Sugar Extraction Study
Round 3!

Initial Study 2010-11, Extractions by each Lab
Second Study 2011-12, All Extractions by one Lab
Third Study 2013, No Extractions, All Pure Standards

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Initial Study 2010-11

- Compared multiple feeds with each lab providing their internal sample preparation methodology.
- Large standard deviations existed in reported data across multiple feed samples.

2nd Study 2011-12, Extraction Study

- Observe agreement between labs from single source extractions
- Compare 50/50 ACN – water vs. Water only extraction.

3rd Study 2013-14
Pure Standards Only

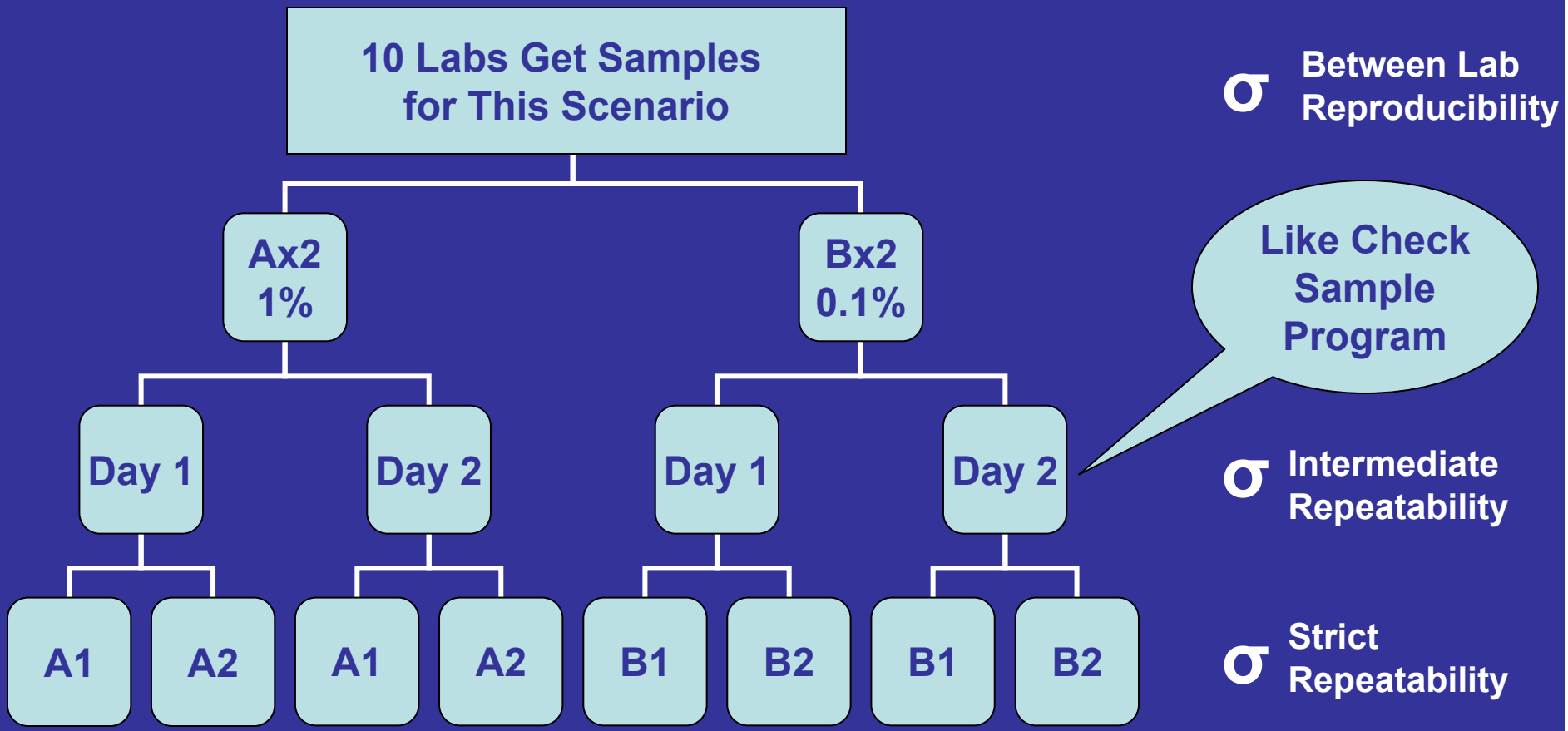
- Discover Best General Precision for Sugar Analysis Under Ideal Conditions and Concentrations
- Identify Best Available Technologies (if possible!)

