Measuring in an uncertain world…

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Canadian Grain Commission

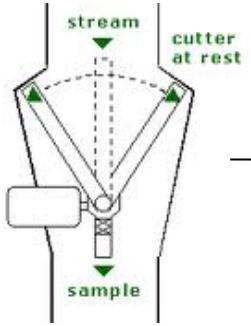
A matter of uncertainty

"All analytical measurements are wrong; its just a matter of how large the errors are, and whether they are acceptable."

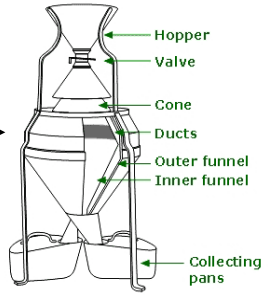
Analytical methods

- An “analytical procedure” is an orderly step-by step instruction designed to ensure operational uniformity and to minimize uncertainty

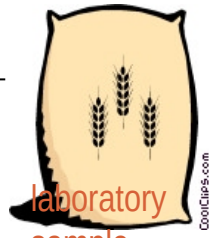
primary sampling



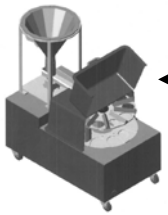
official sample



secondary sampling and sample preparation



laboratory sample



test portion

chemical analysis

Error

- **Error** is defined as the difference between an individual measurement result and the true value

- Error is an ideal concept, and errors cannot be known exactly

Uncertainty

- **Uncertainty** is a parameter associated with a measurement result that characterizes the dispersion of the values that could reasonably be attributed to this mesurand

Sources of Uncertainty

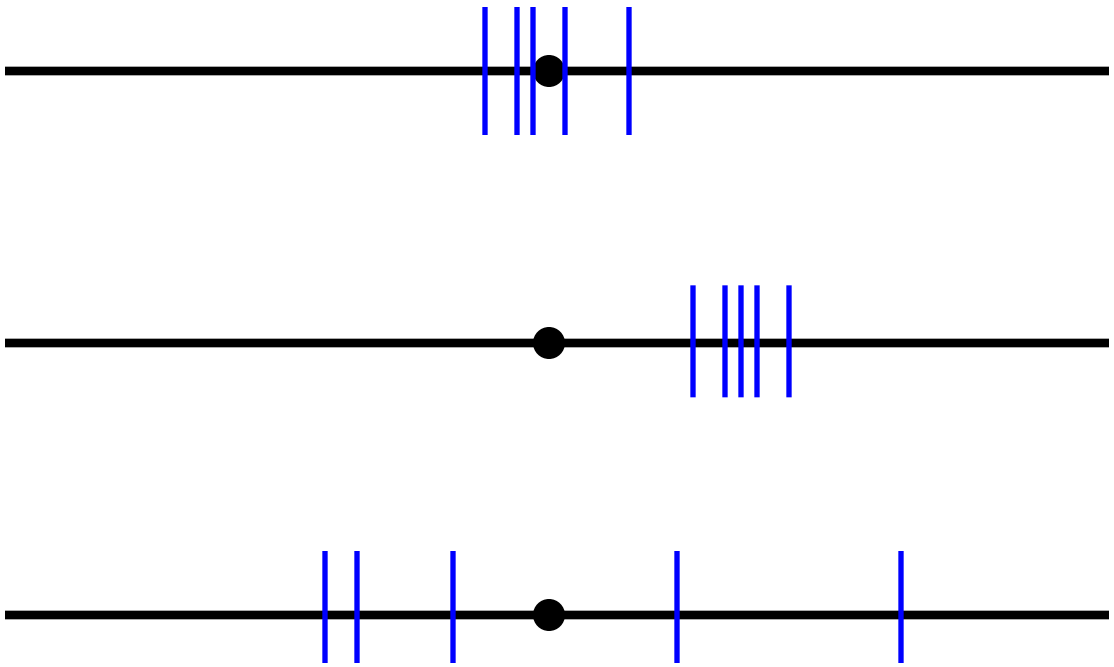
- Analyst
- Instrument long term (drift, maintenance,…)
- Instrument short term (noise, calibration, …)
- dispensing, weighing, etc. . .
- Laboratory environment

Random vs. Systematic

- Random errors usually result from unpredictable variations of parameters that influence the measured result

- Systematic errors, in the course of a number of analyses, remain constant or vary in a predictable way

Accuracy vs Precision



Quantifying uncertainty

- There is always a margin of doubt about any measurement
- We need to ask ‘How big is the margin?’ and ‘How bad is the doubt?’

Repeatability error

- an estimate of the precision that can be expected when one analyst performs a single analysis.

- The repeatability of a method can be determined by monitoring its performance

Standard deviation

- Calculating the SD from a series of single measurements

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

Standard deviation

- Calculating the SD from a series of duplicate measurements

$$s = \sqrt{\frac{\sum d^2}{2n}}$$

mU/g of sample			
310			
312			
310			
299		Mean =	311
312		SD =	5.3
318			
310			
318			
314			
308			
312			
319			
311			
308			
304			

Coefficient of Variation

- Expresses the standard deviation as a percentage of the mean result

$$CV = \frac{S}{\overline{X}} \times 100\%$$

Repeatability Value

- Two results obtained in the same lab by the same operator should not differ by more than this amount

