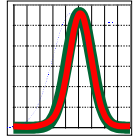


Sample Homogeneity Testing

Following the Protocol Outlined in:

“The International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories”, 2006 (IHP), MICHAEL THOMPSON, STEPHEN L. R. ELLISON AND ROGER WOOD

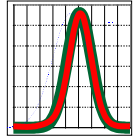


Heterogeneity between the sample units ($\sigma^2_{\text{Sampling}}$) can inflate the spread in sample results. This can mask the true Lab Bias and interferes with Z scores.

Sources of Variance for Check Sample Results can be expressed as follows:

$$\sigma^2_{\text{SampleResults}} = \sigma^2_{\text{Analytical}} + \sigma^2_{\text{Labs}} + \sigma^2_{\text{Sampling}}$$

Ideally, to test for sample homogeneity we would like to minimize the analytical variance ($\sigma^2_{\text{Analytical}}$) and the Lab Bias (σ^2_{Labs}) and isolate the variance due to the sample units ($\sigma^2_{\text{Sampling}}$) .

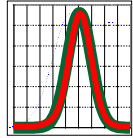


So, how do we minimize the analytical variance ($\sigma^2_{\text{Analytical}}$) and the Lab Bias (σ^2_{Labs}) and isolate the variance due to the sample units ($\sigma^2_{\text{Sampling}}$).

- Randomly select 10 sample units from a batch for analysis.
 - Represents the sampling bias we wish to measure.
- Select a single expert lab (we used an ISO 17025 accredited lab using methods reported by AAFCO participant labs).
 - Should remove inter Lab bias between the samples.
- Choose a method with very low analytical variance.
 - Minimize analytical variance.

A Quote from the IHP:

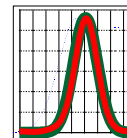
“Homogeneity tests should be regarded as essential, but not foolproof, safeguards.”



Let's look at a Dataset for 10 sample units analyzed in duplicate at one lab.

- A Cochran test checks for outliers in duplicates.
- **Subtracting duplicates removes the sampling bias** in each sample unit.
- **Adding the duplicates generates twice this bias.**

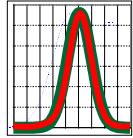
Sample Code (Sample Unit)	Moisture (%)			
	Dup 1	Dup 2	Difference	Sum
1	5.69	5.60	0.09	11.29
2	5.84	5.72	0.12	11.56
3	5.59	5.54	0.05	11.13
4	5.70	5.69	0.01	11.39
5	5.69	5.56	0.13	11.25
6	5.72	5.65	0.07	11.37
7	5.54	5.53	0.01	11.07
8	5.58	5.42	0.16	11.00
9	5.52	5.65	-0.13	11.17
10	5.64	5.65	-0.01	11.29



Now we can Calculate the Sampling Variation

Number of Pairs	10
Grand Average	5.626
Sampling Variation Calculation	
A - Variance of Differences (sampling error removed)	0.00448
B - Variance of Sums (includes A and 2 x sampling error)	0.02766
Variance Attributed to Sampling (B/2-A)/2	0.0047
Repeatability %rsd	1.19%
% RSD of Sample Means	1.48%
$\sigma_{\text{analytical}} / \sigma_{\text{proficiency}}$ (Should be $\sim < 0.5$)	0.3789

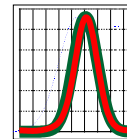
**Next we need to calculate a critical Variance
which should not be exceeded**



Calculating a one sided 95% CI for the Sampling Variation

Number of Pairs	10
Grand Average	5.626
Allowed Variation	
We must decide what dispersion is ffp! Median Robust % RSD from last 19 CSP Samples Chosen to represent the σ_{ffp} for the % Moisture Method	3.14%
SD for Proficiency Testing (σ_{ffp}) 5.626 * 3.14/100.	0.1767
From the IHP: Allowed Variance (30% of target)	0.0028
F1 constant (95% Confidence)	1.8799
F2 constant (95% Confidence)	1.0102
Critical Allowed Variance ($\sigma^2_{Allowed}$)	0.0098

Since **0.0047** is less than **0.0098** there is no evidence of sampling variance.



