Recommendations from JBF discussion group, DG2013-01 on the preparation of calibration standards and QC samples for bioanalysis of small molecular drugs

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Overview

- Practical preparation methods of calibration standards (CS) and QC samples are not covered in FDA/EU/MHLW BMV guidelines.
- Broad range of opinions on the preparation methods of CS and QC samples in the questionnaires survey from 148 bioanalysts in Japan.
- Present our recommendable procedures on the preparation of CS and QC samples with pros and cons .

Focusing on small molecules

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Points to consider

- Sample preparation processes: Accurate and reproducible as possible even in different occasions, analysts or labs
- Analytes dispersion into matrix solution: Uniform as possible
- QC samples: Similar to actual samples as possible

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Process flow

		Points to consider
Before prep	• Pipette check • Standard solution prep	➢Check performance before use ➢Select solvent
In prep	• Mix analyte and blank matrix • Dispense for storage	➢Preparation timing ➢Organic solvent ratio in matrix ➢Adding order / mixing way ➢Storage volume
After prep	• Check appropriateness (concentration, study personnel)	

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Recommendable procedures

Pipette check

- A mechanical pipette used for diluting standard solutions should be checked just before use.
- Systematic error : should be within ±2%
  - To add the analyte accurately and reproducibly as possible in different occasions.
  - In practically, the systematic error to be kept within ±1% in DG 2013-01 member's experience.
- Random error : To set appropriate criteria based on the systematic error

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Solvent choice

- Consider based on the analyte solubility and adsorption , etc.
- Many kinds of solvents can be used, but consider to minimize the protein denaturation as possible:
  - ✓ Mixture of organic solvent and water
  - ✓ Organic solvent (Alcohol > Acetonitrile)

Pros :
- Improve a difficulty of handling organic solvents
- Avoid bacteria growth in aqueous solutions

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Preparation timing

Sample		Recommendable timing	Reason
CS		Prep in use	Multiple prep is effective to avoid the specific bias depending on a single preparation
QC	A&P	Batch prep	To check if QC samples prepared in a batch are measured accurately and reproducibly throughout the study
	Stability		
	Sample analysis		

A&P: accuracy and precision

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Solvent ratio

Sample		Recommendable ratio*	Reason
CS		Not limited	Need QC samples quantified appropriately
QC	A&P[1)]	Within 2% (max 5%)	To be same as actual samples as possible and avoid protein denaturation
	Stability[2)]		
	Sample analysis [1)]		

*Solvent ratio to blank matrix

1) Not limited when prepare in use (e.g. rare matrix)
2) Stability is not evaluated when prepare in use (e.g. rare matrix)

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Adding order, mix, storage volume

Process	Recommendable process	Reason
Adding order	Dispense blank matrix →add ss* to the matrix	To avoid an adsorption of analyte and a contamination to blank matrix
Mix	Vortex mix and then inversion	To avoid insufficient dispersion
Storage volume	Aliquot more than required volume of a sample analysis	Enable to take an aliquot as same as study samples on the day of analysis

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Check after prep

Check point	Sample		Recommended timing to check	Reason
Conc.	QC	A&P	Before use	To check accurately prepared and to avoid a reject of analytical run
		Stability	Just after prep	
		Sample analysis	Before use	
Study personnel*	CS, A&P, etc		Before sample analysis	To verify appropriateness of study personnel

*Note: A partial validation is required if study personnel , who does not perform the validation study, is participated to the sample analysis.

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Summary

Recommendable process

Before prep	• Check pipette just before use, systematic error: ≤ ±2% • SS* prep: consider analyte solubility, adsorption and protein denaturation
In prep	• Prep timing; CS: prep in use, QC: prep in batch • Solvent ratio; CS: depends on QC, QC: ≤ ±2% (max 5%) • Dispense blank matrix→ add ss→mix vortex and inversion • Aliquot more than analytical volume , then store
After prep	• Check conc; QC (stability): just after prep, QC others: before use • Check study personnel: before sample analysis

