Near Infra-red Spectroscopy (NIRS) *versus* Wet Chemistry: Accuracy and Precision in Forage Analysis

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NIRS Consortium Education Series 2025

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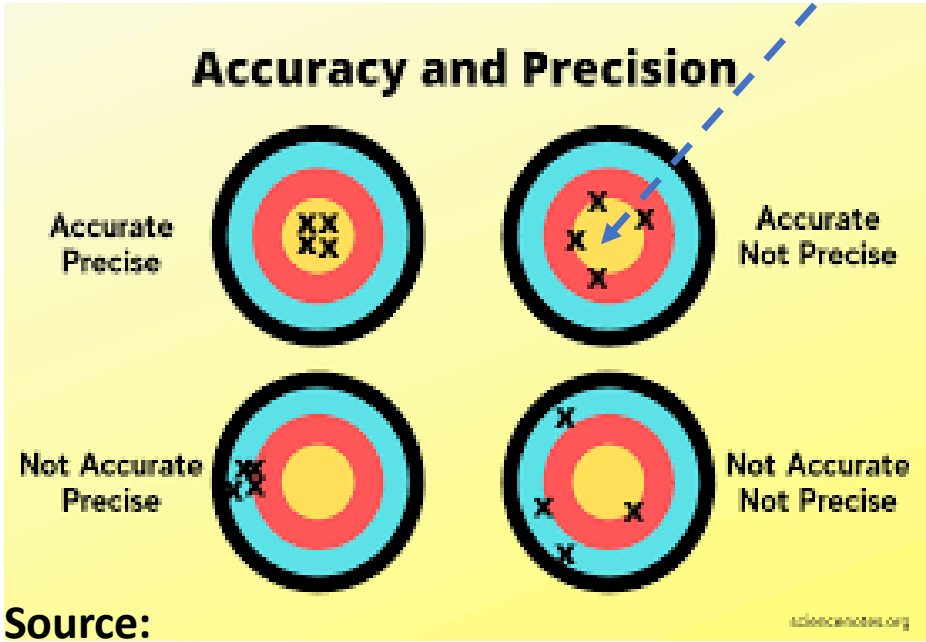
Quality Assurance in Analytical Chemistry

What are two pillars of Quality Assurance?

- **<u>Precision</u> of repeated measures**
  - ✓ Repeatability
  - ✓ Reproducibility
- **Accuracy**



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Precision and Accuracy in Interlaboratory Collaborative Proficiency Testing

Horwitz, Kamps, and Boyer (1980)

- Examination of the results of over 50 interlaboratory collaborative studies
- Conducted by AOAC
- Various commodities
- Numerous analytes
- Mean coefficient of variation (CV or RSD), expressed as powers of 2
- Mean concentration measured (C), expressed as powers of 10 (i.e., Log_{10} C)
- Independent of the determinative method

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Precision and Accuracy in Interlaboratory Collaborative Proficiency Testing

Statistician Jung Keun Lee expressed Horwitz Equation as:

$$RSD_R, \% = 2^{(1 - 0.5 \text{ Log } C)}$$

C = Concentration of analyte expressed as **mass fraction**

RSD_R = Relative Standard Deviation or CV under reproducibility conditions.

Michael Thompson (1999) transformed the equation into:

$$RSD_R, \% = 2C^{-0.15}$$

Or

$$S_R = 2C^{0.85}$$

- **HSD, in % on the NFTA Proficiency Test Report = S_R x 100**
- **C is the RMA (On NFTA PT Reports) expressed as mass fraction (e.g., For 95%, DM; C = 0.95)**

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Horwitz Equation

$$RSD_R, \% = 2^{(1 - 0.5 \log C)} \text{ or } 2C^{-0.15}$$

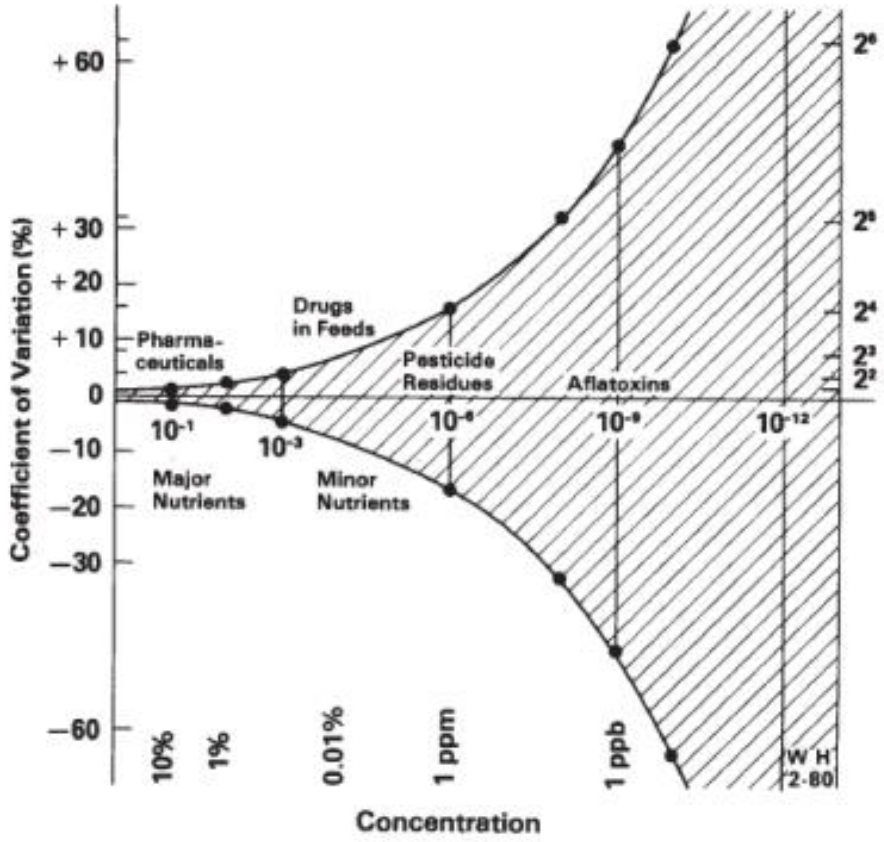

Figure 1. Horwitz Horn, the original curve

Values of Horwitz RSD_R at different concentrations

Analyte concentration	RSD_R
10 %	2.8%
1 %	4.0%
0.1 %	5.7%
0.01 %	8.0%
1 Ppm	16%
1 Ppb	45%
0.1 Ppb	64%

Overestimated?

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Adjustment of Horwitz equation

- **Later, in 2000, Michael Thompson found that precision was overestimated at the extreme low values of C.**

- **As a result, the Horwitz Equation was further adjusted as:**

➢ $S_R = 0.22C$; if C is $<1.2 \times 10^{-7}$ .....(1)

➢ **$S_R = 0.02C^{0.8495}$; if $1.2 \times 10^{-7} \leq C \leq 0.138$.....(2)**

➢ **$S_R = 0.01C^{0.5}$; C > 0.138.....(3)**

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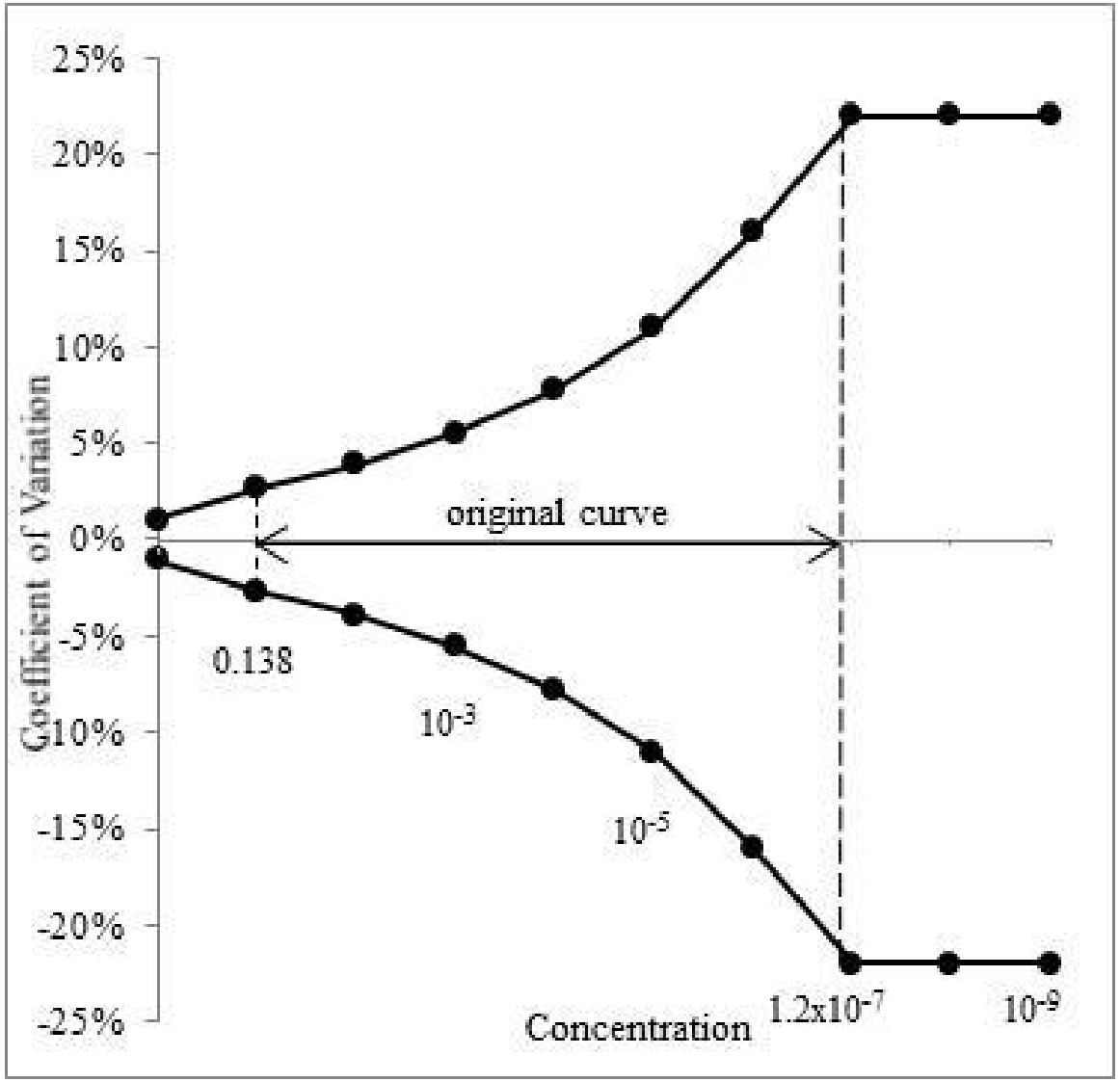
The Horwitz Curve Adjusted for Concentrations



