

	10	20	30	40	50	60	70	80	90	100
hRSV_F	MELLILKANAI	TILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNCNGTDAKVKLIKQELDKYKNAVT	ELQLLMQST							
hMPV_F	.SW---	VVI.FSL.IT----	PQH.--LK.SYLEES..TITE....V.....N.F.L.VGDVENLT.ADGP---S...T...LT.S.LR..RTVSADQ							
	110	120	130	140	150	160	170	180	190	200
hRSV_F	QATNNRARRELPRFMNYTLNNAKKTNTLSKRRKRRF-LGFL-LGV--GSAIASGVAVSKVLHLEGEVNIKSALLSTNKAVVSL	NSNGVSVLTSKVLDLK								
hMPV_F	L.REEQIEN-----	P.QP..V..AIA...ATAA.VTA...IA.TIR..S..TA..N..KK..E..ST.G...R..ATA.RE..								
	210	220	230	240	250	260	270	280	290	300
hRSV_F	NYIDKQLLP	IVNKQSCSISNIETVIEFQQKNNRLL	LEITREFSVNAGVTTPVSTYMLTNS	SELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEV						
hMPV_F	DFVS.N.TRAI..NK.D.ADLKMAVS.S.F.R.F.NVV.Q..D...I.PAI.LDLM.DA..ARAVSN..TSAG.I...LE.RAM..RKGFG.LIGVYGSS									
	310	320	330	340	350	360	370	380	390	400
hRSV_F	LAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTL	PSEVNLCNVDIFNPKYDCKIM								
hMPV_F	VI.M....IF.....IVKAA.S.SGKKG--NYA..L.E.Q....Q....TVYY.NEKD.ETRGDH.....AAGINVAEQSKE..IN.STTN.P..VS									
	410	420	430	440	450	460	470	480	490	500
hRSV_F	TSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGI	IKTFSNGCDYVSNKGVDTVSVGNTLYYV	NKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVN							
hMPV_F	.GRHPI.MVALSP...L.A..KGV.SIGSNRV....QLNK..S.IT.QDA...TID..V.QLS.V..EQHVI..R.VSSSF..VK..E.Q.NVALD..F									
	510	520	530	540	550	560	570	580		
hRSV_F	EKINQSLAFIRKSDELLHNVNAGKSTTNIMITII	IVIIIVILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNI	AFSN							
hMPV_F	.S.EN.Q.LVDQ.NRI.SSA--E.GN.GFI.VI.L.AVLGSTMI.VS.FIIIKTKKP.G---APPE...VT.NG.IPHN									

Figure S1.- Sequence alignment of the F proteins from hMPV strain NL/1/00 and hRSV F strain A2 used for variability studies. Identical amino acids are represented by dots, gaps are represented by dashes.