SS*: standard solution

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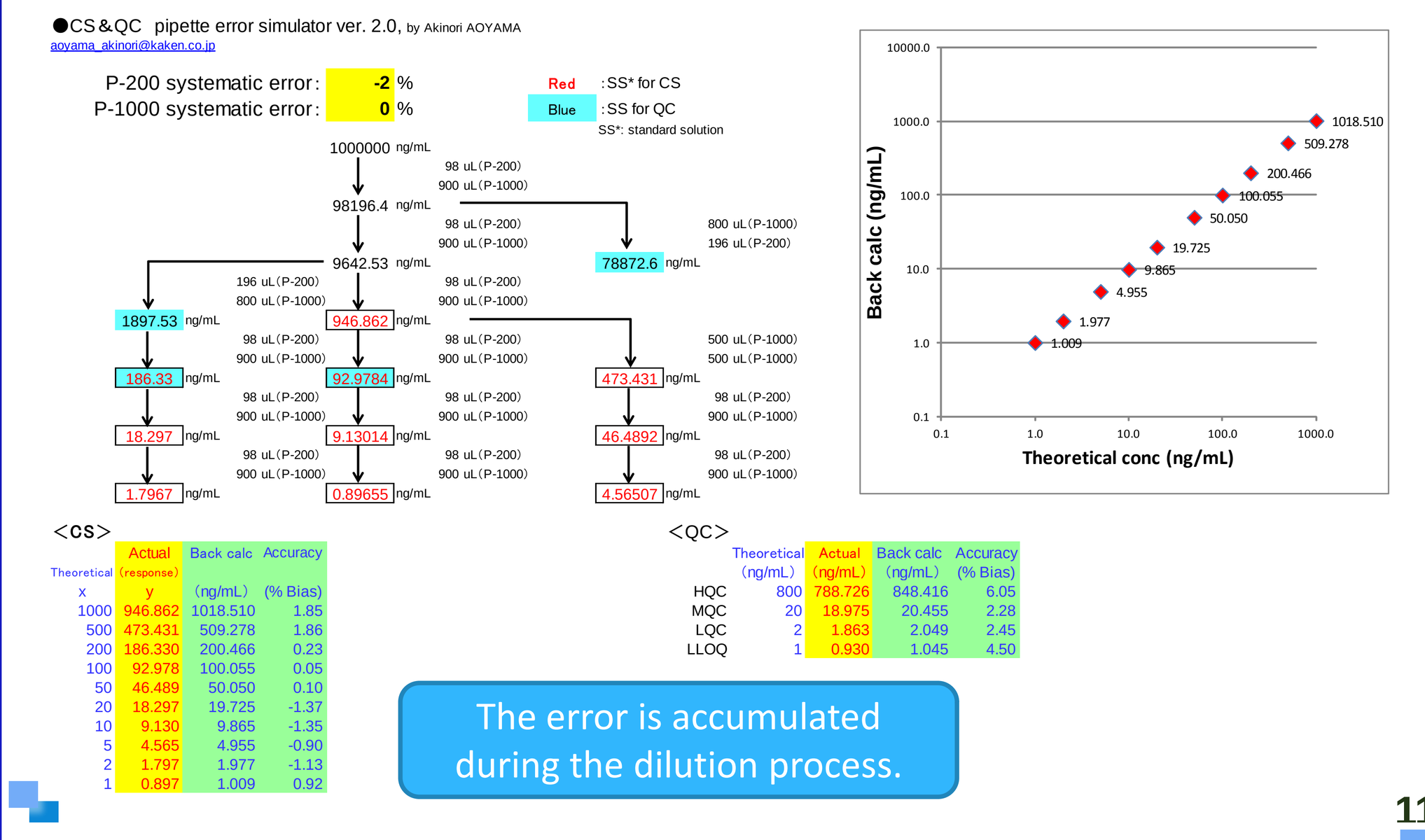
Questionnaires and discussion