Experimental Design

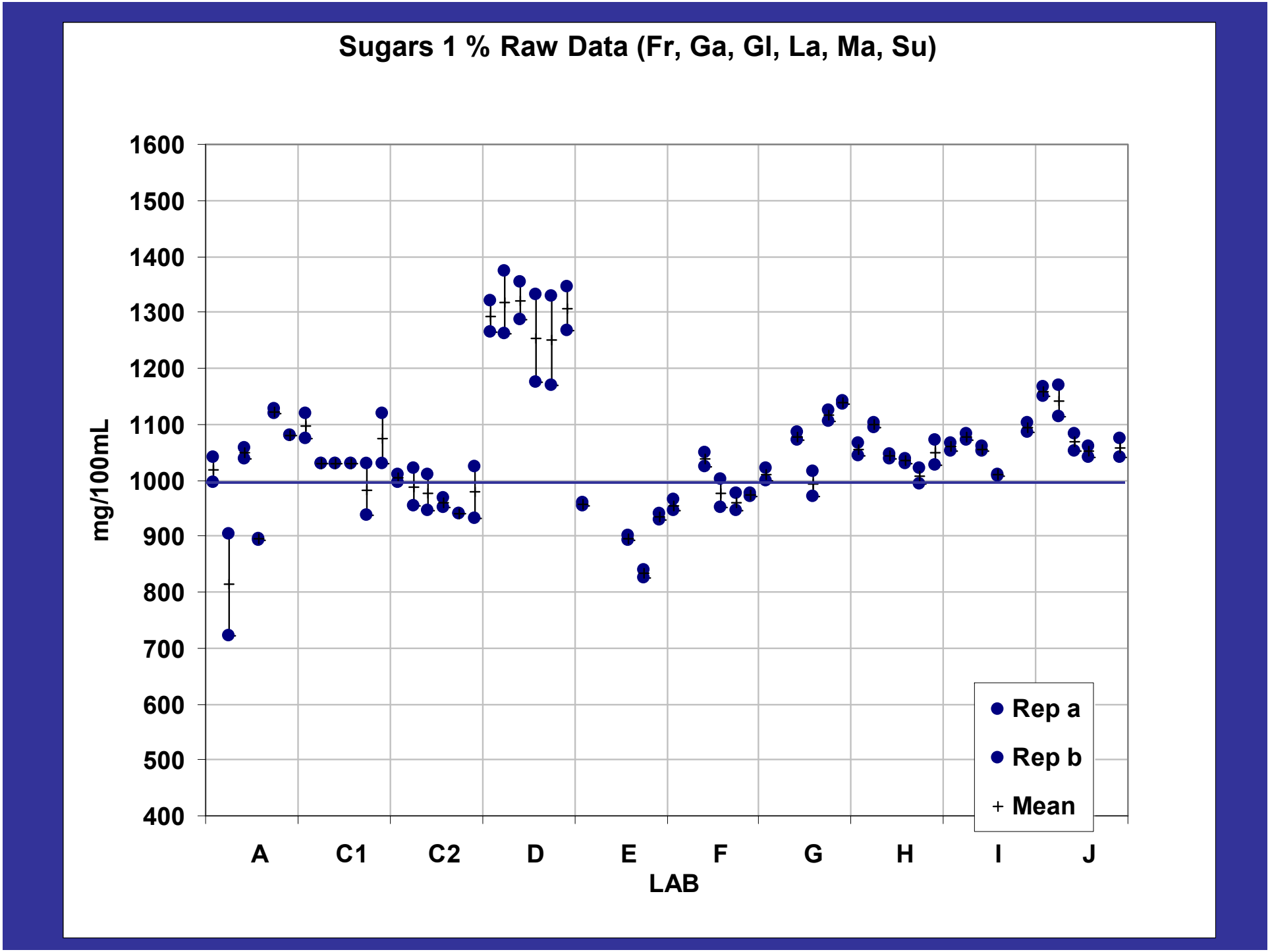
Fructose, Galactose, Glucose, Lactose, Maltose and Sucrose Standards.

