in various biofluids (matrices). It also adds flexibility to test various parameters simultaneously in early method development and increases the number of samples analyzed in regulated studies. Furthermore, singlicate analysis optimizes resource use and enhances workflow efficiency in the laboratory. It also allows us to make informed decisions early in the drug discovery stage when a large data set is required. By focusing bioanalytical efforts on individual samples, we can streamline the use of precious study samples (volume, rare sample), reduce consumable costs, and accelerate data generation for a large number of study samples without compromising robustness and quality.

In GLP and non-GLP studies, adherence to rigorous regulatory standards and scientific principles is fundamental to ensure the integrity and credibility of the bioanalytical data collected.

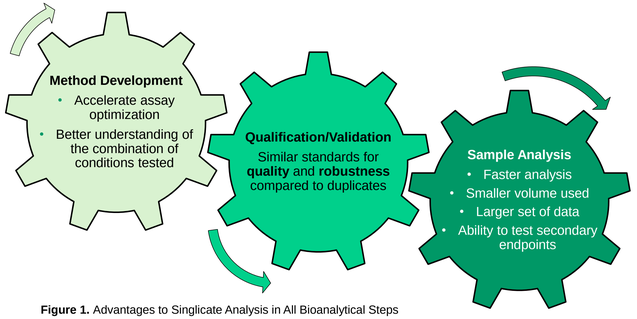


Figure 1. Advantages to Singlicate Analysis in All Bioanalytical Steps

From discovery to regulated clinical studies

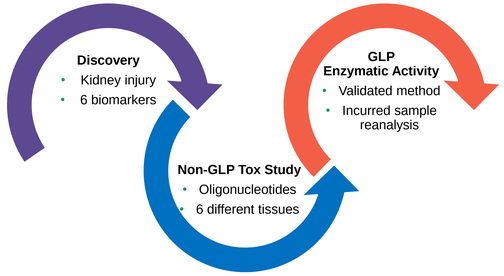


Figure 2. Singlicate Analysis at All Drug Development Stages

Drug Discovery: Biomarkers

Urine Rat Sample

Not a primary endpoint
Evaluated with 190 selected study samples

A total of 6 biomarkers:
One aliquot with limited volume available (limit F/T cycle). Evaluated using four commercial kits:
- two colorimetric assays
- single MSD assay
- 4plex MSD assay

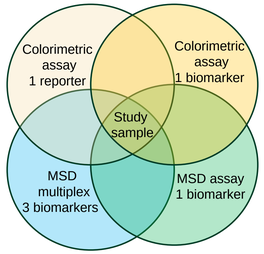
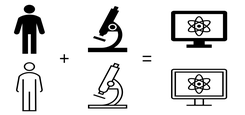


Figure 3. Combined Strategy for Kidney Injury Biomarker Evaluation in Rat Urine Sample

Quality: Assay Performance Evaluation

- Coordinate kit availabilities
- Assay performed by a different analyst
- Different days



Results of Evaluations Performed

Table 1. Overall Precision (% CV) and Accuracy (%bias) of STDs and QCs With Each Kit

Category	Assay type	All STD Run 1 Mean		All STD Run 2 Mean		All QC Run 1 Mean		ALL QC Run 2 Mean	
		%CV	%bias	%CV	%bias	%CV	%bias	%CV	%bias
Reporter	Colorimetric	1.3	0.0	**8.3**	0.0	1.3	-2.7	2.3	-3.6
Biomarker 1	Colorimetric	4.1	**0.4**	2.6	-0.2	**3.3**	**4.4**	1.8	0.2
Biomarker 2	MSD assay	2.0	-0.2	**4.0**	-0.3	4.3	-1.7	2.6	0.5
Biomarker 3	MSD plex	2.1	**2.7**	2.5	0.0	4.8	-3.0	1.3	-9.3
Biomarker 4		2.5	0.0	2.9	0.0	1.3	**-9.3**	2.1	-7.4
Biomarker 5		**9.6**	1.0	3.9	0.1	2.2	-3.9	**4.9**	-2.7

Colorimetric assays:
- STDs: 0.4 %bias and 8.3 %CV
- QCs: 4.4 %bias and 3.3 %CV

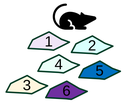
MSD assays:
- STDs: 2.7 %bias and 9.6 %CV
- QCs: -9.3 %bias and 4.9 %CV

Study Sample Analysis

- 190 x 6 samples ≤ 4 F/T cycle
- 12 total runs
- 2 analysts
- Each biomarker analyzed in 2 working days (WD)
- QCed data provided in 11 WD
- Complete BioA final report 39 WD