Simulation: accumulating error caused by pipette error



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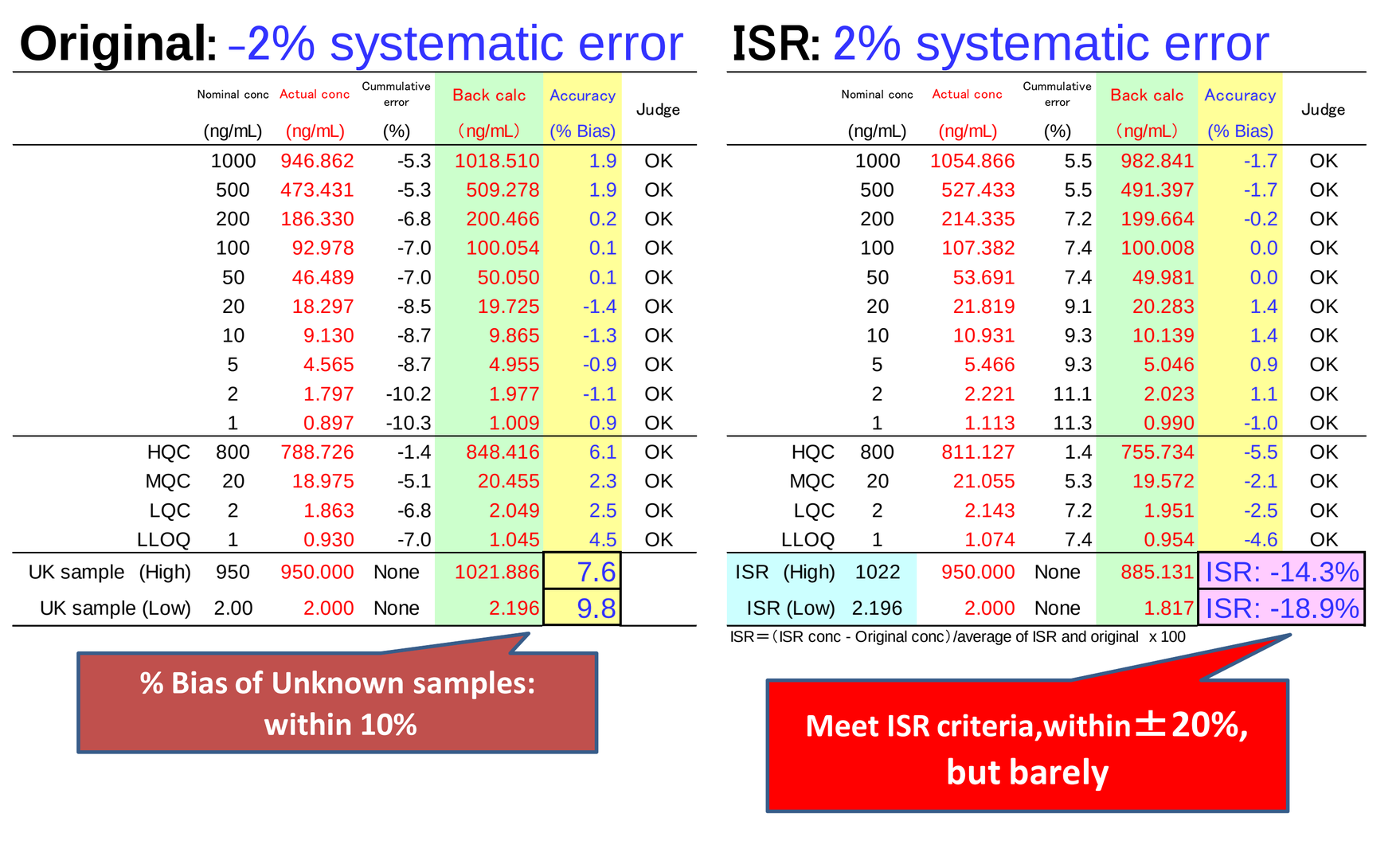
Simulation : ±2% systematic error