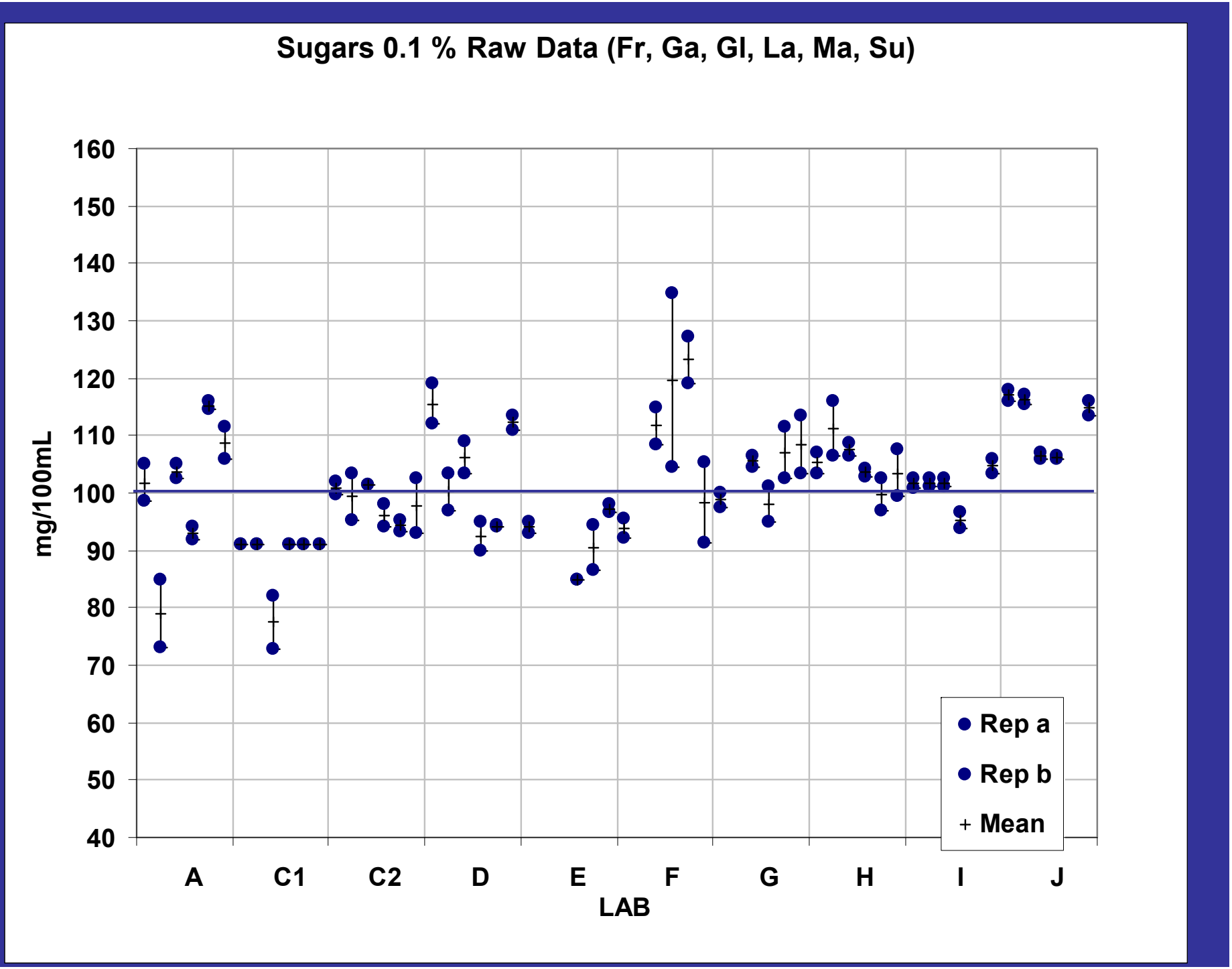
- 10 Labs x 6 Sugars x 2 Concentrations
- Each Lab gets 4 Samples, 2 A and 2 B
  - A contains each Sugar at 1% Solution
  - B contains each Sugar at 0.1% Solution
- On the first day run 1 A and 1 B both in duplicate under strict repeatability conditions.
- On another day run the remaining A and B samples in duplicate under strict repeatability conditions.

