Supplementary material

Table S1. Sequences of LAMP primers used in this study

Primer		Primer sequence (5'-3')	
RKN	RKN-F3	CTGCCCTTTGTACACACC	[1]
	RKN-B3	GACACCAGCGACAGCCGTT	
	RKN-FIP	CTGCGATTAAATTGGTTTCCATCAACGGGACTGAGCCATTTCG	
	RKN-BIP	GCTTGAACCGGGCAAAAGTCCATAAAGTAATGATCCAGCAGC	
	RKN-LB	GTAACAAGGTAGCTGTAGGTGAAC	
Mh	Mh-F3	GAATATGAGGTGACATGTTAGG	[2]
	Mh-B3	TCAATGTTTCTGCAGTTCG	
	Mh-FIP	TGAAAAAAATATTGCTGGCGTCCACCTTAATCGGGTTTAAGACT	
	Mh-BIP	TCTATCCTTATCGGTGGATCACTCCACAAATTATCGCAGTTAGCT	
	Mh-LB	GGCTCGTGGATCCATGAAGAACG	
HSP	F3	GGTGCTGACATCAGTATGATT	This study
	B3	GTCATCTCCGGATCAACG	
	FIP	GTGACGATGACGCGGTCAGGTCAATTCGGTGTTGGAT	
	BIP	CACAATGACGACGACTGCCATCGAATGATGAAGGAACCAC	
	Loop-F	CAACCAAGAAAGCAGAGTAGA	
	Loop-B	TCAATGGGAATCTTCTGCTG	

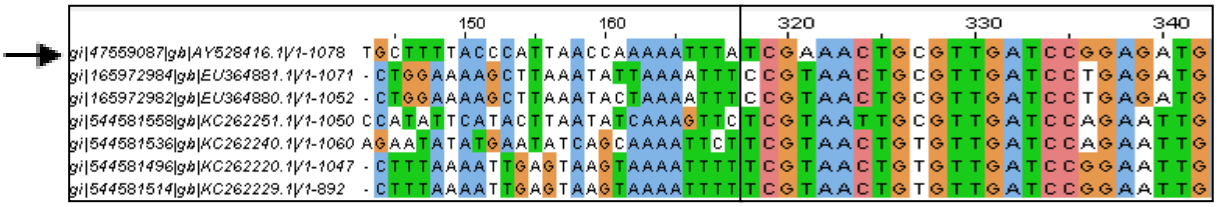


Figure S1. DNA sequences of *hsp90* gene from *M. hapla* (arrow) and 6 other RKN (*M. arenaria*, *M. chitwoodi*, *M. fallax*, *M. incognita*, *M. minor*, *M. naasi*) used for designing the HSP-LAMP primers.

Table S2. Soil chemical characteristics and root-knot nematode (RKN) density per 250 g^{-1} soil

	Country	RKN	Soil pH	OM	Clay	Sand	C-tot [5]
		(250 g^{-1} soil)		(%)	(%)	(%)	(Ma. % air dw)
RKN free soil[1]							
NF1–17	Sweden	0	6.1	1.2	6.8	84	0.9
NF2–17	Sweden	0	6.6	3.2	47	11	3.1
RKN infested soil[2]							
NI1–19	Denmark	110	N.a[4]	1.9	6.8	78	1.5
NI2–19	Denmark	78	N.a	1.5	9.2	67	1.1
NI3–19	Denmark	0	N.a	1.6	8.8	75	0.8
NI4–19	Sweden	2	N.a	3.8	10.0	62	2.4
NI5–19	Sweden	0	N.a	1.5	9.2	67	1.1
NI6–19	Sweden	261	N.a	3.8	5.1	89	2.3
NI7–19	Sweden	2	N.a	1.4	3.5	89	1.0
NI8–19	Sweden	4	N.a	14	8.4	75	9.0
NI9–19	Sweden	4	N.a	3.6	12.0	54	2.1
NI10–19	Sweden	5	N.a	3.0	8.0	78	1.7
NI11–19	Sweden	9	N.a	1.4	2.0	93	1.2
NI12–19	Sweden	3	N.a	2.9	11.0	71	1.7
NI13–19	Sweden	110	N.a	9.9	9.6	69	6.5
NI14–19	Sweden	8	N.a	1.7	4.3	81	3.1
NI15–19	Sweden	3	N.a	2.7	10.0	78	2.1
NI16–19	Denmark	8	N.a	N.a	N.a	N.a	N.a
NI17–19	Denmark	10	N.a	2.1	8.0	70	1.3
NI18–19	Denmark	38	N.a	1.8	6.8	70	1.1
NI19–19	Denmark	2	N.a	N.a	N.a	N.a	N.a
NI20–19	Sweden	18	N.a	3.2	7.6	80	1.9
RKN infested soil[3]							
NI1–20	Sweden	217	6.7	1.9	12.0	64	1.3[4]
NI2–20	Sweden	45	6.9	2.1	10.0	70	1.3
NI3–20	Sweden	140	5.9	7.1	9.5	76	4.3
NI4–20	Sweden	119	6.8	6.4	5.6	83	4.5
NI5–20	Sweden	185	6.2	2.2	6.4	81	1.6
NI6–20	Sweden	344	6.3	1.4	7.9	81	1.1