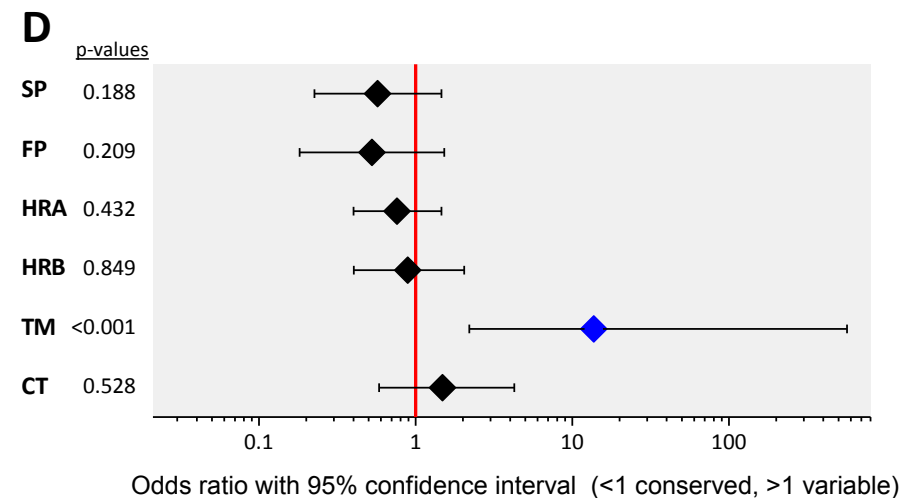
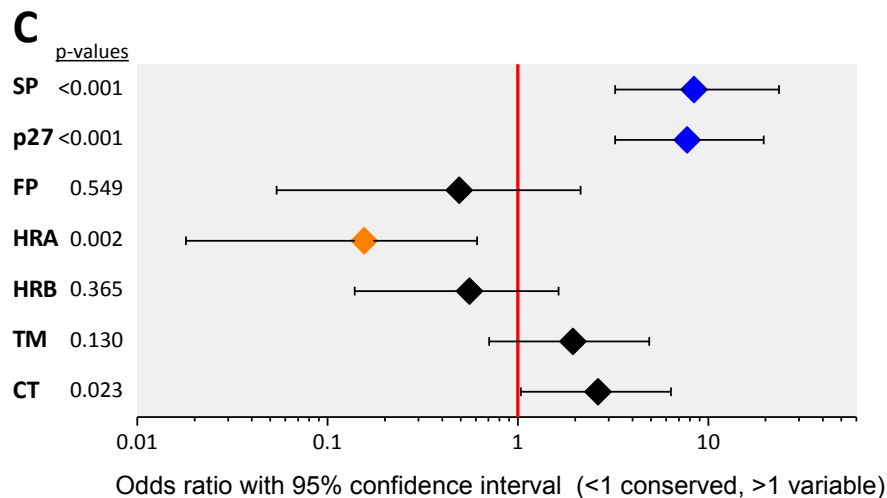
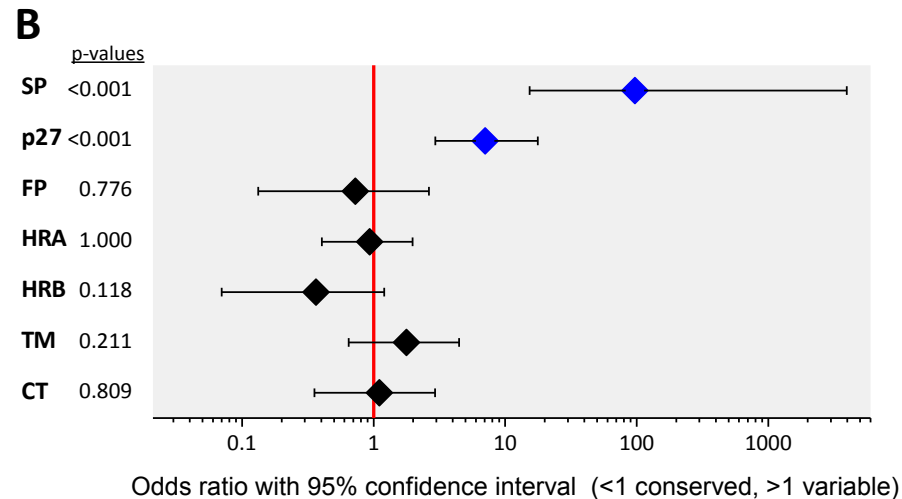
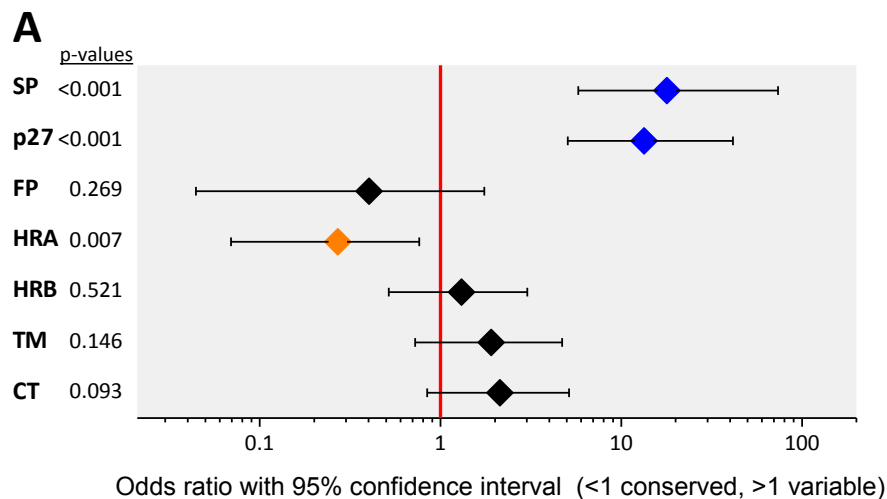


Figure S2.- Probability of mutation in F regions of hRSV group A (A), hRSV group B (B), bRSV (C) and hMPV (D) sequences using the strain A2 as reference sequence . Data are presented as odds ratios with 95% confidence intervals. Significantly ($p < 0.05$) increased or reduced values are displayed in blue and orange, respectively, with non-significant odds ratios shown in black.