Let's Talk About σ_{ffp} ...

We need our best estimate of the usual and expected dispersion for the analysis with respect to our sample matrices and concentration types.

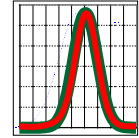
In the CSP we calculate σ_{ffp} for each sample from participants data.

This is not practical for Homogeneity testing. We need to pick a σ_{ffp} appropriate to a particular analyte/method but across all our samples.

I looked back over the last 21 CSP samples and selected the Median Reproducibility %RSD for each Analyte used in the Homogeneity test.

This is a first approximation which we can refine as we move forward with more samples.

I favor using %RSD as it reflects variance dependent on concentration.

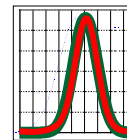


For example, we ran 2 Check Samples for Homogeneity Testing:

- Medicated Chicken Starter (201326)
- Soya Flour (201342)

And requested the following analyses:

Analyte	Method	CSP Method #
Moisture	Moisture-Forced Draft (Food Products) - AOAC 930.15	011.01
Protein	Nitrogen by Combustion - AOAC 990.03	002.06
Ca	Calcium by ICP - AOAC 965.17 / 985.01 mod.	019.41/019.44
Fe	Iron by ICP - AOAC 965.17 / 985.01 mod.	025.41
Mg	Magnesium by ICP - AOAC 965.17 / 985.01 mod.	027.41/027.44
Mn	Manganese by ICP - AOAC 965.17 / 985.01 mod.	028.41/028.44
P	Phosphorus by ICP - AOAC 965.17 / 985.01 mod.	031.41/031.44
K	Potassium by ICP - AOAC 965.17 / 985.01 mod.	032.41
Na	Sodium by ICP - AOAC 965.17 / 985.01 mod.	035.41
Zn	Zinc by ICP - AOAC 965.17 / 985.01 mod.	037.41/037.44

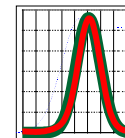


Medicated Chicken Starter (201326)

Expert Lab Sample Code		Moisture (%)		Protein (%)		Ca (%)		P (%)	
#	Ending in:	1	2	1	2	1	2	1	2
1	6040235	11.11	11.59	18.63	18.88	0.9068	0.9097	0.7447	0.7471
2	6040236	11.13	11.52	18.56	18.63	0.9417	0.9101	0.7613	0.7667
3	6040237	11.23	11.54	18.75	18.63	0.9009	0.9175	0.7698	0.7548
4	6040238	11.13	11.44	18.63	18.63	0.9048	0.9238	0.7677	0.7675
5	6040239	11.12	11.39	18.75	18.75	0.9088	0.9284	0.7487	0.7665
6	6040240	11.08	11.40	18.56	18.69	0.9077	0.9340	0.7663	0.7655
7	6040241	11.09	11.32	18.81	18.94	0.9093	0.9248	0.7576	0.7606
8	6040242	11.02	11.25	18.56	18.94	0.9206	0.9195	0.7623	0.7668
9	6040243	11.09	11.32	18.75	18.63	0.9143	0.9243	0.7589	0.7663
10	6040244	11.27	11.28	18.81	18.50	0.9127	0.9122	0.7715	0.7650
Outlier Test		PASS		PASS		PASS		PASS	
Allowed Variation									
Median % RSD from CSP		3.14%		1.58%		4.20%		4.11%	
Critical variance		0.0671		0.0333		0.0004		0.0002	
Actual Variation									
Variance Attributed to Sampling		0.0000		0.0000		0.0000		0.0000	
Homogeneity Decision		PASS		PASS		PASS		PASS	



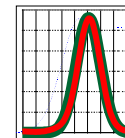
Soy Flour (201326)



