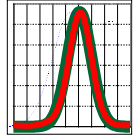


Sample Stability Testing

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

Warning: There will be math!



What do we mean by STABILITY ??

From the IHP:

“Materials distributed in proficiency tests must be sufficiently stable over the period in which the assigned value is to be valid.”

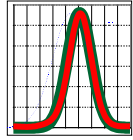
The Assigned Value is decided by Consensus.

We typically see about 90 analytes. That’s ~300 Assigned Values!

Sufficiently stable means that all Z scores are not significantly affected.

Do all the Analyte values remain constant over a two month period?

Has the distribution unit “Sample” changed in any significant way?



STABILITY is a Tricky Problem to measure correctly!

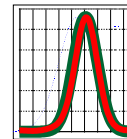
A sample is usually split and one half, the “Control” is analyzed. Then a period of time passes and the second half, the “Test” is analyzed.

Our sources of variance now has a new player, “Intermediate Variance”. This is all the stuff that happens between the “Control” and the “Test” including an instability.

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

We can control for:

- Analytical variance ($\sigma_{\text{Analytical}}^2$) by using a very precise method.
 - Lab Bias (σ_{Labs}^2) by using one expert lab.
 - Sampling ($\sigma_{\text{Sampling}}^2$) after a successful Homogeneity test.
-



How do we control for Intermediate Variance?

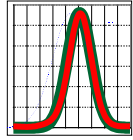
If an Instability exists, its variance is confounded with other contributors to Intermediate variance which are due largely to variation in the efficacy of the Analytical Method over time.

- Different days
- Different techs
- Different reagents/standards/solvents/buffer preps.
- Different instruments

--- A veritable *potpourri* of uncontrolled variance ---

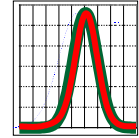
Of course, there are ways to isolate the confounding factors and assess only the variance attributed to an Instability.

However, they require very careful experimental control, can be costly, not very practical in the real world and in the end do we actually believe the result.



So we do the simple (fiscally prudent) experiment and look only for gross Instability effects.

- Our Homogeneity Study provides the stability “Control” or week 0 data.
 - 8 weeks later 3 more replicates are analyzed in duplicate. We call this the stability “Test”.
1. Using the IHP procedure, first we calculate the absolute difference between the average at 8 weeks (V_8) and the control average (V_0).
 2. Using a t-Test we establish if there is a significant difference.
 3. If **Not Significant** then we can not detect a difference, sample is stable (?).
 4. If **Significant** we compare the absolute difference ($|V_0 - V_8|$) with a difference we can tolerate.
 5. If the absolute difference is less than our tolerance the sample is stable.
-



Swine Grower/Finisher, Medicated (201328)

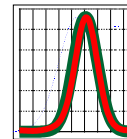
In this example the probability of t is < 0.05
and therefore significant

So now we see if we can tolerate this
difference.

And to do that we first convert this
difference into a Threshold Passing %RSD.

This is a passing criteria for comparison
with our selected tolerance.

Protein (%)			
Week 0		Week 8	
18.1	18.3	18.7	18.3
18.3	18.1	18.6	18.4
18.3	18.3	18.8	18.6
18.8	17.9		
18.1	18.3		
18.0	18.1		
18.5	18.1		
18.0	18.2		
18.1	17.9		
18.0	18.0		
Average		18.12	18.54
Difference ($V_0 - V_8$)			
- 0.42			
t-Test (p) between Week 0 and 8 Weeks			
0.0099			



What Can We Tolerate? Calculating a Threshold Passing %RSD From Our Study

- 1) From the IHP to establish Stability we compare:

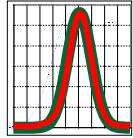
$$|V_0 - V_8| \Leftrightarrow 0.3 \times \sigma_{ffp} \quad (\text{IHP Tolerance, Magic Numbers!})$$

- 2) Then by equating we define the threshold condition:

$$\text{Threshold Passing \%RSD} = 100 \times \frac{|V_0 - V_8|}{0.3 \times V_0}$$

- 3) From our Protein Example:

$$\text{Threshold Passing \%RSD} = 100 \times \frac{|0.42|}{0.3 \times 18.12} = 7.72 \%$$



Magic Numbers!