[1]NF: root-knot nematode free soil collected in 2017; [2]NI: naturally nematode infested soil collected in 2019 (Oostenbrink elutriator); [3]NI: naturally nematode infested soil collected in 2020 (Baermann funnel method); [4]Not analyzed; [5]total carbon.

Table S3. Specificity of the HSP-LAMP primer set (designed in this study) and the published LAMP primers RKN [1] and Mh [2] using colorimetric and real-time LAMP

Nematode species	Source	Colorimetric LAMP			Real-time LAMP (Ct-value)[6]	
		HSP	RKN	Mh	HSP	Mh
Globodera spp	HS[1] Nematode Laboratory	-	N.a[5]	N.a	-	-
Heterodera schachtii	HS Nematode Laboratory	-	N.a	N.a	-	45 ± 2.2
Longidorus spp	HS Nematode Laboratory	-	N.a	N.a	-	-
Meloidogyne arenaria	ILVO[2]	-	N.a	-	-	58 ± 0.1
Meloidogyne chitwoodi	HS Nematode Laboratory	-	N.a	+	-	51 ± 0.2
Meloidogyne chitwoodi	Intertek Scanbi Diagnostics	-	N.a	-	-	-
Meloidogyne fallax	Intertek Scanbi Diagnostics	-	N.a	-	-	-
Meloidogyne incognita	ILVO	-	N.a	-	-	-
Meloidogyne javanica	ILVO	-	N.a	-	-	-
Meloidogyne hapla	HZPC[3]	+	+	+	38 ± 0.08	51 ± 0.2
Pratylenchus penetrans	SLU[4]	-	+	N.a	-	47 ± 1.6
Trichodorus spp	HS Nematode Laboratory	-	N.a	N.a	-	-

[1]Husållningssällskapet; [2]Instituut voor Landbouw-, Visserij- en Voedingsonderzoek, Belgium; [3]HZPC, Netherlands ; [4]Swedish University of Agricultural Sciences [5]N.a: not analyzed; [6]cycle threshold of real-time LAMP reactions conducted in a real-time PCR machine; negative DNA amplification (-); positive DNA amplification (+). Means ± Standard Error (SE).