Expert Lab Sample Code		Moisture (%)		Protein (%)		Ca (%)		P (%)	
#	Ending in:	1	2	1	2	1	2	1	2
1	6040245	5.69	5.60	50.44	50.38	0.2705	0.2683	0.6982	0.7001
2	6040246	5.84	5.72	50.00	50.00	0.2662	0.2724	0.7024	0.7019
3	6040247	5.59	5.54	50.56	50.56	0.2659	0.2687	0.6996	0.6965
4	6040248	5.70	5.69	50.06	50.44	0.2650	0.2686	0.7022	0.7039
5	6040249	5.69	5.56	50.75	51.19	0.2669	0.2723	0.6976	0.7032
6	6040250	5.72	5.65	50.38	50.56	0.2686	0.2706	0.7019	0.6992
7	6040251	5.54	5.53	50.75	50.81	0.2604	0.2708	0.6934	0.6949
8	6040252	5.58	5.42	50.06	50.31	0.2668	0.2686	0.6961	0.7014
9	6040253	5.52	5.65	51.06	50.75	0.2620	0.2703	0.6978	0.7008
10	6040254	5.64	5.65	50.81	50.75	0.2642	0.2701	0.6980	0.7081
Outlier Test		PASS		PASS		PASS		PASS	
Allowed Variation									
Median % RSD from CSP		3.14%		1.58%		4.20%		4.11%	
Critical variance		0.0098		0.1351		0.0000		0.0001	
Actual Variation									
Variance Attributed to Sampling		0.0047		0.0929		0.0000		0.0000	
Homogeneity Decision		PASS		PASS		PASS		PASS	

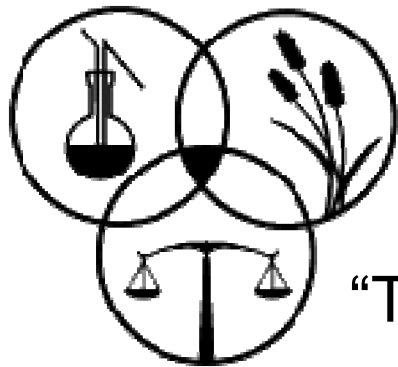
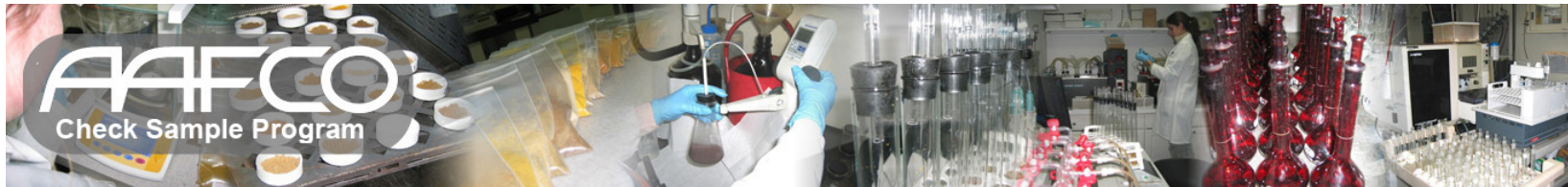


Soy Flour (201326)



Expert Lab Sample Code		Fe (%)		Mg (%)		Mn (ppm)		K (%)		Na (%)*		Zn (ppm)	
#	Ending in:	1	2	1	2	1	2	1	2	1	2	1	2
1	6040245	0.0071	0.0072	0.2898	0.2935	33.28	32.84	2.3078	2.2967	0.0031	0.0032	46	46
2	6040246	0.0071	0.0071	0.2870	0.2909	32.54	32.73	2.2889	2.2632	0.0026	0.0032	46	45
3	6040247	0.0070	0.0071	0.2887	0.2910	32.97	32.47	2.3172	2.2846	0.0055	<0.002	45	45
4	6040248	0.0071	0.0071	0.2876	0.2906	32.58	32.51	2.3162	2.3006	0.0024	0.0044	45	45
5	6040249	0.0071	0.0071	0.2889	0.2901	32.76	32.59	2.3029	2.3024	0.0033	0.0032	45	48
6	6040250	0.0070	0.0072	0.2871	0.2902	32.72	32.64	2.2905	2.2968	0.0028	0.0035	45	48
7	6040251	0.0070	0.0070	0.2851	0.2891	32.59	32.58	2.3039	2.2784	0.0022	0.0033	46	46
8	6040252	0.0071	0.0071	0.2861	0.2917	32.36	32.58	2.2937	2.3073	0.0035	<0.002	47	45
9	6040253	0.0071	0.0072	0.2835	0.2872	32.58	32.53	2.2670	2.2966	0.0033	<0.002	46	45
10	6040254	0.0072	0.0073	0.2899	0.2882	32.45	32.74	2.2924	2.3365	0.0036	<0.002	46	45
Outlier Test		PASS		PASS		PASS		PASS		No Test		PASS	
Allowed Variation													
Median % RSD from CSP		5.73%		4.56%		4.52%		4.77%		5.06%		6.32%	
Critical variance		0.0000		0.0000		0.4016		0.0023		0.0000		2.6772	
Actual Variation													
Variance Attributed to Sampling		0.0000		0.0000		0.0097		0.0000		0.0000		0.0000	
Homogeneity Decision		PASS		PASS		PASS		PASS		FAIL		PASS	