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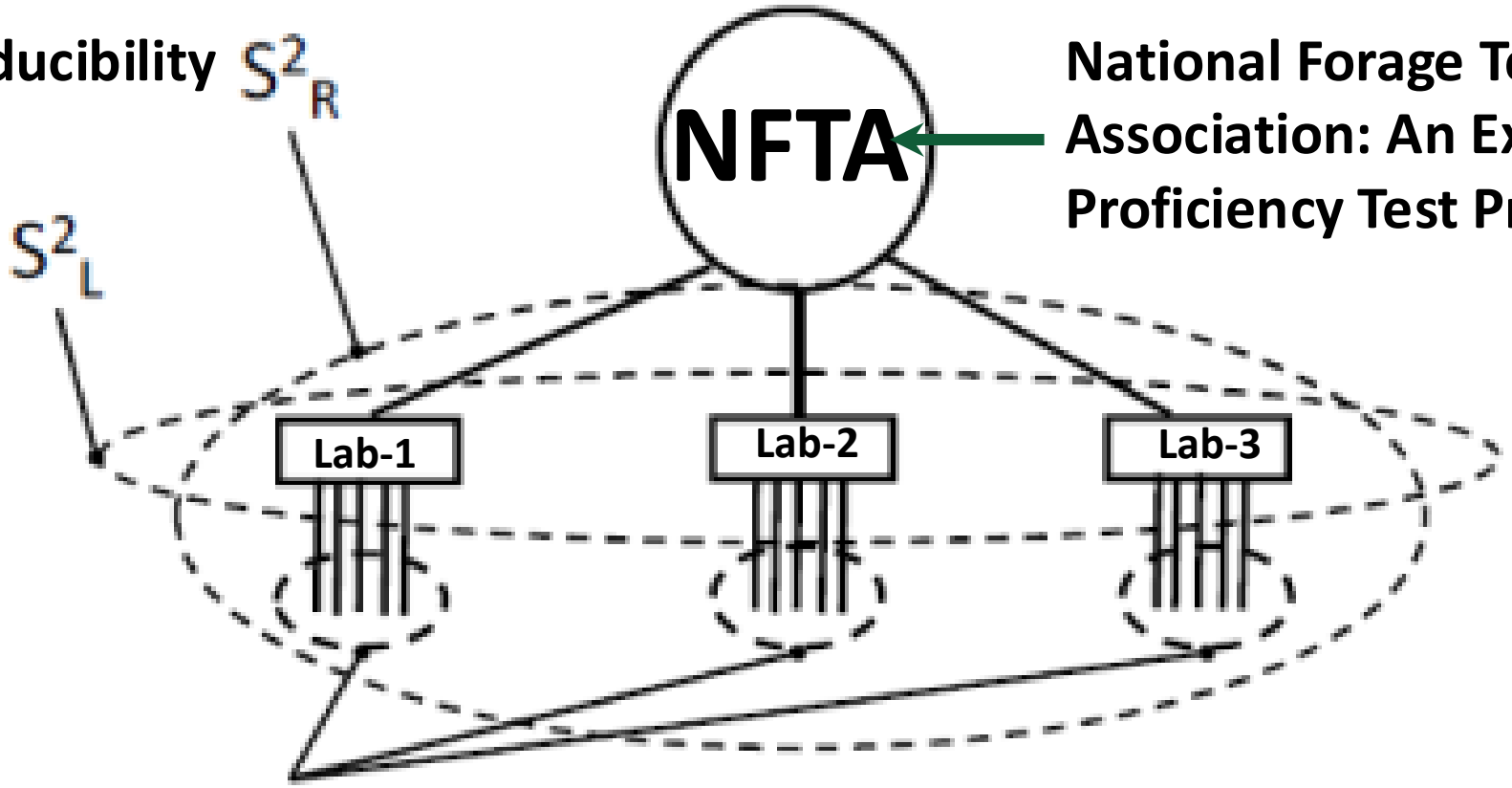
Precision: Repeatability and Reproducibility

3. Method's Reproducibility Variance S^2_R

2. Inter-Laboratory Variance S^2_L



National Forage Testing Association: An Example Proficiency Test Provider

1. Intra-Laboratory Variance S^2_r

$$S^2_R = S^2_r + S^2_L$$

$$S_R = \sqrt{S^2_r + S^2_L}$$

The Equations

1) $$S_r = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \cdots + (n_k - 1)s_k^2}{n_1 + n_2 + \cdots + n_k - k}}$$

2) $$RSD_r = \sqrt{\frac{(n_1 - 1)RSD_1^2 + (n_2 - 1)RSD_2^2 + \ldots + (n_k - 1)RSD_k^2}{n_1 + n_2 + \ldots + n_k - k}}$$

$RSD_i = (S_i \div Mean_i) \times 100$

3) $$S_L = \sqrt{\frac{1}{k-1}\sum_{i=1}^{k}(Xmean_i - Grand\ Mean)^2}$$

4) $$S_R = \sqrt{S^2_r + S^2_L}$$

5) $$RSD_R = \frac{S_R}{\text{Grand Mean}} \times 100$$

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Horwitz Ratios or "HorRat" for Precision

$$\text{Repeatability } \mathbf{HorRat_r} = \frac{\mathbf{RSD_r}}{\mathbf{PRSD_r}}$$

- *Acceptable* ***HorRat$_r$*****: ≤1.3**

$$\text{Reproducibility } \mathbf{HorRat_R} = \frac{\mathbf{RSD_R}}{\mathbf{PRSD_R}}$$

- *Acceptable* ***HorRat$_R$*****: ≤2.0**

- **RSD** is calculated from the results reported by the participating labs in replicates
- **PRSD** is the **RSD** predicted from Horwitz Equation

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Accuracy Using "Z-Score" Based on Horwitz Function

$$Z = \frac{\left| \text{XmeanL} - \mu \text{ (or RMA)} \right|}{S_{\text{Horwitz}} \text{ (or HSD)}}$$

- RMA is the Reference Method (i.e., wet chem.) Average
- $Xmean_L$ is the mean value of replicated measurements reported by a given laboratory

- HSD = $0.02C^{0.8495}$; if $1.2 \times 10^{-7} \leq C \leq 0.138$ (or 13.8%)
- HSD = $0.01C^{0.5}$; $C > 0.138$

Z-score ≤3.0: Satisfactory Accuracy } NFTA Passing Grades

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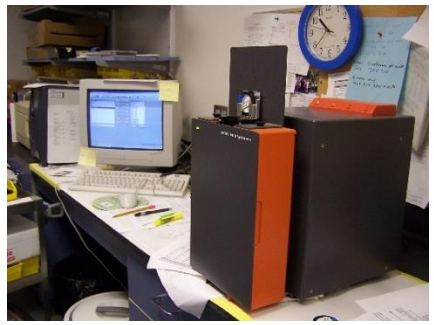
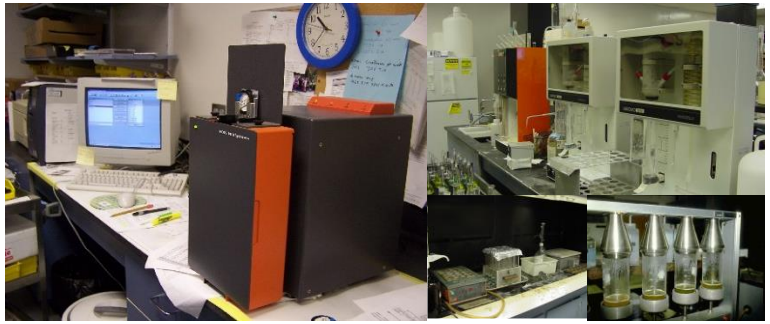
This Study

- **NFTA Proficiency Test Reports from 6 Laboratories**



All 6 laboratories provided NIRS results



4 laboratories provided both NIRS and Wet Chemistry results

- **33 NFTA Alfalfa Hay PT rounds during 2013-2019**

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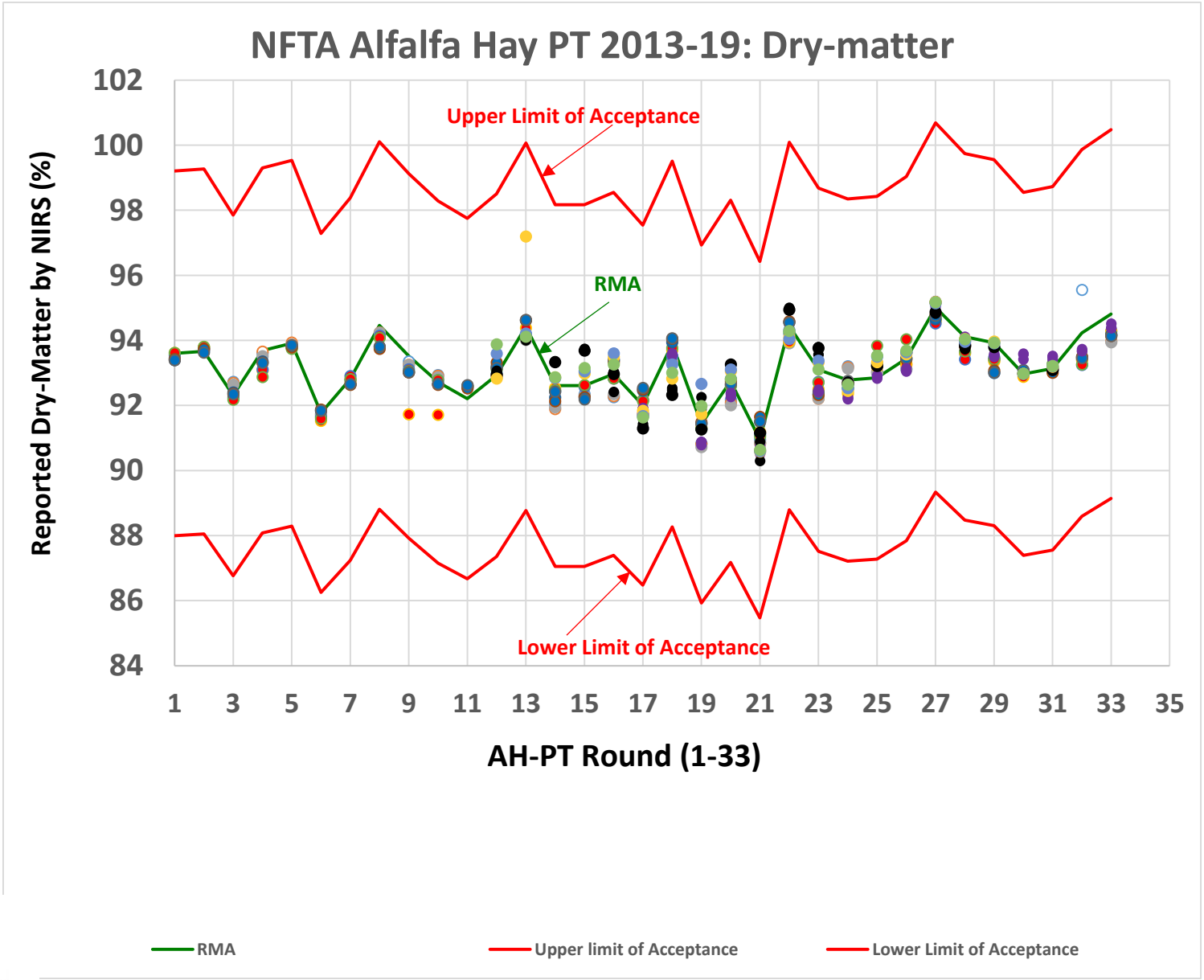
NIRS is an Accurate Forage Testing Method