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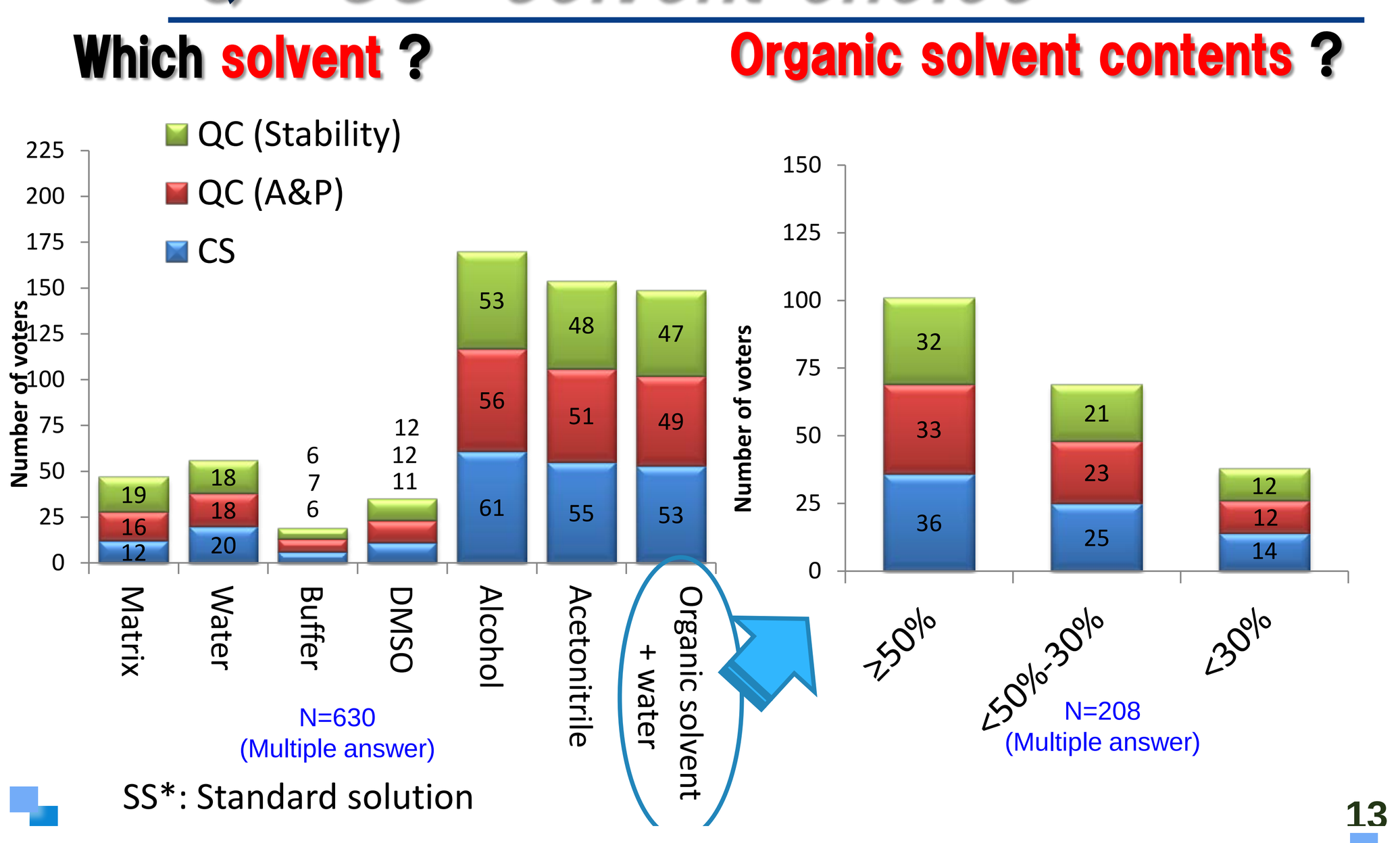
Q : SS* solvent choice



SS*: Standard solution

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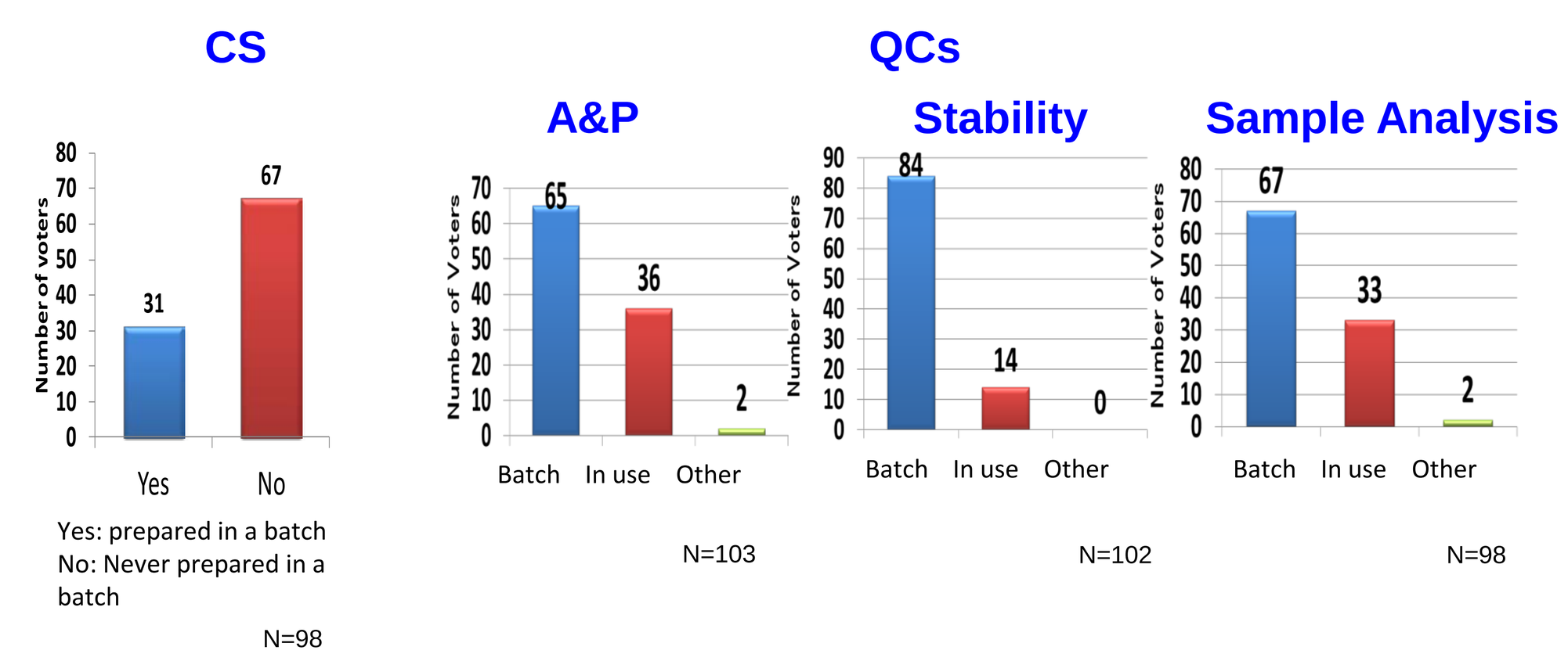
Q : Prepare Batch or In Use?