$$r_{95} = 2.8s$$

mean =	311
SD =	5.3
CV% =	1.7
r_{95} =	15

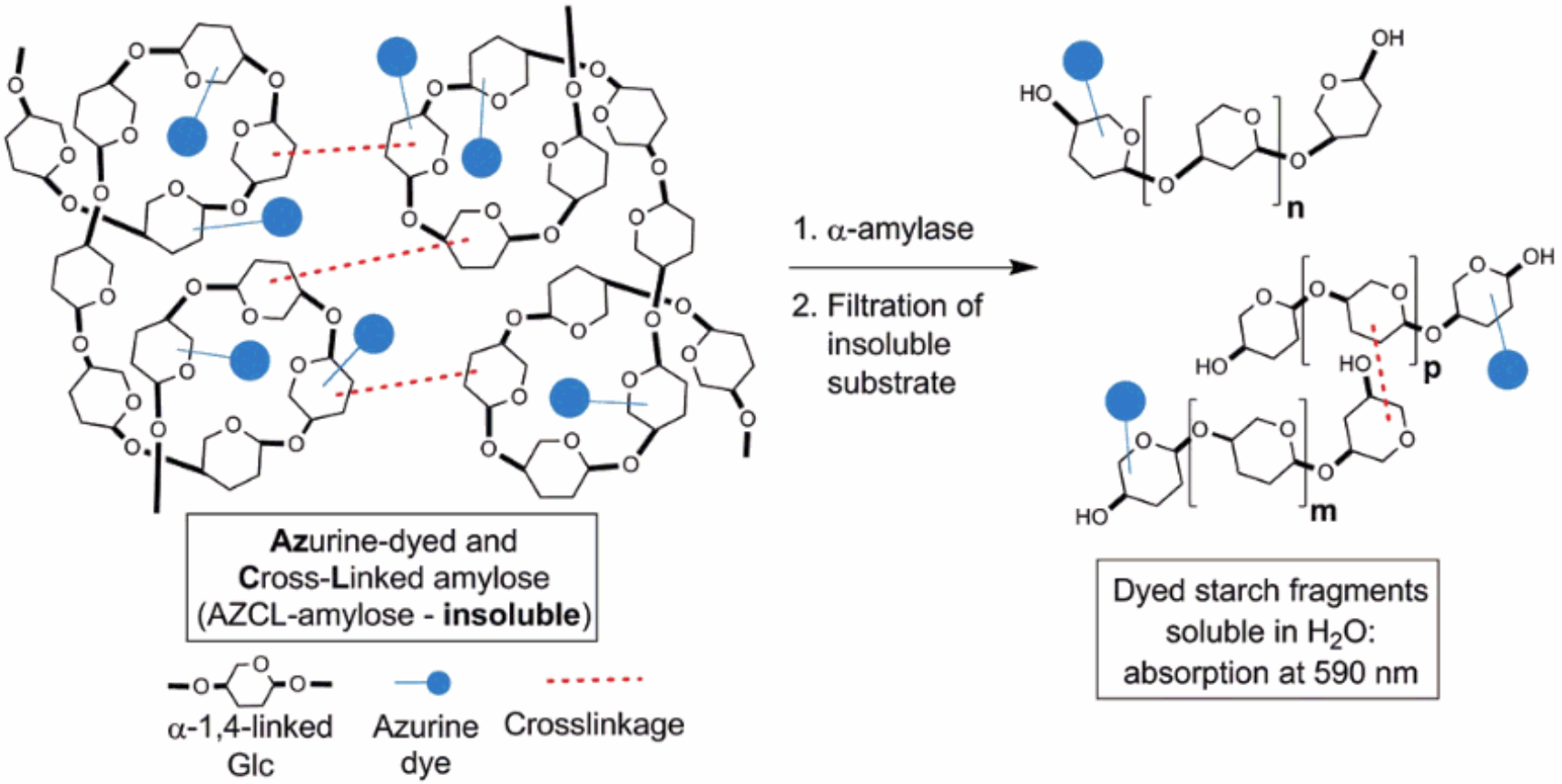
Standard Error

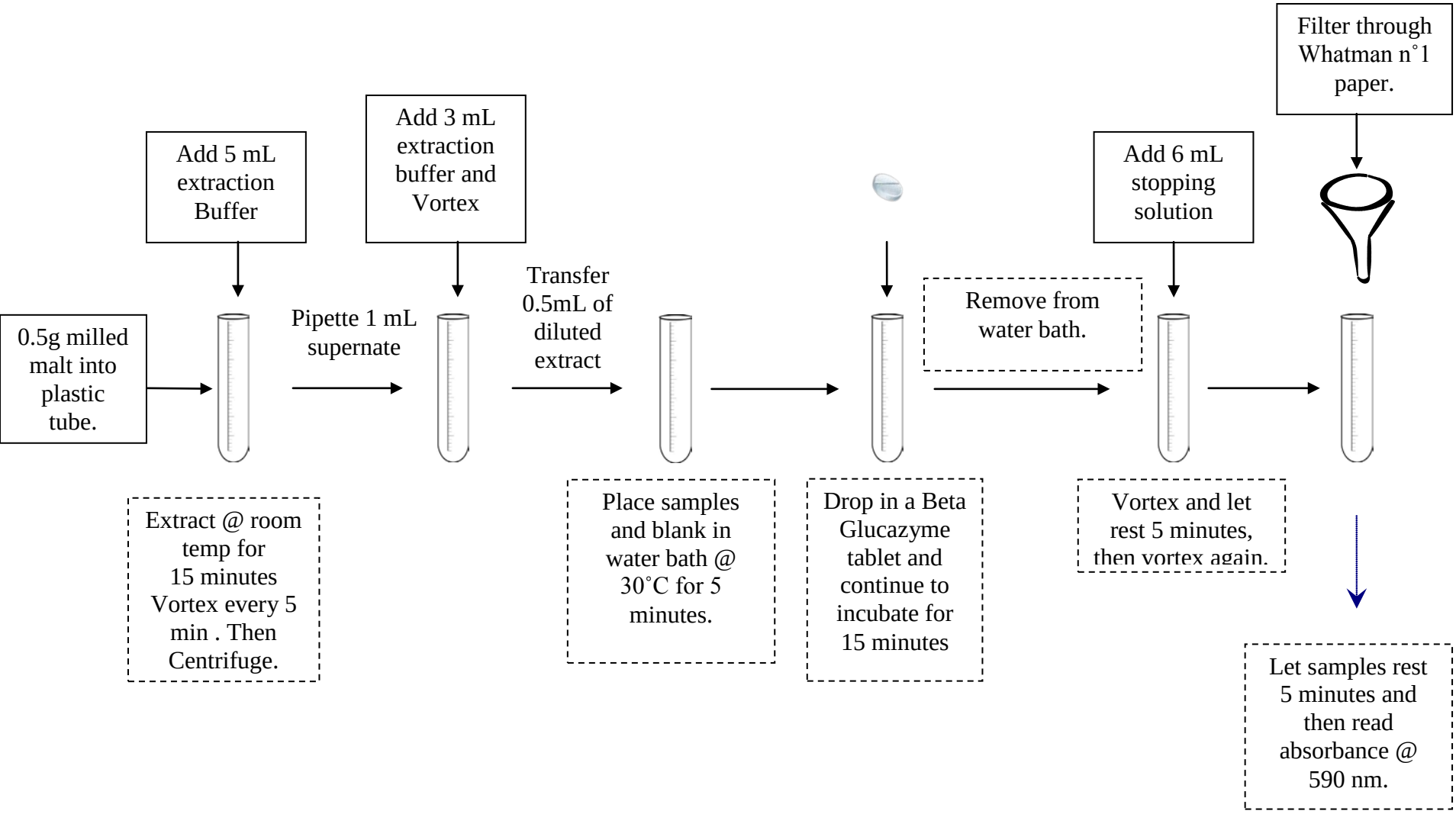
- the more measurements you take, the better the estimate you will have of the ‘true’ value.

$$SE = \frac{s}{\sqrt{n}}$$

Identify sources of variation

- Replicate measurements at selected stages of a method can help identify the steps associated with the largest sources of error

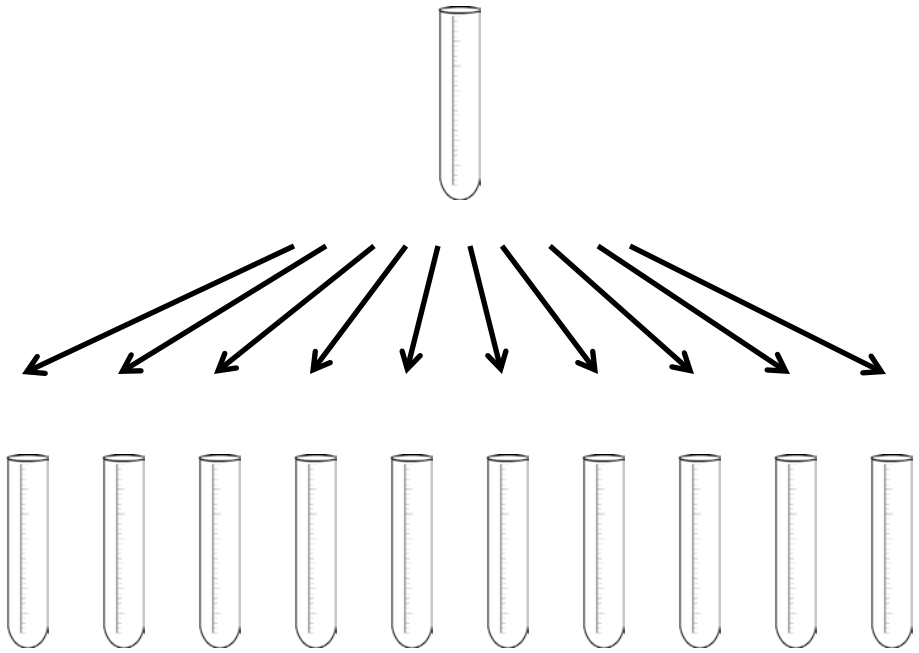

1. α-amylase
2. Filtration of insoluble substrate
Azurine-dyed and **C**ross-**L**inked amylose (AZCL-amylose - **insoluble**)
α-1,4-linked Glc
Azurine dye
Crosslinkage
Dyed starch fragments soluble in H_2O: absorption at 590 nm


0.5g milled malt into plastic tube.
Add 5 mL extraction Buffer
Extract @ room temp for 15 minutes Vortex every 5 min . Then Centrifuge.
Pipette 1 mL supernate
Add 3 mL extraction buffer and Vortex
Transfer 0.5mL of diluted extract
Place samples and blank in water bath @ 30˚C for 5 minutes.
Drop in a Beta Glucazyme tablet and continue to incubate for 15 minutes
Remove from water bath.
Add 6 mL stopping solution
Vortex and let rest 5 minutes, then vortex again.
Filter through Whatman n˚1 paper.
Let samples rest 5 minutes and then read absorbance @ 590 nm.