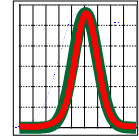
*A review of the failure for Na reveals concentrations too close to the reporting limit, $\sigma^2_{Analytical}$ not consistent.



Sample Homogeneity Testing

“The Remarkable Case of Combustion Nitrogen”

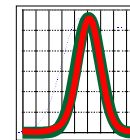
Protein by Combustion Nitrogen (# 002.06) Method Parameters		Assigned Value - Robust Mean	# Labs Included in Calculations	Between Labs %RSD	Repeatability %rsd	Reproducibility %RSD	sR/sr	Modified Horwitz %RSD
201341	Chicken Meal	64.93	48	1.02%	0.75%	1.27%	1.69	1.24
201342	Soya Flour	50.61	39	0.97%	0.41%	1.05%	2.59	1.41
201230	Beef Feedlot special, Medicated	43.41	140	1.86%	0.45%	1.91%	4.30	1.52
201223	Beef Feedlot special, Medicated	41.18	141	0.82%	0.72%	1.10%	1.51	1.56
201225	Dry Cat Food	32.47	137	1.08%	0.58%	1.22%	2.12	1.75
201222	Senior Pig Starter, Medicated	23.37	140	1.07%	0.76%	1.31%	1.73	2.07
201323	Swine Pre-starter, Medicated	22.94	133	1.51%	0.66%	1.65%	2.50	2.09
201229	Swine Pre-starter, Medicated	22.58	139	1.40%	0.62%	1.53%	2.46	2.10
201226	Dairy Herd & Beef Calf Milk Replacer, Medicated	19.48	140	2.09%	0.85%	2.26%	2.65	2.27
201321	Dry Dog Food	19.07	152	1.47%	0.61%	1.59%	2.61	2.29
201322	Chicken Starter/Grower, Medicated	18.85	122	1.70%	0.60%	1.81%	3.00	2.30
201326	Chicken Starter/Grower, Medicated	18.57	129	1.23%	0.69%	1.41%	2.05	2.32
201232	Calf Sarter/Grower, Medicated	18.10	145	1.46%	0.71%	1.63%	2.29	2.35
201221	Chicken Sarter/Grower, Medicated	17.81	142	1.59%	0.84%	1.80%	2.14	2.37
201325	Pelleted Beef Special, Medicated	17.14	128	1.75%	0.72%	1.89%	2.63	2.42
201224	Chicken Starter/Grower, Medicated	17.12	143	1.63%	0.80%	1.81%	2.26	2.42
201228	Beef Cattle Pellet, Medicated	15.44	134	1.86%	0.95%	2.09%	2.19	2.55
201227	Sheep and Goat Grower/Finisher, Medicated	15.29	141	2.10%	0.94%	2.30%	2.44	2.56
201324	Sheep & Goat Grower/Finisher, Medicated	15.04	140	1.81%	0.83%	1.99%	2.39	2.58
201231	Preconditioning cattle starter, Medicated	12.81	144	3.27%	1.21%	3.49%	2.89	2.72
201298	Feed grade monocalcium phosphate	0.83	17	47.15%	5.65%	47.48%	8.41	4.12



Can We Estimate Homogeneity From the CSP Data?

