Validation of NemaGENE SCN – *A rapid method for soybean cyst nematode (SCN) enumeration equivalent to the industry standard that enables routine, actionable decision-making for SCN management*

Introduction

Soybean cyst nematode (SCN, Heterodera glycines) continues to be the primary yield-limiting pathogen in North American soybean production. While morphological quantification via the traditional sieving method is the established benchmark, its low throughput and requirement for taxonomic expertise hinder expanded soil testing. This study evaluates NemaGENE SCN, a rapid PCR-based alternative for detection and enumeration of SCN eggs derived from cysts. The study results demonstrate that this genetic assay provides equivalent diagnostic value to microscopy while streamlining laboratory workflows and accelerating the delivery of actionable data.

Comparison Methodology

Eighteen (18) bulk soil samples (encompassing variable soil types of clay, loam, silt, and sand) sampled across southern Minnesota (>1 kg) with unknown SCN counts were dried and ground according to standard physical and chemical soil processing methods. To account for natural non-homogeneity of nematode distributions, 4-6 unpaired replicate assessments for both tradtional sieving reference method and the NemaGENE assays were performed for each bulk soil sample. The traditional enumeration method was completed at two independent laboratories (Minnesota Valley Testing Labs – MVTL - and Iowa State University), while all NemaGENE SCN assay data was generated at MVTL.

Average enumeration data generated for each sample by the two methods was compared (Fig. 1a), and a Deming regression analysis was completed to determine any statistical difference or enumeration bias between the methods (Fig. 1b).

See appendix for method parameters for both the traditional wet sieiving and microscopy method and the NemaGENE SCN assay.

Results

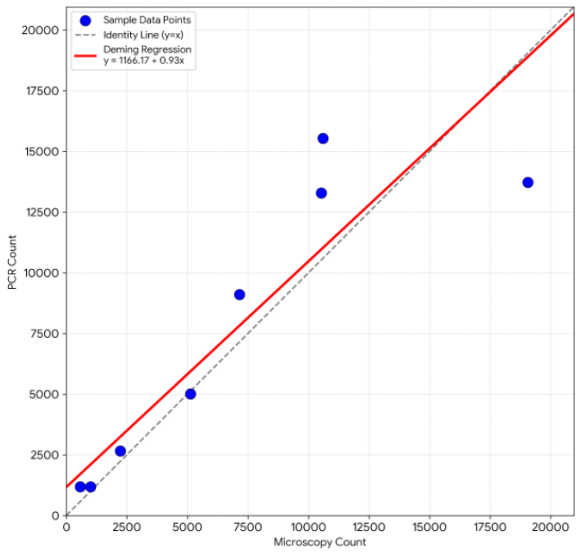
Comparative Analysis of Eight Samples Demonstrates NemaGENE Assay Performs Consistently with Traditional Microscopy Method

To evaluate the enumeration accuracy of the rapid NemaGENE assay, eight bulk samples were analyzed and compared against the reference microscopy method. PCR assessments were conducted at MVTL, while microscopy enumeration was performed at Iowa State University. The comparative data for both methods are presented in Figure 1.

A

ID	PCR Count (100cc)	Iowa Scope Count (100cc)	Average PCR	Average Microscopy Count	Difference
1	24019	27800	13724	19050	-5326
	14591	18800			
	14203	14900			
	13476	14700			
	13257				
	12731				
	9701				
	7812				
9	20199	13500	13288	10525	2763
	15496	10000			
	14957	9700			
	13194	8900			
	10590				
	5289				
10	25273	15600	15531	10600	4931
	18289	9700			
	17274	9100			
	15094	8000			
	12300				
	4953				
14	15607	10000	9105	7150	1955
	8617	9200			
	8470	5100			
	8134	4300			
	7107				
	6696				
15	9176	6300	5011	5125	124
	7141	6000			
	6937	4900			
	2961	3300			
	1934				
	1914				
17	2305	1400	1044	1000	44
	1894	1000			
	1454	800			
	994	800			
	374				
	218				
	66				
18	4880	3000	2653	2225	428
	4826	2500			
	2870	1900			
	2407	1500			
	662				
	271				
20	2294	900	1183	575	608
	1435	700			
	1099	400			
	1096	300			
	663				
	510				

B



Slope 0.93, 95% Ci (0.6307, 1.5142)

Figure 1 – Comparative evaluation of enumeration values generated by the standard microscopy method and the NemaGENE rapid PCR method across 8 bulk soil samples. A) Enumeration values produced by the NemaGENE SCN assay and reference microspcpy method. B) Deming regression where each average value from each method were plotted against each other (y values for the PCR enumeration, and x-values for the reference microscopy method). A slope of 0.93 (where 1 is a perfect match between methods) demonstrates good agreement between methods, and a confidence interval that includes 1 demonstrates there is no statistical difference between the methods.