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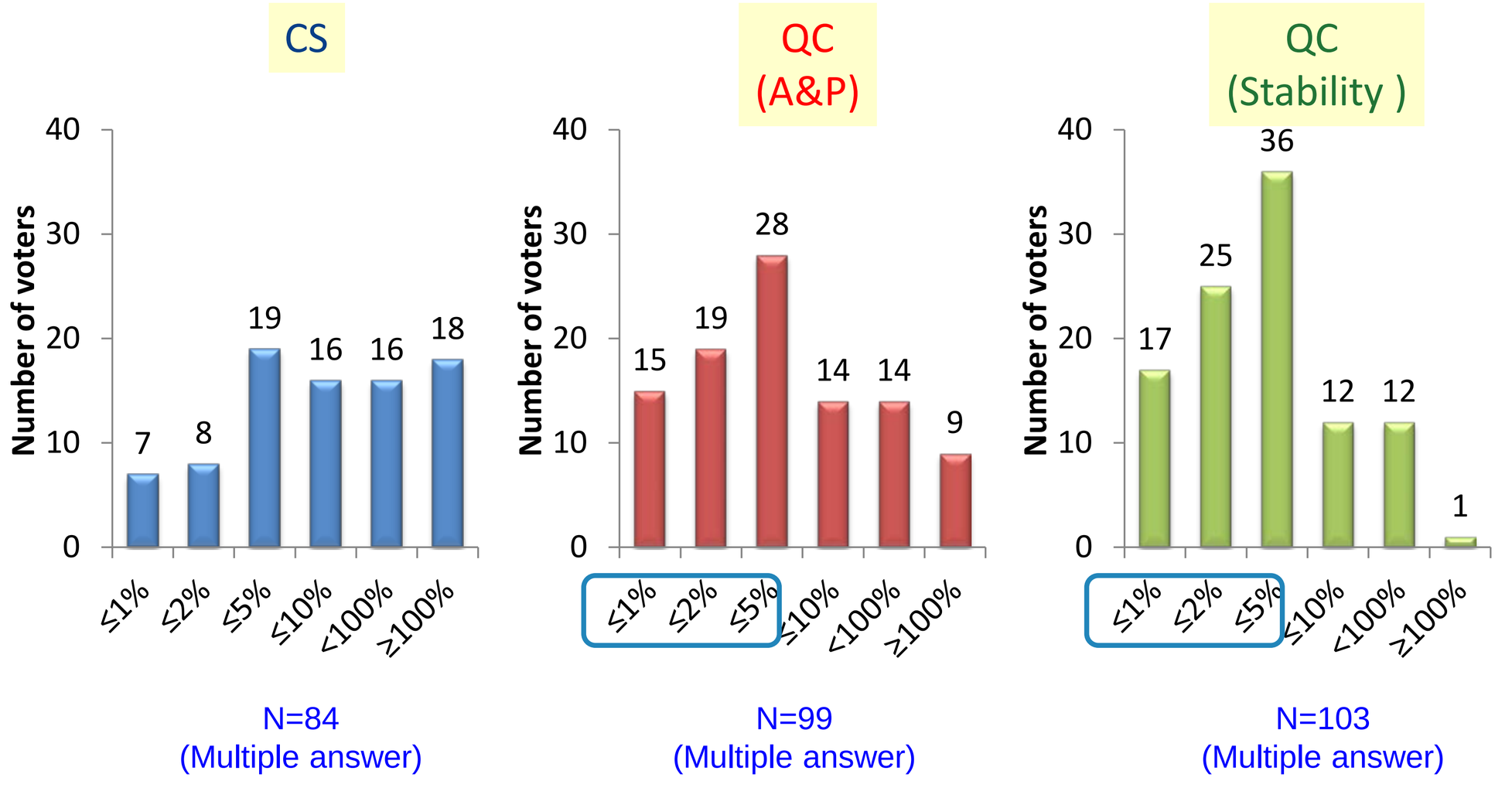
Q : SS* Ratio to blank matrix?