Combining uncertainties

$$\sigma^2_{method} = \frac{\sigma^2_{extraction}}{n_{extractions}} + \frac{\sigma^2_{assay}}{n_{assays}}$$

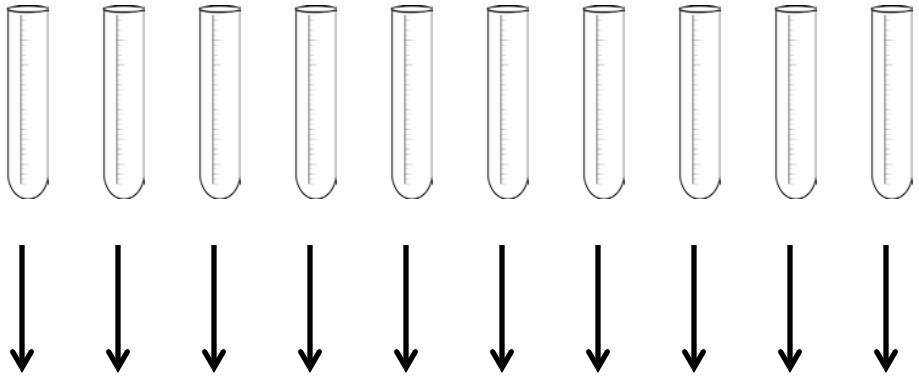
Extraction



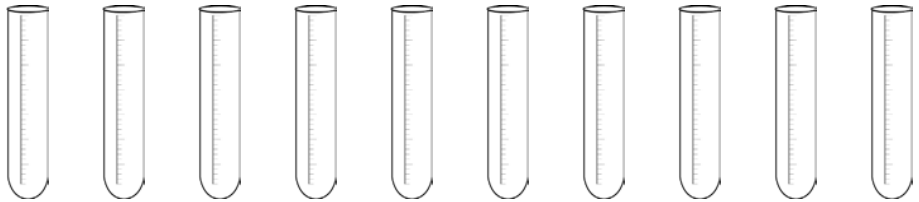
Assay

$$s = 0.6\ \mathrm{Ug}$$

Extraction



Assay



$$s = 5.4\ \mathrm{Ug}$$

eg. single extraction, duplicate assays

$$\sigma_{method} = \sqrt{\frac{\sigma^2_{extraction}}{n_{extractions}} + \frac{\sigma^2_{assay}}{n_{assays}}}$$

$$= \sqrt{\frac{(5.4)^2}{1} + \frac{(0.6)^2}{2}}$$

$$= \sqrt{\frac{29.16}{1} + \frac{0.36}{2}}$$

eg. single extraction, duplicate assays

$$\sigma_{method} = \sqrt{29.16 + 0.18}$$

$$= \sqrt{29.34}$$

$$= 5.42$$

eg. duplicate extraction, single assays

$$\sigma_{method} = \sqrt{\frac{\sigma^2_{extraction}}{n_{extractions}} + \frac{\sigma^2_{assay}}{n_{assays}}}$$

$$= \sqrt{\frac{(5.4)^2}{2} + \frac{(0.6)^2}{1}}$$

$$= \sqrt{\frac{29.16}{2} + \frac{0.36}{1}}$$

eg. single extraction, duplicate assays

$$\sigma_{method} = \sqrt{14.58 + 0.36}$$

$$= \sqrt{14.94}$$

$$= 3.86$$

eg. duplicate extractions, duplicate assays

$$\sigma_{method} = \sqrt{\frac{\sigma^2_{extraction}}{n_{extractions}} + \frac{\sigma^2_{assay}}{n_{assays}}}$$

$$= \sqrt{\frac{(5.4)^2}{2} + \frac{(0.6)^2}{2}}$$

$$= \sqrt{\frac{29.16}{2} + \frac{0.36}{2}}$$

eg. single extraction, duplicate assays

$$\sigma_{method} = \sqrt{14.58 + 0.18}$$

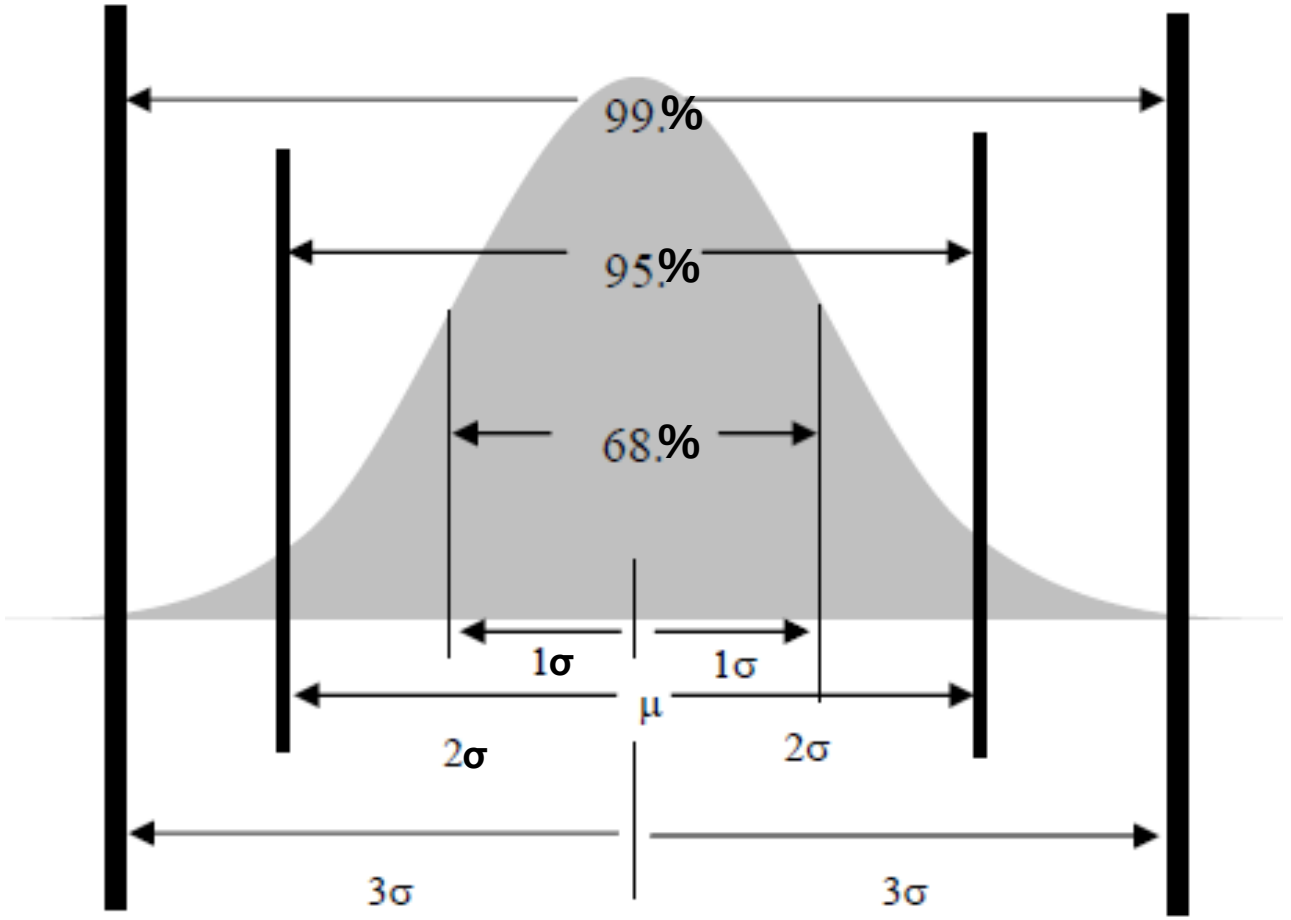
$$= \sqrt{14.76}$$

$$= 3.84$$

Take home lesson

- Do replication where you will get the biggest bang for your buck!

