Guidelines for Accuracy and Consistency

Sample handling and variation in results, NIRSC equation use

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"I am always ready to learn although I do not always like being taught."

Winston Churchill

>Please try to cover these points:
>1. All samples should be prepared by labs as described by the equation
>provider (that's us) 2. All incoming samples should be dried before
>grinding 3. Run down the QA committee's list of sample handling steps
>and rank what will mess up the lab most if perform the step improperly.
>Where is the most dramatic variation/culprit for creating variation in
>results? The list of sample handling steps is:
> (1) sample collection
> (2) subsampling the original/raw sample
> (3) drying
> (4) grinding
> (5) mixing the ground sample
> (6) packing and scanning a sample
> (7) using the NIRSC equation to predict
> (8) sample storage
>4. What have been issues in the past for labs in NIRSC equation use?
>How can someone recognize if something is going wrong when using an
>equation? How does sample handling affect the NIRS outcome? Give
some
>examples to illustrate this.
>5. 95% of problems on farms are attributed to dry matter problems.
>Show how accurate DM determination is important and how sample
handling
>can affect DM prediction/results.

Outline

- General principles
- From farm to report
  1. Sampling
  2. Sample preparation
  3. Sample presentation
  4. Use of NIRSC equations
- Importance of DM for Farm use;
- NIRSC equations

General principles

Principle #1:
The sign "±" is deceiving

"Errors always grow….never cancel out"

Overall error (σ^2) = Sampling errors (σ^2) +
Prep. errors (σ^2) +
Analytical error (σ^2)

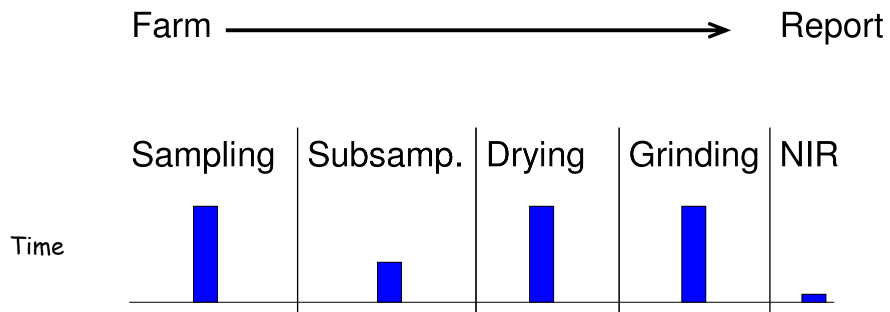
General principles

Principle #2:

"The larger are particles the greater has to be the (sub)sample mass"

1. Sampling at the farm
2. Preparation (sampling at the lab)
3. Presentation (sampling at the instrument).

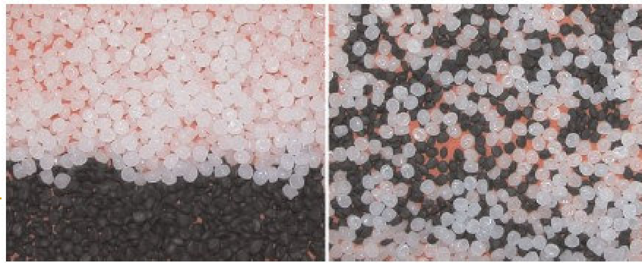
Sample prep and NIR



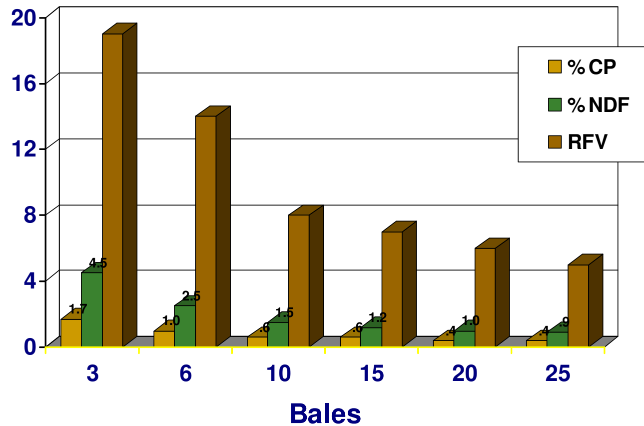
Sample collection

Principle #3: The final analytical result is as good as the sample taken.

