Accuracy and Precision in Bioanalysis: Review of Case Studies

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Outline-Part I

- Overview of accuracy and precision from the May 2018 Bioanalytical Method Validation Guidance
- Use of freshly prepared calibrators and QCs for accuracy and precisions evaluations
- Acceptance criteria of calibration curves
- Use of separate stock solutions to prepare calibrators and QCs for accuracy and precision

Overview of accuracy and precision from the May 2018 Bioanalytical Method Validation Guidance

2018 BMV Guidance

- The May 2018 Bioanalytical Method Validation Guidance describes the Agency's current thinking of the topic and should be viewed as recommendations

- The recommendations in the guidance can be modified and justification should be provided

What is Accuracy and Precision?

- Accuracy is the proximity of measurement results to the true value (nominal)

- Precision is the proximity of measurement results to each other

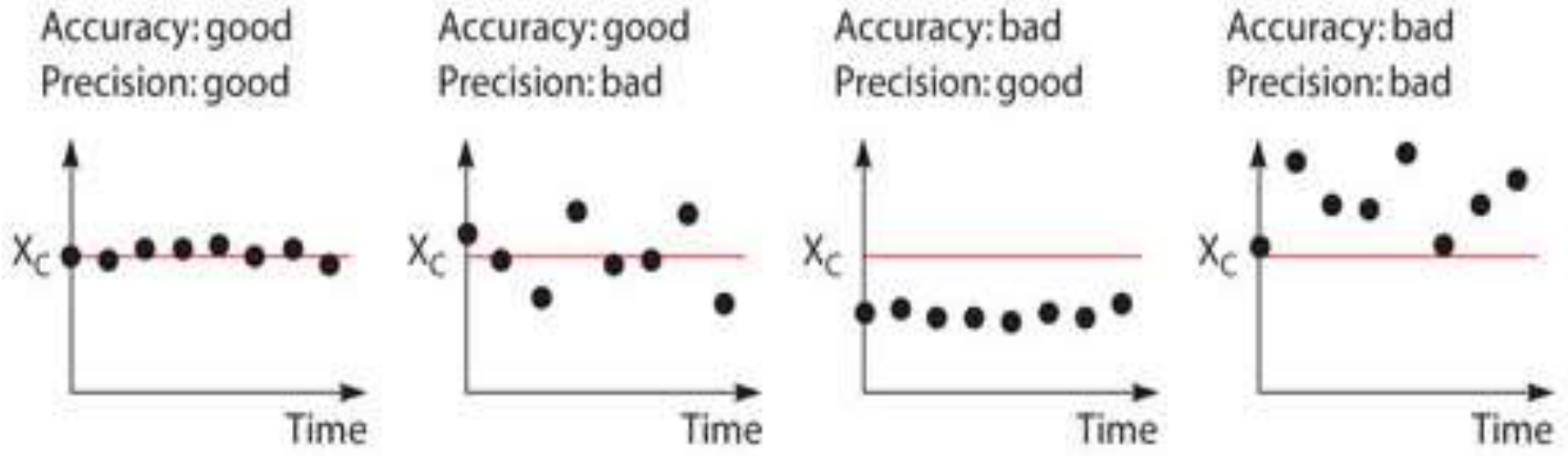


Figure source: http://www.edn.com/design/test-and-measurement/4388718/Manage-your-measurement-errors

Accuracy and Precision - Overview

- A&P evaluated by analyzing replicate QCs at multiple concentrations across the proposed assay range
- Chromatographic Assays (2001 BMV Guidance)
  - Accuracy should be measured using a minimum of five determinations per concentration
  - A minimum of three concentrations in the range of expected concentrations is recommended

Accuracy and Precision - Overview

- Chromatographic Assays (2018 BMV Guidance)
  - Should include 4 QC levels (LLOQ QC, Low QC, Mid QC, and High QC) per run
  - At least five replicates per QC level
  - At least three independent A&P runs

Accuracy and Precision - Overview

Chromatographic Assays

- Accuracy: ±15% of nominal concentration, ±20% of nominal concentration at LLOQ
- Precision: ≤15% CV, ≤20% CV at LLOQ

Accuracy and Precision - Overview

- Ligand Binding Assays
  - Should include 5 QC levels (LLOQ QC, Low QC, Mid QC, High QC, and ULOQ QC) per run
  - At least three replicates per QC level
  - At least six independent A&P runs

Accuracy and Precision - Overview

Ligand Binding Assays

- Accuracy: ±20% of nominal concentration, ±25% of nominal concentration at LLOQ, ULOQ
- Precision: ≤20% CV, ≤25% CV at LLOQ, ULOQ

Accuracy and Precision - Overview

- Although the accuracy and precision expectations are provided in the guidance, QCs that perform consistently “close” to the acceptance criteria may indicate analytical issues and an investigation should be performed

Accuracy and Precision - Overview

- 2001 BMV guidance states that at least four of every six QCs should be ±15% of nominal (chromatographic) or ±20% of nominal (LBA)
- 2018 BMV guidance states that ≥67% of QCs should be ±15% of nominal (chromatographic) or ≥67% of QCs should be ±20% of nominal (LBA)
- Does that mean that 5/6 QCs need to pass? Recommend that 4/6 (66.67%) of QCs need to meet the acceptance criteria

Accuracy and Precision - Overview

- ISR verifies the reliability of the reported study sample analyte concentrations
- At least 67% of samples should be ±20% of the mean
- If ISR meets the acceptance criteria yet shows large differences between results for multiple samples, this may indicate analytical issues and it is advisable to investigate

Use of freshly prepared calibrators and QCs for accuracy and precisions evaluations

A&P - Cals and QCs

Method Development

- 2018 BMV Guidance states that freshly prepared QCs are recommended for A&P evaluations during method development
  - Stability data are generally not available during method development

A&P - Cals and QCs

Method Validation

- 2018 BMV Guidance states that the sponsor should prepare calibrators and QCs from separate stock solutions
- Freshly prepared calibrators and QCs in all A&P runs is preferred; however, if this is not possible, the sponsor should use freshly prepared QCs in one or more A&P runs

A&P - Cals and QCs

- Not unusual for other tests (e.g., bench-top stability, F/T stability, short-term stability) to be included on A&P runs during method validation

- If other tests on A&P runs include stability evaluations, can frozen calibrators and/or frozen QCs be used in later A&P runs?

A&P - Cals and QCs

Method Validation

- OSIS has audited method validations where freshly prepared calibrators or freshly prepared QCs were used in A&P runs
- If the QCs are within ±15% for accuracy and ≤15% precision, what additional justification is necessary if not using freshly prepared calibrators and QCs?

Acceptance criteria of calibration curves

Calibration Curve

Calibration curve acceptance criteria (2001 BMV Guidance)

- Chromatographic Assays
  - 75%, or a minimum of six standards, when back-calculated (including ULOQ) should fall within ±15%, except for LLOQ, when it should be ±20% of the nominal value. Values falling outside these limits can be discarded, provided they do not change the established model.

