Assessing the Accuracy of On-Farm Rapid Testing Kits for Nitrate-N Concentration in Forage

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Introduction

- Ruminants, particularly cattle, are at risk of nitrate toxicity when forage nitrate levels are high – a concern in Alberta due to factors like excessive fertilization and environmental stressors (hail damage, drought & hard frost).
- Traditional lab analysis for nitrates is perceived as time-consuming and costly, leading to producer reluctance.
- There is need to encourage the use of rapid, on-farm nitrate testing methods. Assessing the accuracy of commercially available rapid test kits is essential to ensure producers have reliable tools to reduce the risk of nitrate poisoning.

Objective

Verify the accuracy of rapid nitrate test kits – Horiba and NECi nitrate tests – in measuring forage nitrate concentration compared to industry-standard wet chemistry lab analysis.

Materials & Methods

Sample Collection

- Collected 2021 to 2022 across various Alberta farms.
- Targeted forage crops and hay bales at risk of nitrate accumulation due to hard frost (<5°C), drought or hail damage.

Sample types:

- Standing forage (n=52): Including alternative forages (n=4), cereals only (n=5), grasses only (n=5), multispecies mix (n=18), legumes only (n=9), and legume-grass mix (n=7).
- Dry forage (n=29): Hay samples comprising legume-grass mix (n=16), grass only (n=10), and cereals only (n=3).

Rapid Nitrate Analysis

- For sap-available standing forages, two tests were used. The NECi Green Forage Nitrate Test utilizes an enzyme-based method suitable for green forages. The Horiba meter uses an ion-selective electrode (ISE) method, which directly measures nitrate ion concentration in the plant sap and provides an estimate of the plant's Nitrate-N levels.
- For dry hay and non-sap forage, the NECi Dry Forage Nitrate Test was used. This test is designed for dry forage samples, ensuring accuracy in nitrate quantification.
- Manufacturer protocols were followed. Results were converted to Nitrate-N for uniformity.

NECi Green Forage Nitrate Test

Horiba Nitrate Test

NECi Dry Forage Nitrate Test

Laboratory Analysis

- All forage samples were analyzed using traditional wet chemistry methods at a commercial lab in Alberta involving precise liquid-based chemical processes.

Statistical Analysis

- The final analysis included 81 forage samples.
- The Paired Wilcoxon signed rank test was used to compare lab analysis with rapid test results.
- Samples were categorized by their Nitrate-N concentration.

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Results & Discussion

Standing Forage Samples:

- The Horiba and Green Forage NECi Nitrate rapid tests generally aligned with wet chemical analysis for standing fresh forage samples in terms of nitrate concentration (Table 1; $P > 0.05$).
- Despite the wet chemical analysis indicating all samples were below 350 ppm, the rapid tests identified some fresh samples with higher Nitrate-N levels. This discrepancy suggests a potential over-sensitivity in the rapid tests or under-detection in lab analysis.

Table 1: Comparative analysis of Nitrate-N concentrations in standing forage samples.

Tests	Lab wet-chemical analysis	Horiba test	Green Forage NECi test
Number of Samples	48	48	43
Mean Nitrate-N (ppm)	10.63	258.91	359.62
Samples in Nitrate-N Ordinal Category			
1 (<350 ppm)	48	43	39
2 (350-1000 ppm)	0	0	1
3 (1000-2500 ppm)	0	5	3
4 (>2500 ppm)	0	0	0
Wilcoxon Signed Rank Test (Ordinal)			
Lab vs. Horiba	P = 0.062		
Lab vs. Green NECi	P = 0.125		
Horiba vs. Green NECi	P = 0.500		

Dry Forage Samples:

- The Dry Forage NECi kit tended to overestimate Nitrate-N in dry hay or dead plant samples compared to wet chemical analysis (Table 2; $P < 0.05$), but there is a need to verify if the lab underestimated Nitrate-N levels in these samples.

Table 2: Comparative analysis of Nitrate-N concentrations in dry forage samples.

Tests	Lab wet-chemical analysis	Dry Forage NECi test
N	32	32
Mean Nitrate-N (ppm)	19.38	154.06
Samples in Nitrate-N Ordinal Category		
1 (<350 ppm)	32	24
2 (350-1000 ppm)	0	8
3 (1000-2500 ppm)	0	0
4 (>2500 ppm)	0	0
Wilcoxon Signed Rank Test (Ordinal)		
Lab vs. Dry Forage NECi	P = 0.008	

Conclusion

- **Speed vs. Accuracy:** Both rapid tests, although significantly faster than lab analysis, exhibit variances in Nitrate-N sensitivity which shows a need for further testing.
- **Practicality:** NECi tests took 15-20 minutes per sample which may be less practical for producers. The Horiba test stands out for its speed, requiring only 1-2 minutes per sample.
- **Overall Impact:** This shows potential of rapid on-farm nitrate tests to aid Alberta producers in managing forage sources, but also indicates the need to further explore hand-held devices for dry forage nitrate quantification.

Next Steps

- **Phase 2:** In collaboration with Union Forage and Blue Rock, this will focus on validating the Horiba test across Alberta (excluding the NECi tests to streamline Horiba's applicability and performance).
- **Cross-Laboratory Validation:** To better understand the observed discrepancies in nitrate analysis, samples will be sent for comparative testing at two labs, providing insight into variations in lab testing methodologies and results.