Normal distribution of errors



Confidence intervals

- estimated range of values which is likely to include the measured result

$$x \pm ks$$

Coverage factor

- the value of the coverage factor *k* is chosen on the basis of the desired level of confidence

  k = 1, 68% confidence range

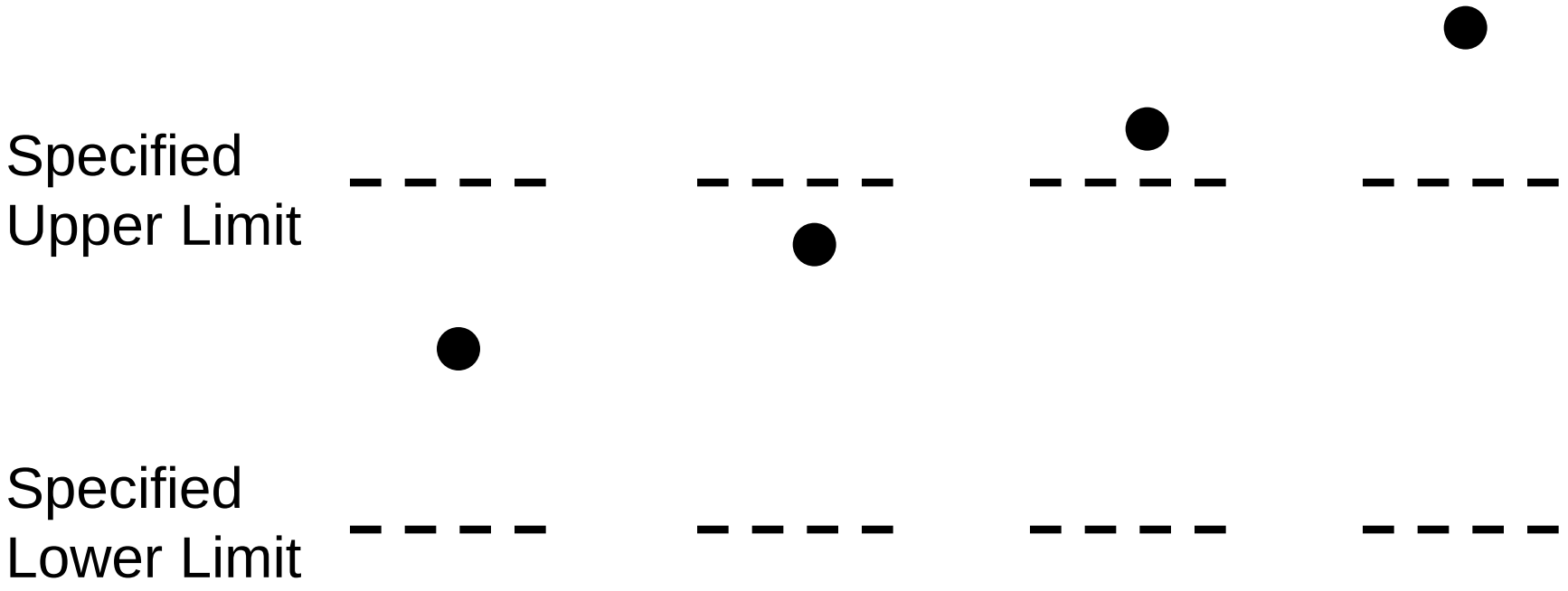
  k = 2, 95% confidence range

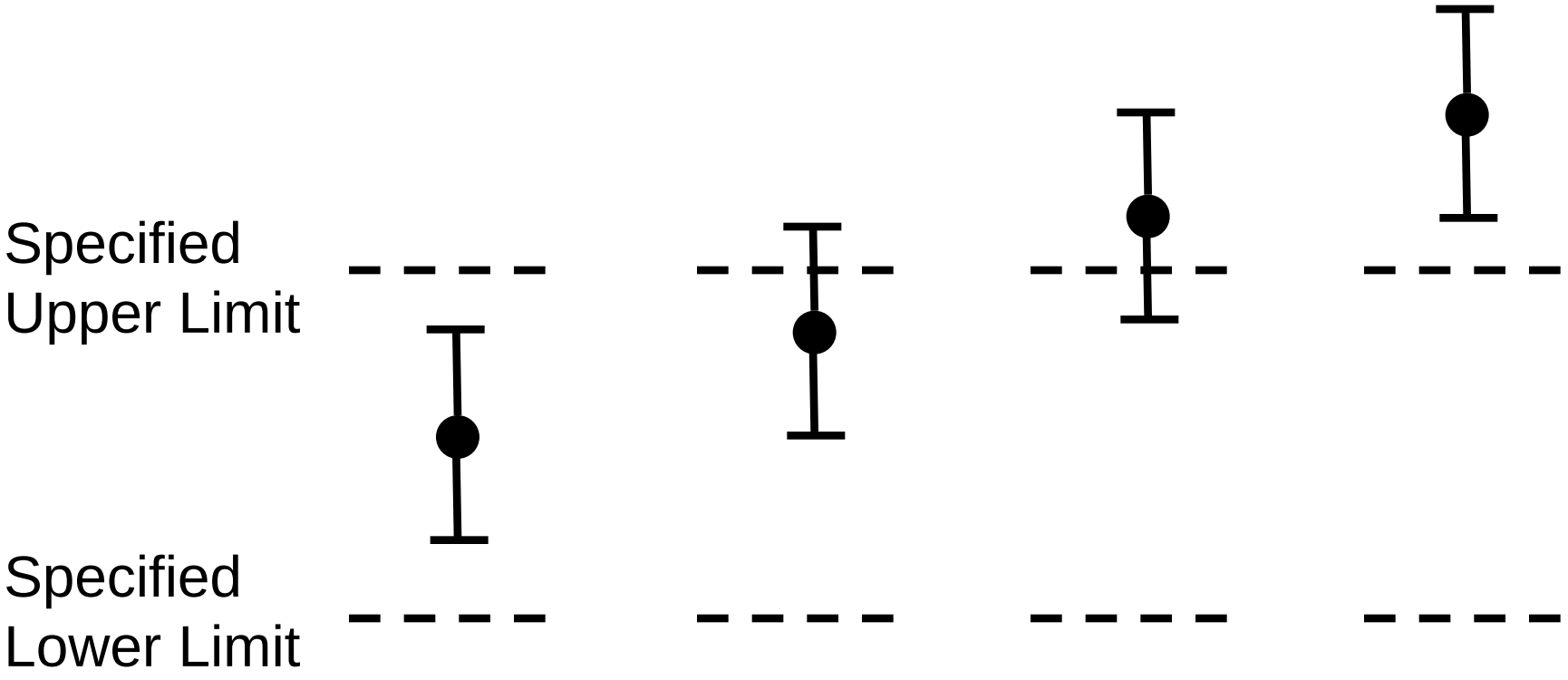
  k = 3, 99% confidence range

eg. $311 \pm 2(5.3) = 311 \pm 10.6$

Confidence intervals

- 68% confidence range = 306 to 316
- 95% confidence range = 300 to 321
- 99% confidence range = 295 to 327


Specified
Upper Limit
Specified
Lower Limit


Specified
Upper Limit
Specified
Lower Limit

Practical significance

- How big a difference do you need to detect?
- What is a meaningful difference in your application?
- What magnitude of result or variance would trigger an action?