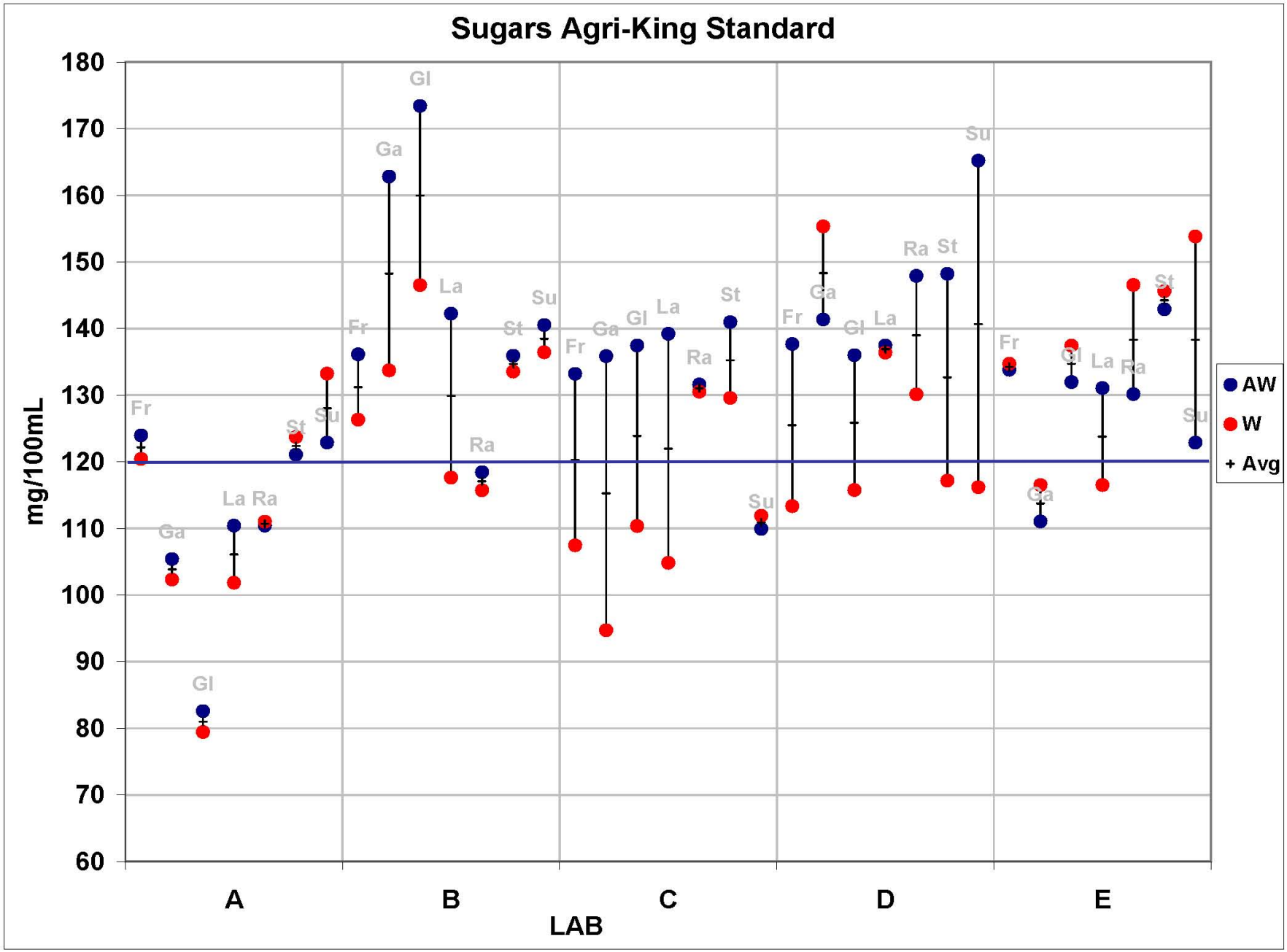
Experimental Design



10 Labs x 2 Days x 2 Concentrations x 6 Sugars x 2 Duplicates
= 480 Analytical Measurements

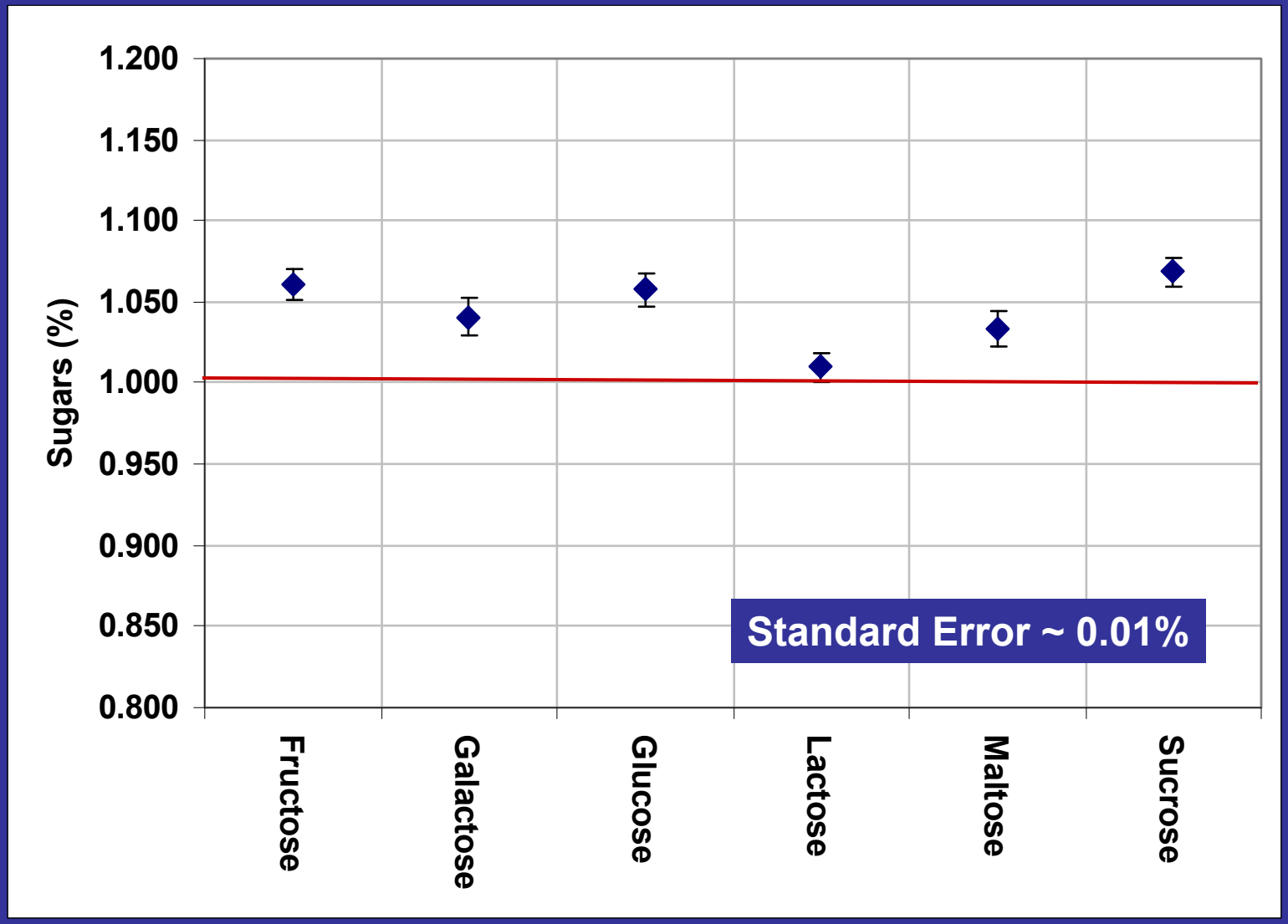

Sugars 1 % Raw Data (Fr, Ga, Gl, La, Ma, Su)
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
mg/100mL
A
C1
C2
D
E
F
G
H
I
J
LAB
Rep a
Rep b
Mean


Sugars 0.1 % Raw Data (Fr, Ga, Gl, La, Ma, Su)
160
150
140
130
120
110
100
90
80
70
60
50
40
mg/100mL
A
C1
C2
D
E
F
G
H
I
J
LAB
Rep a
Rep b
Mean


Sugars Agri-King Standard
mg/100mL
180
170
160
150
140
130
120
110
100
90
80
70
60
A
B
C
D
E
LAB
Fr
Ga
Gl
La
Ra
St
Su
AW
W
+ Avg