*SS: Standard solution

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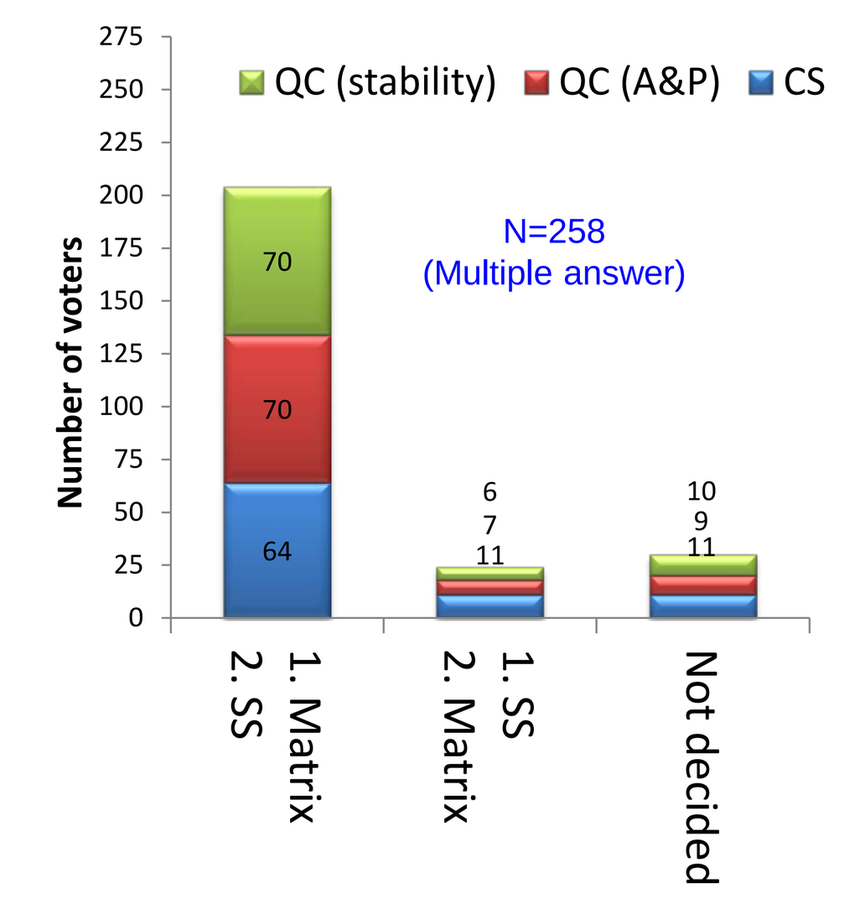
Q : Which first?



Order	Pros	Cons
Matrix first	✓Avoid blank matrix contamination ✓Avoid an analyte adsorption to the tube	NA
SS * first	✓Dry up standard solution before adding matrix	✓Possibility of blank matrix contamination