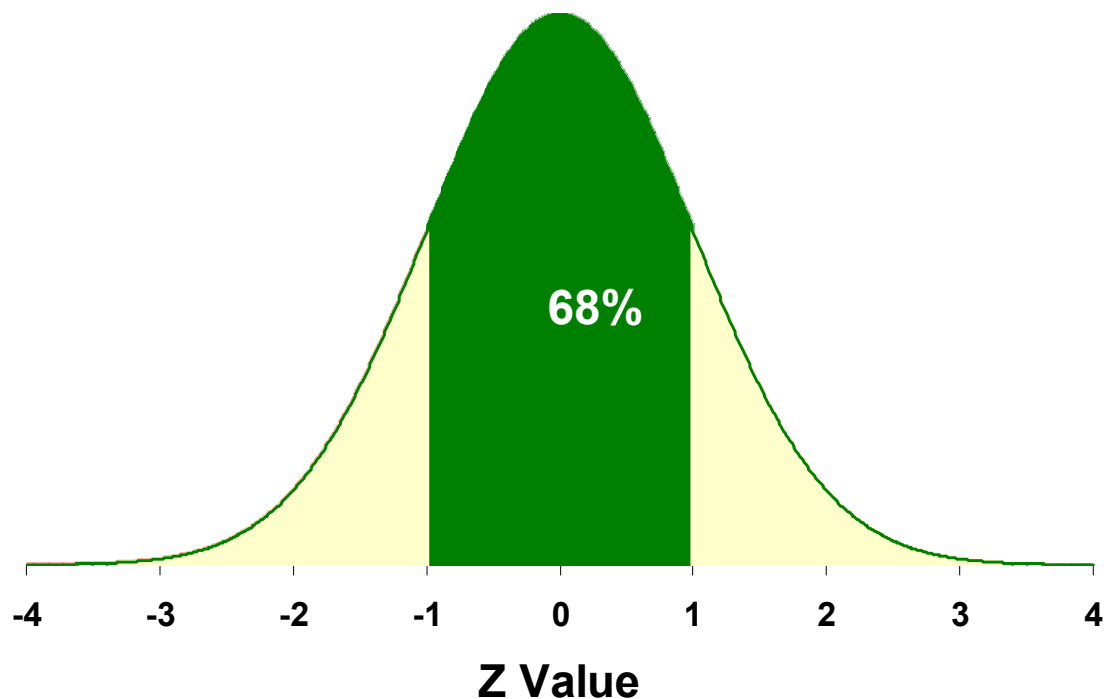
- In “Protein by Combustion N₂” we have a very precise method with over 140 Analysts consistently reporting Reproducibility < 2% (%RSD) and Repeatability < 1% (%rsd) for each sample.
- This is an extremely narrow dispersion for so many Labs.
- So looking at our sources of variance again:
 - Reproducibility is heavily reflected in σ_{Labs} and is low!
 - Repeatability is essentially the $\sigma_{\text{Analytical}}$ and is low!

$$\sigma_{\text{SampleResults}}^2 = \sigma_{\text{Analytical}}^2 + \sigma_{\text{Labs}}^2 + \sigma_{\text{Sampling}}^2$$



Can We Estimate Homogeneity From Protein Data?

So if I take the center portion of the data (Z between ± 1 , $\sim 68\%$ representing typically 140 labs), these Labs should begin to approach the data from a Homogeneity study.



Let's call this the "Z" Cut – the "filet mignon" of the data, if you will.

Now let's compare the Soya Flour Homogeneity report with the "Z" Cut samples run by different labs/analysts from the CSP.

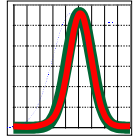
Soya Flour (201342)	Expert Lab	CSP 002.06
Homogeneity Decision	PASS	PASS
Number of Pairs (samples)	10	88 of 120 (Z Cut)
Grand Average	50.53	50.59
Allowed Variation		
Median % RSD from CSP	1.58%	1.58%
SD for Proficiency Testing	0.7984	0.7993
Allowed Variance (30% of target)	0.0574	0.0575
F1 constant	1.8799	1.2618
F2 constant	1.0102	0.2120
Critical variance	0.1351	0.0809
Actual Variation		
Variance of Differences	0.02695	0.03949
Variance of Sums	0.42535	0.19100
Variance Attributed to Sampling	0.0929	0.0280
Repeatability %rsd ($\sigma_{\text{analytical}}$)	0.32%	0.39%
% RSD of Sample Means	0.65%	0.43%
Outlier Test	PASS	PASS

Medicated Chicken Starter (201326) Homogeneity report with the “Z” Cut samples run by different labs/analysts from the CSP.