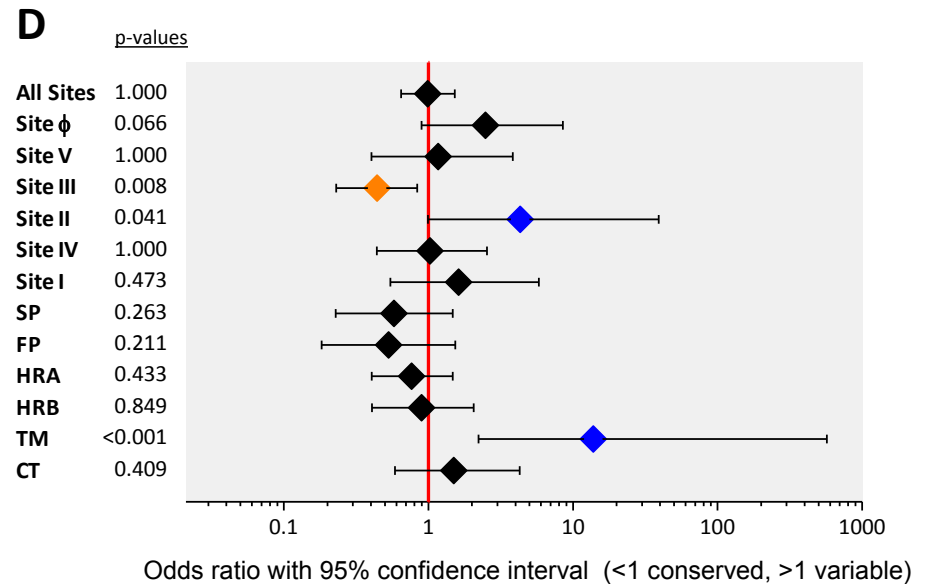
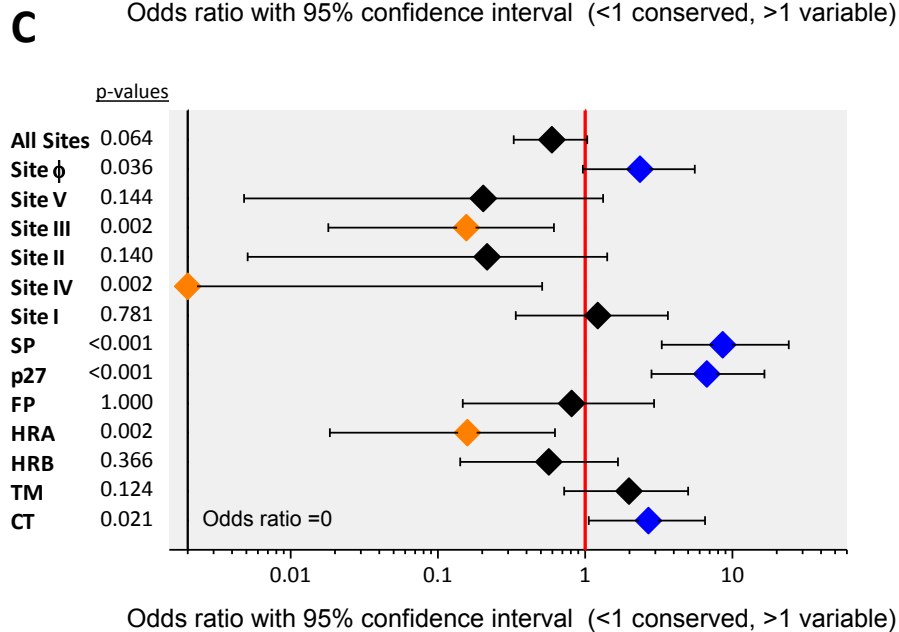
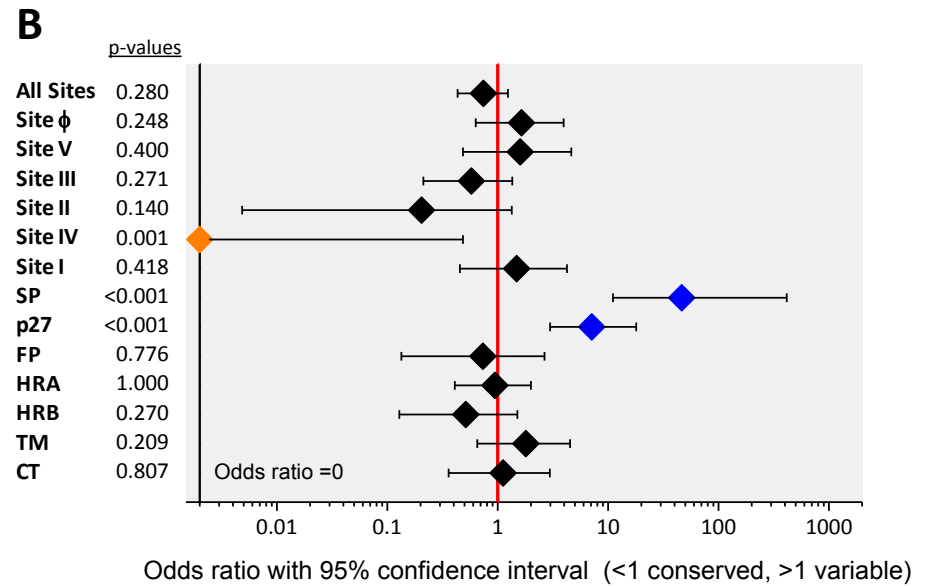
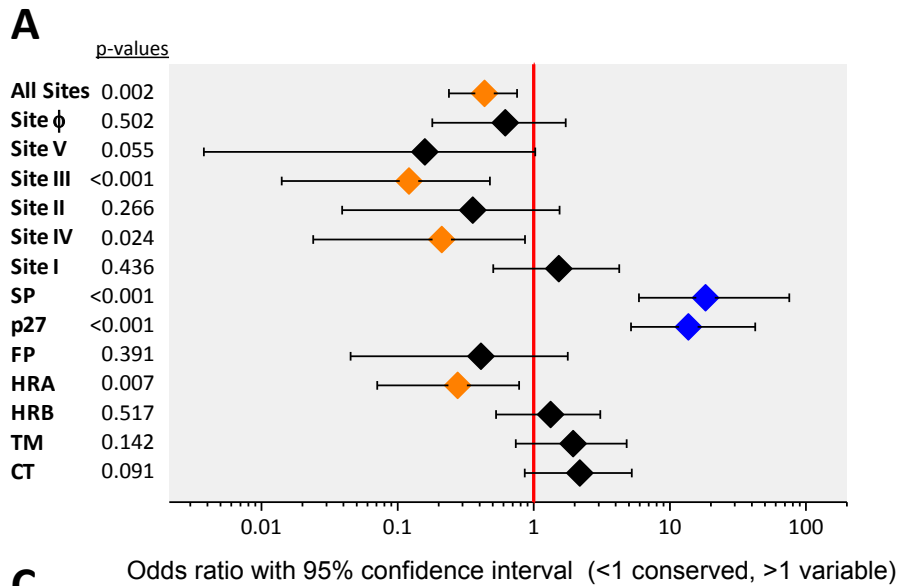