- 6 Laboratories
- Triplicate analysis
- 33 PT rounds

Acceptance Envelope:

- **RMA ± 3HSD**

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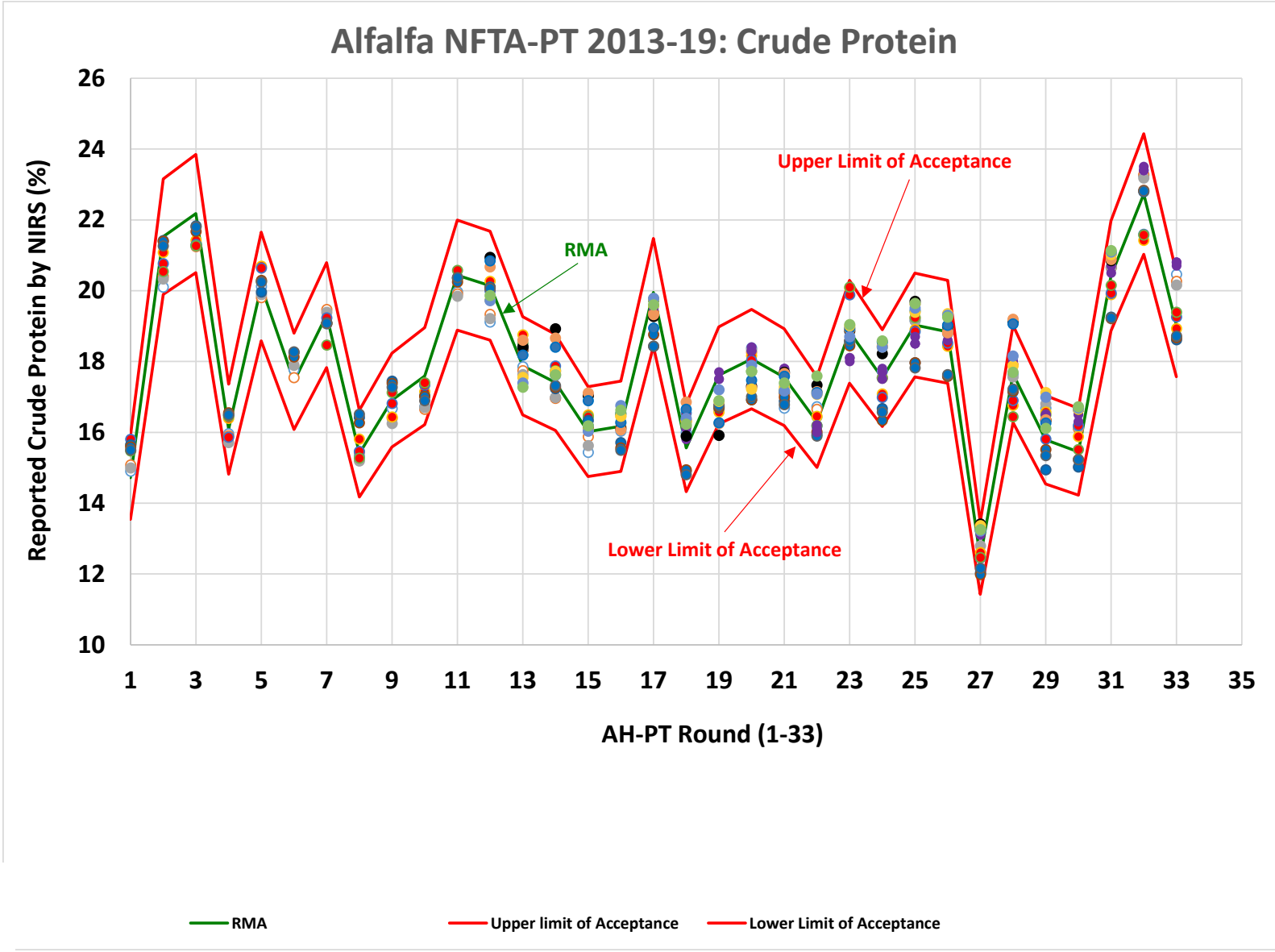

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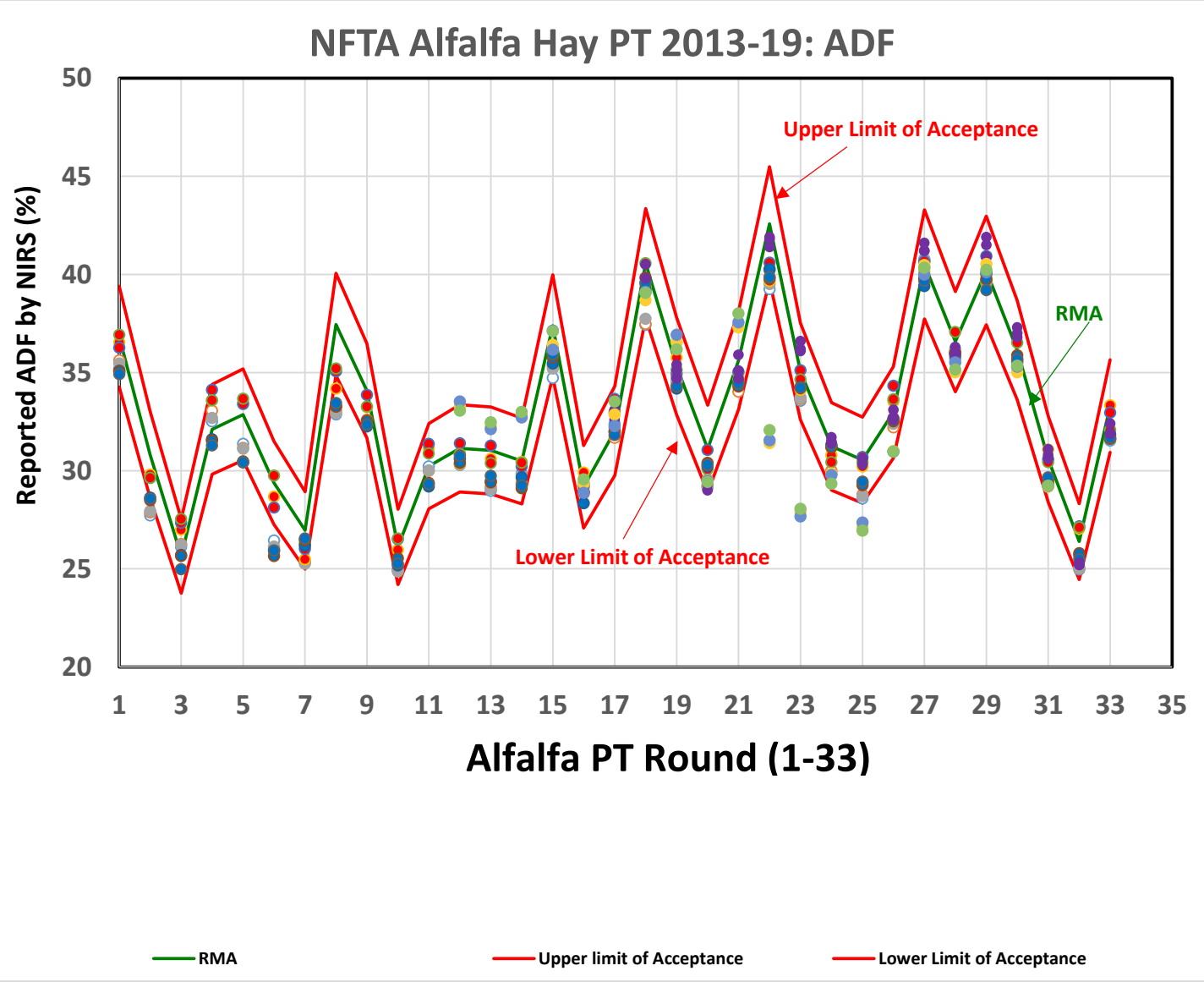