Calibration Curve

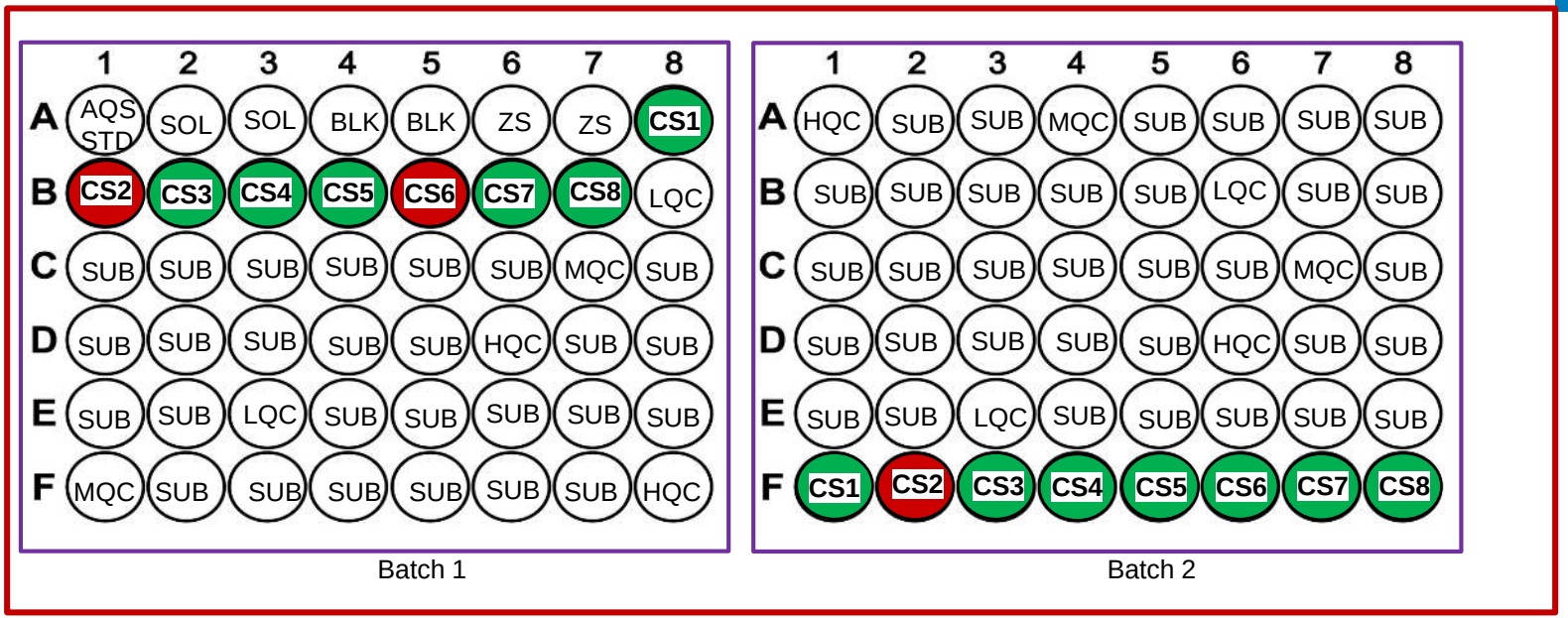
Calibration curve acceptance criteria (2018 BMV Guidance)

- Chromatographic Assays
  - Non-zero calibrators should be ±15% of nominal (theoretical) concentrations, except at LLOQ where the calibrator should be ±20% of the nominal concentrations in each validation run
  - 75% and a minimum of six non-zero calibrator levels should meet the above criteria in each validation run

Calibration Curve

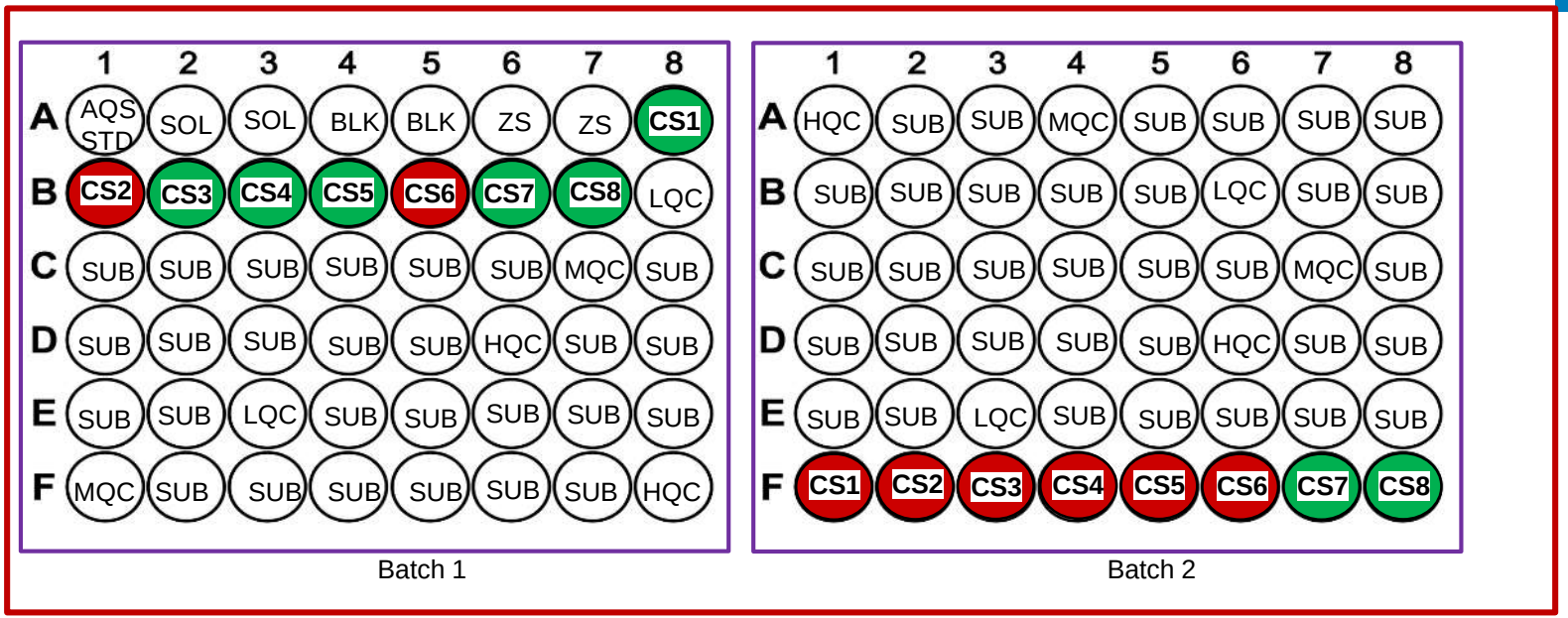
- 2018 BMV Guidance doesn’t discuss the use of two calibration curves
- If two calibration curves are used for regression, the guidance doesn’t provide criteria for the individual curves