Figure S3.- Probability of mutation in F regions and Ag Sites of hRSV group A (A), hRSV group B (B), bRSV (C) and hMPV (D) sequences using the Long strain as reference sequence. Results are shown as in Figure S2.

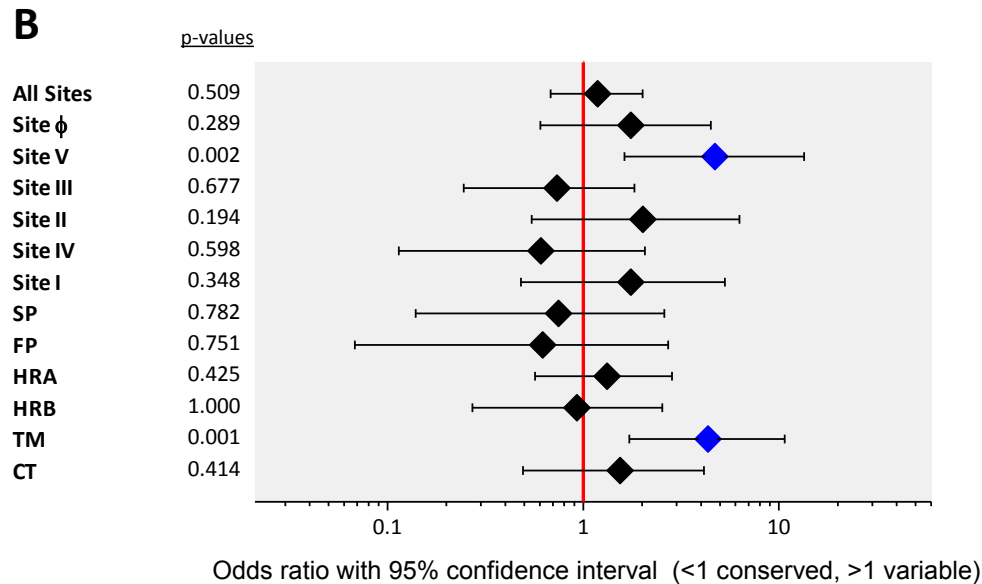
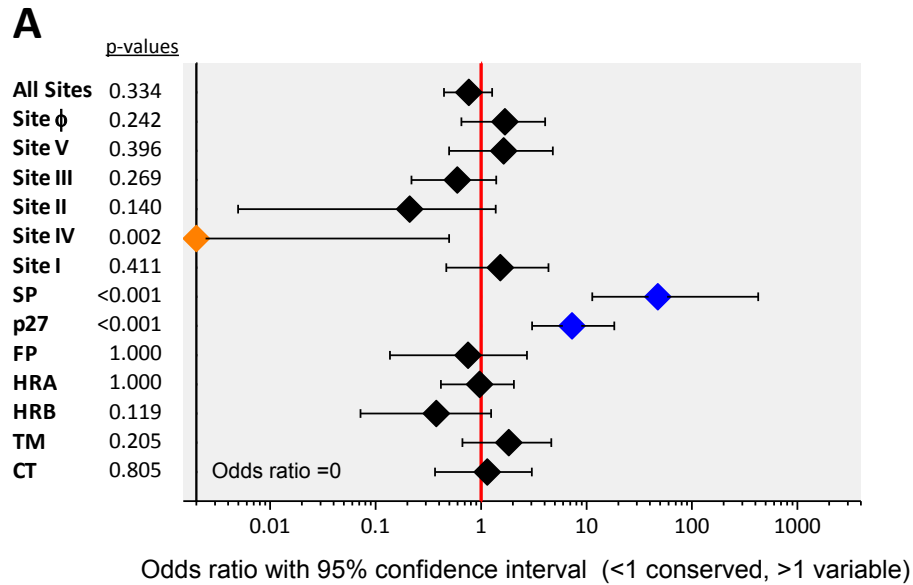


Figure S4.- Probability of mutation in F regions and Ag Sites of hRSV group B using CH-18537 strain as reference sequence (A) and of hMPV sequences using as reference the TN/82/5-18 strain as reference sequence (B).Results are shown as in Figure S2.