201326	Expert Lab	CSP 002.06
Homogeneity Decision	PASS	PASS
Number of Pairs	10	133 (Z Cut)
Grand Average	18.7	18.55
Allowed Variation		
Median % RSD from CSP	1.58%	1.58%
SD for Proficiency Testing	0.2955	0.2930
Allowed Variance (30% of target)	0.0079	0.0077
F1 constant	1.8799	1.2107
F2 constant	1.0102	0.1660
Critical variance	0.0333	0.0131
Actual Variation		
Variance of Differences	0.01836	0.0223
Variance of Sums	0.02795	0.0576
Variance Attributed to Sampling	0.0000	0.0033
Repeatability %rsd ($\sigma_{\text{analytical}}$)	0.72%	0.80%
% RSD of Sample Means	0.45%	0.65%
Outlier Test	PASS	PASS

Z Cut Pseudo Homogeneity Testing at 3.00 %RSD to Calculate σ_p

Sample # and Sample Name		Decision	n_z	Mean	Critical	Allowed
201321	Dry Dog Food	PASS	172	19.10	0.0371	0.0155
201322	Chicken Starter/Grower, Medicated	PASS	141	18.87	0.0381	0.0102
201323	Swine Pre-starter, Medicated	PASS	147	22.95	0.0549	0.0107
201324	Sheep & Goat Grower/Finisher, Medicated	PASS	152	15.04	0.0238	0.0226
201325	Pelleted Beef Special, Medicated	PASS	127	17.11	0.0324	0.0148
201326	Chicken Starter/Grower, Medicated	PASS	133	18.55	0.0374	0.0033
201341	Chicken Meal	PASS	100	64.93	0.4598	0.0582
201342	Soya Flour	PASS	88	50.59	0.2699	0.0280
201221	Chicken Sarter/Grower, Medicated	PASS	141	17.82	0.0344	0.0045
201222	Senior Pig Starter, Medicated	PASS	141	23.37	0.0564	0.0069
201223	Beef Feedlot special, Medicated	PASS	158	41.17	0.1690	0.0210
201224	Chicken Starter/Grower, Medicated	PASS	158	17.14	0.0305	0.0196
201225	Dry Cat Food	PASS	145	32.49	0.1077	0.0031
201226	Dairy Herd & Beef Calf Milk Replacer, Medicated	PASS	141	19.45	0.0392	0.0206
201227	Sheep and Goat Grower/Finisher, Medicated	PASS	156	15.30	0.0251	0.0206
201228	Beef Cattle Pellet, Medicated	PASS	144	15.45	0.0255	0.0139
201229	Swine Pre-starter, Medicated	PASS	158	22.60	0.0520	0.0117
201230	Beef Feedlot special, Medicated	PASS	156	43.42	0.1900	0.0170
201231	Preconditioning cattle starter, Medicated	FAIL	159	12.84	0.0225	0.0300
201232	Calf Sarter/Grower, Medicated	PASS	169	18.10	0.0344	0.0133
201298	Feed grade monocalcium phosphate	FAIL	13	0.84	0.0011	0.0319



Recommendation:

- I run a “Z” Cut pseudo Homogeneity test on “Protein by Combustion Nitrogen” data for each sample.
- If it passes the pseudo Homogeneity test at say 3.0 %RSD then we can assume acceptable homogeneity for our purposes.
- If it does not pass we will examine the sample data more closely before reporting a possible homogeneity issue.

This does not substitute for a legitimate Homogeneity study.

But, I think we can make a good case that it is a very reasonable (cost effective!) sample to sample homogeneity flag.