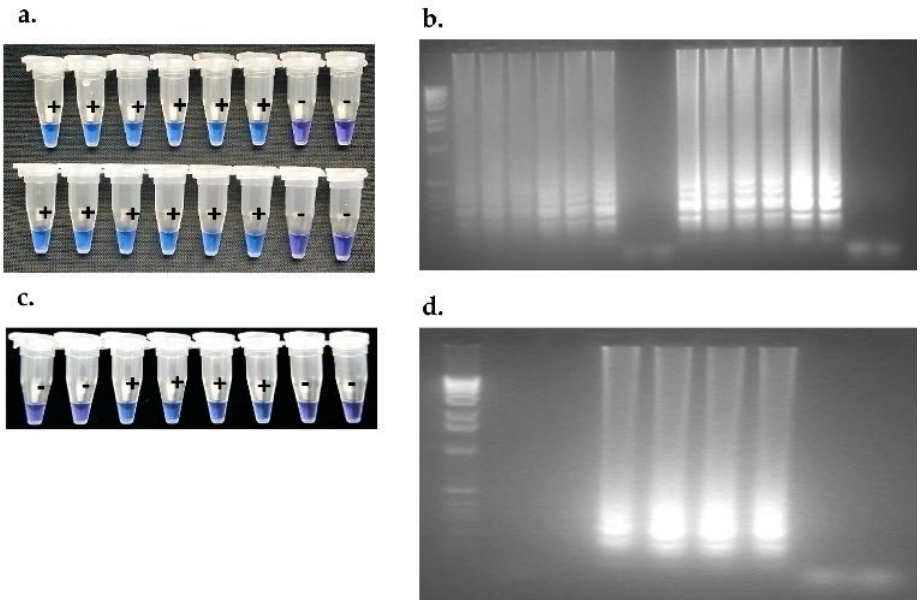


Figure S2. Specificity tests of RKN primers with colorimetric LAMP; a: *Meloidogyne hapla* DNA extracted from 2–64 J2s (n = 2); negative DNA amplification in water control (-); positive DNA amplification (+); b: corresponding gel electrophoresis analysis; c: *Pratylenchus penetrans* DNA extracted from 2–64 nematodes (n = 1); d: corresponding gel electrophoresis analysis.

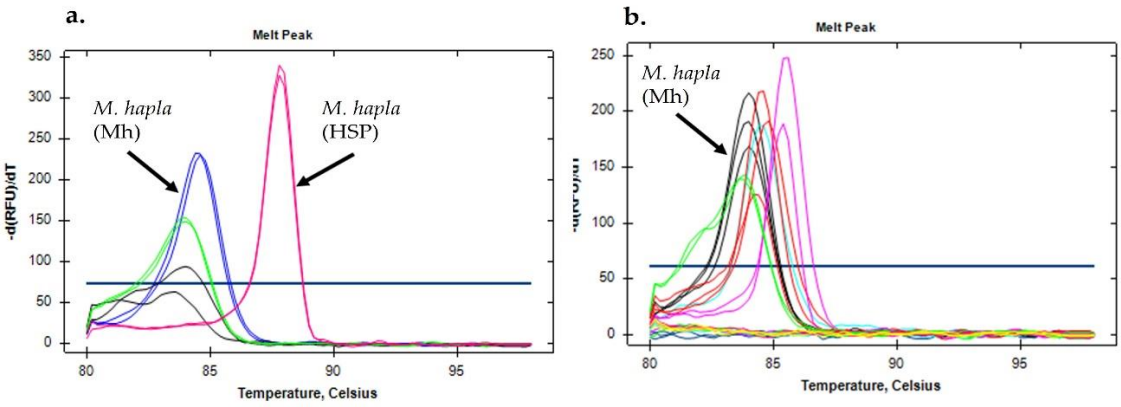
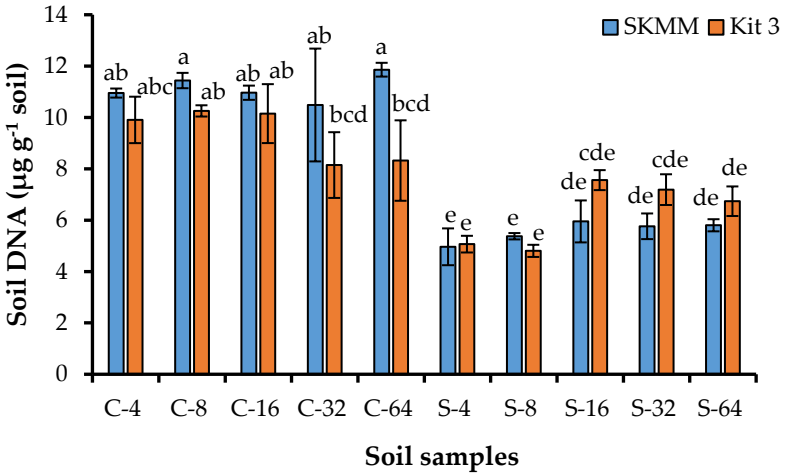
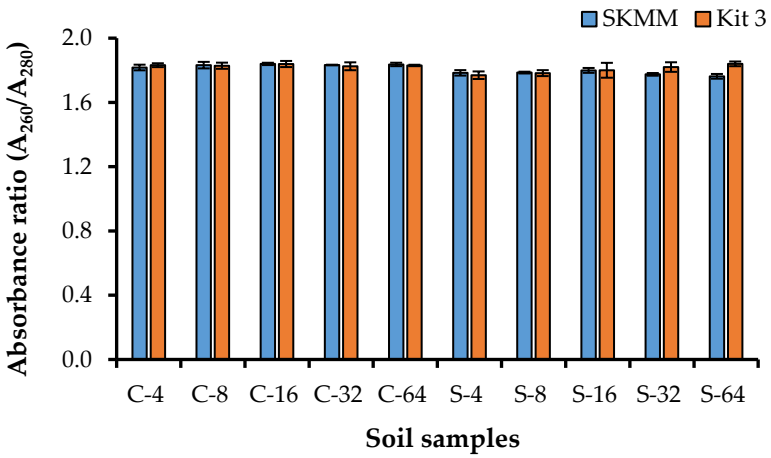