Chromatographic Assays



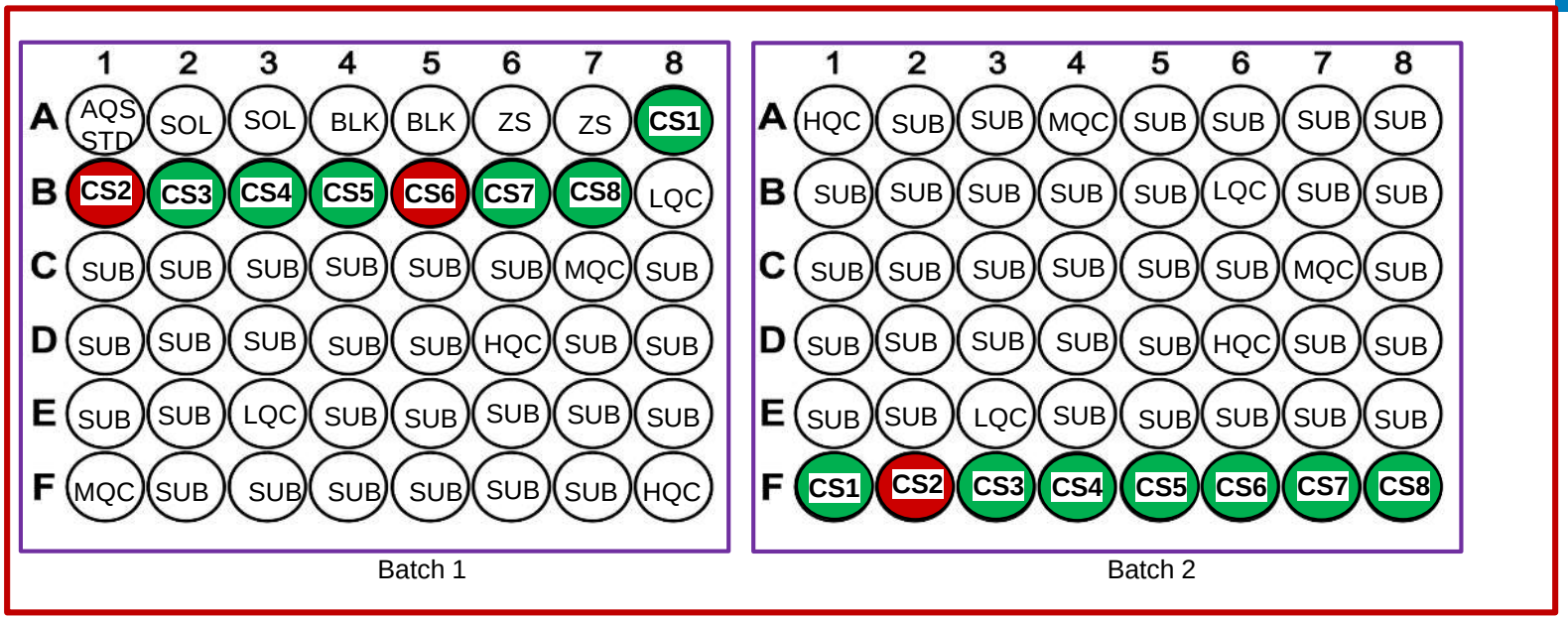
Run Acceptance (2001 Guidance) - 75%, or a minimum of six standards (13/16=81%) ✓

Chromatographic Assays



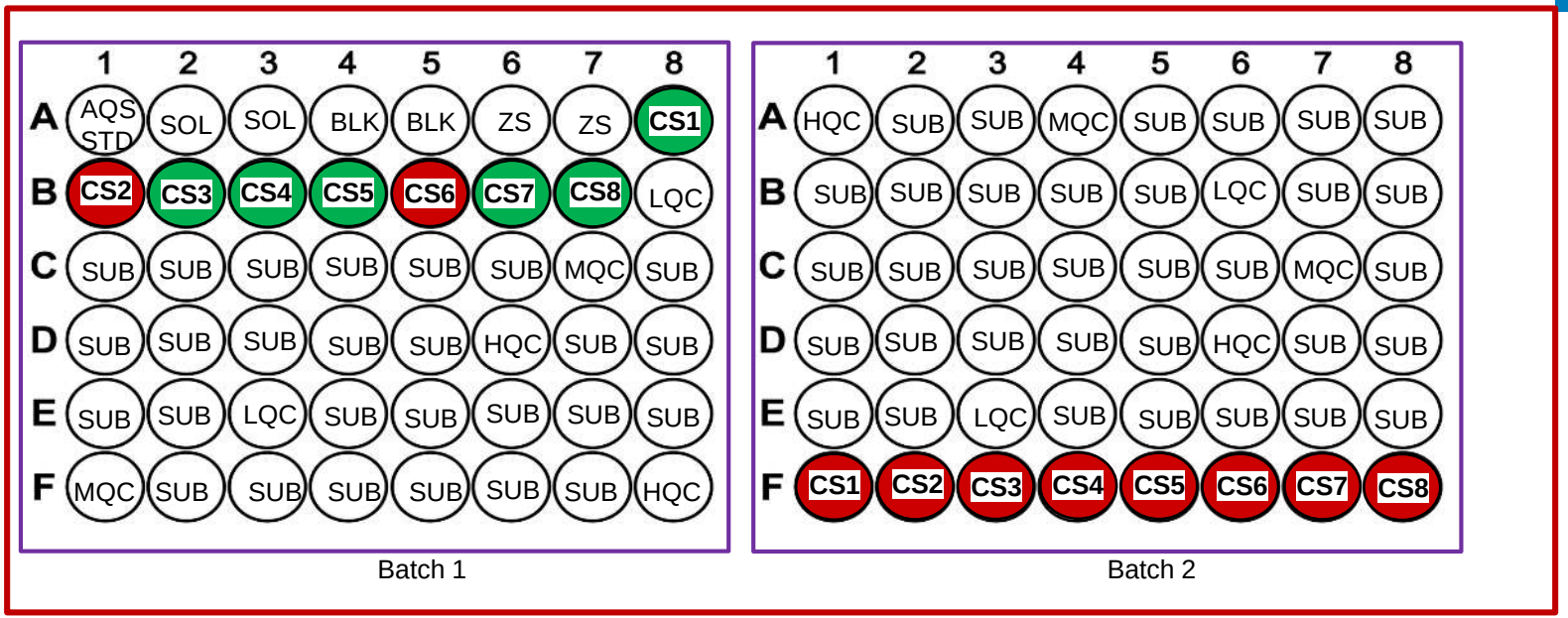
Run Acceptance (2001 Guidance) - 75%, or a minimum of six standards (8/16=50%)

Chromatographic Assays



Run Acceptance (2018 Guidance)-75% and a minimum of six non-zero calibrator levels (6/8=75%) ✓

Chromatographic Assays



Run Acceptance (2018 Guidance)-75% and a minimum of six non-zero calibrator levels (6/8=75%) ✓

Calibration Curve

- If two calibration curves were included in a run, are both curves included in the regression? If so, how is the regression being calculated?
- Even if the second calibration curve is not included in the regression, should we be concerned if the majority of the calibrators fail?

Use of separate stock solutions to prepare calibrators and QCs

A&P - Stock Solutions

- 2001 BMV Guidance states that calibration standards and QC samples can be prepared from the same spiking stock solution, provided the solution stability and accuracy have been verified

A&P - Stock Solutions

- 2018 BMV Guidance states that the sponsor should prepare any calibration standards and QCs from separate stock solutions.

- However, if the sponsor can demonstrate the precision and accuracy in one validation run using calibrators and QCs prepared from separate stock solutions, then the sponsor can use calibrators and QCs prepared from the same stock solution in subsequent runs.

A&P - Stock Solutions

- Accuracy of stock solutions are commonly evaluated with a stock check (comparison of peak areas from both stock solutions)

- During inspections, OSIS has observed CROs making two stock solutions, performing a stock check, and if peak areas within 5%, discarding one of the stock solutions

A&P - Stock Solutions

- How should the accuracy of stock solutions be evaluated?
- If the A&P of calibrators and QCs prepared from separate stock solutions are evaluated in a validation run, does that demonstrate that the stock solutions were accurately prepared?

A&P - Stock Solutions

- Performing a stock check or preparing calibrators and QCs from separate stock solutions in a validation run supports reproducibility

- Justification should be provided if a single stock solution is used to prepare calibration standards and QCs

Part II

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Outline-Part II

- Run acceptance of Precision and Accuracy runs during method validation - is there such a thing?
- Use of acceptance criteria for processing batches-do we use overall run acceptance criteria or acceptance criteria of each processing batch?
- QCs meet the 67% overall and 50% acceptance criteria at each concentration: Will there be any concerns?

Run acceptance of Precision and Accuracy runs during method validation - is there such a thing?

How do you measure Accuracy and Precision?