- Sampling error= $\frac{\sigma}{\sqrt{n}}$



Sample collection



Martin, 2001

(sub)Sampling at the lab (before any prep)

- If possible dry and grind the entire sample (Think about Principle #2)
- Sample reduction: it is time consuming, but keep in mind Principle #3
  - Quartering
  - Tabling

Drying (55-60 °C)

- Our EQAs are all developed with dried samples (even hays)
- They work best in the range of 4-10% moisture.
- If hay samples are in that range they don't need to be dried
- MW vs. oven:
  - MW is variable between labs
  - Oven is required with Dig. predictions

Comparing NDF Prediction of Oven and Microwave Samples Hay - LAB1

HAY	Spectra of Oven vs. Microwave			
NDF Equations	Oven	Micro	SED	Bias
No Rep	46.1	49.0	3.80	-2.90
Lab 1 rep	47.2	47.9	1.49	-0.68
Lab 2 rep	45.7	47.9	3.00	-2.20
Lab 3 rep	46.0	48.8	3.80	-2.80
All Labs rep	46.2	47.9	2.66	-1.70

Selman and Berzaghi, 2003

Comparing NDF Prediction of Oven and Microwave Samples Haylage – LAB2

HAYLAGE	Spectra of Oven vs. Microwave			
NDF Equations	Oven	Micro	SED	Bias
No Rep	40.0	41.8	2.10	-1.83
Lab 1 rep	40.5	41.9	1.75	-1.39
Lab 2 rep	40.1	40.9	1.10	-0.79
Lab 3 rep	39.2	40.7	1.90	-1.50
All Labs rep HY	39.4	41.4	2.20	-1.98
All Labs rep HG	40.2	41.3	1.50	-1.10

Selman and Berzaghi, 2003

Grinding

- One of the most critical step in the lab.
- Grind the entire dried sample
- For NIR, finer is better, but consistency of grinding is also extremely important.
- Recommendation:
  - Udy, Cyclotec, Christy
  - 1mm
- We can minimize, but not cancel particle size effect by calibration math treatments.

At the NIR instrument

- Mixing the ground sample
  - Subsampling a ground sample is a smaller issue (principle #2), but samples can stratify and tumbling and mixing is highly recommended.
- Packing and scanning.
  - Fill up cups to about ½ --¾ of their capacity and spread the sample over the entire surface of the window.
  - Press it with card board that need to lock.
  - Brush off dust

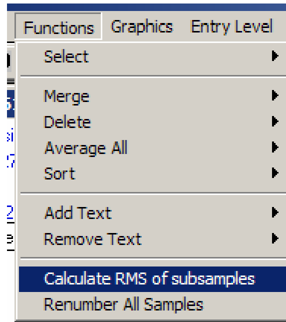
At the NIR instrument

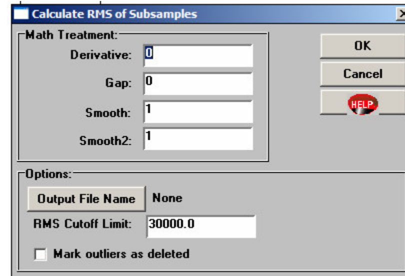
Do you want to check consistency of your scan?