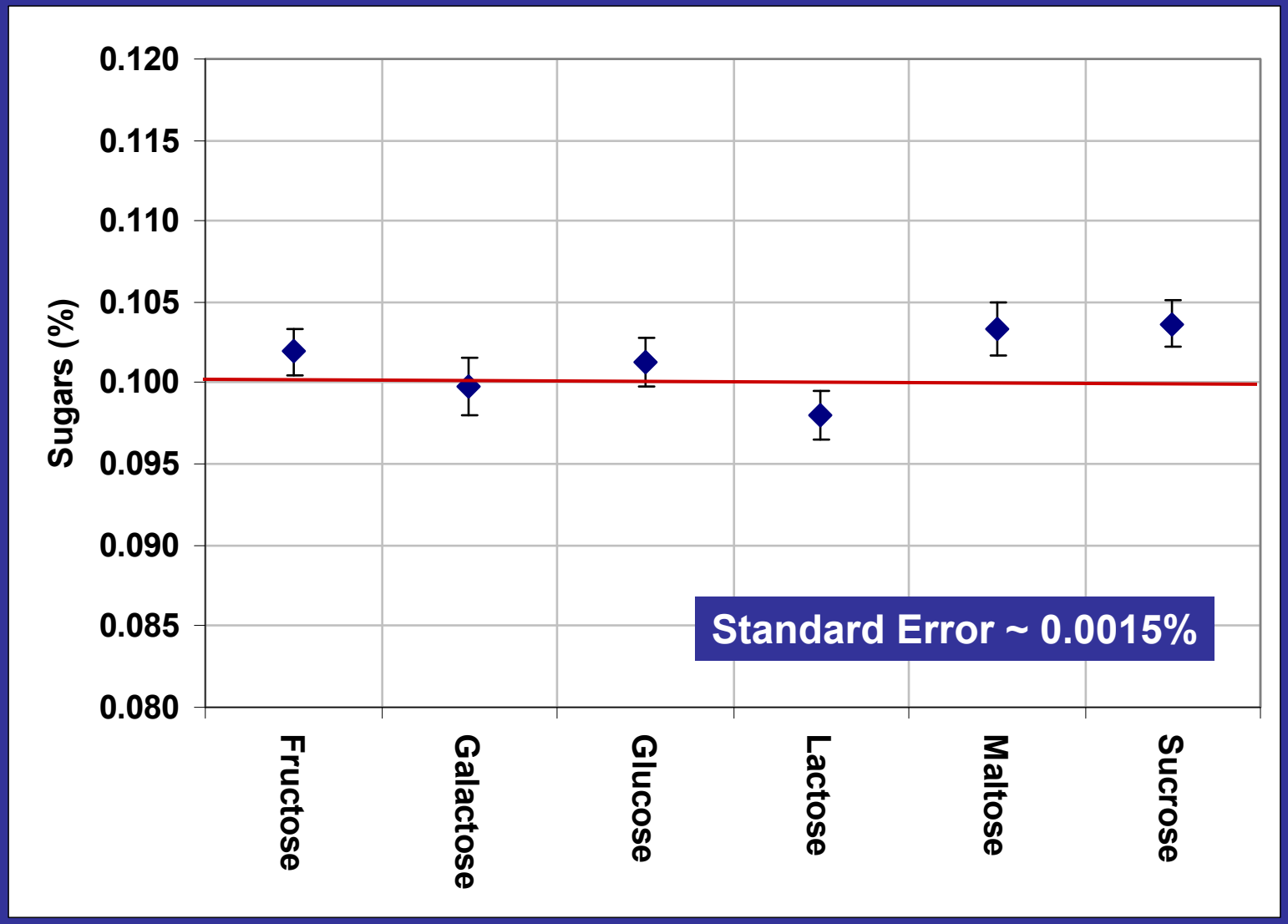
ANOVA

Observed Mean Sugar Concentrations Compare with Expected (1 %)



ANOVA

Observed Mean Sugar Concentrations Compare with Expected (0.1 %)