- The run should meet the calibration curve acceptance criteria and include the LLOQ calibrator
- Determined by replicate analysis of samples containing known amounts of analyte
- The deviation of the mean from the nominal value (relative error) serves as the measure of accuracy
- The imprecision determined as coefficient of variation (CV) at each concentration level and serves as measure of precision

A&P runs have has no QC acceptance criteria. Why?

- A&P runs are **predictive** of how well the method being validated will perform during sample analysis.
- QCs in A&P runs serve to mimic study samples
- Not including “bad” QC data and including only “good” QC data during A&P assessment gives a false estimate of Accuracy and Precision of the method
- Method may seem better than it actually is

Accuracy and Precision run

EXPERIMENT NAME/ NUMBER			A&P 01 June 1, 2019		
ANALYTE NAME			DRUG X		
Batch	Concentration			pg/mL	
	Quality control samples ID				
	HQC	MQC	M1QC	LQC	LLOQ QC
A&P 01 June 1, 2019	10716.636	3234.383	2120.424	236.731	83.212
	11810.294	3247.814	2311.186	235.935	80.000
	11172.564	3274.028	2087.116	236.423	87.573
	11069.598	3127.053	2131.921	232.577	71.661
	10617.270	3178.977	2082.714	238.313	76.000
	10289.890	3168.921	2162.111	213.073	89.467
Mean	10946.0420	3205.1960	2149.2453	232.1753	81.3188
SD ±	530.04970	55.70576	84.62574	9.54634	6.81754
Precision (%CV)	4.8	1.7	3.9	4.1	8.4
Nominal value	11999.647	3009.705	2031.551	224.394	74.985
Accuracy (%)	91.2	106.5	105.8	103.5	108.4
n	6	6	6	6	6

Accuracy ± 15% of Nominal (± 20% at LLOQ)

Precision ± 15% of Nominal (± 20% at LLOQ)

All QCs have to be included In the analysis

Case Study

- During an inspection of a bioequivalence study, the following findings were noted for an experiment to estimate accuracy and precision
- QCs were at 5 levels including LLOQ QC, Low, Mid 1, Mid and High QC levels
- Some replicate QCs at various levels were excluded from analysis
- The accuracy and precision calculated from the included QCs were within acceptable limits

Accuracy and Precision run-Case Study

EXPERIMENT NAME/ NUMBER	A&P 01 June 1, 2019				
ANALYTE NAME	DRUG X				
Batch	Concentration pg/mL				
	Quality control samples ID				
	HQC	MQC	M1QC	LQC	LLOQ QC
A&P 01 June 1, 2019	10716.636	3400.383	-	-	87.212
	-	3247.814	2311.186	235.935	-
	11172.564	-	2087.116	-	87.573
	11069.598	3127.053	2131.921	232.577	85.661
	10617.270	3178.977	-	238.313	80.000
	10289.890	3168.921	2162.111	-	-
Mean	10773.1916	3224.6296	2173.0835	235.6083	85.1115
SD ±	356.52685	107.39597	97.08666	2.88192	3.50716
Precision (%CV)	3.3	3.3	4.5	1.2	4.1
Nominal value	11999.647	3009.705	2031.551	224.394	74.985
Accuracy (%)	89.8	107.1	107.0	105.0	113.5
n	5	5	4	3	4

Case Study

- The site was asked to recalculate the accuracy and precision after including all QC values

Accuracy and Precision run-Case Study

EXPERIMENT NAME/ NUMBER	A&P 01 June 1, 2019				
ANALYTE NAME	DRUG X				
Batch	Concentration pg/mL				
	Quality control samples ID				
	HQC	MQC	M1QC	LQC	LLOQ QC
A&P 01 June 1, 2019	10716.636	3400.383	1500.424	325.731	87.212
	7000.294	3247.814	2311.186	235.935	105.000
	11172.564	4000.714	2087.116	350.145	87.573
	11069.598	3127.053	2131.921	232.577	85.661
	10617.270	3178.977	2500.714	238.313	80.000
	10289.890	3168.921	2162.111	175.124	50.250
Mean	10144.3753	3353.9770	2115.5787	259.6375	82.6160
SD ±	1572.94262	331.07645	337.11991	65.50008	17.94665
Precision (%CV)	15.5	9.9	15.9	25.2	21.7
Nominal value	11999.647	3009.705	2031.551	224.394	74.985
Accuracy (%)	84.5	111.4	104.1	115.7	110.2
n	6	6	6	6	6

Case study

Observation

Failure to report all validation data for estimation of assay Accuracy and Precision. Inclusion of unreported results led to failure of the experiment to meet acceptance criteria

Recommendations

- All data generated for estimation of accuracy and precision should be included in the analysis except those where errors are documented due to “assignable causes”

- Assessment of A&P should include minimum of 3 (chromatographic) and 6 (LBA) experiments; however, ALL runs should be included in global assessment of A&P if more than 3 (chromatographic) and 6 (LBA) experiments are conducted

- “Within run” and “ Between run” accuracy and precision should be determined for each QC level including LLOQ QC

Use of acceptance criteria for processing batches- do we use overall acceptance criteria or acceptance criteria of each processing batch?

Recommendations from BMV

- Bioanalytical method may necessitate separation of the overall analytical run into distinct processing batches

- Samples in the run exceeds the capacity of a 96-well plate or when a solid phase extraction manifold cannot accommodate all samples

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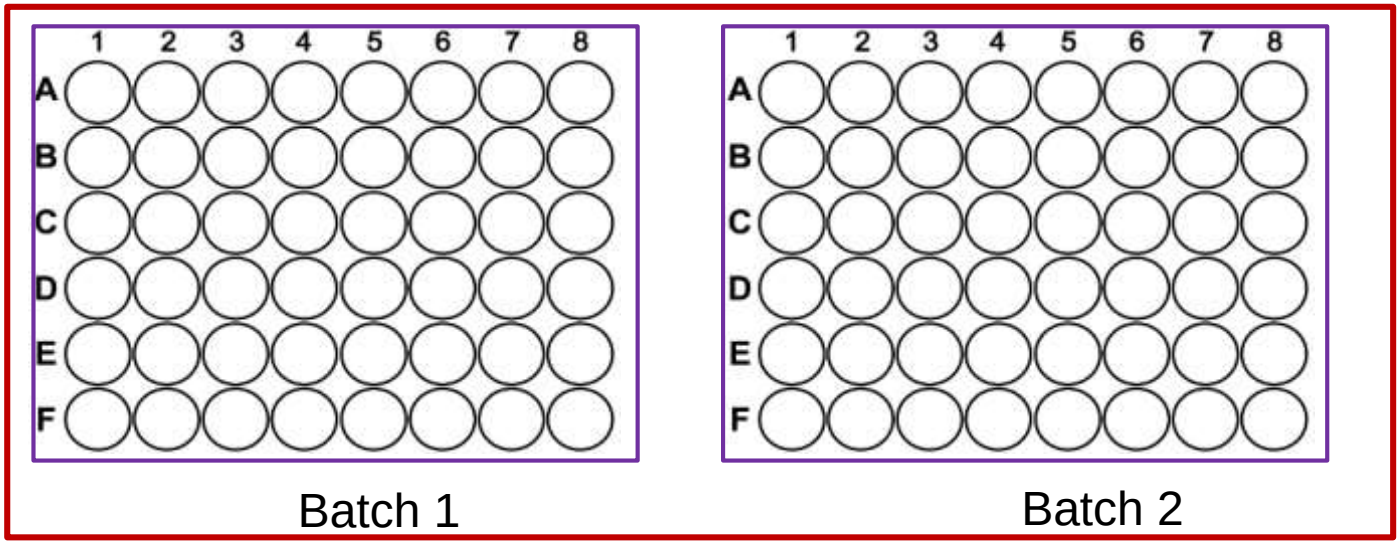
- During sample analysis, each distinct process batch should contain duplicate QCs at all levels (e.g., low, mid, high) along with the study samples

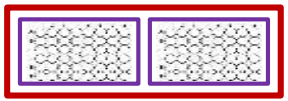
- During sample analysis, a distinct batch or batches in an analytical run may be rejected when it fails to meet QC acceptance criteria; however, remaining batches may pass provided that the analytical run meets the overall QC acceptance criteria

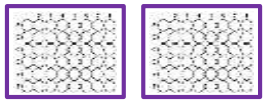
Questions

- What is the acceptance criteria of an analytical run?
- What is the acceptance criteria for a batch?
- Can we reject individual batch/es when the analytical run meets acceptance criteria?