Precision

Based on a collaborative study, repeatability and reproducibility coefficients of variation can be expected to range from 1.1 to 2.0% and 5.0 to 6.5%, respectively, over typical FAN concentrations encountered in wort.

References

1. American Society of Brewing Chemists. Report of Subcommittee on Free Amino Nitrogen. *Journal* 33:88, 1975.
2. American Society of Brewing Chemists. Report of Subcommittee on *Methods of Analysis* Wort Review. *Journal* 68:222, 2010.
3. American Society of Brewing Chemists. Report of Subcommittee on Determination of Free Amino Nitrogen in Wort by Segmented Flow Analysis. *Journal* 69:295, 2011.

Determination of Free Amino Nitrogen in Wort by Segmented Flow Analysis

Subcommittee Members: A. MacLeod, *Chair*; C. Adam; A. Budde; T. Chicos; K. Churchill; S. Jenson; B. Johnson; R. Joy; C. Marker; M. Schmitt (EBC); R. Schuba; A. Stern; T. Whittaker; and R. Jennings (*ex officio*)

Keywords: FAN, SFA

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for determination of free amino nitrogen (FAN) by segmented flow analysis (SFA) ranged from 1.1 to 2.0% and 5.0 to 6.3%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that determination of FAN in wort by SFA be included in the ASBC *Methods of Analysis* (1).
2. Discharge the subcommittee.

This is the subcommittee's first year of existence. The subcommittee was formed on the recommendation of the Subcommittee on *Methods of Analysis* Wort Review (2). SFA is commonly used for the determination of FAN in wort.

PROCEDURE

A total of eight barley malt samples representing four sample pairs (similar but distinct) with a range of FAN levels were sent to each collaborator. For each sample, each collaborator prepared a Congress wort according to ASBC Method Malt-4 and measured FAN using segmented flow instrumentation. Collaborators were also

erence method, at 2.5% and 9.3%, respectively, which was determined by a previous collaborative study (3).

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*. Statistical Analysis-4 Youden unit block collaborative testing procedure; Wort-12 Free amino nitrogen. The Society, St. Paul, MN, 2009.
2. American Society of Brewing Chemists. Report of the Subcommittee on *Methods of Analysis* Wort Review. *J. Am. Soc. Brew. Chem.* 68:222-223, 2010.
3. American Society of Brewing Chemists. Report of the Subcommittee on Free Amino Nitrogen. *Proc. Am. Soc. Brew. Chem. 1975*, pp. 88-91.

TABLE I
Free Amino Nitrogen in Wort (ppm) Determined by Segmented Flow Analysis

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	162	158	208	213	192	191	212	215
2	162	161	212	208	189	188	213	213
3	171	171	220	223	200	201	230	227
4	154	155	205	197	183	183	213	217
5	176	177	228	230	208	213	237	239
6	173	175	227	230	206	208	237	238
7	133[a]	116[a]	156[a]	183[a]	162[a]	165[a]	185[a]	199[a]
8	177	179	222	236	209	215	228	241
9	187	188	230	223	210	211	231	232
10	172	179	226	219	202	202	229	232
11	164	168	219	216	199	197	224	226
12	186	185	238	232	212	211	237	240
13	167	170	219	216	194	185	222	219
14	163	168	212	212	190	186	210	213

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Collaborator	A	B	C	D	E	F	G	H
1	162	158	208	213	192	191	212	215
2	162	161	212	208	189	188	213	213
3	171	171	220	223	200	201	230	227
4	154	155	205	197	183	183	213	217
5	176	177	228	230	208	213	237	239
6	173	175	227	230	206	208	237	238
7	133[a]	116[a]	156[a]	183[a]	162[a]	165[a]	185[a]	199[a]
8	177	179	222	236	209	215	228	241
9	187	188	230	223	210	211	231	232
10	172	179	226	219	202	202	229	232
11	164	168	219	216	199	197	224	226
12	186	185	238	232	212	211	237	240
13	167	170	219	216	194	185	222	219
14	163	165	212	212	190	186	210	213
15	151	155	200	205	196	194	203	215
Mean[b]	168.9	170.4	219.0	218.6	199.3	198.9	223.3	226.2
Grand mean[b]	169.7		218.8		199.1		224.8	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Calculated excluding outliers.

TABLE III
Statistical Summary of Results[a]

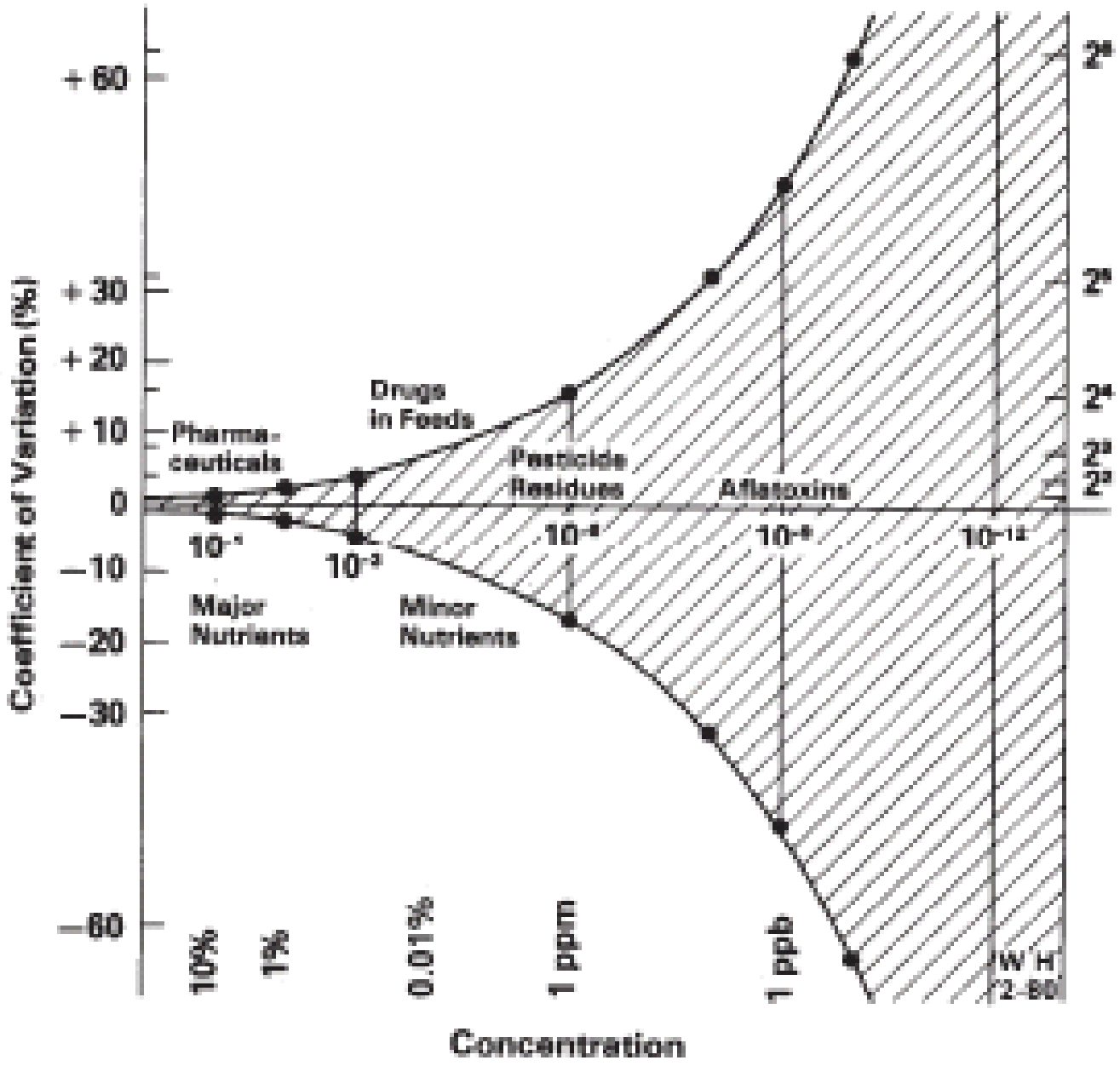
Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
A/B	14	169.7	1.9	1.1	5.2	10.7	6.3	29.8
C/D	14	218.8	4.4	2.0	12.2	10.9	5.0	30.5
E/F	14	199.1	2.6	1.3	7.2	10.2	5.1	28.6
G/H	14	224.8	2.7	1.2	7.5	11.2	5.0	31.3