Why is an extra 30% of σ_{ffp}
An Acceptable Tolerance?

$$Z = \frac{(X_{Lab} - \bar{X}_{AllLabs})}{\sigma_{ffp}}$$

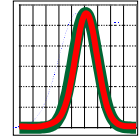
$$Z_{Instability} = \frac{(X_{Lab} - \bar{X}_{AllLabs})}{\sqrt{\sigma_{ffp}^2 + (0.3\sigma_{ffp})^2}}$$

$$Z_{Instability} = \frac{(X_{Lab} - \bar{X}_{AllLabs})}{\sqrt{\sigma_{ffp}^2 \times (1 + 0.09)}}$$

$$Z_{Instability} = \frac{(X_{Lab} - \bar{X}_{AllLabs})}{\sigma_{ffp}} \times \frac{1}{\sqrt{1 + 0.09}}$$

**We can tolerate a 4%
reduction in Z**

$$Z_{Instability} = \frac{(X_{Lab} - \bar{X}_{AllLabs})}{\sigma_{ffp}} \times 0.96$$

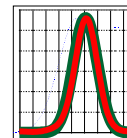


I Chose This Example to Represent an All Too Common Situation

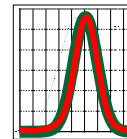
Swine Grower/Finisher, Medicated (201328)

Data Source for Protein %RSD Estimation	% RSD
Threshold Passing %RSD from Stability Study	7.72%
Fit-For-Purpose %RSD from median %RSD in CSP	1.58%
Very Pertinent Additional Information! Sample 201328 Actual Robust %RSD from 297 Labs	1.65%

Let's look at the three stability tests we ran and interpret in light of this finding.

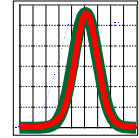


Analyte Tested	Stability Concern (Not Analytical Concern!)
Protein (%)	Represents the organic component - usually decay (N?).
Moisture (%)	Indicator of desiccation or hygroscopic effects.
Ca (%)	<p>Elements in food are usually of the stable variety.</p> <p>Any gross instability in the sample should affect all elements equally.</p>
Fe (%)	
Mg (%)	
Mn (ppm)	
P (%)	
K (%)	
Na (%)	
Zn (ppm)	



AAFCO Check Sample Program - Sample Stability Testing
Swine Grower/Finisher, Medicated (201328)

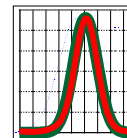
Analyte	Mean At 0 Weeks	Mean at 8 Weeks	t-Test (p) between 0 and 8 Weeks	$ A_0 - A_8 $	Threshold Passing %RSD From Study	Actual %RSD from CSP	# Labs	Significant Difference ?	Actual %RSD > Study %RSD	Action
Protein (%)	18.12	18.54	0.0099	0.420	7.72%	1.65%	297	YES	NO	
Moisture (%)	10.54	10.73	0.0786	0.191	6.04%	3.34%	154	NO		
Ca (%)	0.74	0.75	0.0001	0.0119	5.39%	5.14%	165	YES	NO	
Fe (%)	0.0295	0.0299	0.0101	0.00034	3.86%	6.45%	85	YES	YES	Review
Mg (%)	0.218	0.215	0.0050	0.0031	4.75%	4.64%	80	YES	NO	
Mn (ppm)	98.9	99.2	0.7531	0.267	0.90%	6.09%	89	NO		
P (%)	0.601	0.612	0.0001	0.0112	6.20%	3.95%	158	YES	NO	
K (%)	0.672	0.684	0.0008	0.0119	5.89%	4.30%	86	YES	NO	
Na (%)	0.234	0.238	0.0000	0.0035	4.97%	5.62%	109	YES	YES	Review
Zn (ppm)	225.3	236.2	0.0000	10.87	16.08%	5.68%	105	YES	NO	