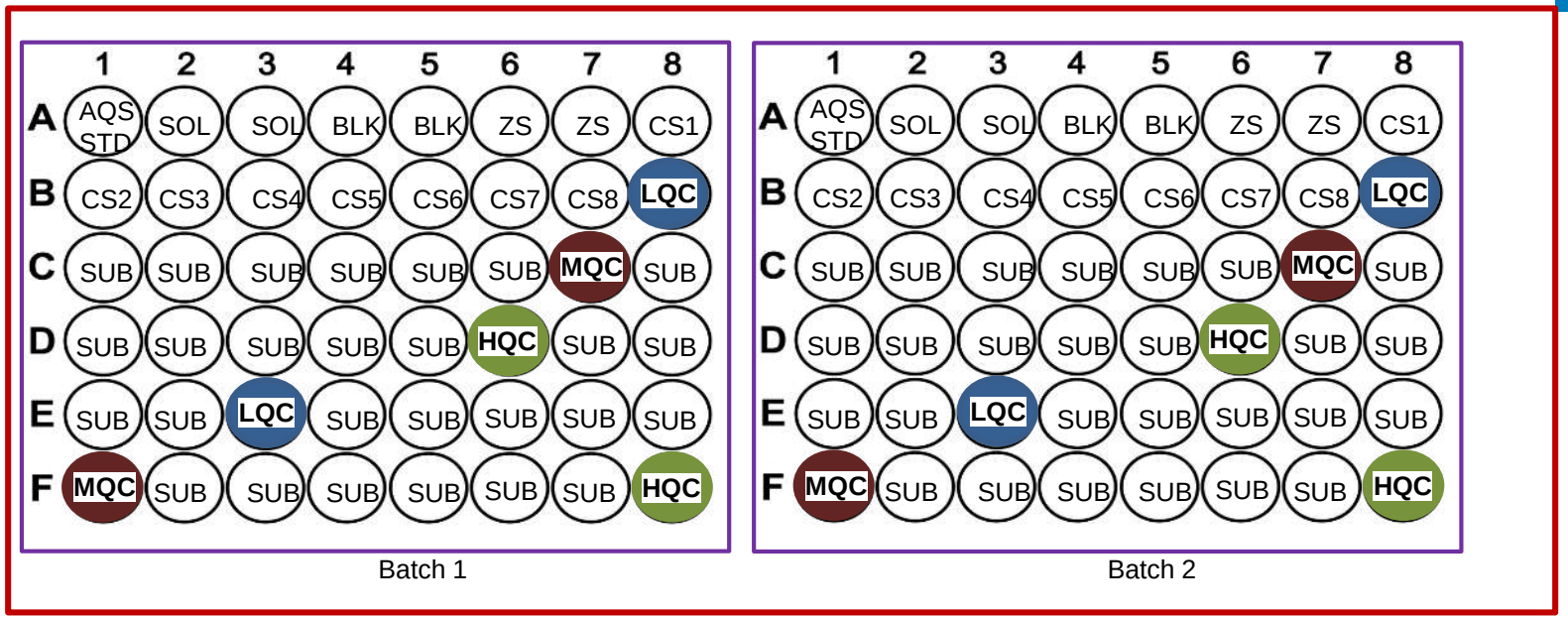
Batch vs Run



 = RUN

 = Batch

Chromatographic Assays



Batch Acceptance Criteria

67% of QCs should be ± 15% of Nominal
≥ 50% of QCs per level should be ± 15% of their nominal

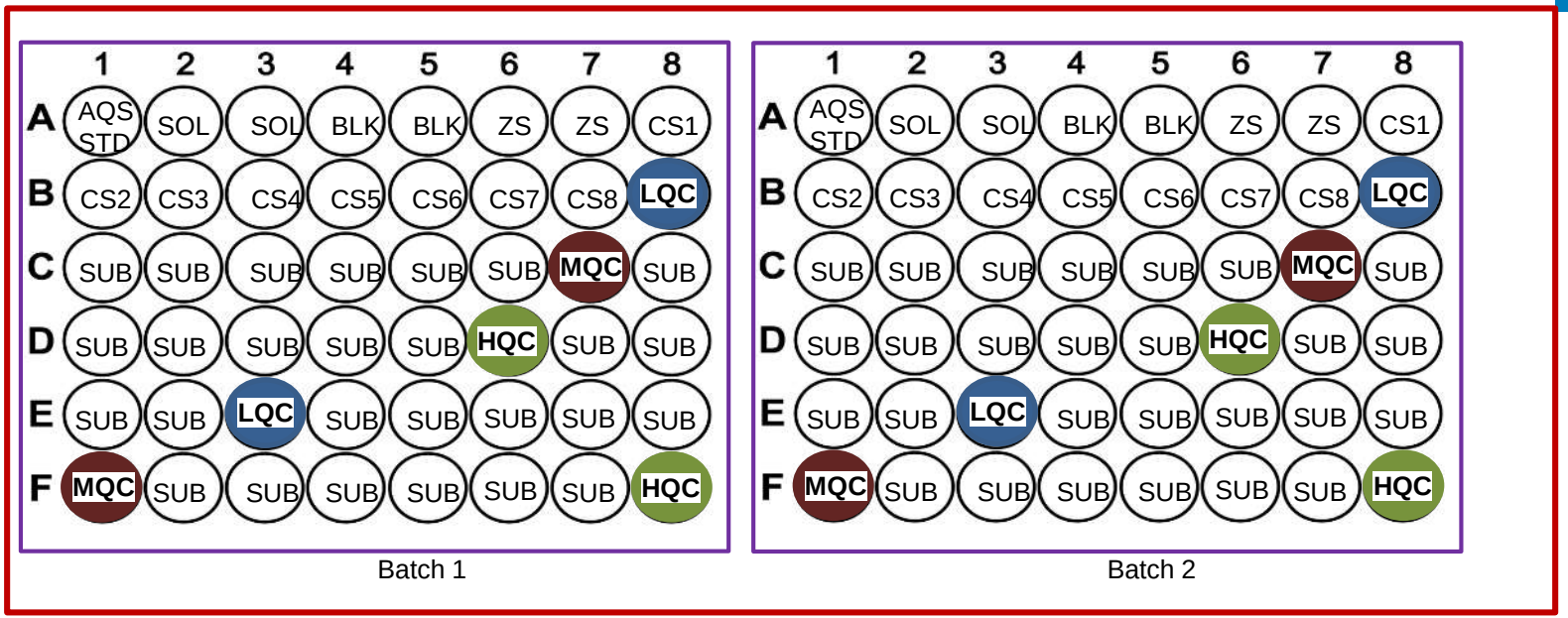
4/6 4/6

Run Acceptance Criteria

67% of QCs should be ± 15% of Nominal
≥ 50% of QCs per level should be ± 15% of their nominal