Comparative Analysis of 18 Samples Demonstrates NemaGENE Assay Performs Consistently with Traditional Microscopy Method

To further evaluate the accuracy robustness of the rapid NemaGENE assay, results from an expanded 18 bulk sample set were analyzed at MVTL. The study data, presented in Figure 2, compare the average enumerated values between the reference microscopy method and the rapid assay.

A

ID	Average PCR Count (100cc)	Average MVTL Scope Count (100cc)	Difference
1	13724	19646	-5922
3	7310	9113	-1803
4	8905	11183	-2279
5	6641	10216	-3575
6	4970	5720	-750
7	3520	5616	-2096
8	24903	24163	739
9	13287	10876	2412
10	15530	10695	4835
11	3661	2487	1174
12	2289	2228	62
13	10168	4111	6056
14	9105	7188	1917
15	5010	4492	519
16	335	598	-263
17	1044	2750	-1706
18	2653	2967	-314
20	1183	814	369

B

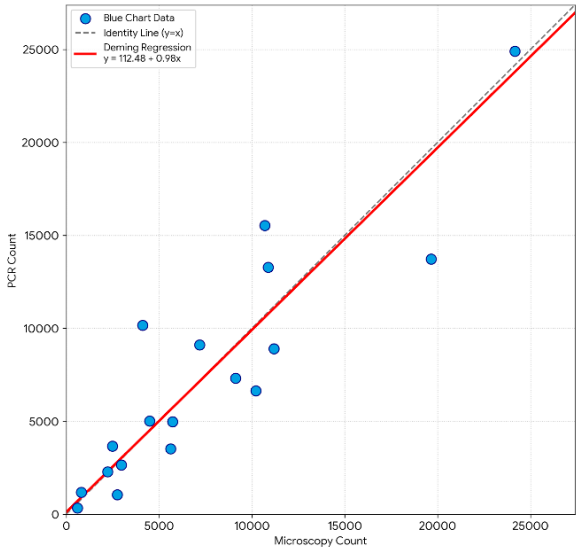


Figure 2 – Comparative evaluation of enumeration values generated by the standard microscopy method and the NemaGENE rapid PCR method across 18 bulk soil samples. A) Average enumaration values produced by the NemaGENE SCN Assay and reference microspcpy method. B) Deming regression where each average value from each method were plotted against each other (y values for the PCR enumeration, and x-values for the reference microscopy method). A slope of 0.98 (where 1 is a perfect match between methods) shows good agreement between methods, and a confidence interval that includes 1 demonstrates there is no statistical difference between the methods.

Conclusion

This independent study confirms that the NemaGENE SCN PCR assay is performance-equivalent to the traditional wet sieving and microscopy method for both detection and counting. Using Deming regression analysis across 18 soil samples, no significant statistical difference between the two approaches was observed, validating NemaGENE as a scientifically robust alternative.

By allowing for a standardized sample size of 20cc, objective results that discimrinate against morphologically similar nematodes, a rapid turnaround time (under three hours) and reducing manual labor requirements, the NemaGENE assay eliminates several logistical barriers that previously prevented routine SCN testing. The result is a high-throughput, accurate diagnostic tool that lowers per-sample costs and enables more frequent, data-driven decisions at the farm level.

Appendix

NemaGENE Assay Method Summary

Sample Preparation Procedure

1. Combine 20cc soil with 40 mL suspension liquid and vortex.
2. Centrifuge sample for 5 minutes at 500xG to pellet soil. Decant supernatant from conical tube through exclusion and inclusion filter.
3. Elute capture filter into mechanical lysis tube, and vortex for 20 minutes.
4. Centrifuge sample and combine 50 uL of the lysate Diluent A, vortex to mix, and proceed to PCR Amplicfication and Data Analysis.

PCR Amplification and Data Analysis

1. Transfer 5 uL from cleaned DNA sample to a thawed NemaGENE SCN Mastermix tube.
2. Transfer to Compatible Thermal Cycler and initial PCR program.
3. Analyze run to generate Cq values.
4. Transfer FAM channel Cq Values to SCN Calculator for enumeration.

Traditional SCN Microscopy Method Summary

Sampling

1. Measure 100 cc of soil into a beaker using water displacement.
2. Allow the sample to settle for 15 minutes followed by thorough soil mixing.
3. Settle sample again for an additional 15 minutes.

Sieving and Cyst Collection

1. Pour the soil suspension through a #20 sieve (850 µm) stacked over a #60 sieve (250 µm).
2. Rinse the material retained on the #20 sieve with water and discard the debris.
3. Backwash the material retained on the #60 sieve into a 100 mL plastic grinding tube, ensuring cysts and fine sediments are collected.

Cyst Disruption and Egg Recovery

1. Assemble a #200 sieve (75 µm) over a #500 sieve (25 µm) positioned into the funnel below the rubber stopper.
2. Grind the collected material to release eggs from cysts, rinsing periodically to ensure complete transfer through the sieves.
3. Rinse and collect the material retained on the #500 sieve into a clean beaker.

Egg Quantification

1. Analyze the recovered suspension under a microscope and count nematode eggs.