AAFCO Check Sample Program - Sample Stability Testing
Swine Grower/Finisher, Medicated (201328)

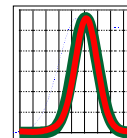
Analyte	Mean At 0 Weeks	Mean at 8 Weeks	t-Test (p) between 0 and 8 Weeks	$ A_0 - A_8 $	Threshold Passing %RSD From Study	Actual %RSD from CSP	# Labs	Significant Difference ?	Actual %RSD > Study %RSD	Action
Protein (%)	18.12	18.54	0.0099	0.420	7.29%	3.34%	297	YES	NO	
Moisture (%)	10.54	10.73	0.0786	0.191	3.34%	3.34%	154	NO		
Ca (%)	0.74	0.75	0.0001	0.0119	3.34%	3.34%	155	YES	NO	
Fe (%)	0.0295	0.0299	0.0101	0.00034	3.86%	3.86%	85	YES	YES	Review
Mg (%)	0.218	0.215	0.0050	0.003	4.64%	4.64%	50	YES	NO	
Mn (ppm)	98.9	99.2	0.7531	0.267	1.90%	1.90%	89	NO		
P (%)	0.601	0.612	0.0001	0.0112	6.25%	6.25%	158	YES	NO	
K (%)	0.672	0.684	0.0008	0.0119	5.89%	4.30%	86	YES	NO	
Na (%)	0.234	0.238	0.0000	0.0035	4.97%	5.62%	109	YES	YES	Review
Zn (ppm)	225.3	236.2	0.0000	10.87	16.08%	5.68%	105	YES	NO	





AAFCO Check Sample Program - Sample Stability Testing
Soya Flour (201342)

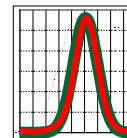
Analyte	Mean At 0 Weeks	Mean at 8 Weeks	t-Test (p) between 0 and 8 Weeks	$ A_0 - A_8 $	Threshold Passing %RSD From Study	Actual %RSD from CSP	# Labs	Significant Difference ?	Actual %RSD > Study %RSD	Action
Protein (%)	50.53	50.34	0.3540	0.188	1.24%	0.86%	129	NO		
Moisture (%)	5.63	5.94	0.0838	0.309	18.31%	4.95%	108	NO		
Ca (%)	0.268	0.270	0.0057	0.0022	2.73%	7.98%	11	YES	YES	Review
Fe (%)	0.007	0.007	0.8521	0.0000	1.09%	10.76%	15	NO		
Mg (%)	0.289	0.292	0.0156	0.0033	3.80%	2.78%	17	YES	NO	
Mn (ppm)	32.7	32.9	0.2420	0.243	2.48%	6.96%	14	NO		
P (%)	0.700	0.692	0.0268	0.0075	3.59%	3.39%	17	YES	NO	
K (%)	2.297	2.336	0.0222	0.0384	5.58%	3.74%	17	YES	NO	
Na (%)	0.003	0.004	0.7606	0.0002	22.54%	107.03%	8	NO		
Zn (ppm)	45.8	44.8	0.4083	0.917	6.68%	16.07%	12	NO		



AAFCO Check Sample Program - Sample Stability Testing
Soya Flour (201342)

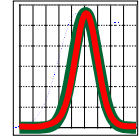
Analyte	Mean At 0 Weeks	Mean at 8 Weeks	t-Test (p) between 0 and 8 Weeks	$ A_0 - A_8 $	Threshold Passing %RSD From Study	Actual %RSD from CSP	# Labs	Significant Difference ?	Actual %RSD > Study %RSD	Action
Protein (%)	50.53	50.34	0.3540	0.188	1.24%	0.86%	129	NO		
Moisture (%)	5.63	5.94	0.0838	0.309	1.21%	4.95%	108	NO		
Ca (%)	0.268	0.270	0.0657	0.002	7.98%	7.98%	11	YES	YES	Review
Fe (%)	0.007	0.007	0.8511	0.0000	1.09%	10.76%	15	NO		
Mg (%)	0.289	0.292	0.0115	0.0033	3.80%	2.78%	17	YES	NO	
Mn (ppm)	32.7	32.9	0.2422	0.200	6.96%	6.96%	14	NO		
P (%)	0.700	0.692	0.0268	0.0075	3.51%	3.39%	17	YES	NO	
K (%)	2.297	2.336	0.0222	0.039	5.59%	3.74%	17	YES	NO	
Na (%)	0.003	0.004	0.7606	0.0002	22.54%	107.03%	8	NO		
Zn (ppm)	45.8	44.8	0.4083	0.917	6.68%	16.07%	12	NO		