Figure S3. Specificity tests of Mh primers and HSP-primers in rt-LAMP performed with qPCR machine and shown as melt curves, a: analysis with Mh primers and DNA from *Meloidogyne arenaria* (green lines), *M. chitwoodi* (not detected), *M. fallax* (not detected), *M. incognita* (not detected), *M. javanica* (black lines), *M. hapla* (blue lines), as well as DNA analyzed with HSP-primers *M. arenaria* (not detected), *M. chitwoodi* (not detected), *M. fallax* (not detected), *M. incognita* (not detected), *M. javanica* (not detected), *M. hapla* (pink lines) (n = 2), b: analysis with Mh primers and DNA from *Trichodorus* spp (not detected), *Longidorus* spp (not detected), *M. chitwoodi* (green lines), *Heterodera schachtii* (pink lines), *Pratylenchus penetrans*(red lines), *Globodera* spp (not detected) and *M. hapla* (black lines) (n = 3).

a.



b.



c.

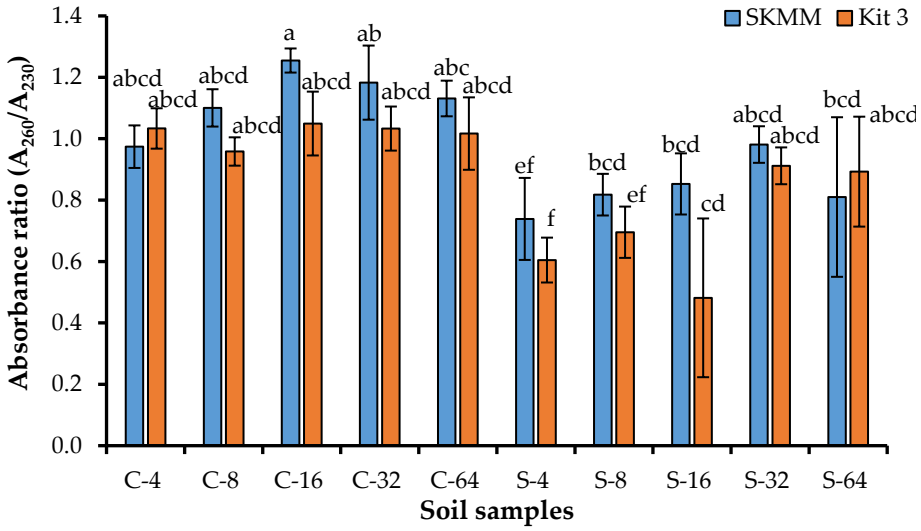
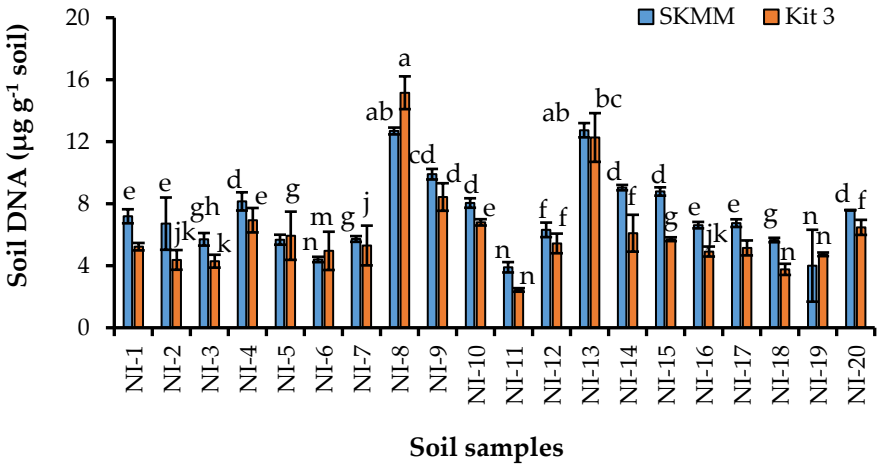
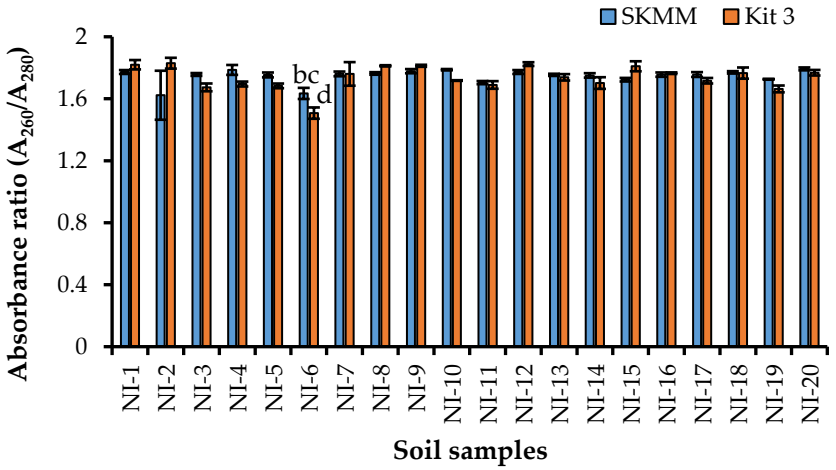


Figure S4. Soil DNA extraction from nematode-free sand (S; NF1–2017) and clay (C; NF2–2017) soils inoculated with 4; 8; 16; 32 and 64 J2 250 g^{-1} soil, DNA extracted with SKMM procedure and FD kit (kit 1) followed by DNA purification with Wizard DNA (kit 2) and MicroSpin S-300 HR Columns (kit 3) commercial kits, a: DNA concentration (µg g^{-1} soil); b: absorbance ratio A_{260}/A_{280}; c: Absorbance ration A_{260}/A_{230}.

a.



b.



c.