8/12

Ligand Binding Assays



Batch Acceptance Criteria

67% of QCs should be ± 20% of Nominal

≥ 50% of QCs per level should be ± 20% of their nominal

4/6 4/6

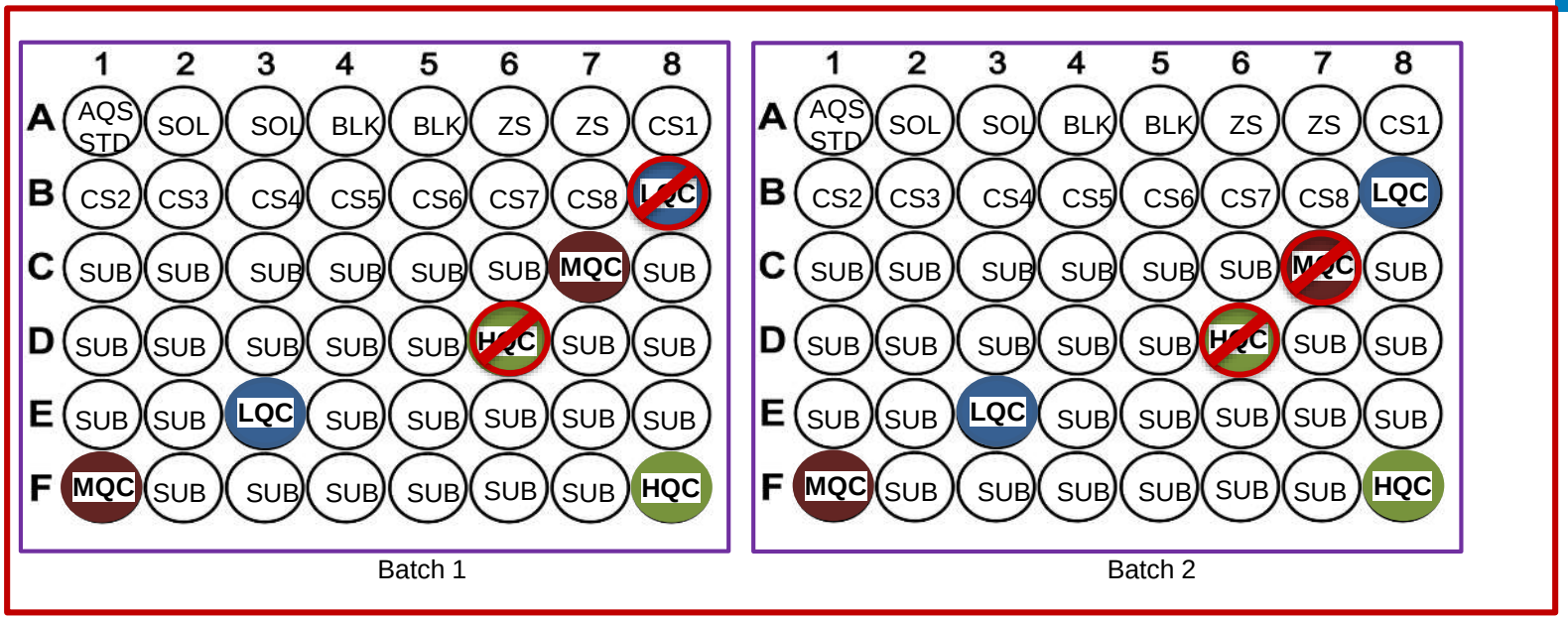
Run Acceptance Criteria

67% of QCs should be ± 20% of Nominal

≥ 50% of QCs per level should be ± 20% of their nominal

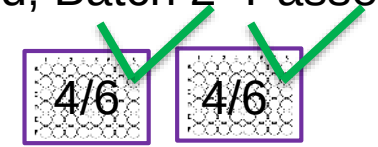
8/12

Chromatographic Assays



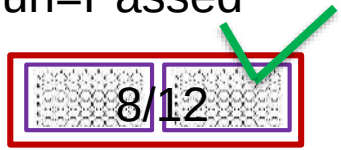
Batch Acceptance Criteria

Batch 1=Passed; Batch 2=Passed

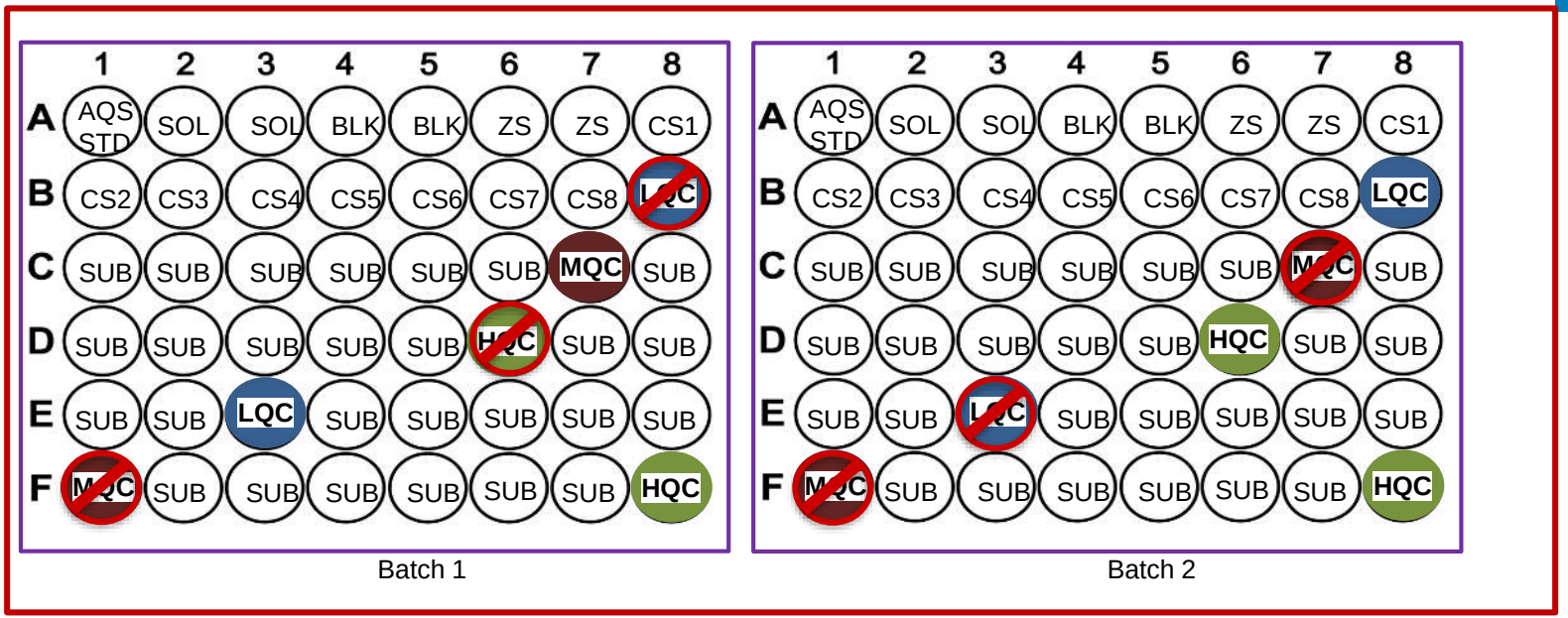


Run Acceptance Criteria

Run=Passed



Chromatographic Assays



Batch Acceptance Criteria

Batch 1=Failed; Batch 2=Failed

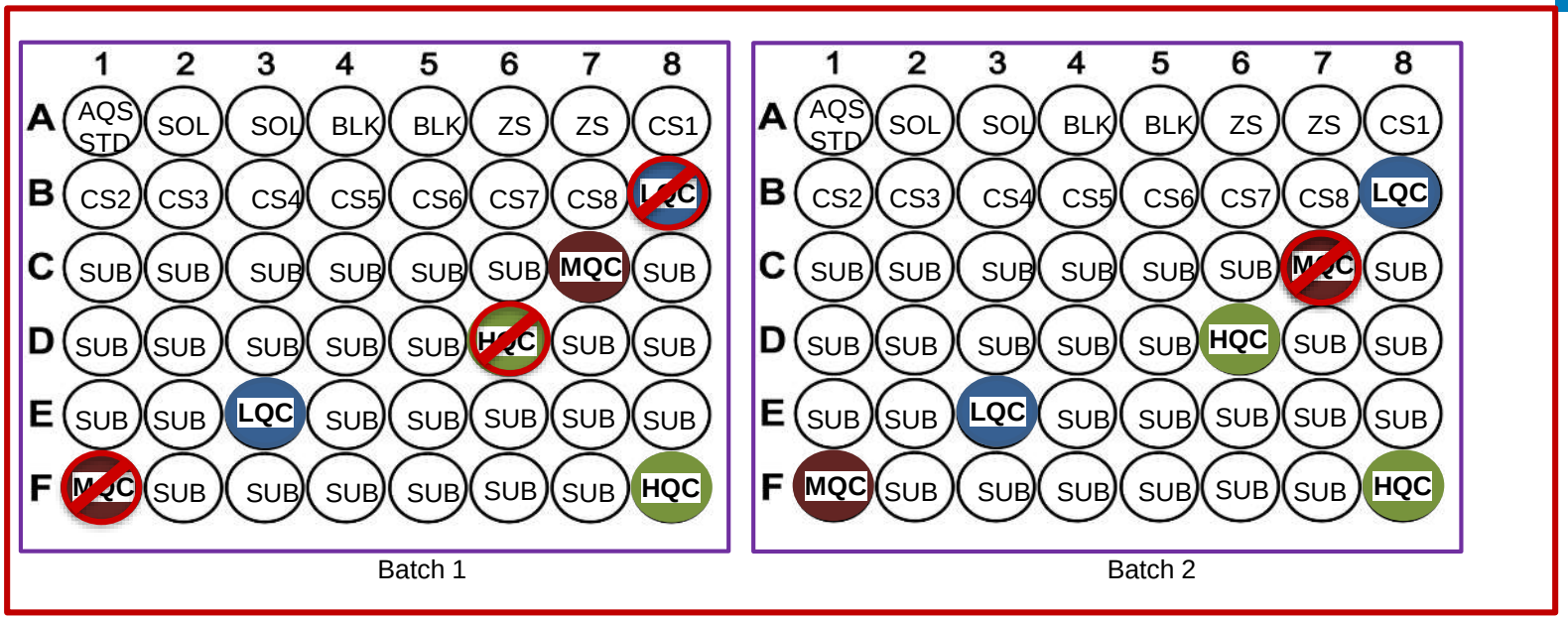
3/6 3/6

Run Acceptance Criteria

Run=Failed

6/12

Chromatographic Assays



