

Singlicate analysis applied to pharmacokinetic ligand binding assays: case studies from a CRO perspective

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Singlicate LBA analysis – Status in 2020

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White Paper

Bioanalysis

European Bioanalysis Forum recommendation on singlicate analysis for ligand binding assays: time for a new mindset

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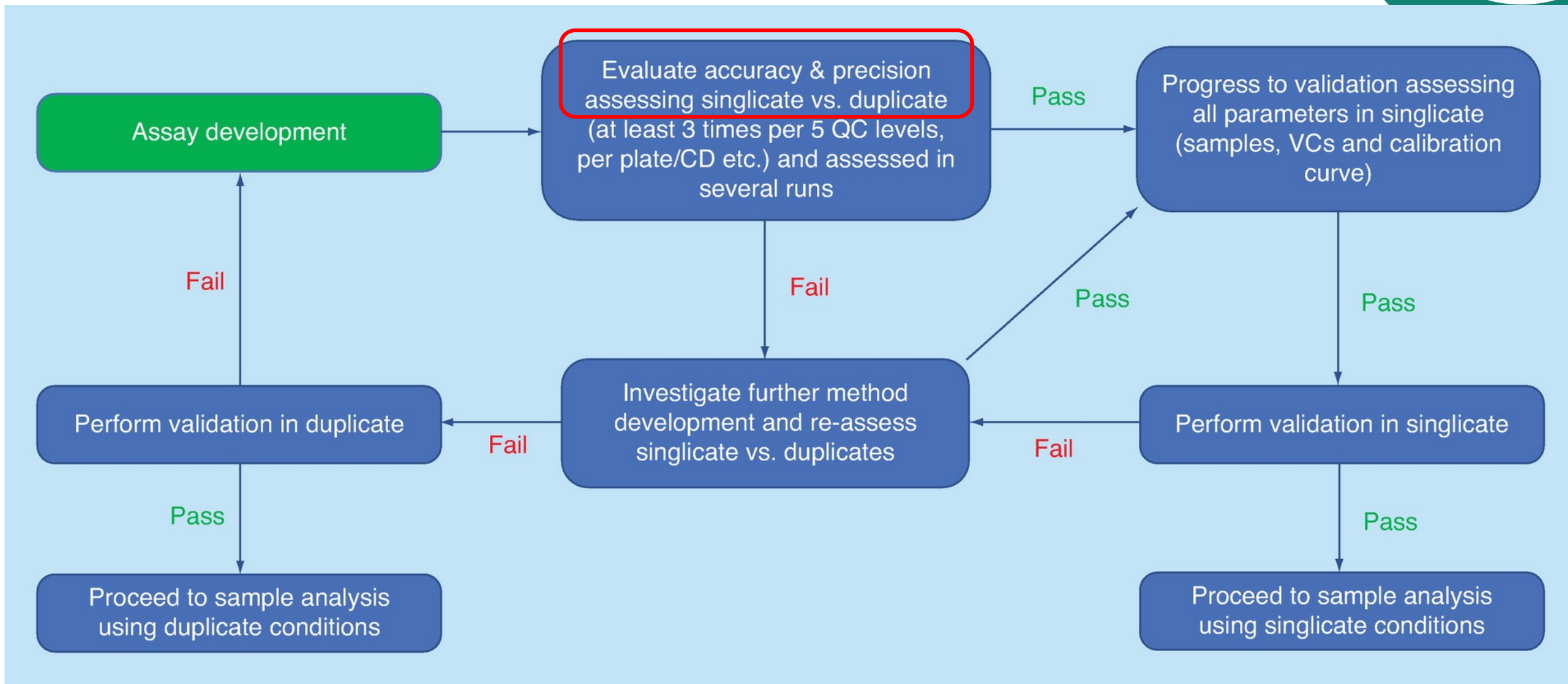
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Conclusions

- TK and PK data show no notable impact on results for C_{max} , AUC and half-life between singlicate and duplicate analyses
- Decision to perform singlicate analysis should be based on validation data
- ISR may be performed to confirm correctness of singlicate analysis

Example workflow for assessing singlicate analysis



Example 1

Duplicate and Singlicate validation

Example 1

Validation

- Original validation performed in duplicate
- Theoretical assessment performed on the original validation data set
- (re)validation performed in singlicate