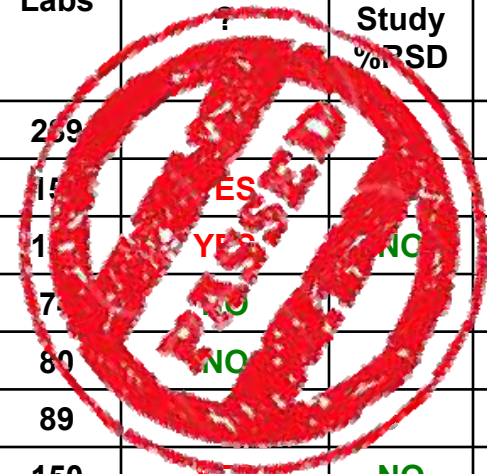
AAFCO Check Sample Program - Sample Stability Testing
Medicated Chicken Starter (201326)

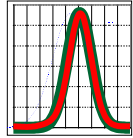
Analyte	Mean At 0 Weeks	Mean at 8 Weeks	t-Test (p) between 0 and 8 Weeks	$ A_0 - A_8 $	Threshold Passing %RSD From Our Study	Actual %RSD from CSP	# Labs	Significant Difference ?	Actual %RSD > Study %RSD	Action
Protein (%)	18.70	18.71	0.9244	0.0083	0.15%	1.47%	289	NO		
Moisture (%)	11.27	11.50	0.0004	0.2307	6.82%	3.31%	156	YES	NO	
Ca (%)	0.92	0.94	0.0036	0.0188	6.85%	5.07%	161	YES	NO	
Fe (%)	0.027	0.027	0.1282	0.0003	3.40%	7.04%	74	NO		
Mg (%)	0.212	0.215	0.0539	0.0027	4.19%	4.04%	80	NO		
Mn (ppm)	92.0	90.8	0.1828	1.1305	4.10%	5.29%	89	NO		
P (%)	0.762	0.754	0.0360	0.0079	3.48%	2.77%	150	YES	NO	
K (%)	0.766	0.792	0.0000	0.0264	11.47%	5.58%	88	YES	NO	
Na (%)	0.219	0.223	0.0094	0.0037	5.59%	6.49%	95	YES	YES	Review
Zn (ppm)	90.0	88.2	0.0001	1.8333	6.79%	6.31%	91	YES	NO	



AAFCO Check Sample Program - Sample Stability Testing
Medicated Chicken Starter (201326)

Analyte	Mean At 0 Weeks	Mean at 8 Weeks	t-Test (p) between 0 and 8 Weeks	$ A_0 - A_8 $	Threshold Passing %RSD From Our Study	Actual %RSD from CSP	# Labs	Significant Difference ?	Actual %RSD > Study %RSD	Action
Protein (%)	18.70	18.71	0.9244	0.0083	0.15%	1.47%	269			
Moisture (%)	11.27	11.50	0.0004	0.2307	6.82%	3.31%	15	YES		
Ca (%)	0.92	0.94	0.0036	0.0188	6.85%	5.07%	1	YES	NO	
Fe (%)	0.027	0.027	0.1282	0.0003	3.40%	7.04%	7	NO		
Mg (%)	0.212	0.215	0.0539	0.0027	4.19%	4.04%	80	NO		
Mn (ppm)	92.0	90.8	0.1828	1.1305	4.10%	5.29%	89			
P (%)	0.762	0.754	0.0360	0.0079	3.48%	2.77%	150	YES	NO	
K (%)	0.766	0.792	0.0000	0.0264	11.47%	5.58%	88	YES	NO	
Na (%)	0.219	0.223	0.0094	0.0037	5.59%	6.49%	95	YES	YES	Review
Zn (ppm)	90.0	88.2	0.0001	1.8333	6.79%	6.31%	91	YES	NO	





Do the Samples Exhibit Any Gross Instabilities?