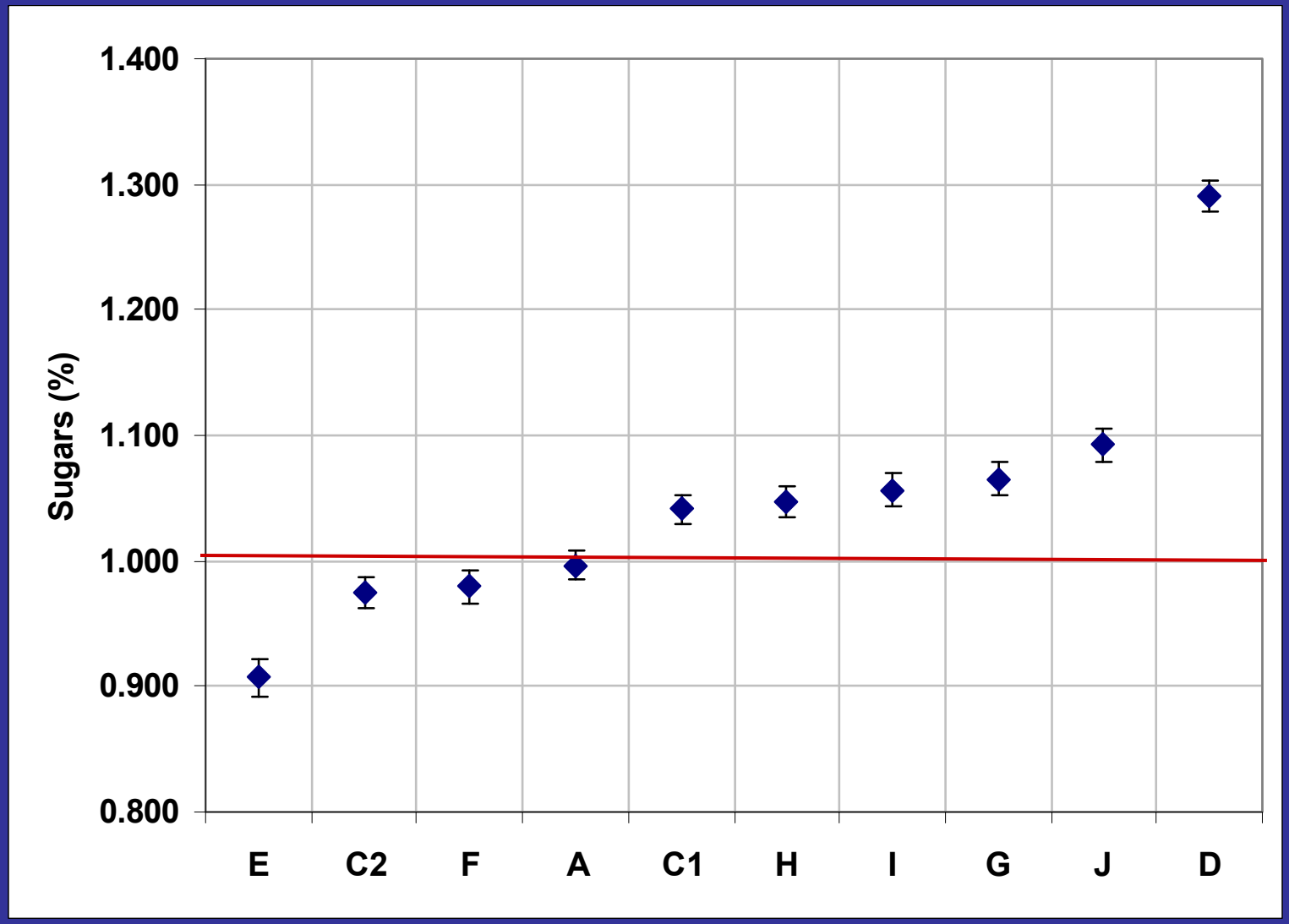
Which Technology is Better?

Within Technology Precision at 1% Sugar				
Lab	Technology	Mean	Strict %rsd	Day %rsd
A	HPLC (ELSD)	1.00	3.5%	5.4%
C-1	GC (FID)	1.04	1.8%	3.8%
C-2	HPAEC (PAD)	0.97	1.0%	4.0%
D	HPLC (ELSD)	1.29	3.0%	6.2%
E	UPLC (RI)	0.91	1.1%	0.8%
F	HPLC (RI)	0.98	2.1%	2.2%
G	HPLC (RI)	1.07	1.2%	1.6%
H	HPLC (Post Column FLD)	1.05	1.4%	1.6%
I	IC (EC detection)	1.06	0.4%	0.8%
J	IC (EC detection)	1.10	4.7%	2.2%

Which Technology is Better?