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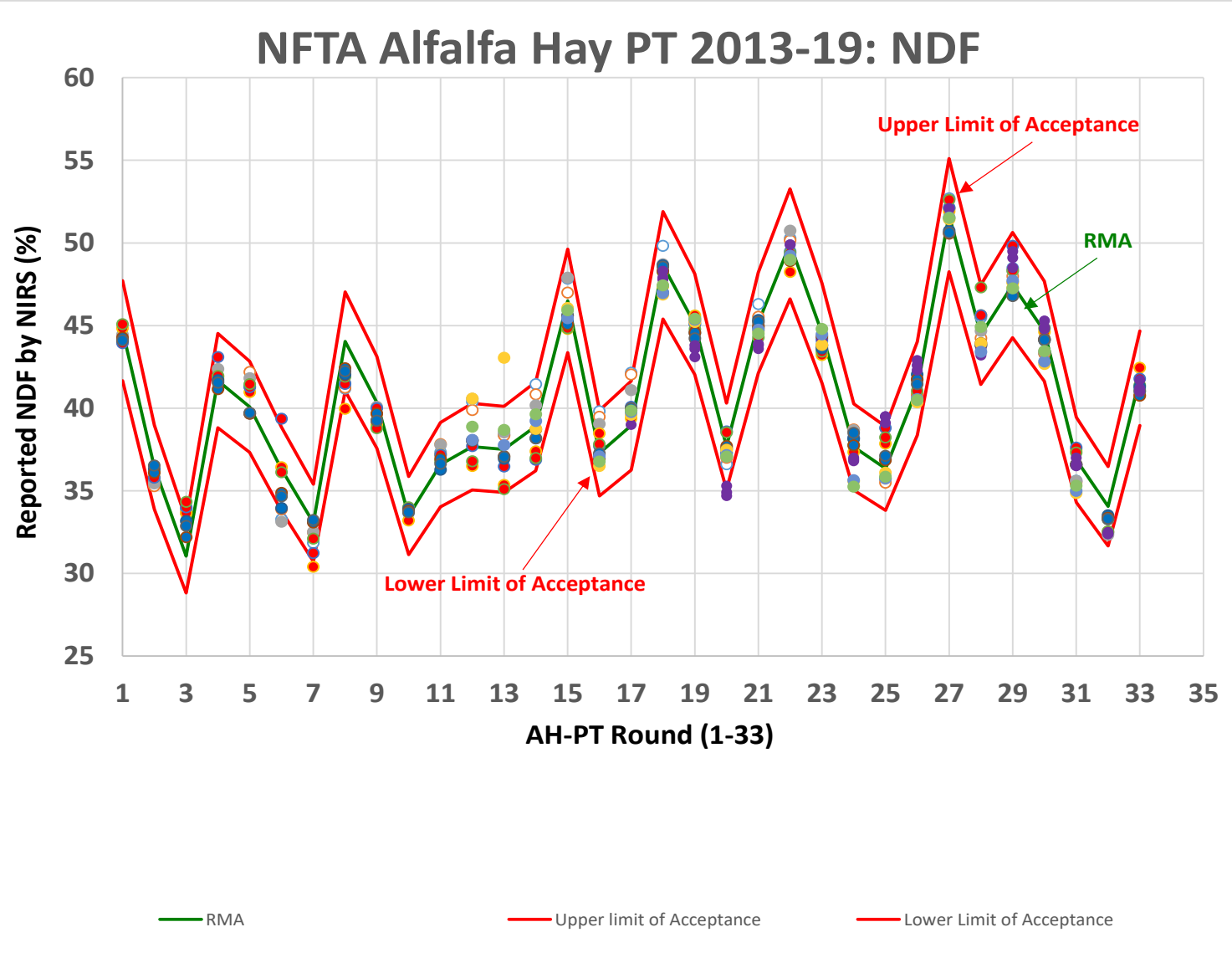

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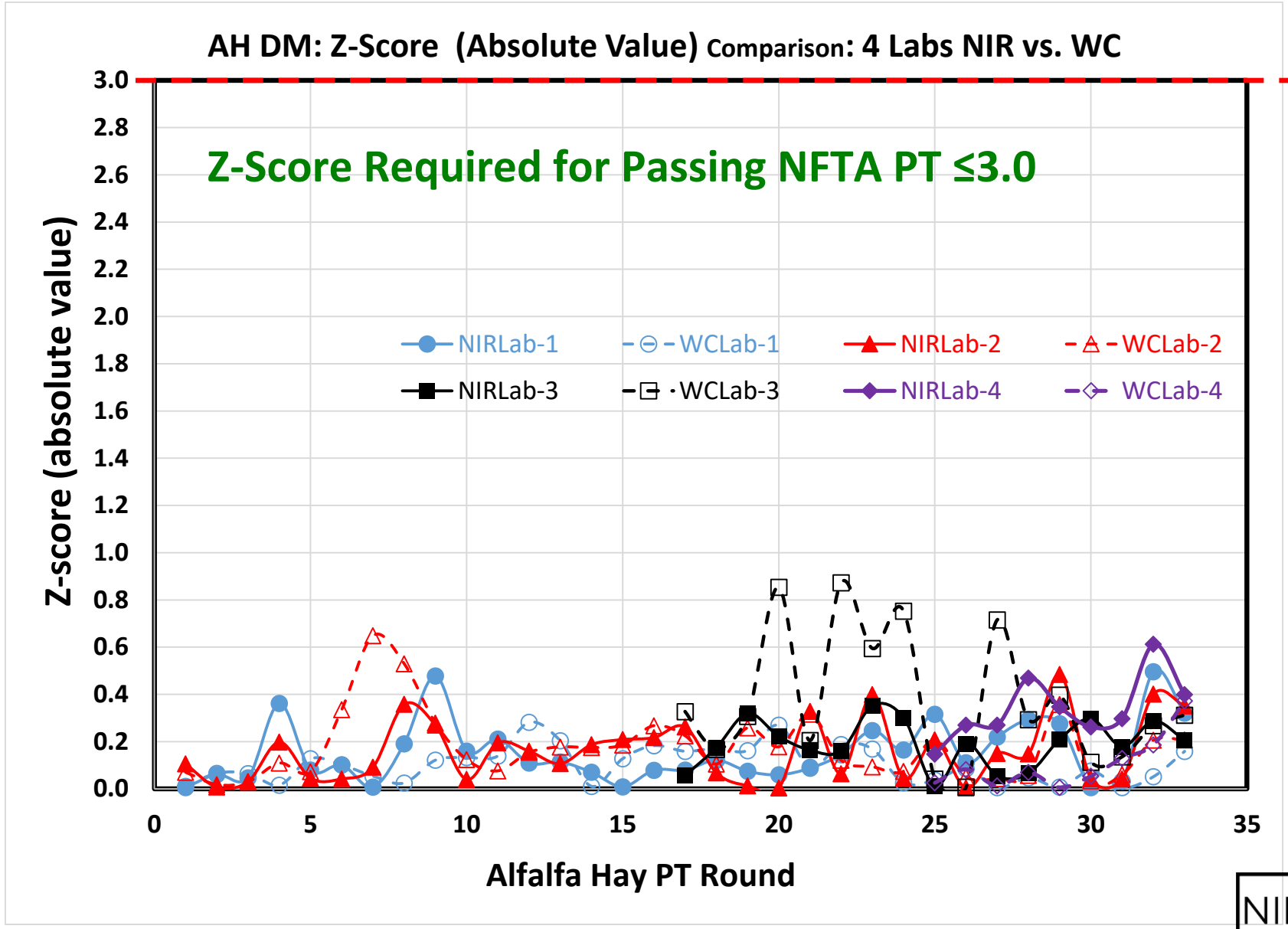
Forage DM Testing Accuracy: NIRS vs. WC



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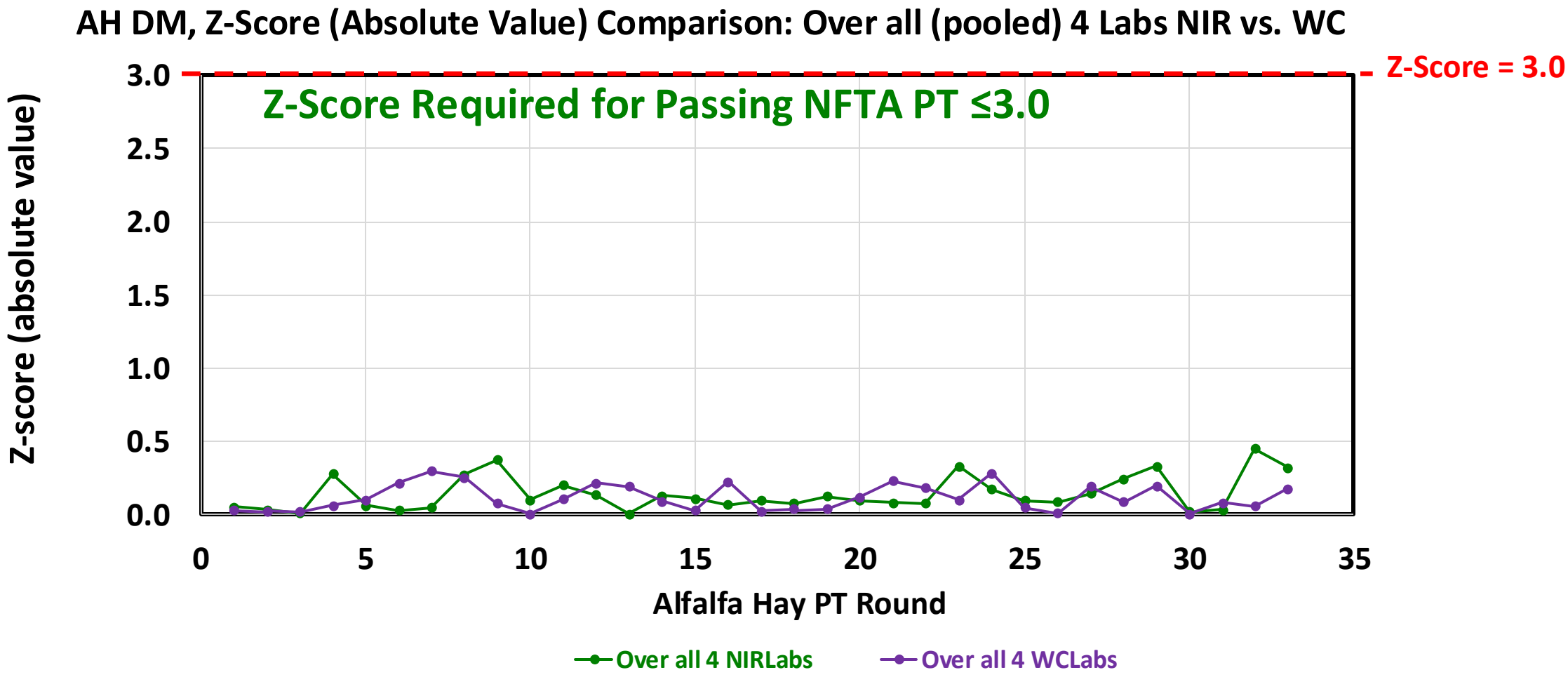
Forage DM Testing Accuracy: NIRS vs. WC