Bioanalysis

- Performed in duplicate

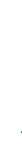
Example 1

Duplicate and Singlicate validation

Validation

Duplicate measurement	LLOQ	Low	Medium	High	ULOQ
Mean (µg/mL)	9.5	17.7	52.3	106	516
Overall bias (%)	-6.5	-12.3	4.4	5.5	-6.7
Total CV (%)	12.8	13.6	15.7	10.8	11.5
Total Error (%)	19.2	25.8	20.1	16.2	14.5
Theoretical singlicate measurement 1	LLOQ	Low	Medium	High	ULOQ
Overall bias (%)	-7.3	-13.3	5.0	4.3	1.4
Total CV (%)	14.3	15.7	17.0	11.0	12.3
Total Error (%)	21.5	28.9	21.9	22.1	13.6
Theoretical singlicate measurement 2	LLOQ	Low	Medium	High	ULOQ
Overall bias (%)	-5.8	-11.4	3.7	5.0	1.2
Total CV (%)	12.2	12.4	14.9	13.8	17.8
Total Error (%)	17.9	23.6	18.5	18.7	18.4

P&A data on theoretical singlicate assessment not robust



Perform validation in singlicate

Example 1

Duplicate and Singlicate validation

Actual singlicate measurement	LLOQ	Low	Medium	High	ULOQ
Overall bias (%)	-0.4	-5.5	-3.3	-0.3	-2.6
Total CV (%)	15.1	18.3	20.2	18.9	21.2
Total Error (%)	15.5	23.7	23.4	19.1	23.7

**Singlicate analysis impacted
the quality of the data**



**Performed bioanalysis in
duplicate**

Example 2

Duplicate and theoretical singlicate validation



Example 2

- **Validation**
- Original validation performed in duplicate
- Theoretical assessment performed on the original validation data set

- **Bioanalysis**
- Sample analysis in singlicate

Duplicate vs Singlicate A&P data in Validation

Validation

Duplicate measurement	LLOQ	Low	Medium	High	ULOQ
Mean ($\mu\text{g/mL}$)	4.89	14.8	146	2841	5121
Overall bias (%)	-2.7	-1.8	-3.7	2.6	2.5
Total CV (%)	5.7	6.2	2.6	6.0	9.7
Total Error (%)	8.2	7.9	6.2	8.5	12.2
Theoretical singlicate measurement	LLOQ	Low	Medium	High	ULOQ
Overall bias (%)	-2.9	-2.4	-3.9	3.3	2.4
Total CV (%)	5.5	6.4	2.6	5.8	9.8
Total Error (%)	8.3	8.7	6.4	9.0	12.1

Minimal impact of singlicate analysis in validation



Perform bioanalysis in singlicate

Other validation parameters (theoretical assessment) with singlicate analysis were all within acceptance criteria:

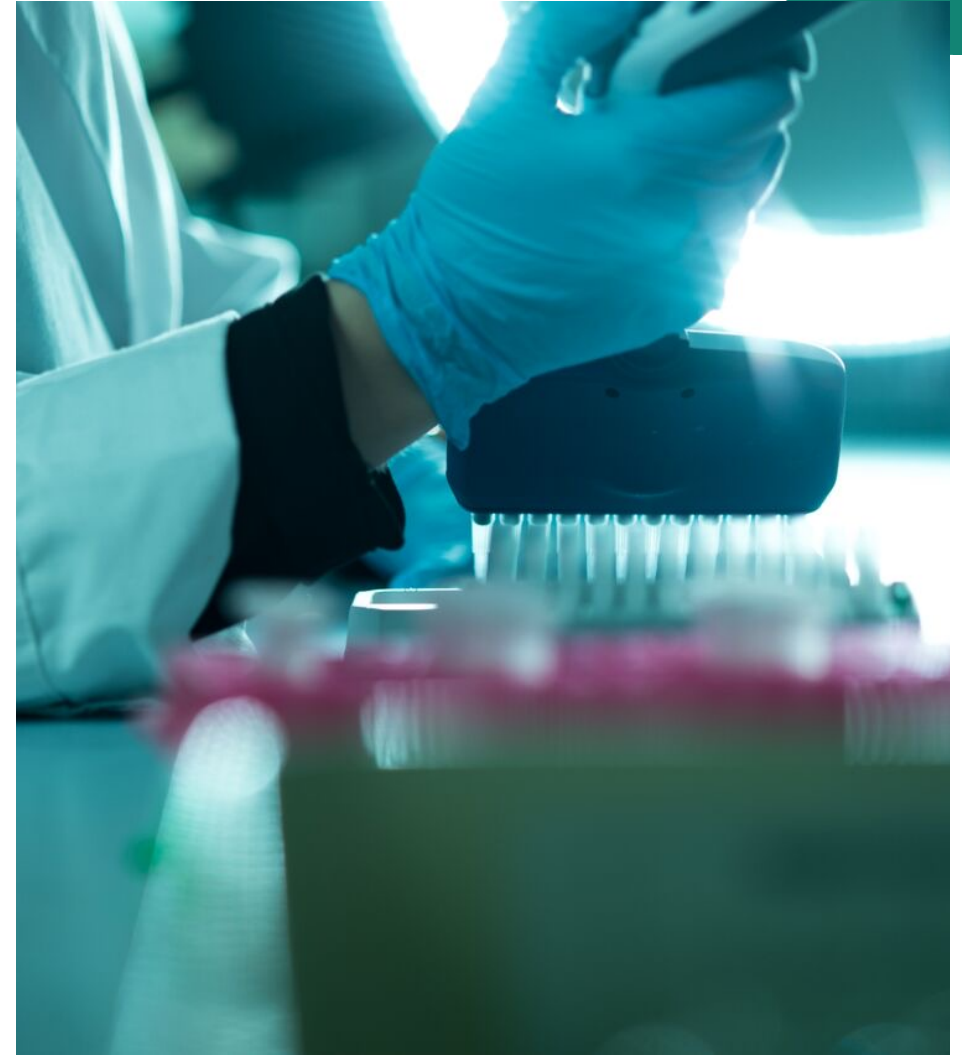
- Dilution linearity and Hook effect
- Selectivity in healthy and diseased matrix
- Freeze-thaw stability
- Bench-top stability
- Long term stability