The Horwitz equation

- There are natural limits on the Reproducibility of chemical measurement methods

$$cv_R = 2C^{-0.15}$$

- where C is the concentration in mass fraction


+60
+30
+20
+10
0
−10
−20
−30
−60
Coefficient of Variation (%)
Pharma-
ceuticals
Drugs
in Feeds
Pesticide
Residues
Aflatoxins
Major
Nutrients
Minor
Nutrients
10%
1%
0.01%
1 ppm
1 ppb
W H
2-60
Concentration

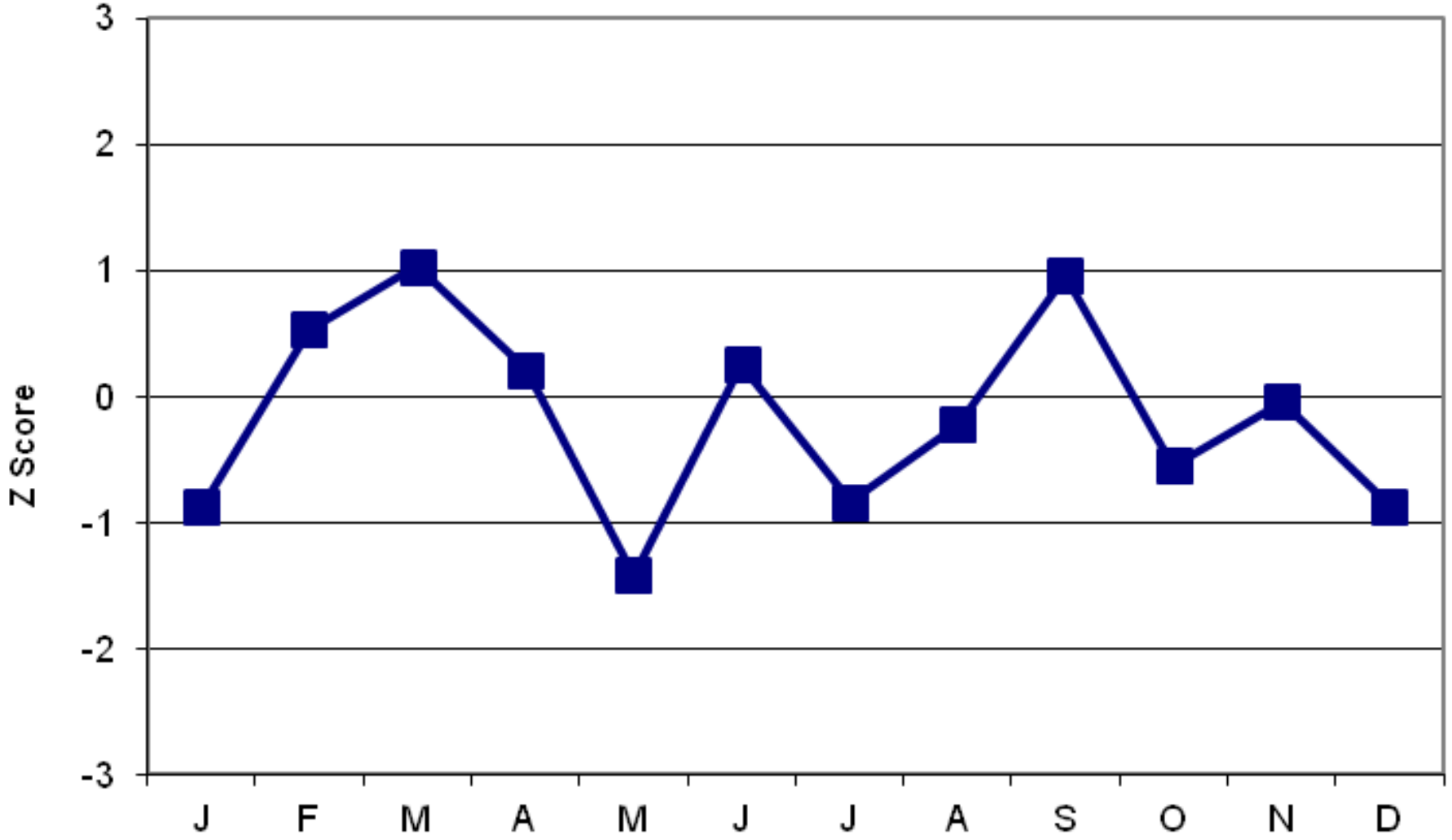
Proficiency testing schemes

- ASBC
  - Barley, Malt, Beer, Hops, Mycotoxins
- Campden BRI
  - Malt (MAPS), Beer (BAPS)

Z-scores

- Z is a measure of how far an individual lab result is from the mean (in units of standard deviation)

