

Figure S1: Antisera immunogenicity characterization and hybridoma production and selection. **A)** Western blots to check the reactivity of **five** antisera (3221, 3222, 3223, 3224 and 3225) from **five** mice immunized with the selected ToBRFV CP peptide. The antisera and commercial ToBRFV antibody (DSMZ) were challenged against extracts from healthy plant (lane 1) and tomato plants infected with ToBRFV (lane 2), TMV (lane 3) or ToMV (lane 4). **B)** Western blots to check the reactivity of 20 hybridoma cell lines. Lanes 1 of each blot were loaded with healthy plant extract, lanes 2 with an extract from tomato plants infected with TMV, and lanes 3 with a 1:100 diluted extract from tomato plants infected with ToBRFV. **C)** Western blots to check the reactivity of 10 hybridoma subclone culture supernatants from **five** parental clones (2F7, 3B1, 4A6, 4B10 and 5A6) to ToBRFV-infected tomato leaf extracts in Western blot. Lanes 1 of each blot were loaded with healthy plant extract, lanes 2 with extract from tomato plants infected with TMV, and lanes 3 with a 1:100 diluted extract from tomato plants infected with ToBRFV. The subclones chosen for the monoclonal antibody production were 4B10-1 and 5A6-1.

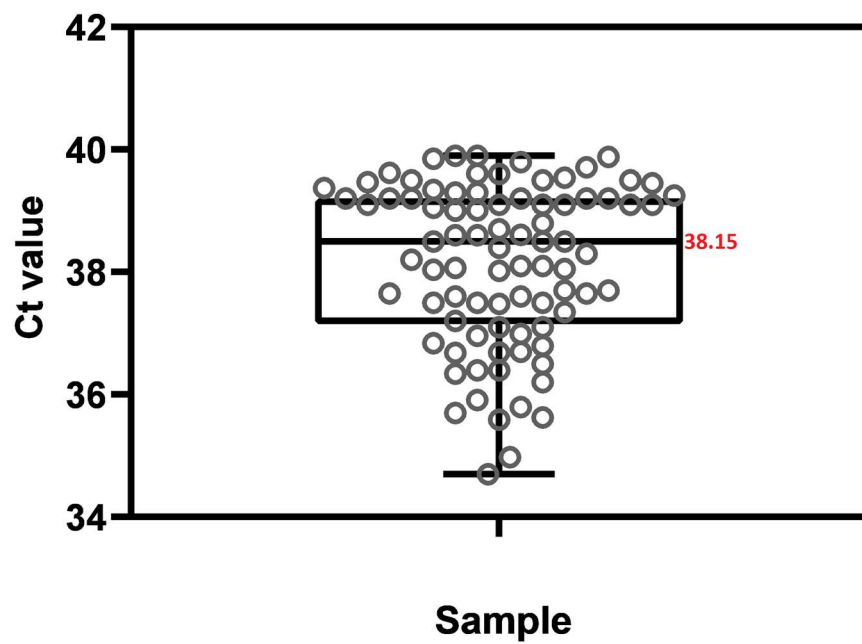


Figure S2: Estimation of the Abiopep RT-qPCR test threshold. Meta-analysis gathering all the negative samples analyzed by Abiopep's diagnostic service in 2020, using the Abiopep RT-qPCR test. The plot contains 85 values and the Ct median resulted in 38.15 and the lower value in 34.7.