*SS: standard solution

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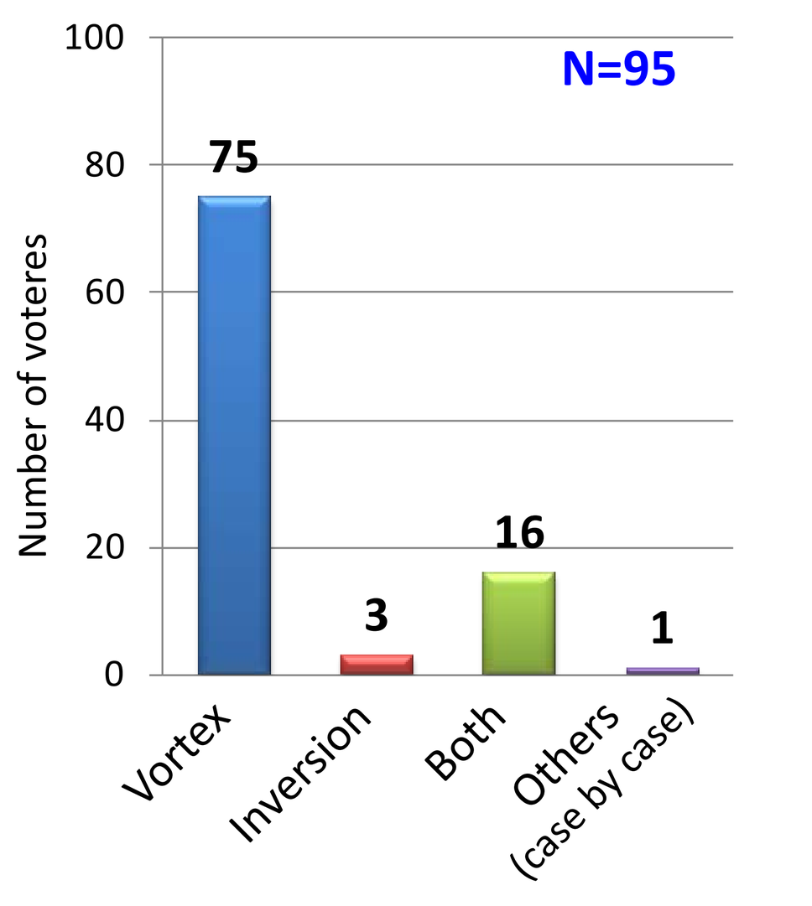
Q: How do you mix?



➢Vortex mix isn't adequate to fully disperse analytes in matrix solutions.

➢The insufficient dispersion causes a large variability in measured concentrations

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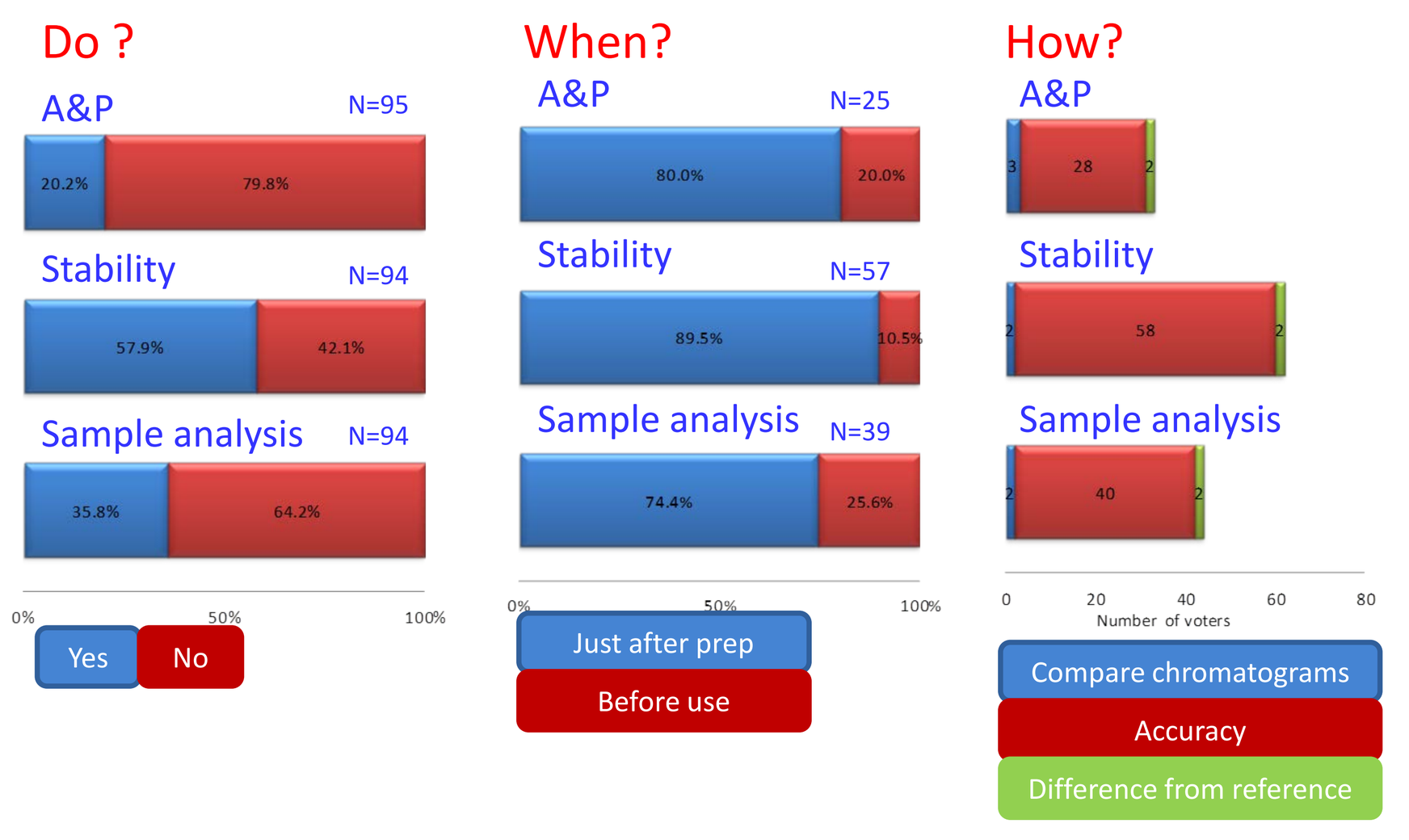
Q: Check after prep