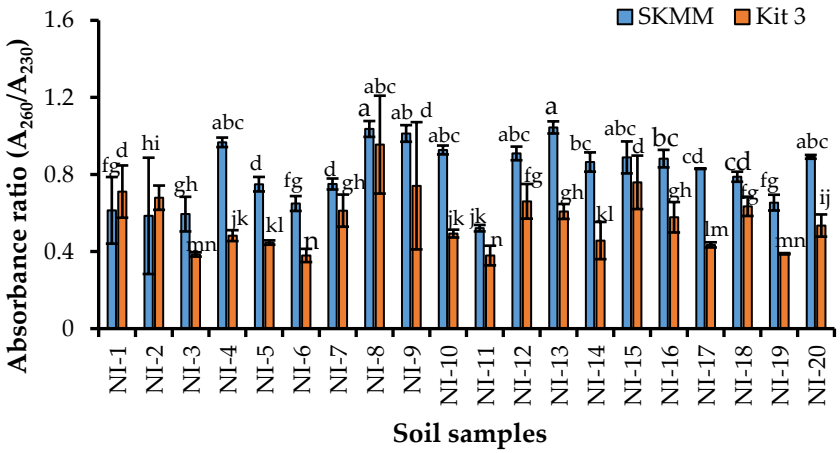
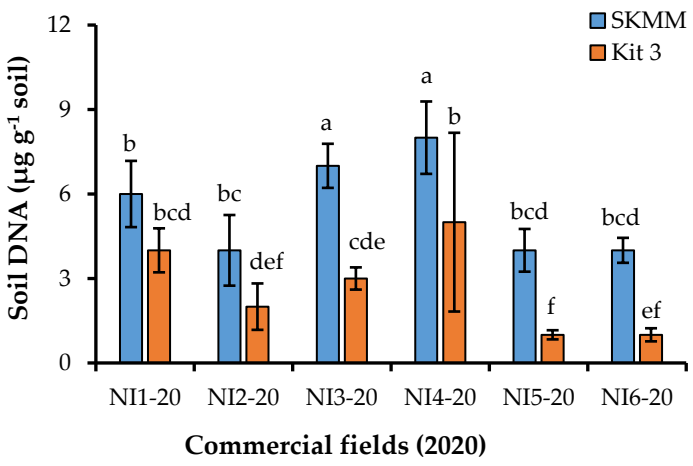
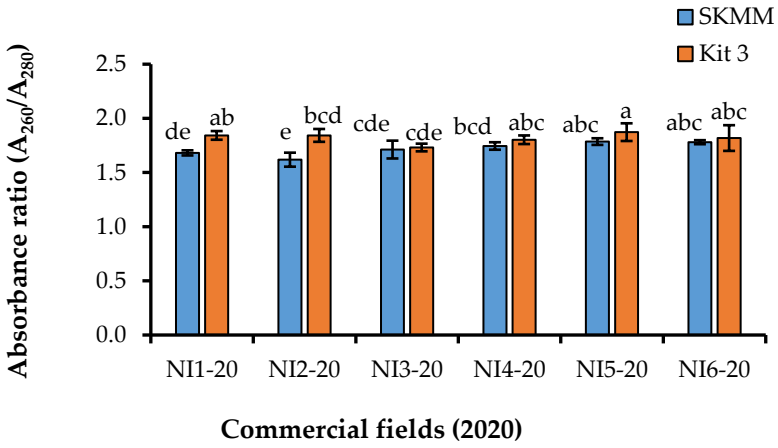


Figure S5. Soil DNA extraction from naturally infested (NI) soil samples collected in 2019 from 20 different commercial fields in Sweden and Denmark. DNA extracted with SKMM procedure and FD kit (kit 