- Run duplicate scans
- Check RMS
- Check prediction of duplicates

At the NIR instrument

- Check RMS





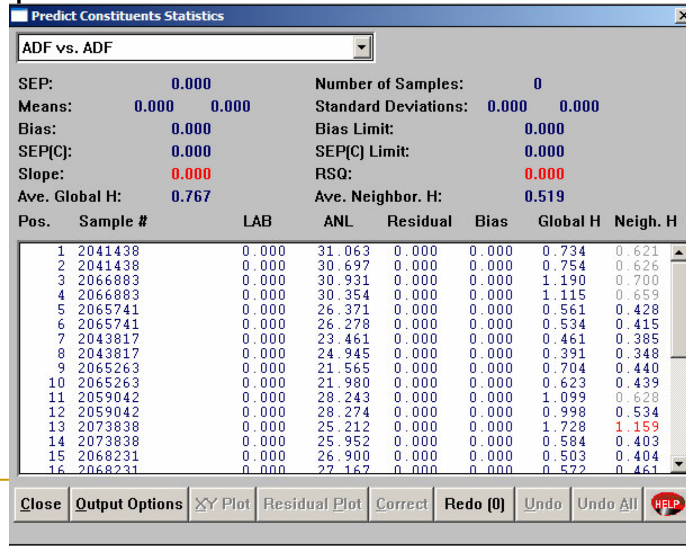
At the NIR instrument

- Check RMS –between 2000 and 3000 it is normal

SAMPLE #	MEAN	STD	RMS 1	
2041438	358	379	358	358
2066883	787	500	787	787
2065741	735	505	735	735
2043817	2820	3885	2820	2820
2065263	719	505	719	719
2059042	2545	2813	2545	2545
2073838	2500	2652	2500	2500
2068231	2635	3146	2635	2635
2067883	1232	239	1232	1232
2060746	1551	188	1551	1551
2063711	682	504	682	682
2067327	1060	387	1060	1060
2052970	1057	389	1057	1057
2056078	2120	1450	2120	2120
2056108	3340	6322	3340	3340
Overall mean:	1609	1591		

At the NIR instrument

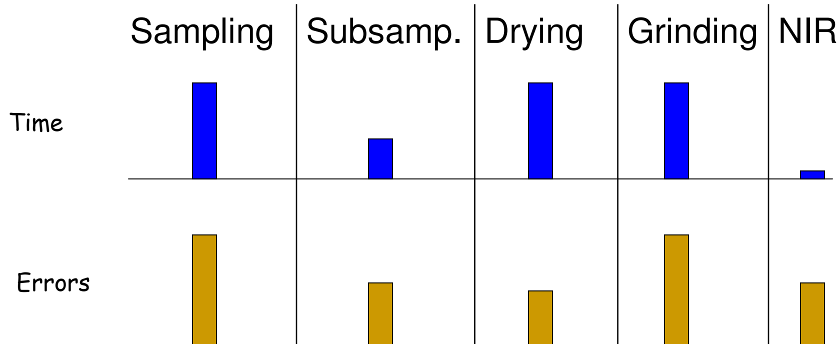
Check predictions



Pos.	Sample #	LAB	ANL	Residual	Bias	Global H	Neigh. H
1	2041438	0.000	31.063	0.000	0.000	0.734	0.621
2	2041438	0.000	30.697	0.000	0.000	0.754	0.626
3	2066883	0.000	30.931	0.000	0.000	1.190	0.700
4	2066883	0.000	30.354	0.000	0.000	1.115	0.659
5	2065741	0.000	26.371	0.000	0.000	0.561	0.428
6	2065741	0.000	26.278	0.000	0.000	0.534	0.415
7	2043817	0.000	23.461	0.000	0.000	0.461	0.385
8	2043817	0.000	24.945	0.000	0.000	0.391	0.348
9	2065263	0.000	21.565	0.000	0.000	0.704	0.440
10	2065263	0.000	21.980	0.000	0.000	0.623	0.439
11	2059042	0.000	28.243	0.000	0.000	1.099	0.628
12	2059042	0.000	28.274	0.000	0.000	0.998	0.534
13	2073838	0.000	25.212	0.000	0.000	1.728	1.159
14	2073838	0.000	25.952	0.000	0.000	0.584	0.403
15	2068231	0.000	26.900	0.000	0.000	0.503	0.404
16	2068231	0.000	27.167	0.000	0.000	0.572	0.461

Sample prep and NIR

Farm → Report



How to recognize something is wrong

Sampling	Subsamp.	Drying	Grinding	NIR -Instr -calibr.

If something doesn't look right what you do?

Blame someone that is not under your control

Sampling	Subsamp.	Drying	Grinding	**NIR** -Instr **-calibr**.

How to recognize something is wrong

Sampling	Subsamp.	Drying	Grinding	NIR -Instr -calibr.