3D+ Number of variants:								3D- Number of variants:			
1 (53 Residues)	Ag Site	2 (7 Residues)	Ag Site	3 (0 Residues)	Ag Site	4 (0 Residues)	Ag Site	1 (42 Residues)	2 (25 Residues)	3 (7 Residue)	4 (0 Residues)
35 (S, A)	I	63 (N, S, T)	0					2 (E, D)	4 (L, P, S)	19 (T, S, L, I)	
44 (Y, F)		74 (A, T, V)						7 (K, N)	5 (I, T, V)	23 (A, V, T, S)	
45 (L, F)	III	152 (V, I, T)						9 (N, K)	6 (L, F, I)	101 (P, Q, S, T)	
59 (I, V)		165 (N, D, K)						14 (I, V)	8 (A, T, I)	102 (P, A, S, T)	
66 (E, K)	0	255 (S, G, N)	II					15 (L, F)	10 (A, V, S)	119 (L, P, F, I)	
68 (K, M)	0	326 (T, I, K)						20 (F, L)	12 (T, A, I)	124 (K, N, T, Y)	
76 (V, G)		384 (V, I, T)	I					22 (F, L)	13 (T, A, I)	125 (T, N, I, A)	
85 (K, R)								24 (S, F)	16 (T, A, I)		
90 (V, I)								99 (S, N)	17 (A, V, T)		
91 (T, I)								100 (T, S)	18 (V, A, I)		
96 (L, F)								105 (N, S)	25 (G, S, N)		
144 (V, F)								111 (L, I)	103 (T, A, S)		
169 (S, N)	V							112 (P, S)	107 (A, T, S)		
183 (N, T)								113 (R, K)	114 (F, S, V)		
199 (I, M)								115 (M, R)	117 (Y, H, N)		
206 (I, V)	0							116 (N, D)	120 (N, K, D)		
213 (S, R)								118 (T, I)	122 (A, T, N)		
215 (S, L)								121 (N, S)	123 (K, E, N)		
228 (N, S)								126 (N, D)	127 (V, A, I)		
240 (N, K)								128 (T, I)	129 (L, V, S)		
276 (N, S)	II							130 (S, G)	131 (K, R, N)		
283 (Q, R)								514 (H, Y)	524 (N, K, S)		
292 (I, V)								515 (N, H)	540 (S, L, A)		
295 (E, D)	0							516 (V, A)	558 (T, S, I)		
300 (V, I)								518 (A, V)	574 (N, S, K)		
316 (L, I)								519 (G, V)			
324 (T, I)								525 (I, V)			
328 (E, V)								529 (T, I)			
334 (L, I)								533 (V, I)			
356 (E, D)								535 (I, V)			
371 (N, S)								541 (L, S)			
376 (P, S)								543 (A, V)			
377 (S, N)	I III							544 (V, I)			
378 (E, D)	I							547 (L, F)			
379 (I, V)								550 (C, F)			
380 (N, D)	I							551 (K, R)			
381 (L, F)	I							553 (R, K)			
389 (P, S)	I							554 (S, N)			
414 (V, M)								555 (T, A)			
436 (S, T)	IV							560 (S, N)			
445 (K, R)								562 (D, E)			
446 (G, E)	IV							564 (L, M)			
447 (M, V)											
466 (S, N)											
472 (E, K)											
477 (F, Y)											
482 (V, L)											
486 (D, N)											
490 (A, T)											
494 (Q, L)											
495 (V, I)											
509 (S, Y)											
510 (D, N)											

Table S1.- Amino acid sequence changes between A2 strain and hRSV group A F protein sequences: For each residue, the original and the new amino acid are shown at left and right between parenthesis, respectively. Residues in the left part of the Table correspond to those resolved in the 3D protein structure (3D+), indicating in different columns whether they have changed 1, 2, 3 or 4 times when all strains are considered. Residues in the right part of the Table and those not resolved in the 3D structure (3D-).