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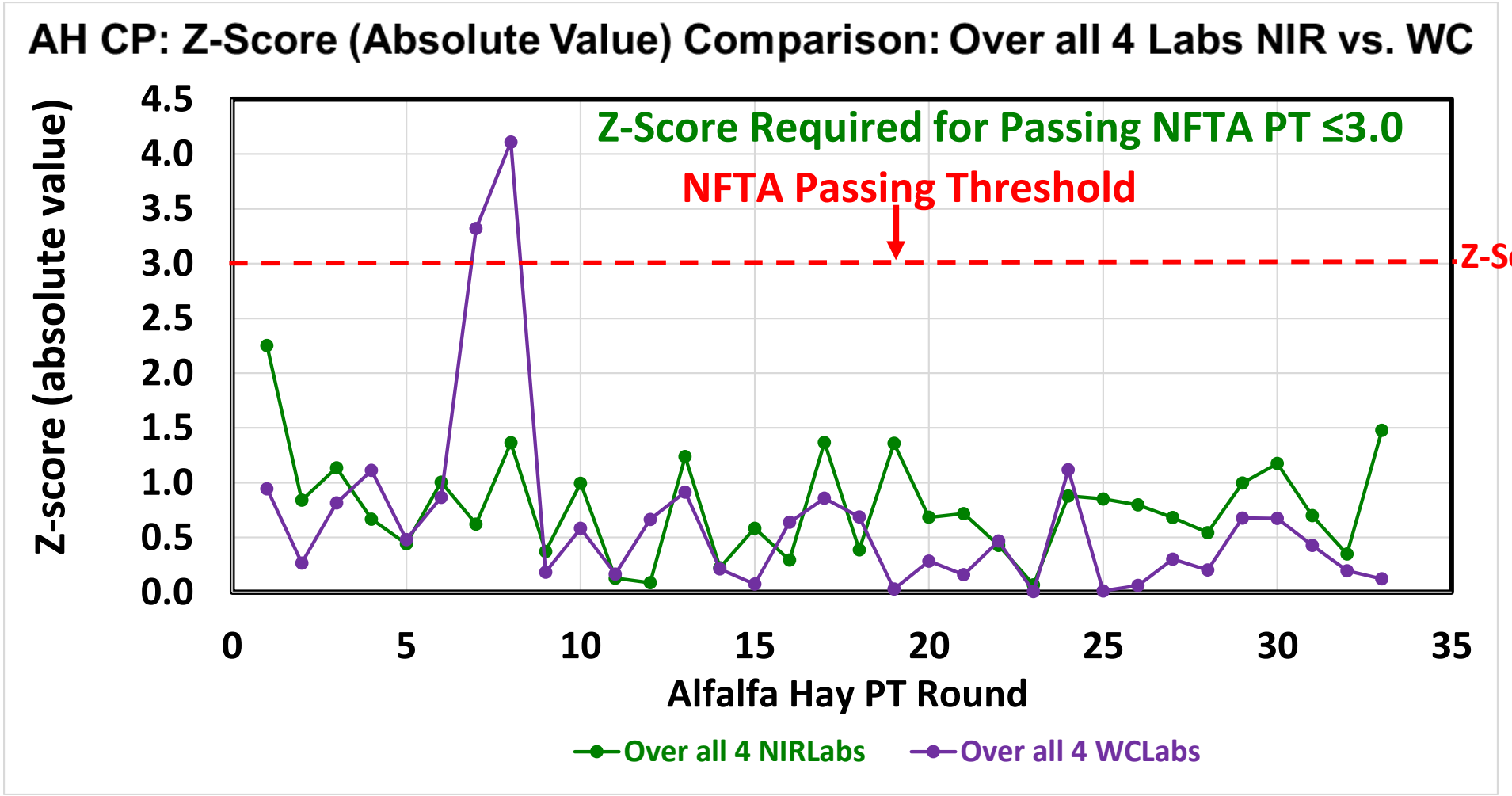
Forage CP Testing Accuracy: NIRS vs. WC



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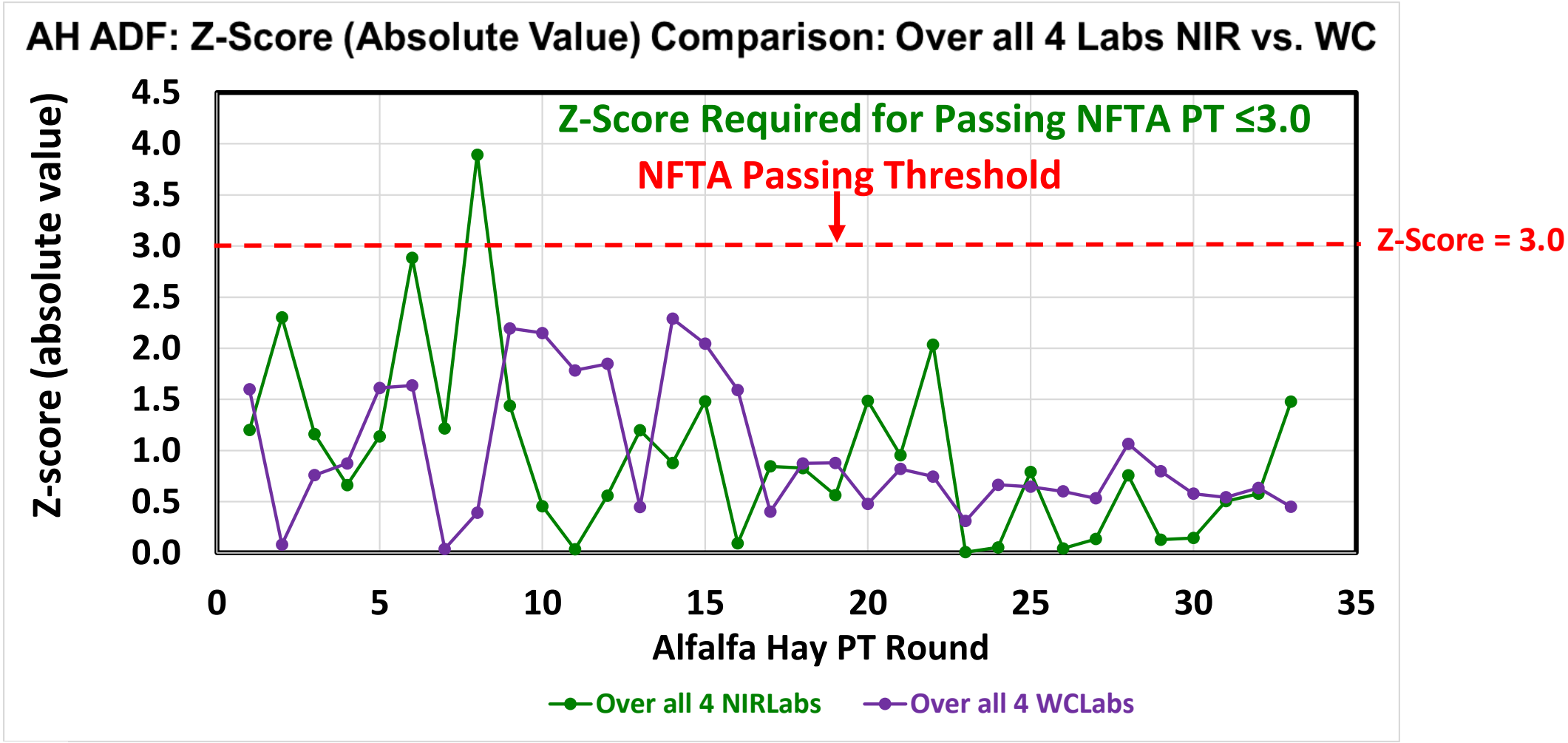
Forage ADF Testing Accuracy: NIRS vs. WC



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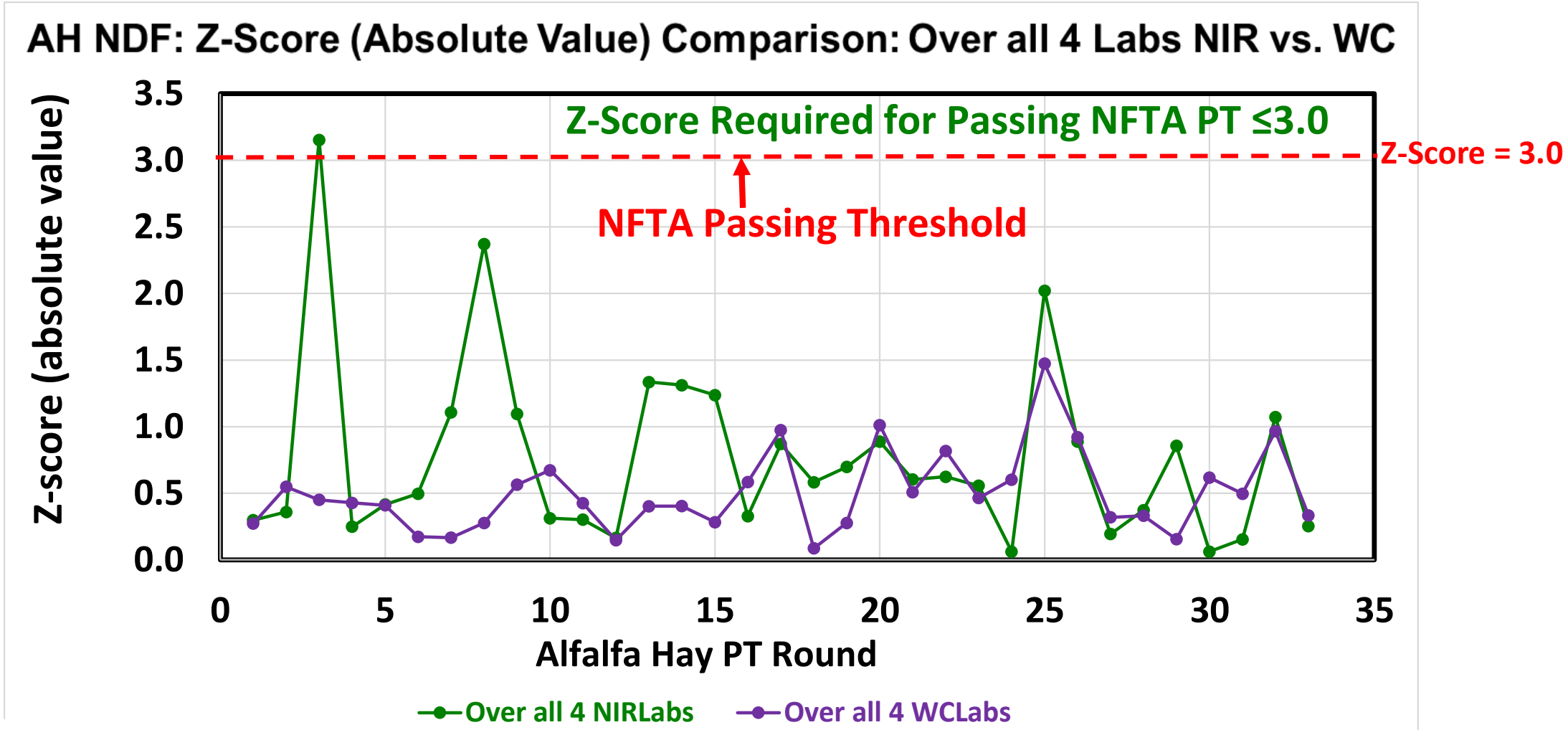
Forage NDF Testing Accuracy: NIRS vs. WC