1) followed by DNA purification with Wizard DNA (kit 2) and MicroSpin S-300 HR Columns (kit 3) commercial kits, a: DNA concentration (µg g^{-1} soil); b: absorbance ratio A_{260}/A_{280}; c: absorbance ration A_{260}/A_{230}.

a.



b.



c.

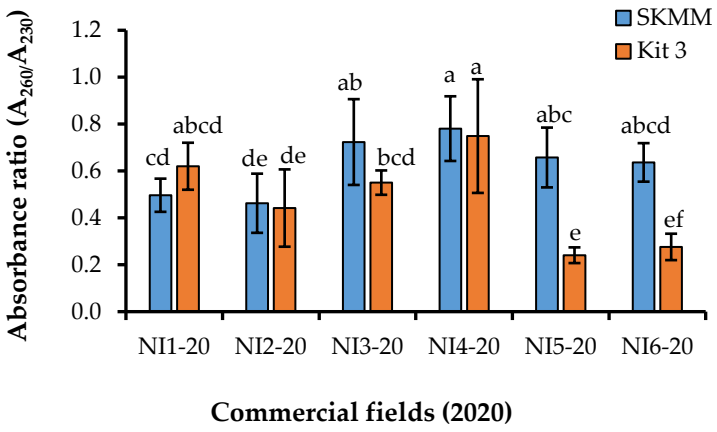
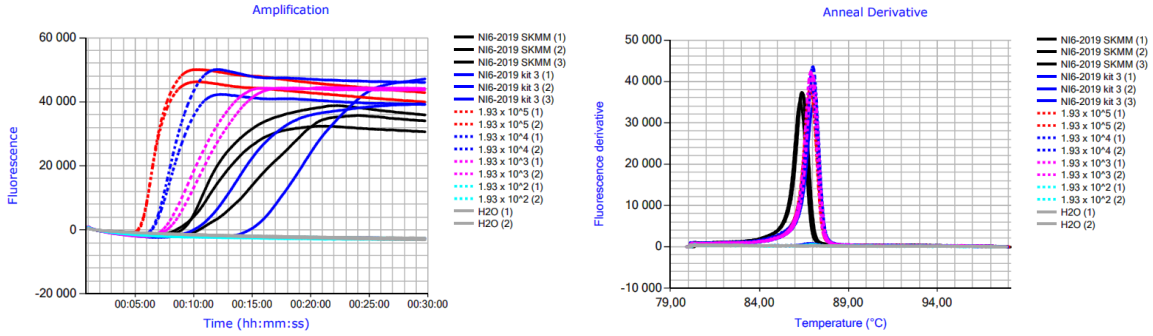