3D+ Number of variants:								3D- Number of variants:			
1		2		3		4		1	2	3	4
(49 Residues)	Ag Site	(12 Residues)	Ag Site	(0 Residues)	Ag Site	(0 Residues)	Ag Site	(31 Residues)	(25 Residues)	(5 Residue)	(1 Residues)
42 (K, R)	I	45 (L, F, I)	III					3 (L, S)	4 (L, M, P)	2 (E, G, K, D)	125 (T, L, P, S, N)
74 (A, T)		65 (K, Q, T)	0					5 (I, V)	7 (K, R, G)	6 (L, H, R, Y)	
77 (K, R)		67 (N, T, I)	0					10 (A, V)	8 (A, S, T)	102 (P, A, V, S)	
80 (K, N)		68 (K, R, N)	0					11 (I, V)	9 (N, S, I)	103 (T, A, V, I)	
84 (D, N)		152 (V, I, M)						16 (T, A)	12 (T, F, L)	123 (K, G, N, R)	
95 (L, Q)		169 (S, N, D)	V					18 (V, N)	13 (T, L, A)		
144 (V, I)		173 (S, A, L)	V					20 (F, L)	14 (I, T, N)		
148 (I, V)		201 (K, N, S)	0 V					21 (C, Y)	15 (L, I, F)		
172 (L, Q)	V	213 (S, R, H)						23 (A, T)	17 (A, I, V)		
178 (V, I)	III V	215 (S, T, F)						25 (G, S)	19 (T, A, S)		
185 (V, I)	III	384 (V, T, I)	I					100 (T, I)	22 (F, L, I)		
187 (V, I)	III	472 (E, D, K)						104 (N, S)	99 (S, N, K)		
200 (D, N)	0 V							105 (N, S)	114 (F, Y, H)		
202 (Q, R)	0							111 (L, A)	117 (Y, H, C)		
203 (L, I)								112 (P, S)	119 (L, I, V)		
207 (V, L)	0							113 (R, Q)	121 (N, T, A)		
209 (K, Q)	0							115 (M, T)	122 (A, T, I)		
211 (S, I)	0							127 (V, A)	124 (K, N, S)		
228 (N, S)								128 (T, S)	129 (L, I, T)		
234 (T, A)								130 (S, I)	518 (A, T, V)		
245 (T, I)								132 (K, R)	529 (T, V, A)		
247 (V, L)								516 (V, I)	540 (S, A, L)		
249 (T, I)								526 (M, I)	542 (I, M, V)		
276 (N, S)	II							527 (I, M)	551 (K, R, N)		
278 (V, A)								537 (I, V)	553 (R, K, Q)		
291 (I, V)								544 (V, I)			
292 (I, M)								547 (L, F)			
305 (L, I)	III							552 (A, T)			
312 (P, H)	I III							554 (S, N)			
315 (K, E)								564 (L, I)			
323 (T, A)								574 (N, K)			
324 (T, I)											
326 (T, I)											
327 (K, R)											
344 (D, N)	I III										
356 (E, D)											
371 (N, Y)											
379 (I, V)											
380 (N, S)	I										
389 (P, S)	I										
402 (V, I)											
447 (M, V)											
462 (Q, L)											
466 (S, N)											
467 (L, F)											
477 (F, Y)											
482 (V, M)											
497 (E, K)											
508 (K, R)											

Table S2.- Amino acid sequence changes between A2 strain and hRSV group B F protein sequences: Results are shown as in Table S1.

3D+ Number of variants:								3D- Number of variants:			
1		2		3		4		1	2	3	4
(53 Residues)	Ag Site	(2 Residues)	Ag Site	(0 Residues)	Ag Site	(0 Residues)	Ag Site	(51 Residues)	(8 Residues)	(0 Residue)	(0 Residues)
42 (K, R)	I	206 (I, K, Q)	0					4 (L, M)	3 (L, T, R)		
57 (I, V)		389 (P, T, A)	I					6 (L, I)	10 (A, F, L)		
63 (N, K)	0							7 (K, S)	15 (L, V, M)		
65 (K, Q)	0							8 (A, I)	100 (T, E, V)		
66 (E, K)	0							9 (N, I)	102 (P, A, T)		
67 (N, D)	0							12 (T, S)	104 (N, F, S)		
68 (K, V)	0							14 (I, Y)	110 (E, S, G)		
70 (N, K)								16 (T, P)	114 (F, L, S)		
71 (G, S)								17 (A, H)			
74 (A, S)								18 (V, I)			
84 (D, E)								20 (F, L)			
85 (K, R)								99 (S, N)			
87 (K, N)								103 (T, S)			
91 (T, V)								105 (N, S)			
95 (L, S)								108 (R, K)			
144 (V, I)								111 (L, I)			
148 (I, V)								113 (R, E)			
169 (S, N)	V							115 (M, I)			
202 (Q, E)	0							116 (N, H)			
209 (K, N)	0							118 (T, A)			
210 (Q, H)	0							119 (L, R)			
211 (S, D)	0							121 (N, S)			
213 (S, R)								122 (A, T)			
216 (N, K)	0							124 (K, R)			
218 (E, A)								125 (T, F)			
234 (T, A)								126 (N, Y)			
243 (V, I)								127 (V, G)			
247 (V, L)								128 (T, L)			
260 (L, I)								129 (L, M)			
276 (N, S)	II							130 (S, G)			
291 (I, V)								515 (N, S)			
292 (I, V)								517 (N, D)			
297 (L, I)								518 (A, V)			
305 (L, I)	III							525 (I, V)			
325 (N, D)								526 (M, V)			
326 (T, N)								535 (I, V)			
327 (K, E)								537 (I, V)			
355 (A, T)								538 (L, I)			
377 (S, T)	I III							540 (S, M)			
378 (E, D)	I							543 (A, T)			
379 (I, V)								548 (L, F)			
384 (V, T)	I							552 (A, T)			
402 (V, I)								553 (R, K)			
410 (L, I)								554 (S, N)			
447 (M, V)								557 (V, I)			
462 (Q, L)								558 (T, M)			
466 (S, A)								560 (S, G)			
469 (V, I)								566 (G, S)			
477 (F, Y)								570 (I, L)			
479 (D, N)								571 (A, S)			
493 (S, A)								574 (N, K)			
497 (E, A)											
508 (K, R)											