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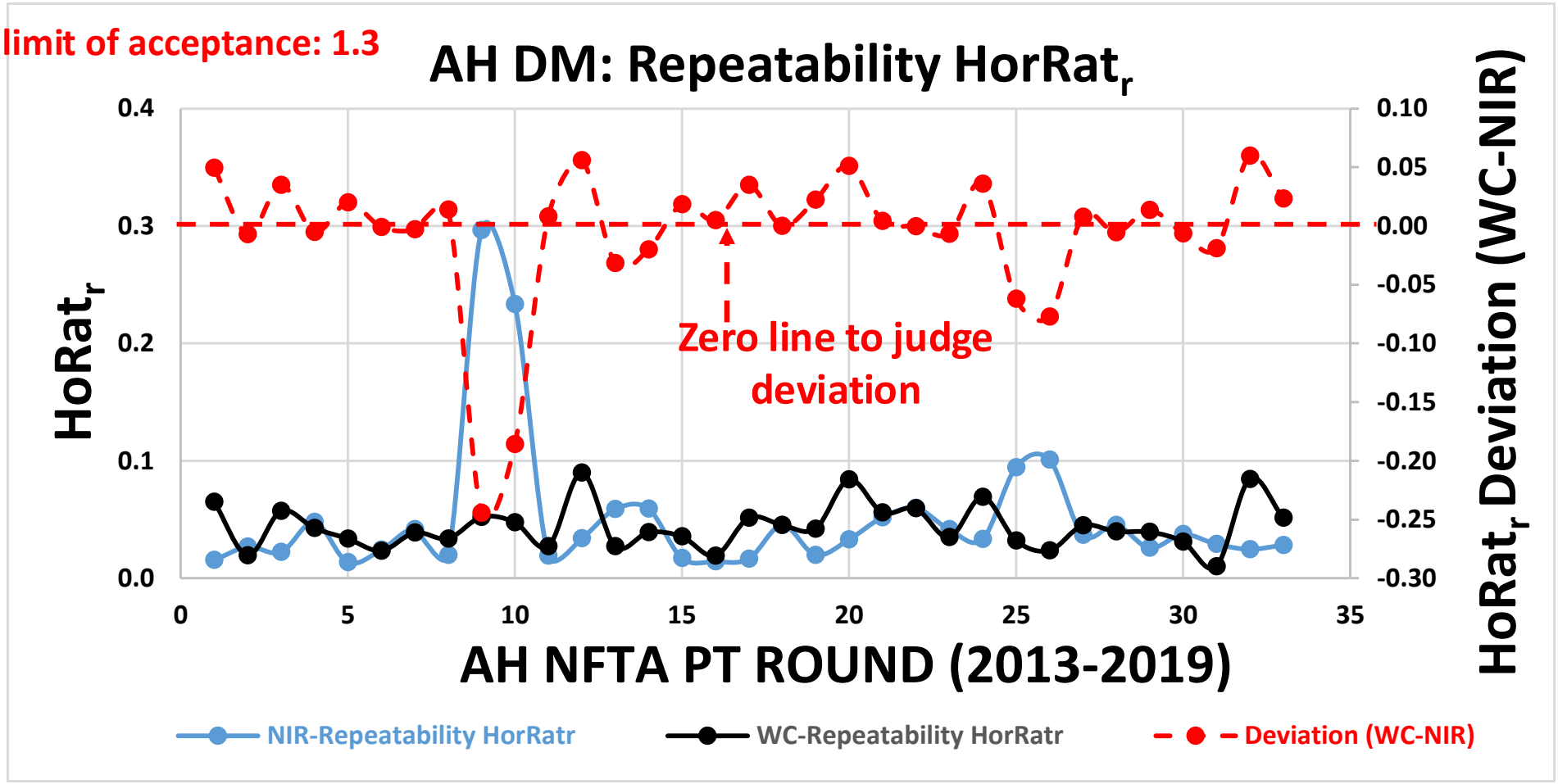
Forage DM Testing Repeatability: NIRS is Better Than WC

Upper limit of acceptance: 1.3



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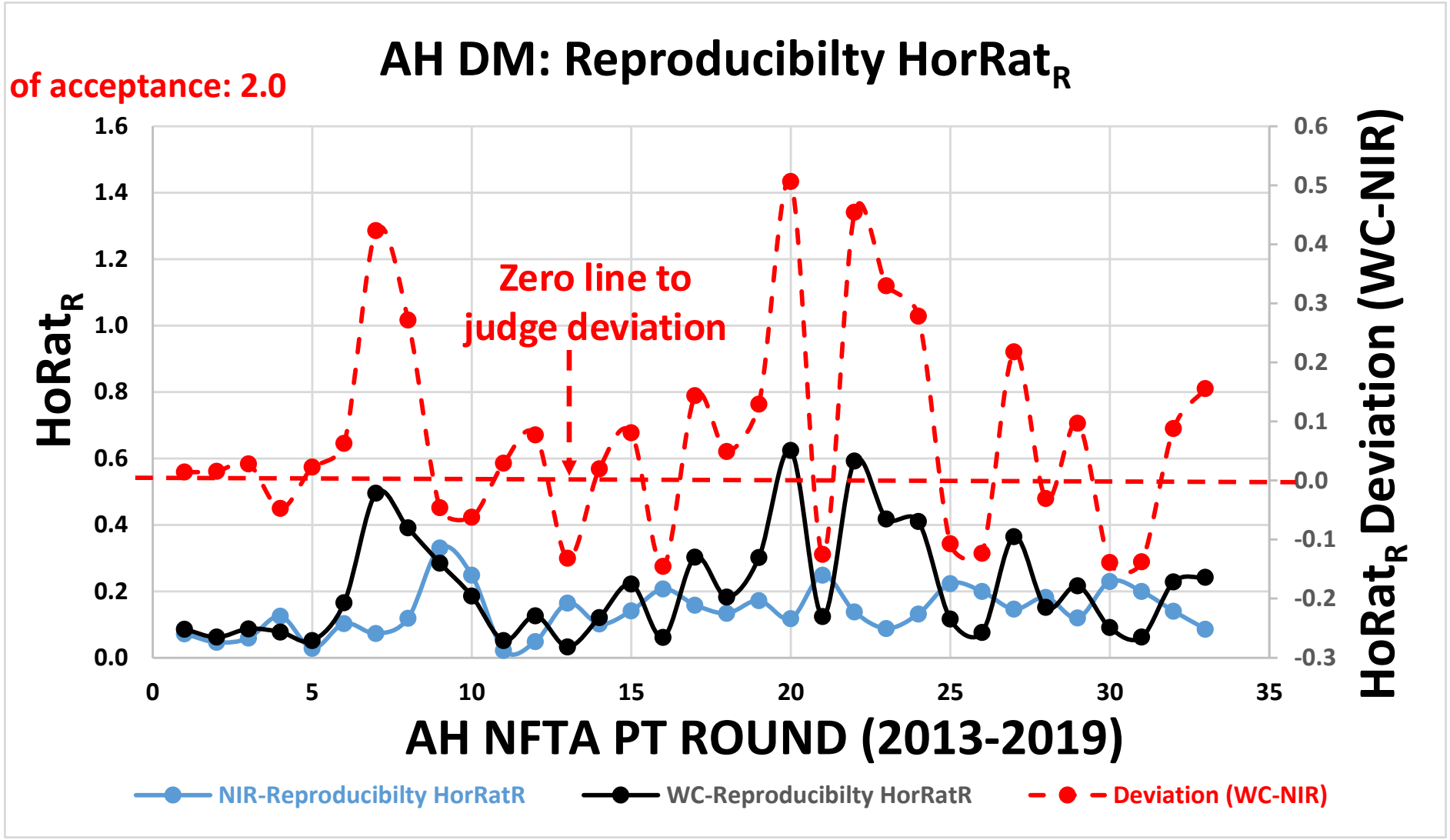
Forage DM Testing Reproducibility: NIRS is Better Than WC

Upper limit of acceptance: 2.0



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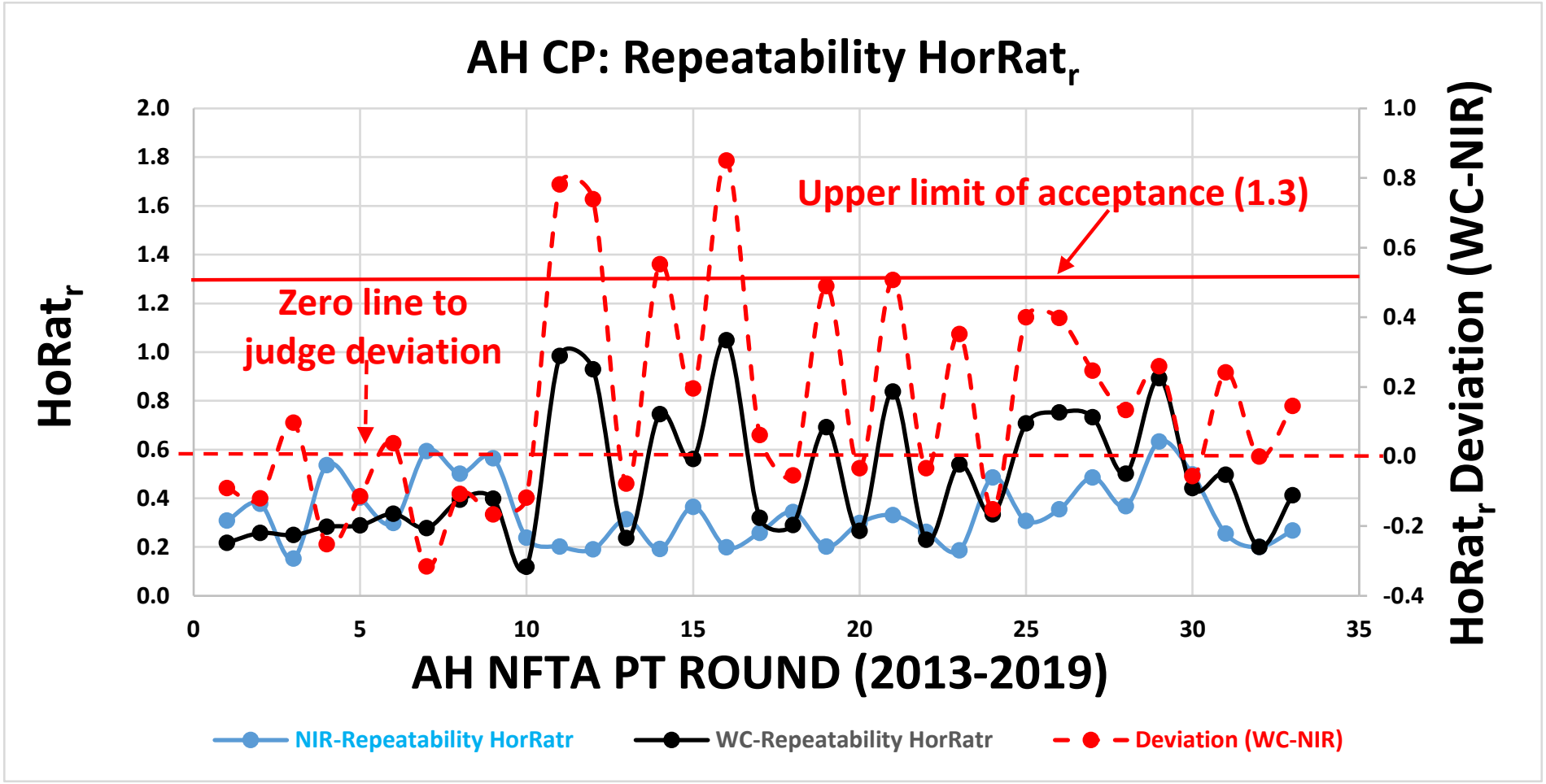