Batch Acceptance Criteria

Batch 1=Failed; Batch 2=Passed

3/6 5/6

Run Acceptance Criteria

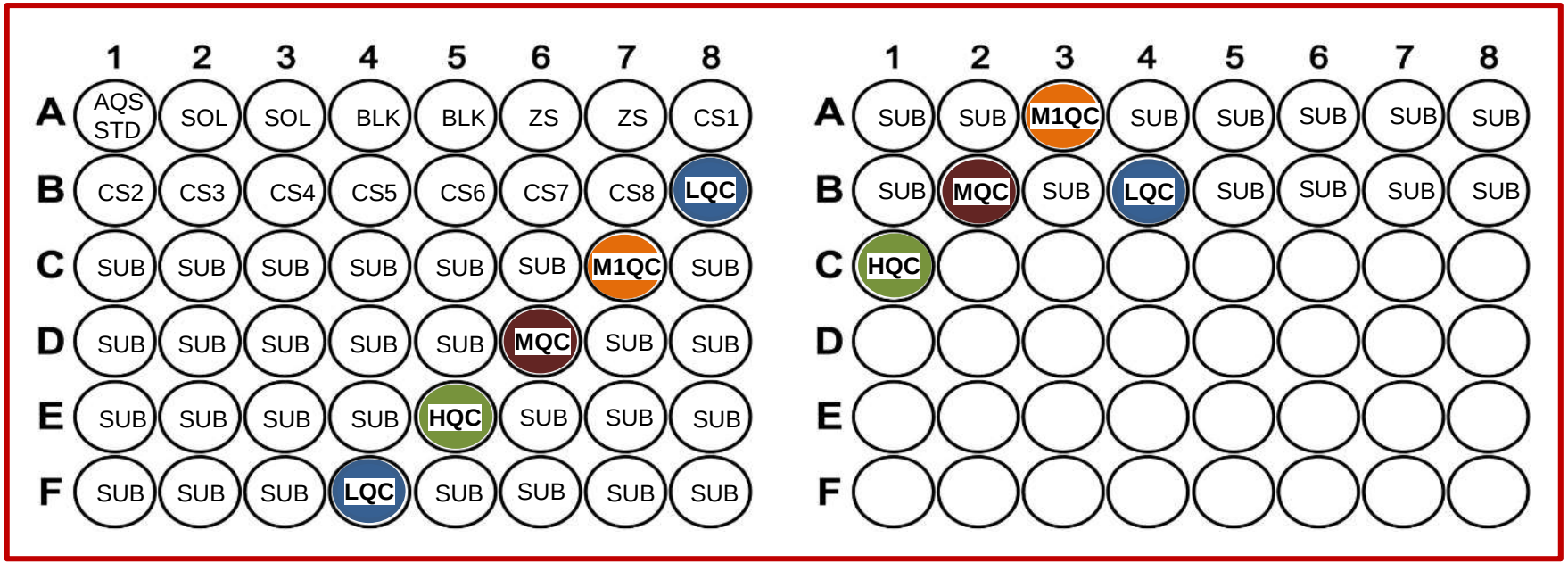
Run=Passed

8/12

Case Study

- An observation was made during inspection of a bioanalytical study and associated method validation
- Solid Phase Extraction (SPE) method used a 48 port SPE manifold
- Injection sequence consisted of 67 samples in two processing batches.
- 9 QCs at 4 QC levels, Low, Mid 1, Mid and High QCs

Organization of SPE manifold



AQS STD= Aqueous Standard
SOL=Solvent
BLK=Blank
ZS=Zero Standard

LQC= Low QC
M1QC=Mid QC1
MQC=Mid QC
HQC=High QC
SUB=Subject sample

Organization of SPE manifold

Concerns with the previous example:

- Although duplicate QCs existed across the run, each processing batch did not have a duplicate set of QCs