Figure S6. Soil DNA extraction from naturally infested soil samples from 6 commercial fields in Sweden collected in 2020. DNA extracted with SKMM procedure and FD kit (kit 1) followed by DNA purification with Wizard DNA (kit 2) and MicroSpin S-300 HR Columns (kit 3) commercial kits, a: DNA concentration (µg g^{-1} soil); b: absorbance ratio A_{260}/A_{280}; c: absorbance ration A_{260}/A_{230}.



(a) (b)

Figure S7. Real-time LAMP analysis of DNA extracted from soil NI6-19; (a): Amplification curves of DNA extracted from sample NI6–19 with SKMM (T_t= 10; 11; 14 min) (black solid lines) and kit 3 (T_t = 14; 17; 0 min) (blue solid lines) (n = 3), gBlock^{HSP} at 193 000 to 193 gene copies 5 µl^{-1} (dashed lines) (n = 2), MQ H_2O (grey solid lines) (n = 2); (b): Anneal derivative curve.

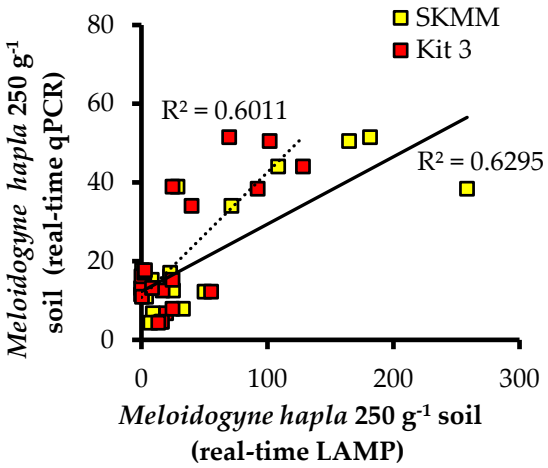


Figure S8. Relationship between qPCR and rt-LAMP of *Meloidogyne hapla* occurrence in soil samples collected from field NI5–20 and NI6–20. In qPCR DNA extracted from nematodes pre-collected in water using Baermann funnel method and extracted by CTAB (ISD, Sweden), and in rt-LAMP (1) DNA extracted from soil and purified with two commercial kits (solid line) and (2) with SKMM manual procedure (dash line) (n = 5) .

References

1. Niu, J-H.; Guo, Q-X.; Jian, H.; Chen, C-L.; Yang, D.; Liu, Q.; Guo, Y-D. Rapid detection of *Meloidogyne* spp. by LAMP assay in soil and roots. *Crop Prot* **2011**, *30*, 1063–1069.
2. Peng, H.; Long, H.; Huang, W.; Liu, J.; Cui, J.; Kong, L.; Hu, X.; Gu, J.; Peng, D. Rapid, simple and direct detection of *Meloidogyne hapla* from infected root galls using loop-mediated isothermal amplification combined with FTA technology. *Sci Rep* **2017**. 7:44853 | https://doi.org/10.1038/srep44853