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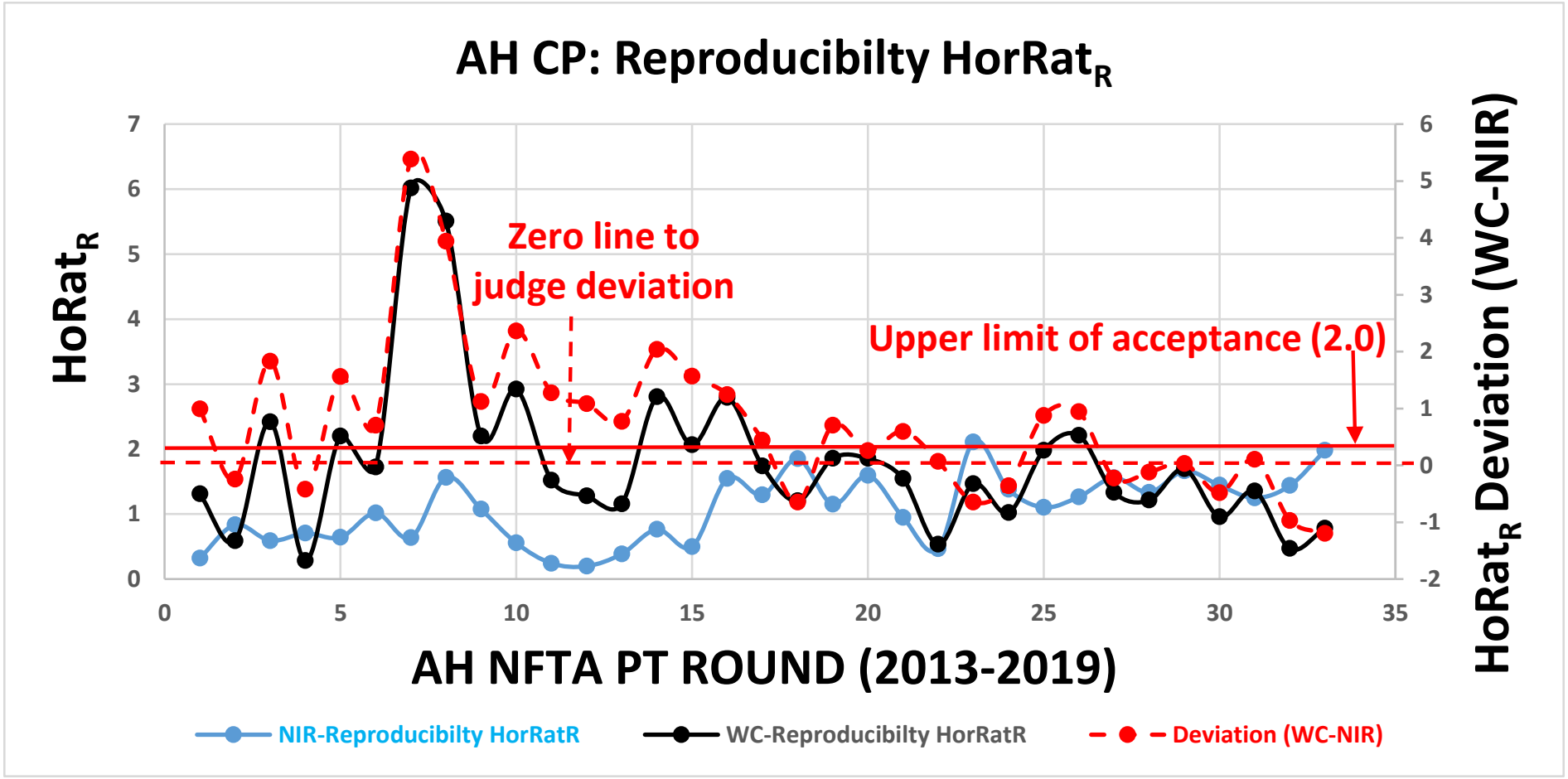

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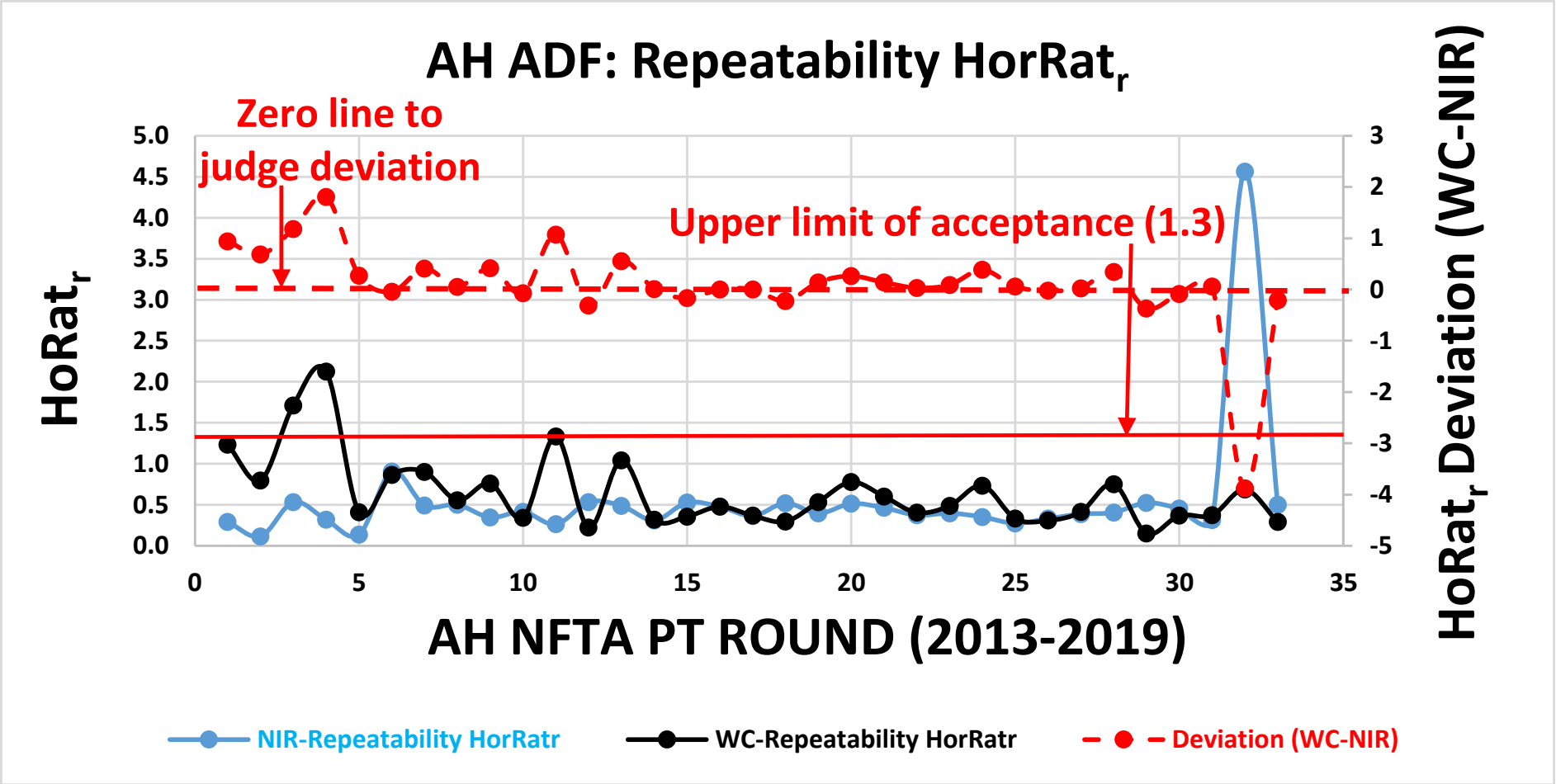
Forage ADF Testing Repeatability: NIRS is Better Than WC