Table S3.- Amino acid sequence changes between A2 strain and bRSV F protein sequences:Results are shown as in Table S1.

3D+ Number of variants:														3D- Number of variants:			
1 (242 Residues)	Ag Site	1 (242 Residues)	Ag Site	1 (242 Residues)	Ag Site	1 (242 Residues)	Ag Site	2 (38 Residues)	Ag Site	3 (8 Residues)	Ag Site	4 (1 Residues)	Ag Site	1 (59 Residues)	2 (16 Residues)	3 (5 Residue)	4 (0 Residues)
26 (Q, H)		189 (T, A)		292 (I, V)		406 (V, A)		27 (N, G, S)		31 (E, S, N, G)	I	326 (T, K, R, D, N)		10 (A, M)	15 (L, V, M)	18 (V, F, L, I)	
28 (I, U)		190 (S, T)		293 (K, Y)		407 (I, L)		29 (T, K, Q)		70 (N, A, S, T)				11 (I, S)	16 (A, I, V)	538 (L, T, S, A)	
32 (F, Y)	I	191 (K, A)	V	295 (E, S)	0	408 (T, S)		94 (Q, R, K)		173 (S, K, T, Q)	V			12 (T, W)	105 (N, E, V)	545 (G, F, L, I)	
33 (Y, L)	I	193 (L, R)		296 (V, S)		409 (S, P)		95 (L, T, P)		209 (K, Q, R, T)	0			13 (T, K)	113 (R, P, S)	557 (V, T, K, A)	
34 (Q, E)	I	194 (D, E)	V	297 (L, V)		413 (I, L)		138 (L, V, I)		215 (S, A, D, N)				14 (I, V)	514 (H, S, N)	570 (I, N, G, S)	
35 (S, E)	I	196 (K, E)	OV	298 (A, I)		415 (S, A)		141 (L, A, T)		324 (T, G, E, K)				17 (A, I)	524 (N, G, S)		
36 (T, S)		198 (Y, F)	0	301 (V, I)		419 (K, G)	IV	169 (S, N, G)	V	428 (N, R, Q, W)	III IV			19 (T, S)	526 (M, I, V)		
39 (A, T)		199 (I, V)		305 (L, I)	III	420 (T, V)	IV	172 (L, K, R)	V	498 (K, S, N, G)				20 (F, L)	529 (T, I, V)		
40 (V, I)	I	200 (D, S)	OV	306 (Y, F)	III	421 (K, S)	IV	197 (N, D, E)	OV					21 (C, L)	532 (I, T, V)		
41 (S, T)		201 (K, R)	OV	315 (K, I)		423 (T, S)	IV	205 (P, R, S)	0					22 (F, L)	537 (I, S, L)		
42 (K, E)	I	202 (Q, N)	0	317 (H, K)		425 (S, G)	III IV	210 (Q, N, D)	0					23 (A, T)	539 (L, M, I)		
47 (A, V)		204 (L, T)	0	318 (T, A)		426 (N, S)	IV	226 (K, F, Y)	V					24 (S, P)	541 (L, P, S)		
55 (S, N)		206 (I, A)	0	319 (S, A)		427 (K, N)	III	259 (S, R, K)	II					25 (S, Q)	548 (L, I, T)		
57 (I, F)		207 (V, I)	0	323 (T, S)		429 (R, V)	III IV	263 (D, N, Y)	II III V					98 (Q, A)	551 (K, T, A)		
59 (I, U)		211 (S, K)	0	325 (N, K)		434 (T, Q)	IV	267 (T, S, A)	II III					99 (S, D)	552 (A, K, R)		
61 (L, V)		213 (R, D)		327 (K, G)		435 (F, L)	IV	269 (D, G, E)	II III					100 (T, Q)	556 (P, Q, T)		
62 (S, G)	0	216 (N, D)	0	330 (N, K)		437 (N, K)	IV	289 (M, L, P)						101 (P, L)			
63 (N, D)	0	217 (I, L)		331 (N, Y)		440 (D, S)	IV	291 (I, G, E)						103 (A, R)			
64 (I, V)	0	218 (E, K)		332 (I, A)		442 (V, I)		294 (E, G, R)	0					104 (N, E)			
65 (K, E)	0	219 (T, M)		335 (T, L)		443 (S, T)		300 (V, M, T)						106 (R, Q)			
66 (E, N)	0	220 (V, A)		337 (T, E)		445 (K, Q)		310 (D, N, G)	II III					107 (A, I)			
67 (N, L)	0	221 (I, V)		338 (D, E)		446 (G, D)	IV	316 (L, V, I)						108 (R, E)			
68 (K, T)	0	222 (E, S)		339 (R, Q)		447 (V, A)		321 (L, S, G)						109 (R, N)			
74 (A, D)		224 (Q, S)		349 (V, T)		451 (S, T)	IV	344 (D, Q, K)	I III					110 (E, P)			