Singlicate LBA analysis Status 2023 - ICH M10

“(...) study samples can be analysed using an assay format of 1 or more well(s) per sample. The assay format should be specified in the protocol, study plan or SOP.

If method development and assay validation are performed using 1 or more well(s) per sample, then study sample analysis should also be performed using 1 or more well(s) per sample, respectively.

**ICH M10
recommendation –
Section 4.2:
Validation**



Example 3

Singlicate validation

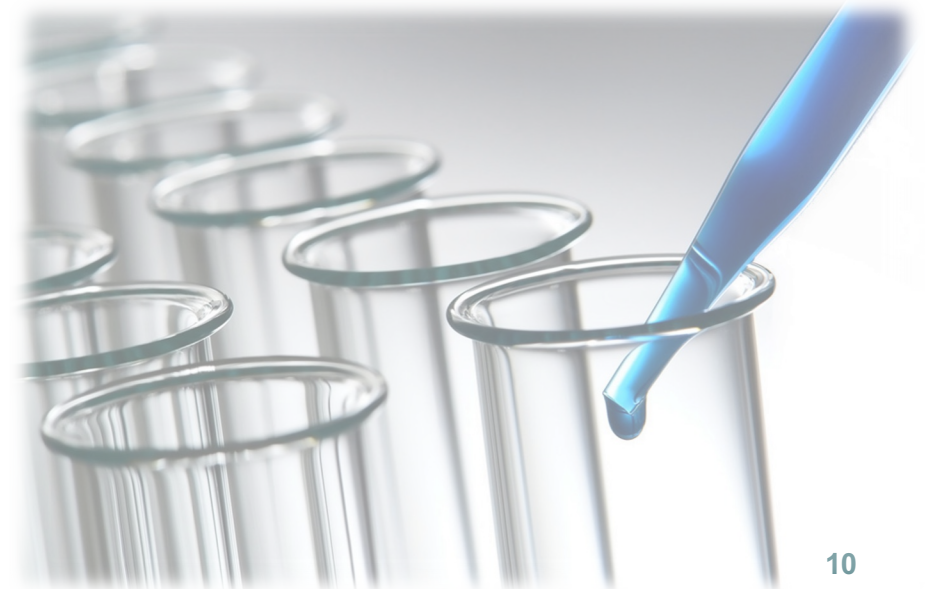
Example 3

Validation

- Validation performed in singlicate, based on scientific evaluation of MD data

Bioanalysis

- Analysis to be performed



ICH M10 approach

Robustness testing in Method Development

Method Development PK compound

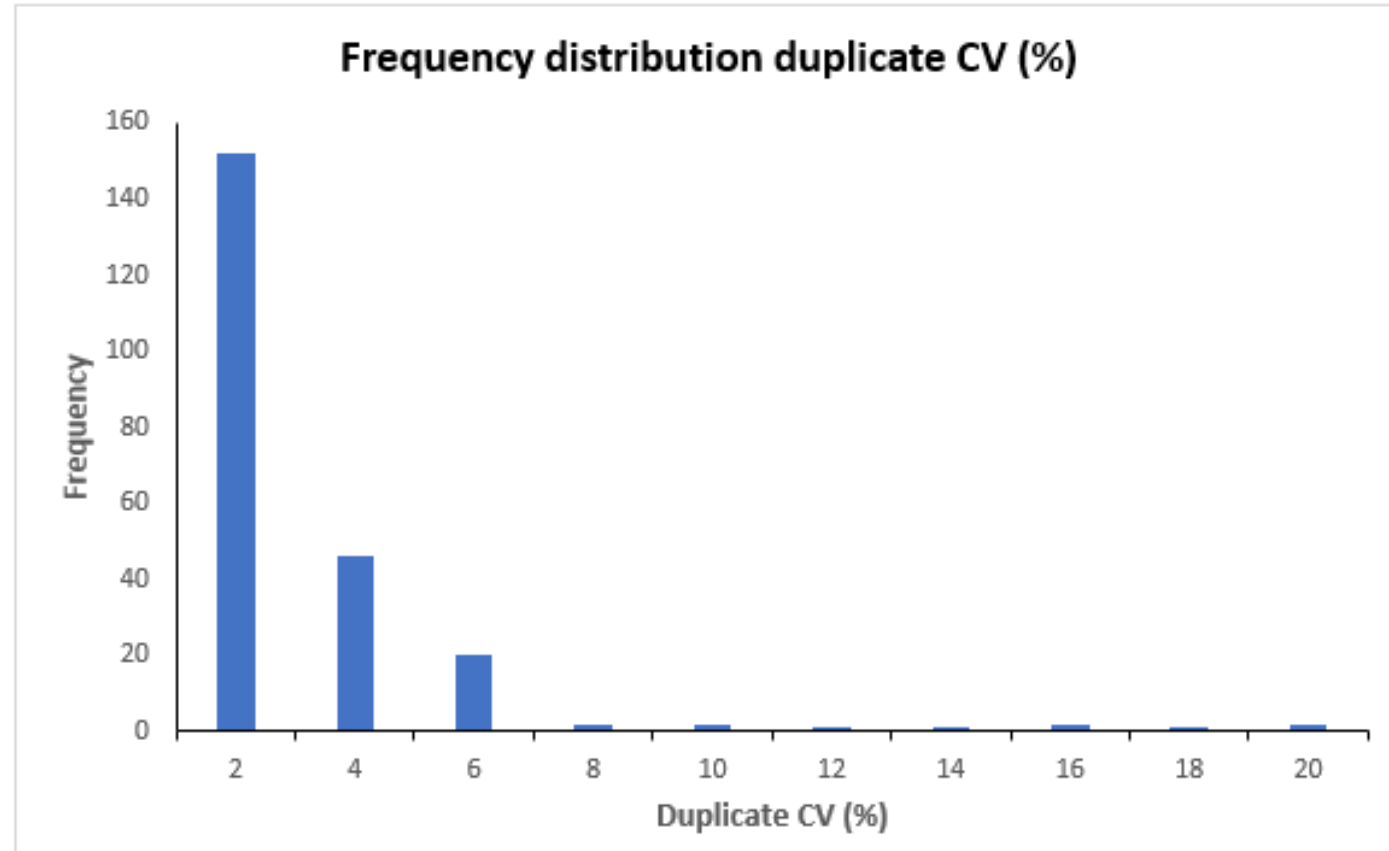
- First 10 MD experiments to be performed in duplicate to allow for an evaluation of %CV over duplicate samples
 - *Result: %CV <15.0% indicates sufficient robustness*
- Confirmation by performing Precision and Accuracy (3 runs) and Dilution Linearity (3 runs) in singlicate and duplicate
 - *In case results are within criteria > perform validation in singlicate*

ICH M10 approach

Evaluation of Robustness using Frequency distribution in Method Development

N:	301
min:	0.0
max:	20

% below 2% CV	66.3
% below 4% CV	86.4
% below 6% CV	95.1
% below 8% CV	96.0
% below 10% CV	96.8



Duplicate vs Singlicate data

Conclusions in Bioanalysis

Singlicate analysis

Samples analyzed	#samples per plate	#plates
2850	78	37

Theoretical duplicate approach

Samples analyzed	#samples per plate	#plates
2850	31	92



Conclusions

- Singlicate analysis increased the samples analyzed per day with approximately 20% (78 vs 62)
- Singlicate approach reduced number of plates by 60% (55 plates less)
- Singlicate approach reduces costs and is more sustainable (less consumables, including coating + detection material)

Take home messages

- Singlicate analysis can impact data quality. Proceeding to bioanalysis in singlicate must always be data driven. Assessment can be made during method development
- Singlicate analysis results in increased efficiency
 - Run more samples in a shorter time frame
 - Reduced time to perform a study
- Singlicate analysis leads to improved sustainability
 - Less reagents, materials and buffers are used
 - Less bridging experiments required (often a critical experiment)

More to gain:

- Develop the assay fit for singlicate analysis (e.g. incubation times, sample dilution steps)
- Automated pipetting in singlicate (high throughput on multiple robots)
- Use 384 well plate for even higher throughput

Questions?

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