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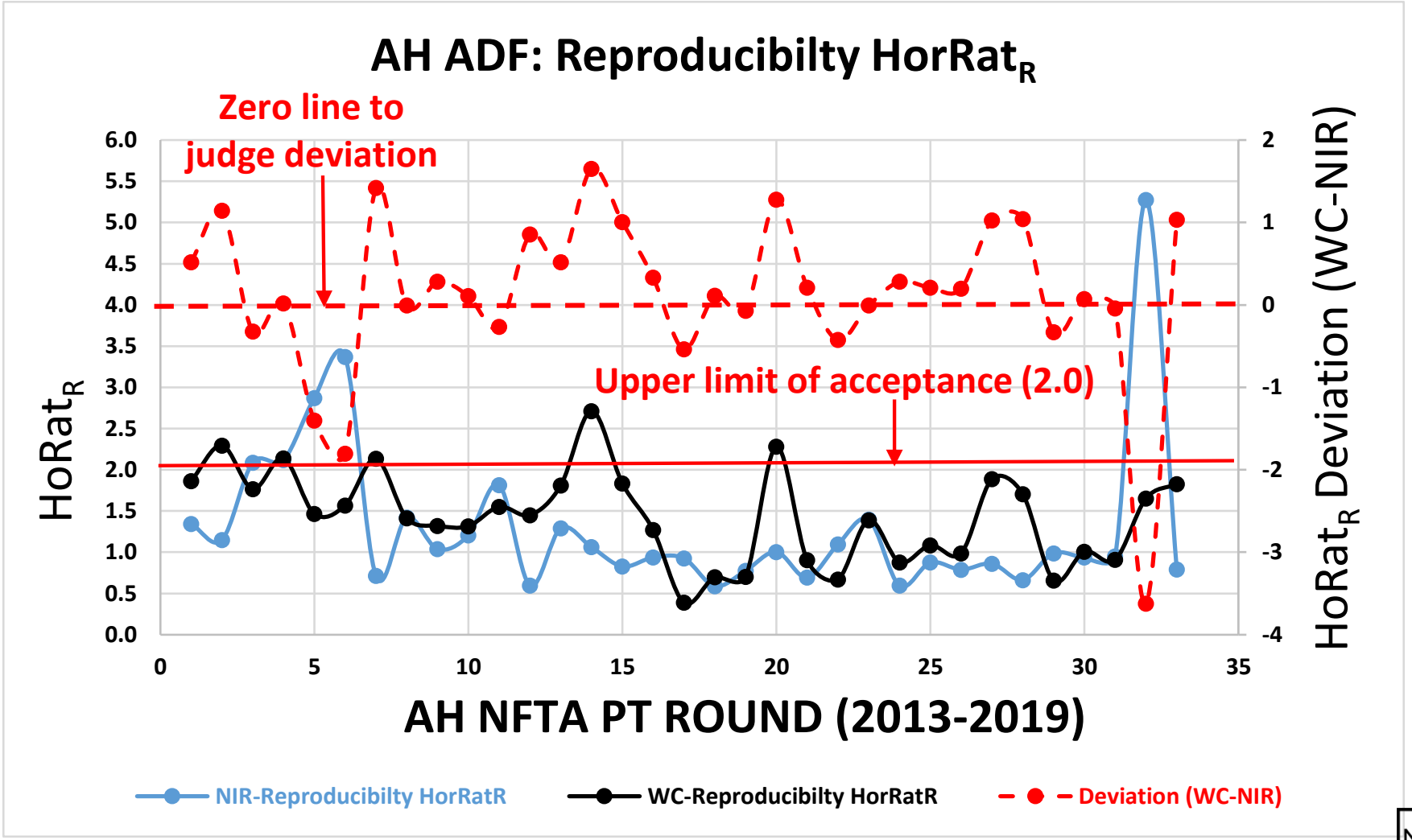

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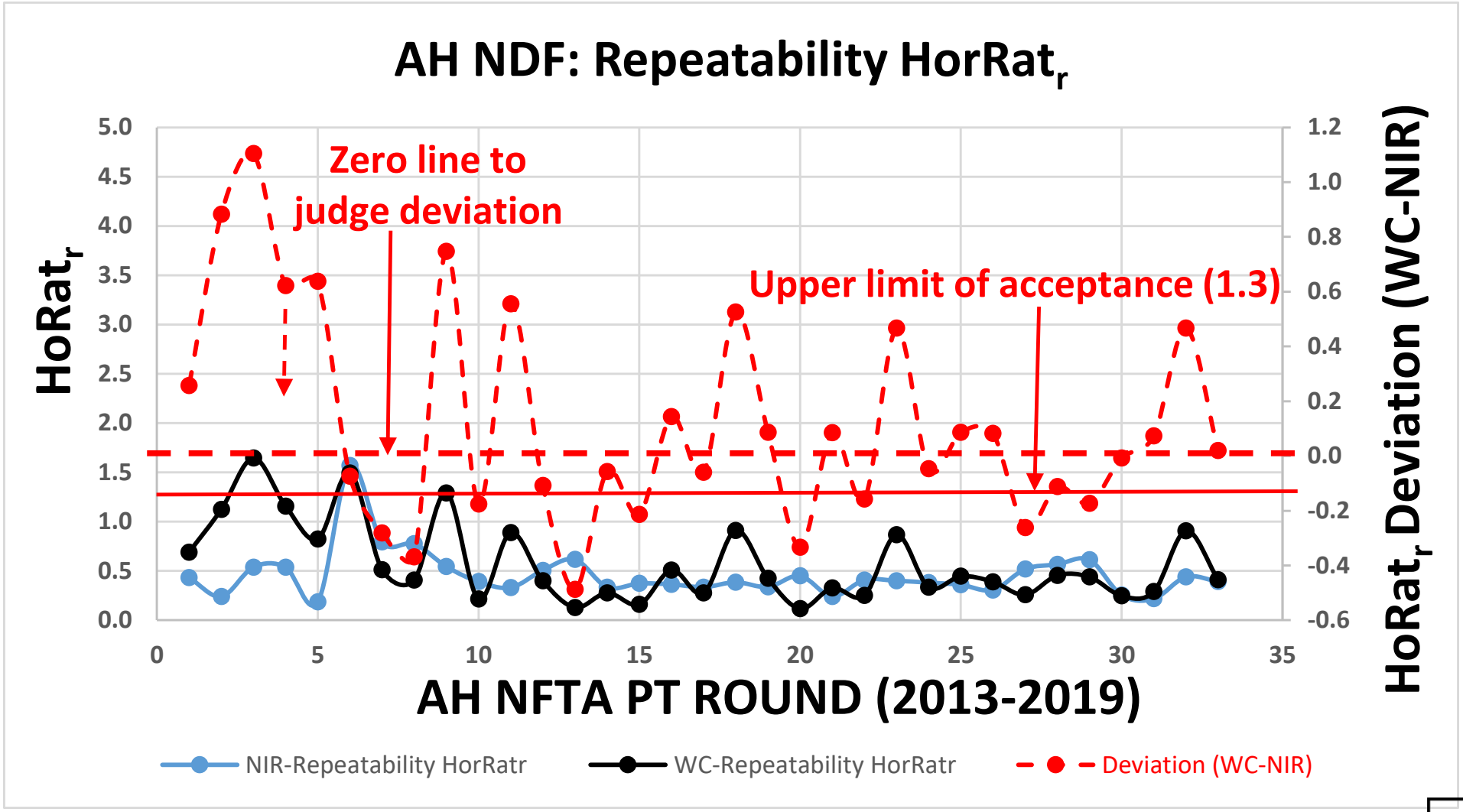
Forage NDF Testing Repeatability: NIRS is Better Than WC



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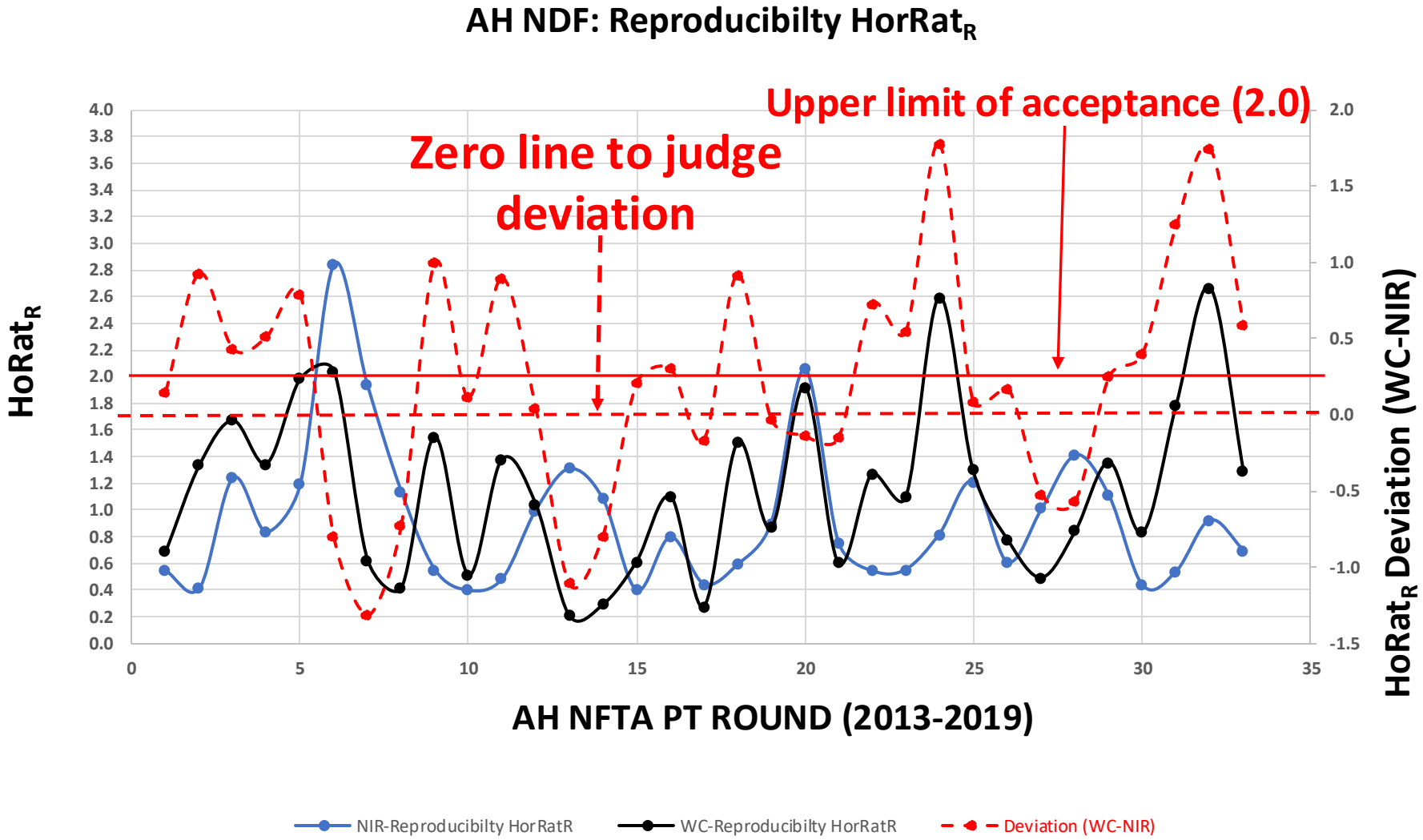

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Conclusions

- **This presentation is a robust demonstration of the FIRST FACT, which is:**

  "When NIR calibration models are developed using good science and applied properly, NIR is as accurate as wet chemistry in forage nutritional analysis."

- **This presentation is also a robust demonstration of the SECOND FACT, which is:**

  "Both intra-laboratory and inter-laboratory precisions of NIR method are better than those of wet chemistry method."

Conclusions

- **Many nutritionists who are quick to brush off NIRS, citing its poor accuracy should be *pleasantly surprised and change their thoughts* by this robust demonstration of similar accuracy of NIRS and WC, and even better precision of NIRS over WC.**

Thank You Very Much?

Questions/Comments/Suggestions

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