They all affect the result

They also affect the spectrum

How to recognize something is wrong

- If the spectra is affected, you may see H outliers:
  - GH >2
  - NH>0.6
- NIR Instrument:
  - Check cell
  - Diagnostic
- LAB environment:
  - T°
  - RH

How to recognize something is wrong

- If the spectra is affected, you may see H outliers:
  - GH >2
  - NH >0.6
- Duplicates are always better than single…… …duplicate scan should be mandatory for H outliers in case there was a subsampling problem.
- Lab QC: keeping track of avg H values may give some indication of changes in the LAB. (same concept of the check cell)

How to recognize something is wrong

Drying	Grinding	NIR -Instr -calibr.

They also affect the spectrum

How to recognize something is wrong

Sampling	Subsamp.	Drying	Grinding	NIR -Instr -calibr.

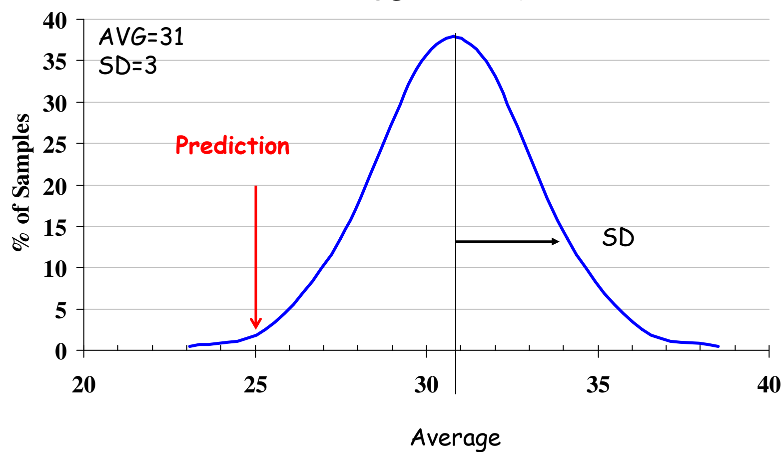
They all affect the result

How to recognize something is wrong

Constituent	As received	dry matter	Global H	Neighbor H	T
DM	96.268	100.000	0.832	0.429	0.009
PROTEIN	10.600	11.011	0.832	0.429	0.953
ADF	30.039	31.204	0.832	0.429	0.822
NDF	53.259	55.324	0.832	0.429	1.228
dNDF48	37.797	39.262	0.832	0.429	2.346
IVTDMD	80.938	84.076	0.832	0.429	0.608
FAT	1.277	1.326	0.832	0.429	-3.519
ASH	5.206	5.408	0.832	0.429	-0.184
Lignin	3.735	3.880	0.832	0.429	0.069
P	0.224	0.233	0.832	0.429	0.232
CA	0.238	0.247	0.832	0.429	-0.258
K	1.485	1.543	0.832	0.429	0.755
MG	0.220	0.229	0.832	0.429	0.255
MOISTURE	3.732	0.000	0.000	0.000	0.000
NEL	0.633	0.657	0.000	0.000	0.000

T test

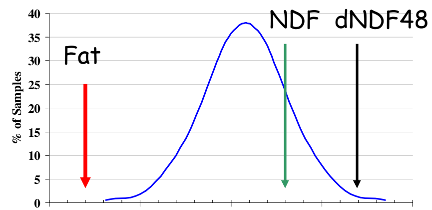
$$T = \frac{(Pred-AVG)}{SD} = \frac{25-31}{3} = -2$$



How to recognize something is wrong

Constituent	Mean	SD	Est.Min	Est.Max	SECV	1-VR
NDF	46.27	7.37	24.15	68.39	2.10	0.92
dNDF48	27.70	4.93	12.91	42.49	1.66	0.89
FAT	2.52	0.34	1.50	3.53	0.13	0.86
Lignin	3.80	1.11	0.49	7.12	0.55	0.75

	AVG	T
Fat	1.28	3.5
NDF	53.3	1.2
dNDF48	37.8	2.3



How to recognize something is wrong

Sampling	Subsamp.	Drying	Grinding	NIR -Instr -calibr.

Is the T test reliable?

It is almost impossible to identify if there was a (sub)sampling problem. The T test is an indicator which just tells us something is outside the normal.

By the way, NORMAL is not always RIGHT

Principle #4: Unbreakable Law of Sampling

- You never know the true value of anything !

hence

- You never know the composition of a feed.
- A feed analysis narrows the confidence range around the expected value.

(St-Pierre and Weiss, 2006)