75 (K, G)		227 (N, T)		350 (S, V)		452 (V, I)	IV	355 (A, E, D)						111 (L, R)			
76 (V, P)		228 (N, R)		351 (F, Y)		453 (G, D)	IV	380 (N, K, R)	I					112 (P, Q)			
77 (K, S)		230 (L, F)		352 (F, Y)		456 (L, V)	III IV	399 (K, R, G)						114 (F, R)			
81 (Q, T)		232 (E, N)		354 (Q, N)		458 (Y, Q)	III	400 (T, H, N)						517 (N, S)			
84 (D, E)		233 (I, V)		356 (K, L)		459 (V, L)		418 (G, K, E)	IV					519 (G, E)			
85 (K, L)		234 (T, V)		357 (T, D)		460 (N, S)		424 (A, I, T)						521 (S, G)			
86 (Y, T)		236 (E, Q)		359 (K, E)		462 (Q, V)		436 (S, N, P)	IV					522 (T, N)			
88 (N, S)		239 (V, D)		360 (V, T)		465 (K, E)		472 (E, R, S)						525 (I, F)			
90 (V, L)		243 (V, I)		361 (Q, R)		466 (S, Q)		477 (F, S, R)						528 (T, V)			
91 (T, R)		245 (T, P)		362 (S, G)		467 (L, H)		481 (L, V, I)						530 (I, V)			
96 (L, V)		246 (P, A)		363 (N, D)		468 (Y, V)		482 (V, K, R)						531 (I, L)			
97 (M, S)		247 (V, I)		364 (R, H)	II III	469 (V, I)		500 (N, E, K)						533 (V, A)			
139 (G, L)		249 (T, L)		370 (M, A)		470 (K, R)		507 (R, D, E)						534 (I, V)			
140 (F, G)		250 (Y, D)		371 (N, A)		473 (P, A)		511 (E, R, K)						535 (I, L)			
142 (L, I)		251 (M, L)		372 (S, G)		474 (I, V)								536 (V, G)			
143 (G, A)		252 (L, M)		373 (L, I)		475 (I, S)								540 (S, I)			
144 (V, L)		253 (T, N)		374 (T, N)		476 (N, S)								542 (I, V)			
146 (S, V)		254 (N, D)		375 (L, V)		478 (Y, F)								543 (A, S)			
148 (I, T)		255 (S, A)	II	376 (P, A)		479 (D, N)								544 (V, I)			
150 (S, A)	III	258 (L, A)	II	377 (S, E)	I III	484 (P, H)								546 (L, I)			
151 (G, A)		260 (L, A)		378 (E, Q)	I	485 (S, E)								547 (L, I)			
152 (T, V)		261 (I, V)	II	379 (V, S)		487 (E, Q)								549 (Y, K)			
153 (A, T)		262 (N, S)	II III	381 (L, E)	I	489 (D, N)								550 (C, K)			
154 (V, A)	III	266 (I, T)	III	384 (V, I)	I	490 (A, V)								553 (R, K)			
155 (S, A)		268 (N, A)	II III	385 (D, N)		491 (S, A)								558 (T, G)			
157 (V, T)		271 (K, I)	III III	387 (F, S)		492 (I, L)								559 (L, A)			
158 (L, I)		275 (S, L)	II	388 (N, T)		493 (S, D)								560 (S, P)			
159 (H, R)		276 (N, E)	II	389 (P, T)	I	496 (N, F)								561 (K, P)			
162 (G, S)		278 (V, R)		390 (K, N)	I	501 (Q, N)								562 (D, E)			
165 (N, T)		279 (Q, A)		392 (D, P)		503 (L, Q)								565 (S, N)			
166 (K, A)		280 (I, M)		395 (I, V)		505 (F, L)								567 (I, V)			
170 (A, S)	V	283 (Q, R)		396 (M, S)		506 (I, V)								568 (N, T)			
176 (K, E)	II V	284 (Q, K)		398 (S, G)		508 (K, Q)								571 (A, G)			
179 (V, S)		285 (S, G)		401 (D, P)		510 (D, N)								573 (S, I)			
180 (S, T)		286 (Y, F)		402 (V, I)		512 (L, I)								574 (N, P)			
182 (S, G)		287 (S, G)		404 (S, M)													
186 (S, R)	III	290 (S, I)		405 (S, V)													

Table S4.- Amino acid sequence changes between A2 strain and hMPV F protein sequences:Results are shown as in Table S1.