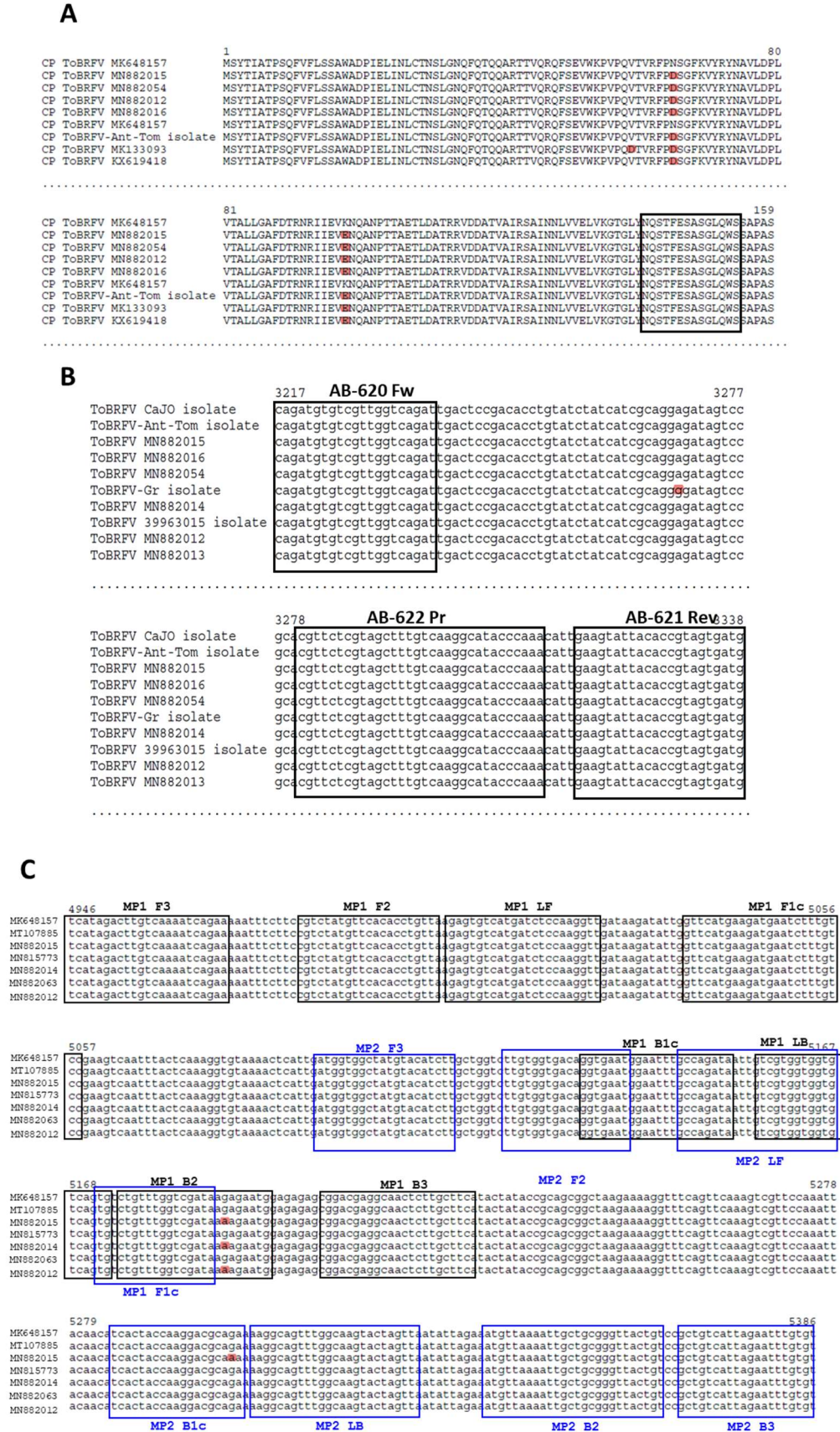


Figure S3: evaluation of the analytical inclusivity of the tests. **A)** MoAbs recognition epitope. **B)** Abiopep's RT-qPCR test oligonucleotides. **C)** MP1 and MP2 LAMP primers. All the sequences shown are from different ToBRFV isolates extracted from NCBI. Alignments were performed using Benchling webtool.

Table S1: detailed Ct values of the RT-qPCR tests shown in Figure 3.

RNA extract concentration (ng/μl)	Mean Ct			SD	
	R1	R2	R3		
Abiopep Primers					
10	14.96	14.70	14.96	0.15	
1	18.36	18.39	18.31	0.04	
1,00E-01	21.96	21.88	21.87	0.05	
1,00E-02	25.36	25.37	25.50	0.08	
1,00E-03	29.25	28.90	28.91	0.20	
1,00E-04	32.36	32.49	32.28	0.10	
1,00E-05	37.42	35.82	36.07	0.86	
1,00E-06	39.35	37.72	39.31	0.93	
1,00E-07	39.14	36.89	38.81	1.22	
WT	39.98	N.D			
Enza Zaden Primers					
10	14.35	14.77	14.40	0.23	
1	17.55	17.72	17.63	0.09	
1,00E-01	20.85	20.98	20.86	0.08	
1,00E-02	24.35	24.24	24.28	0.06	
1,00E-03	27.64	27.54	27.48	0.08	
1,00E-04	30.87	31.20	30.91	0.18	
1,00E-05	33.88	34.43	33.79	0.35	
1,00E-06	N.D	36.96	N.D		
1,00E-07	36.98	36.99	N.D		
WT	N.D	35.92			
CSP Labs Primers					
10	17.42	17.50	17.49	0.04	
1	20.75	20.66	20.66	0.05	
1,00E-01	23.95	24.12	20.08	0.09	
1,00E-02	27.39	27.33	27.34	0.03	
1,00E-03	30.60	30.78	30.49	0.14	
1,00E-04	34.28	35.14	33.62	0.76	
1,00E-05	36.14	N.D	35.96	0.13	
1,00E-06	N.D	N.D	N.D		
1,00E-07	N.D	35.94	37.02		
WT	36.94	N.D			

Ct values from the comparison of the CaTa28, CSP1325 and Abiopep RT-qPCR tests. The Ct value for each technical replicate (R1-R3) and RNA dilution are tabulated together with the standard deviation (SD).