If 95% of 297 Labs can regularly produce a Protein Nitrogen Result Within $\pm 3\%$

Consider Timing and Regions:

USPS and International Couriers all over the US and the World...

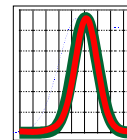
Consider Environments:

Shipping warehouses, Airplanes, Trucks, Loading Docks, Mail Vans, Labs, ...

Consider Temperatures and Humidity:

Northern and Southern Hemispheres, Coastal, Equitorial, Desert, Canada, ...

The Answer is NO!



What do we mean by STABILITY ??

From the IHP:

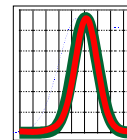
“Materials distributed in proficiency tests must be sufficiently stable over the period in which the assigned value is to be valid.”

Sufficiently stable implies that Z scores are not significantly affected.

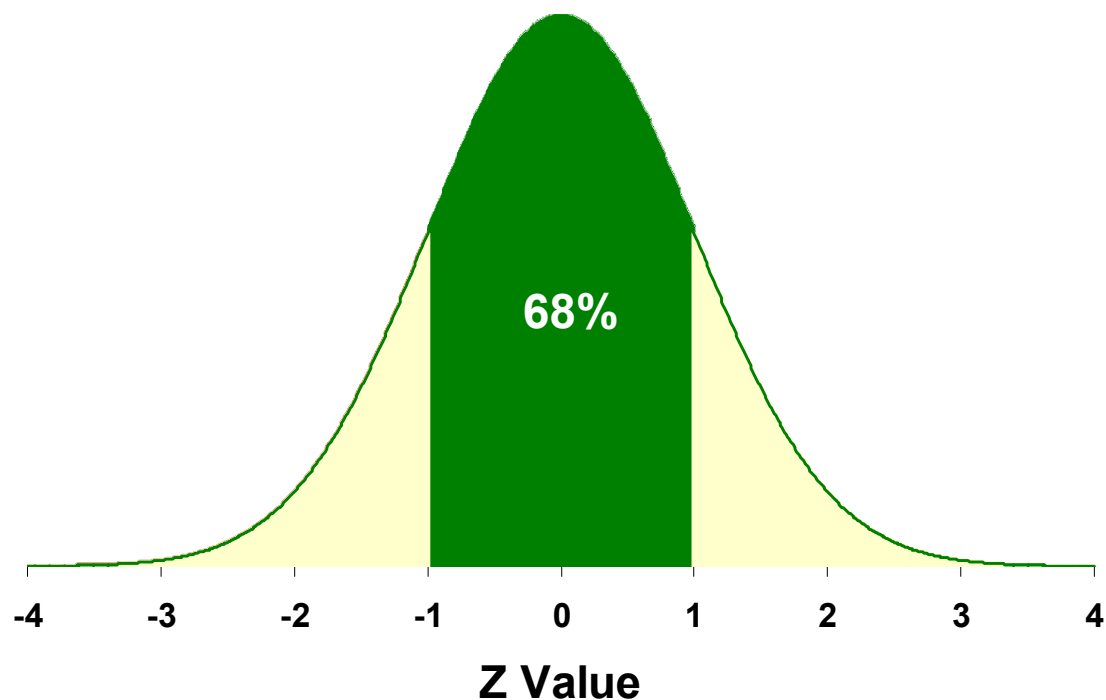
Do the Analyte values remain constant over a two month period?

There are no Gross Instabilities!

Discrete Stability issues are more to do with notoriously Unstable compounds like Vitamin E (α -tocopherol) and we may have to address that sort of problem in a more focused PT situation.



I propose we monitor the Protein Z Cut



And investigate any departures from our experience.

"In Vigilando Victoria"!