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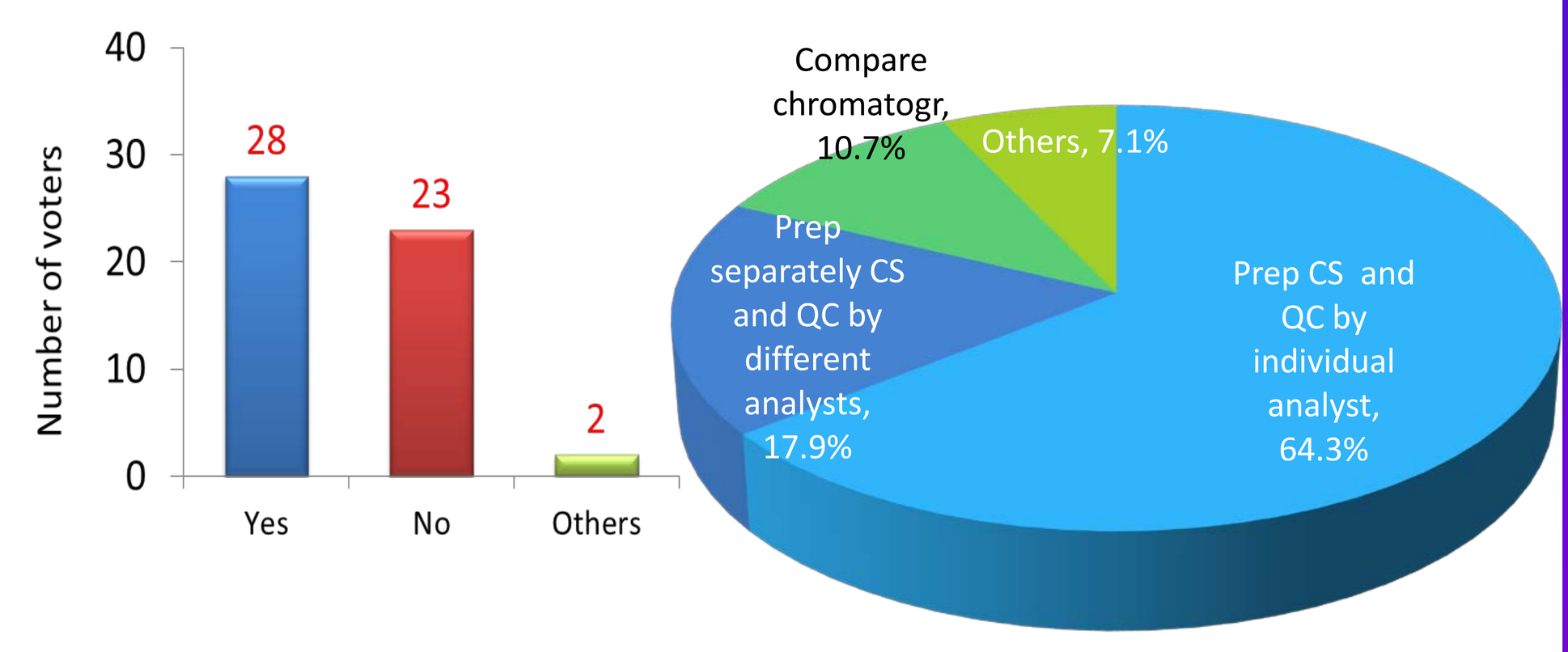
Q: Check personnel



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