Table S2: oligonucleotides used in this study.

Oligonucleotide	Sequence (5'-3')	Notes
F3 MP1	TCATAGACTTGTCAAATCAGAA	RT-LAMP MP1
B3 MP1	GAAGCAAGAGTTGCCTCG	
FIP MP1	GGACAAAGATTTCATCTTCATGAACCCGTCTATGTTACACCTGTT	
BIP MP1	AGGTGAATGGAATTTGCCAGATCATTCTCTTATCGACCAACAG	
LF MP1	ACCTTGAGATCATGACACTCT	
LB MP1	TGTCGTGGTGGTGTCAGTG	
F3 MP2	GATGGTGGCTATGTACATCT	RT-LAMP MP2
B3 MP2	ACACAAATTCCTAATGACAGCG	
FIP MP2	TATCGACCAAAACAGACACTGACACTTGTGGTGACAGGTGAA	
BIP MP2	TCACTACCAAGGACGCGAGAACAGTAACCCGACGCAATT	
LF MP2	CACCACGACAATTATCTGGCA	
LB MP2	AAGGCAGTTTGGCAAGTACTAGTT	
F3 25S	AGCCAAGCGTTCATAGCG	RT-LAMP rRNA25S
B3 25S	GCTTCTAGCCCGGATTCTGA	
FIP 25S	CACGTTCCCTATTGGTGGGTGAATCCTTCGATGTCGGCTCTT	
BIP 25S	CACACAATTGGCCATCGCGCAGTCATAATCCAGCACACGG	
LF 25S	ACTTGGTGAAATCTGCTTCACA	
LB 25S	AAGCCAGTGGCGCGAAG	
CaTa28 Fwd.	GGTGGTGTCTAGTGTCTGTTT	RT-qPCR ISHI veg/ENZA ZADEN ToBRFV primers
Cata28 Rev.	GCGTCCTTGGTAGTGATGTT	
CaTa28 Pr.	/56-FAM/AGAGAATGGAG/ZEN/AGAGCGGACGAGG/3IABKFQ/	RT-qPCR ISHI veg/CSP Labs ToBRFV primers
CSP1325 Fwd.	CATTTGAAAGTGCATCCGGTTT	
CSP1325 Rev.	GTACCACGTGTGTTTGCAGACA	
CSP1325 Pr.	/56-FAM/ATGGTCTCTGC/ZEN/ACCTGCATCTTGAGA/3IABKFQ/	RT-qPCR Abiopep ToBRFV test
AB-620	CAGATGTGTCGTTGGTCAGAT	
AB-621	CATCACTACGGTGTAACTTCT	
AB-622	/56-FAM/CGTAGCTTTGTCA/ZEN/AGGCATACCCAAA/3IABKFQ/	RT-qPCR PMMoV test
AB-233	AGCAGTTCCTCTGAYGTGTGGA	
AB-234	AGAAGTGCCGACACTAGWGAA	
AB-235	/56-FAM/CGACCGCTACAGTTAGATTCTCTGTAC/3IABKFQ/	RT-qPCR TMGMV test
AB-236	GTAGCTATAAGGGCTTCAATCA	
AB-237	TGGTCCARACAAGTCCACTA	
AB-238	/56-FAM/TGAACTGGTTCGTGGAAGTGGCAT/3IABKFQ/	RT-qPCR ToMV test
AB-753	CTCGCAAAGTTTCAACCAA	
AB-754	GACGTCTCGGCTTCATCT	
AB-755	/56-FAM/GTTCAGGCGGA/ZEN/AGGCCTAAACCAA/3IABKFQ/	RT-qPCR TMV test
AB-756	GTTGATGAGTTCATGGAGATGT	
AB-757	ATTTCTATAGTTCTTGTTCGGC	
AB-758	/56-FAM/CGATCTCGAACCGGAA/ZEN/AAAAGAGTGA/3IABKFQ/	