Non-GLP Tox Study for Oligonucleotide Drug

Disease Model



6 different tissues

Laboratory model

Tissue A

Preparation of STDs and QCs

Method Developed

Six precisions and accuracy singlicate runs
Bridging of QCs (6 tissue types) against tissue A standards

Inter-assay performance of QCs
Inter-assay performance of STDs

Figure 4. Strategy for Qualification of Oligonucleotide Assay to Analyze 6 Tissues

Table 2. Inter-Assay Precision and Accuracy of QCs Prepared in Tissue 1 From 6 Runs

	LLOQ QC (0.0300 ng/mL)	LQC (0.0800 ng/mL)	MQC (0.500 ng/mL)	HQC (7.50 ng/mL)	ULOQ QC (10.0 ng/mL)
Mean Concentration	0.0267	0.0758	0.4814	7.0309	9.3984
Inter-run SD	0.00370	0.00814	0.05902	0.95553	1.07032
Inter-run %CV	**13.9**	10.7	12.3	13.6	11.4
Inter-run %Bias	**-11.1**	-5.2	-3.7	-6.3	-6.0
Inter-run %Total Error	25.0	15.9	16.0	19.8	17.4

Table 3. Back-Calculated STD Performance in Precision and Accuracy Runs Prepared in Tissue A from 6 Runs

	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	0.0300	0.0600	0.100	0.400	1.00	4.00	8.00	10.0
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
Mean Conc.	0.0308	0.0564	0.107	0.399	0.990	4.040	8.451	9.629
Inter-Run S.D.	0.00138	0.00356	0.00325	0.0126	0.0383	0.215	0.371	0.422
Inter-Run %CV	4.5	**6.3**	3.1	3.2	3.9	5.3	4.4	4.4
Inter-Run %Bias	-5.4	**7.3**	-2.3	1.1	-3.9	5.8	-2.6	0.1

Table 4. Preliminary Bridging of 4 Tissue Types in Duplicate

QC Level	% C.V range			
	Tissue 1	Tissue 2	Tissue 3	Tissue 4
LLOQ	1.1 – 8.5	2.6 – 3.9	0.4 – 2.6	5.5 – 12.2
HQC	2.1 – 5.6	1.0 – 4.3	0.6 – 4.4	1.3 – 1.6

Singlicate analysis confirmed by:
- 6 PA runs
- Bridging of 4 tissues analyzed in duplicate against Tissue A.

Study Sample Analysis

> 900 samples
- Weighing
- homogenization

- 25 runs
- 2 analyst

- Interim monthly data transfer
- Fast analysis following sample reception

GLP Enzymatic Activity Study

GLP Study

Enzymatic activity
Human serum sample
Colorimetric assay

Method Validation

Six precisions and accuracy runs
- Surrogate matrix
- Human serum

Intra-assay precision of QCs ≤15.0%
Inter-assay precision of QCs

Table 5. Inter-assay Precision and Accuracy of QCs in Human Serum

Run Number	Concentration (nM)				
	LLOQ QC	LQC	MQC	HQC	ULOQ QC
	15000	37522.2	52485.52	97480.68	119991.4
Mean Concentration	15210.16	35025.66	51631.09	94329.67	112505.5
Inter-run SD	2450.61	4038.12	4984.67	9819.72	9274.06
Inter-run %CV	16.1	11.5	9.7	10.4	8.2
Inter-run %Bias	1.4	-6.7	-1.6	-3.2	-6.2
Inter-run %Total Error	17.5	18.2	11.3	13.6	14.5

- Maximum inter %CV at 16.1%
- Maximum inter %bias at -6.2%

Remaining validation evaluation performed in singlicate

Study Sample Analysis

> 600 samples

- 3 analysts
- 13 runs

- Analyzed in 9 WD
- ISR (93.2%) and parallelism evaluations met acceptance criteria

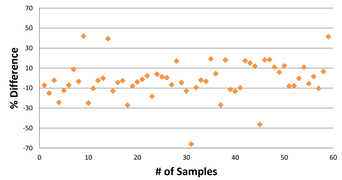


Figure 3. Incurred Sample Reanalysis

CONCLUSION

- The pertinence of singlicate sample analysis in GLP and non-GLP studies with various matrices and drug entities or biomarkers stems from its ability to maintain data reliability, regulatory compliance, and improve operational efficiencies.
- The singlicate analysis evaluation in bioanalytical methods was addressed by assessing %CV and %bias in various precision and accuracy runs.
- A thorough examination of assay performance for robustness evaluation in singlicate analysis is required. Typically, this involves comparing the replicate and singlicate methods to assess precision and accuracy across multiple runs.
- By adopting this bioanalytical strategy, the overall process of bioanalytical method development, validation, and sample analysis can be expedited.