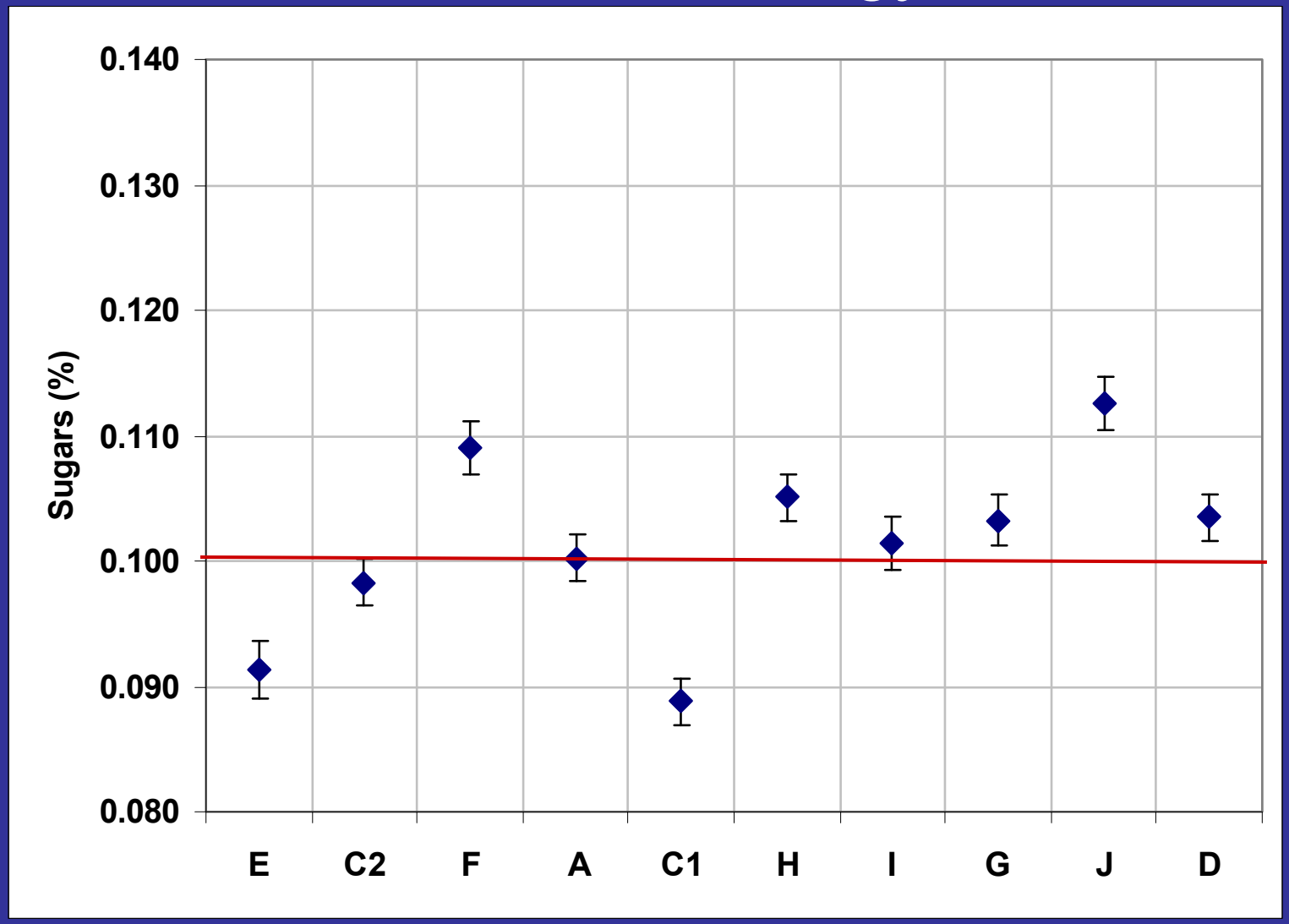
Within Technology Precision at 0.1% Sugar				
Lab	Technology	Mean	Strict %rsd	Day %rsd
A	HPLC (ELSD)	0.100	2.2%	4.4%
C-1	GC (FID)	0.089	0.0%	3.0%
C-2	HPAEC (PAD)	0.098	2.4%	4.0%
D	HPLC (ELSD)	0.104	0.8%	3.4%
E	UPLC (RI)	0.092	6.4%	3.2%
F	HPLC (RI)	0.109	19.0%	10.1%
G	HPLC (RI)	0.104	6.2%	4.6%
H	HPLC (Post Column FLD)	0.105	1.5%	4.0%
I	IC (EC detection)	0.101	0.2%	1.4%
J	IC (EC detection)	0.112	1.3%	1.0%

ANOVA: Which Technology is Better?



*Each Technology represented by only one lab

ANOVA: Which Technology is Better?



*Each Technology represented by only one lab

General Precision for Sugars Analysis Under Ideal Conditions

	Mean	Strict %rsd	Day %rsd	%RSD
1% Sugars	1.05	2.5%	3.7%	10.8%
0.1% Sugars	0.101	6.8%	4.7%	10.1%

Within

Between

Method Needs Statement

Table 1. Recommended Method Performance Characteristics:

	Method LOQ, %	Operational concentration range, %	Accuracy at LOQ	Accuracy at midrange	Repeatability (CVr) at Midrange	Repeatability (CVr) at 2xLOQ
Each compound	0.1%	0.1% – 100%	90% - 108%	92% - 105%	= or < 4%	= or < 5%

LOQ = 100 mg/100mL (0.1 %)
Repeatability (CVr) at 2 x LOQ ≤ 5%

LOQ defined as the lowest concentration at or above which the analyte can not only be reliably detected but at which some predefined goals for bias and precision are met.

What's Next?

Efficacy of Extraction Procedures
Can we retain this Precision?