Recommendations

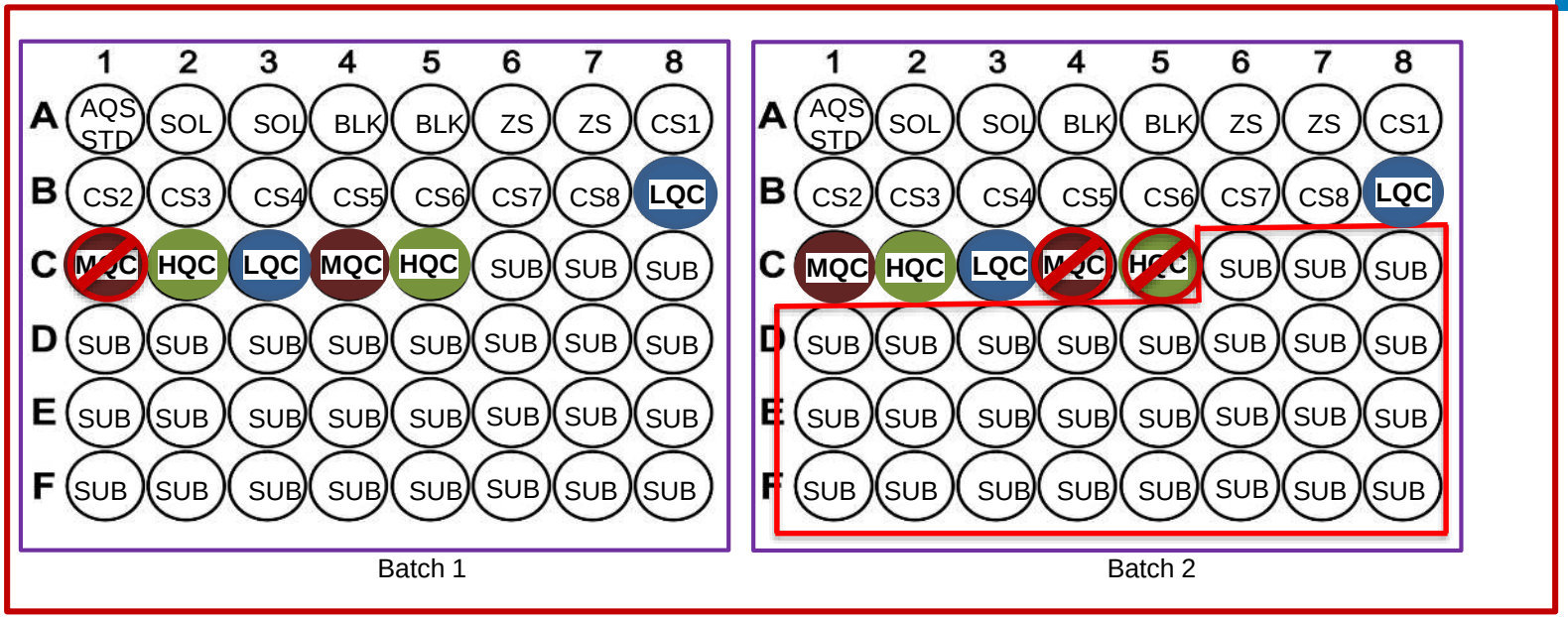
- Separate acceptance criteria can be set for individual batches and overall run
- Full set of QCs in each batch if separate batches are used
- Data from individual batches may be acceptable if overall run meets acceptance

QCs meet the 67% overall and 50% acceptance criteria at each concentration: Will there be any concerns?

What is the role of a Quality Control (QC)?

- QCs are representative samples inserted into the testing process
- QC Samples are treated in the same manner as study samples and by being exposed to the same operating conditions
- QCs mimic subject samples and evaluate reliability (precision and accuracy) of the method
- Acceptable performance of QCs indicate results from study samples are valid and reliable for reporting

What is the role of a QC?



Batch Acceptance Criteria

Batch 1=Passed; Batch 2=Passed

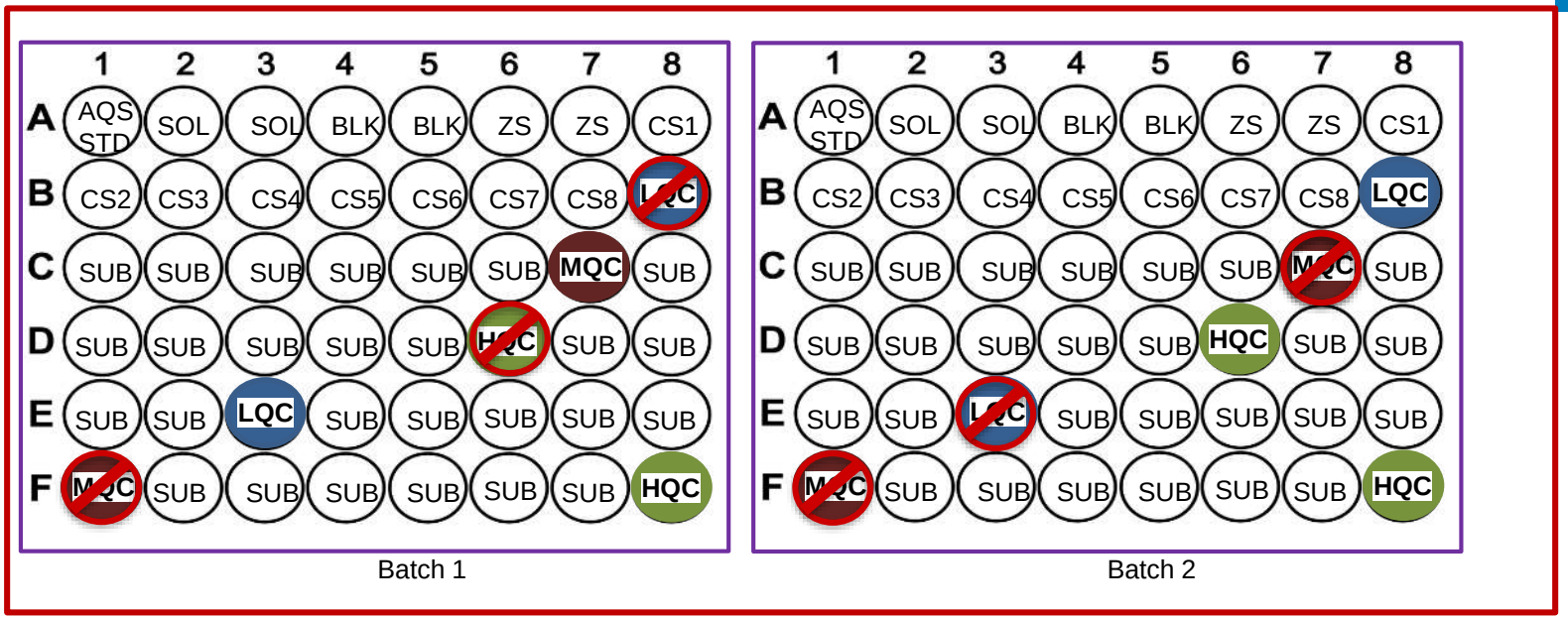
5/6 4/6

Run Acceptance Criteria

Run=Passed

9/12

Chromatographic Assays



Batch Acceptance Criteria

Batch 1=Failed; Batch 2=Failed

3/6 3/6

Run Acceptance Criteria

Run=Failed

8/12

Recommendations

- If the QCs are clustered in a region of a run, multiple QC failures may indicate analytical/instrument issues with the run even if overall run meets acceptance
- It may be advisable to investigate this further
- To alleviate this concern, BMV guidance recommends that QCs are interspersed with study samples during processing and analysis
- Interspersed QCs also allows monitoring of drift and its impact on the accuracy of the measured sample concentrations

Challenge Question 1

Accuracy and Precision evaluations during method validation should use which of the following:

1. Freshly prepared calibrators
2. Freshly prepared QCs
3. Freshly prepared calibrators and QCs
4. Frozen calibrators and QCs

Challenge Question 2

When sample analysis involves multiple and distinct processing batches, QC acceptance criteria should be based on which of the following:

1. Individual batches
2. Entire run
3. Both individual batches and entire run
4. None of the above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