$$Z = \frac{(x - \bar{x})}{s}$$


Z Score
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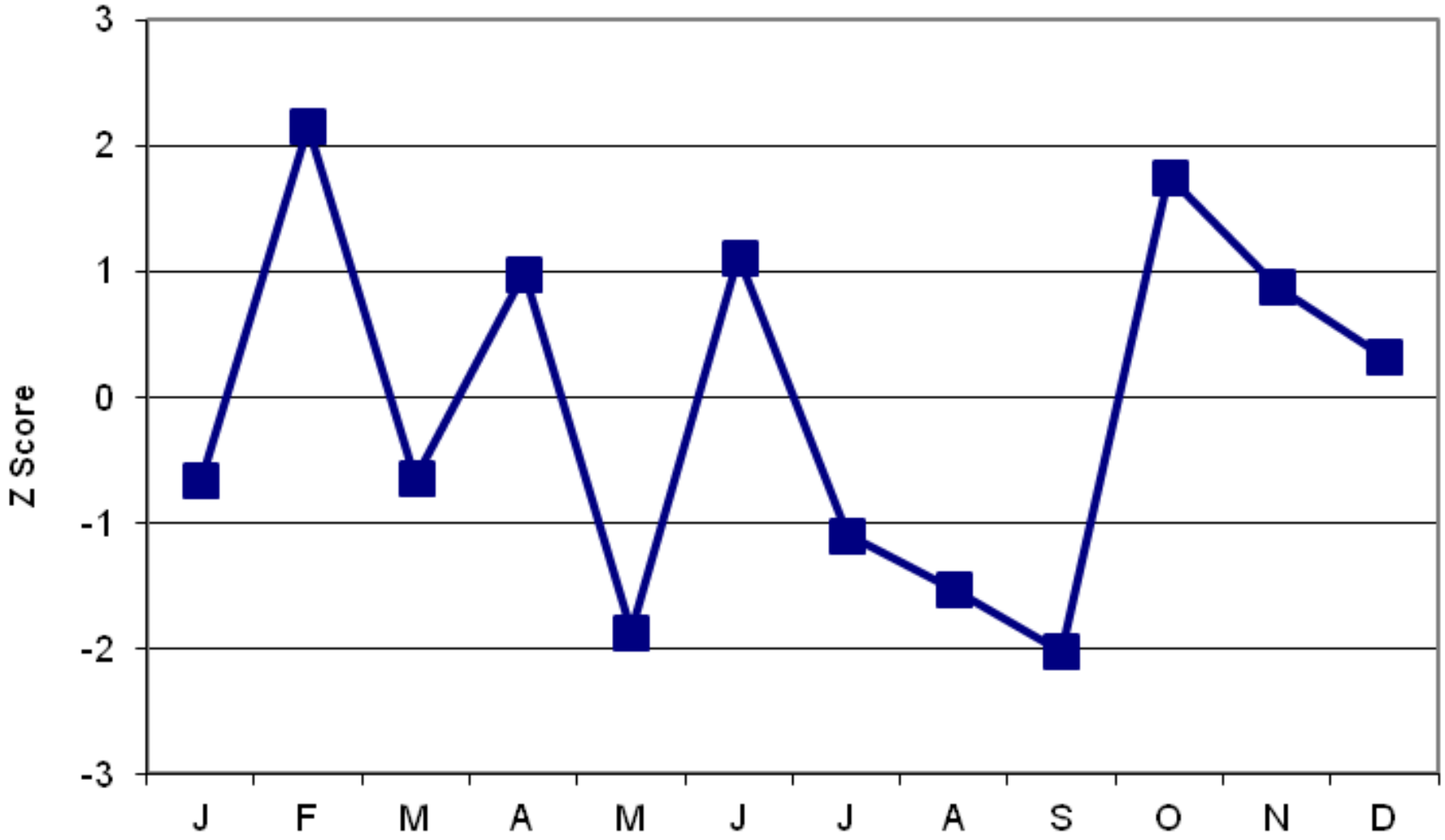
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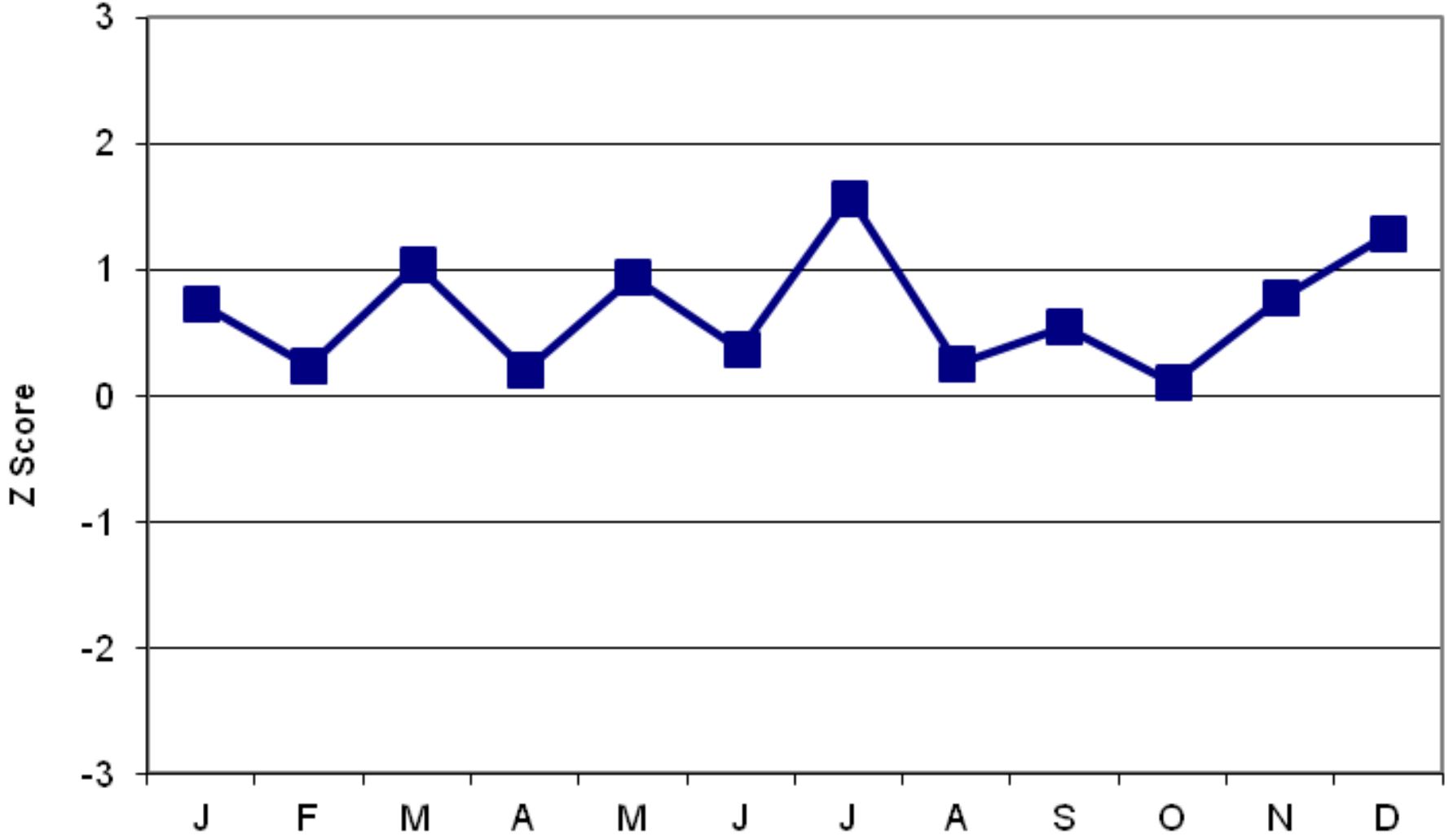
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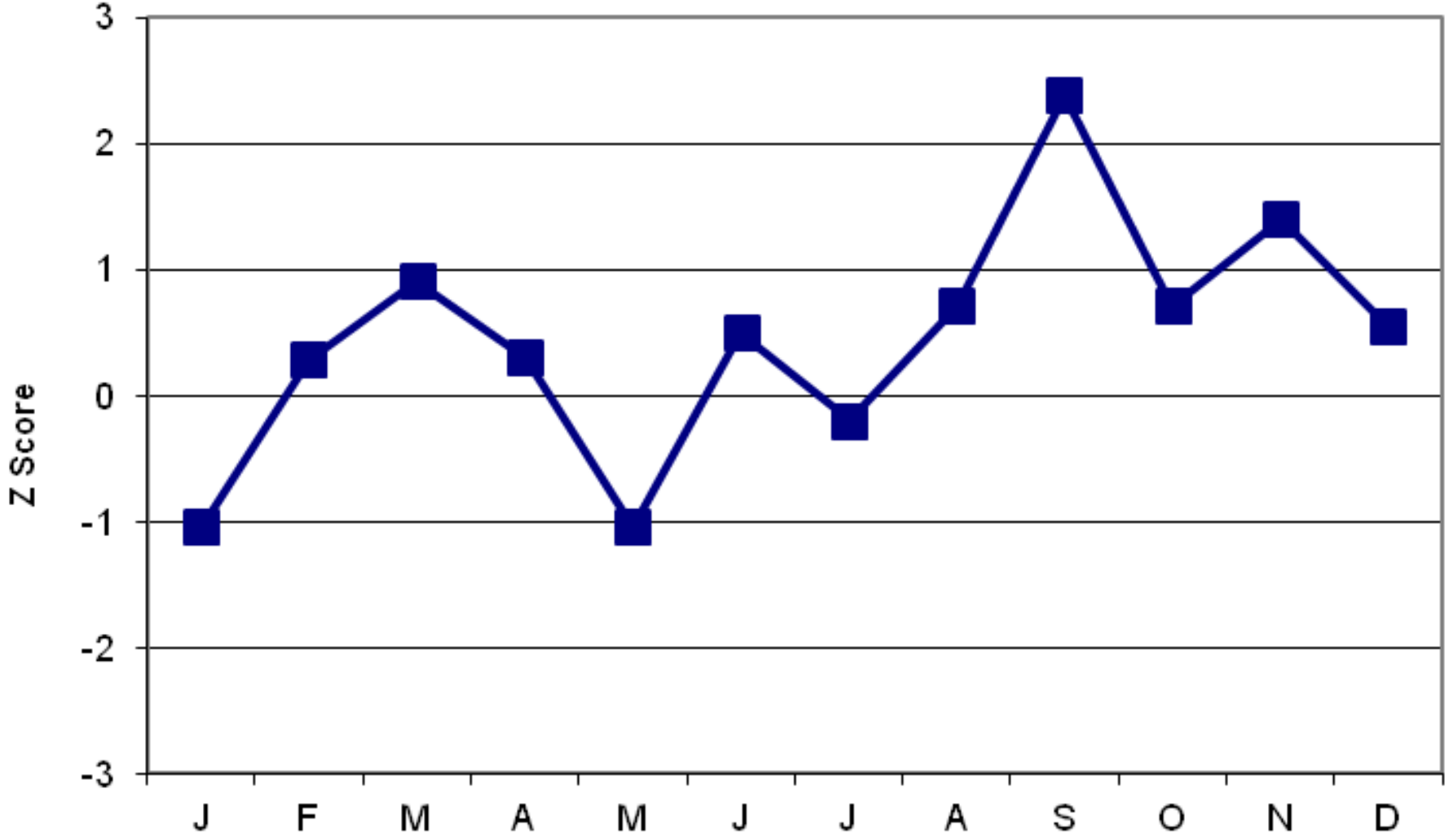
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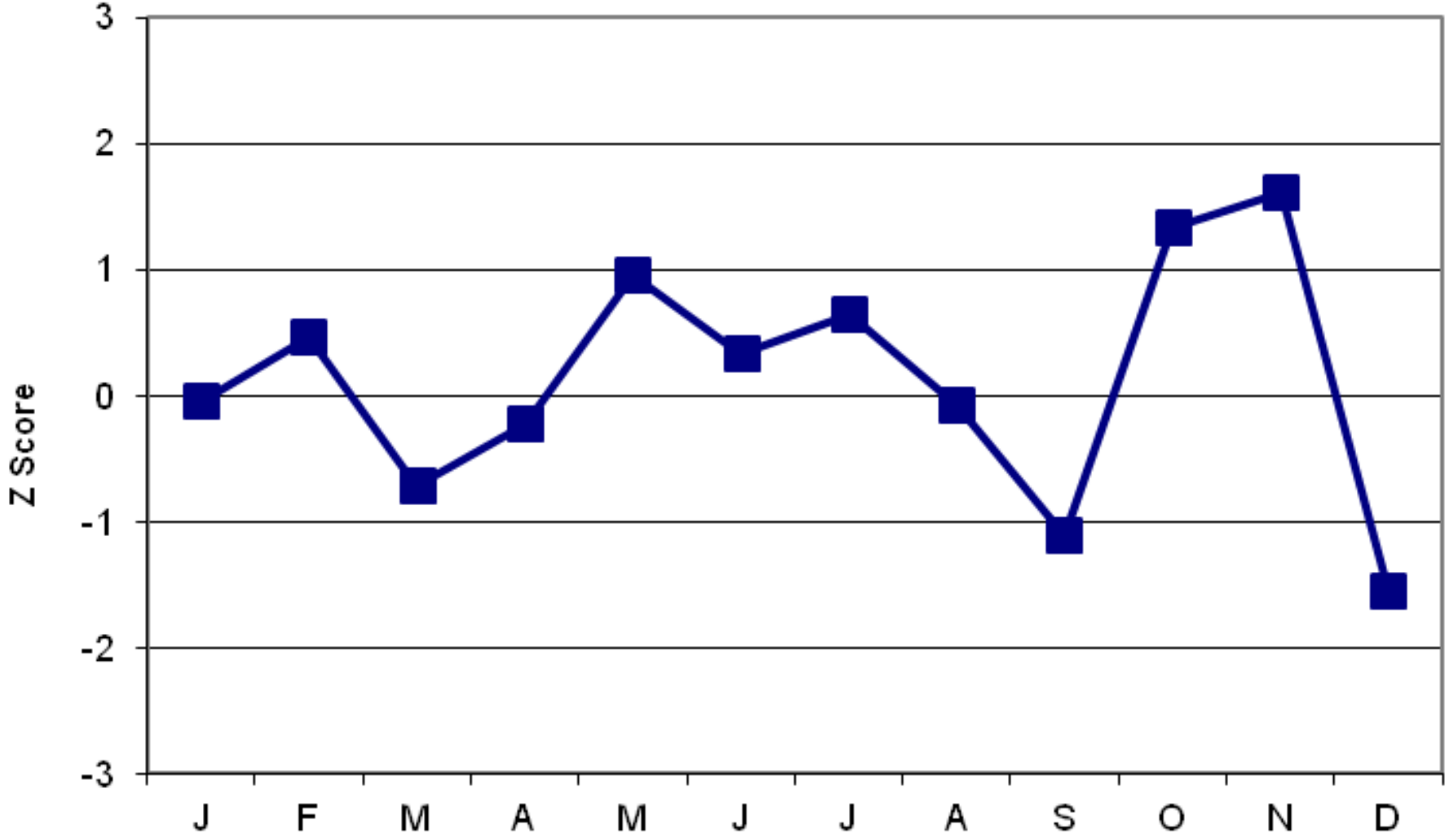
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Test	Within a lab (r_{95})	Between labs (R_{95})
Moisture (%)	0.2	0.8
Extract (%)	0.4	1.4
Friability (%)	3.0	7.0
Color (°ASBC)	0.2	0.5
Diastatic Power (°L)	10	30
Alpha Amylase (DU)	5	15
FAN (mg/L)	7	40
B-glucan (mg/L)	20	50

Standard Reference Material

- SRM should represent the same sample matrix
- SRM must be homogeneous so that test portions are identical for the analyte
- SRM must be stable over time with respect to the analyte concentration

Non parametric data

- *Sensitivity* is the probability that the test will correctly identify a positive sample.

- *Specificity* is the probability that the test will correctly identify a negative sample

	The Truth: Positive	The Truth: Negative
Test Score: Positive	True Positives (TP) — a	False Positives (FP) — b
Negative	False Negatives (FN) — c	True Negatives (TN) — d

$$\textbf{PPV} = \frac{TP}{TP + FP}$$

$$\textbf{NPV} = \frac{TN}{TN + FN}$$

Sensitivity

$$\frac{TP}{TP + FN}$$

Specificity

$$\frac{TN}{TN + FP}$$

Or,

$$\frac{a}{a + c}$$

$$\frac{d}{d + b}$$

- *Sensitivity* = (# of true positives)/(# of true positives + # of false negatives) * 100

- *Specificity* = (# of true negatives)/(# of true negatives + # of false positives) * 100

Good news!

- Good routine QC practices can all be used as tools in the estimation of uncertainty of analytical measurements

- Plan your method development and routine QC program with this in mind

Discussion…