



HAL
open science

Parvovirus 4 in French in-patients: a study of haemodialysis and lung transplant cohorts

Mhammed Touinssi, Martine Reynaud-Gaubert, Carine Gomez, Pascal Thomas, Bertrand Dussol, Yvon Berland, Agnes Basire, Christophe Picard, Jean François Cantaloube, Philippe de Micco, et al.

► **To cite this version:**

Mhammed Touinssi, Martine Reynaud-Gaubert, Carine Gomez, Pascal Thomas, Bertrand Dussol, et al.. Parvovirus 4 in French in-patients: a study of haemodialysis and lung transplant cohorts. *Journal of Medical Virology*, 2011, 83 (4), pp.717-721. 10.1002/jmv.22003 . hal-00614692

HAL Id: hal-00614692

<https://hal.science/hal-00614692>

Submitted on 15 Aug 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**Parvovirus 4 in French in-patients:
a study of haemodialysis and lung transplant cohorts.**

Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-10-2127.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	31-Oct-2010
Complete List of Authors:	Touinssi, Mhammed Reynaud-Gaubert, Martine Gomez, Carine Thomas, Pascal Dussol, Bertrand Berland, Yvon Basire, Agnes Picard, Christophe Cantaloube, Jean François de Micco, Philippe Biagini, Philippe; UMR CNRS 6578 Equipe « Emergence et co-évolution virale », Etablissement Français du Sang Alpes-Méditerranée et Université de la Méditerranée
Keywords:	PARV4, Parvoviridae, hemodialysis, lung transplant, epidemiology

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

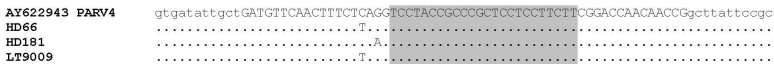


Fig. 1.

297x210mm (200 x 200 DPI)

Review

Parvovirus 4 in French in-patients:

A study of haemodialysis and lung transplant cohorts.

Mhammed Touinssi¹, Martine Reynaud-Gaubert², Carine Gomez², Pascal Thomas²,
Bertrand Dussol³, Yvon Berland³, Agnes Basire⁴, Christophe Picard⁴, Jean-François
Cantaloube¹, Philippe de Micco¹, Philippe Biagini^{1*}.

¹ *Equipe « Emergence et co-évolution virale », Etablissement Français du Sang Alpes-Méditerranée et Université de la Méditerranée, Marseille, France*

² *Service de Pneumologie, Pole Thoracique et Equipe de Transplantation Pulmonaire, CHU Nord, Marseille, France*

³ *Centre de Néphrologie et de Transplantation Rénale, CHU Conception, Marseille, France*

⁴ *Service Immunogénétique-HLA, Etablissement Français du Sang Alpes-Méditerranée, Marseille, France*

* Correspondence: Dr. Philippe Biagini, UMR CNRS 6578 Equipe « Emergence et co-évolution virale », Etablissement Français du Sang Alpes-Méditerranée et Université de la Méditerranée, 27 Bd. Jean Moulin, 13005 Marseille, France.

Tél. : 33.(0)4.91.32.45.53 / Fax : 33.(0)4.91.32.44.95

E-mail : philippe.biagini@efs.sante.fr / philippe.biagini@univmed.fr

Running head: Parvovirus 4 in French in-patients.

1
2
3 27 **ABSTRACT**
4
5
6 28
7

8 29 The epidemiology and the clinical implication of human parvovirus 4 (PARV4) in
9
10 30 human populations is still under evaluation. The distribution of PARV4 DNA was
11
12 31 determined in cohorts of French haemodialysis and lung transplant patients. Plasma
13
14 32 samples (n=289) were tested for PARV4 by real-time PCR assay (ORF2), and
15
16 33 amplification products selected at random were sequenced. Analysis of available
17
18 34 serological and biological markers was also undertaken. Fifty-seven samples out of
19
20 35 185 (30.8%) were positive for PARV4 DNA in the cohort of haemodialysis patients. A
21
22 36 higher prevalence of the virus was identified in patients with markers of HBV
23
24 37 infection. PARV4 was also identified in 14 out of 104 samples (13.5%) from lung
25
26 38 transplant recipients, with no clear-cut association with available clinical markers.
27
28 39 Point mutations located on the zone of real-time detection were identified for some
29
30 40 amplification products. This study describes the detection of PARV4 in the blood of
31
32 41 haemodialysis and lung transplanted patients with significant difference in prevalence
33
34 42 in these two cohorts. Further studies will be needed in order to understand better
35
36 43 both the potential implication in host health and the natural history of this virus.
37
38
39
40
41
42
43
44

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

45
46
47
48

KEY WORDS: PARV4, *Parvoviridae*, haemodialysis, lung transplant, epidemiology, genetic diversity.

For Peer Review

INTRODUCTION

Parvovirus 4 (PARV4) is a virus discovered recently belonging to the family *Parvoviridae* [Jones et al., 2005]. Identified initially in the blood of a patient harbouring acute viral syndrome, this virus had limited sequence homology with parvovirus B19 (<30% aa similarity) despite a conserved genomic organization showing two large non-overlapping ORFs [Simmonds et al., 2008]. First studies revealed that the virus was present in the blood from intravenous drug users and individuals positive for HCV or HIV at prevalence ranging from 6% to 30%, and in cohorts of kidney transplant patients as well [Biagini et al., 2008; Fryer et al., 2006; Fryer et al., 2007; Lurcharchaiwong et al., 2008; Vallerini et al., 2008]. PARV4 had been also identified in individuals without apparent pathology like blood donors (1%-24%), and in blood products negative for parvovirus B19 DNA [Fryer et al., 2007; Lurcharchaiwong et al., 2008; Touinssi et al., 2010; Vallerini et al., 2008]. Genetic diversity of the virus is poorly known, but was extended recently to highly divergent animal variants identified in domestic (cow, pig) and wild (baboon, chimpanzee) species [Adlhoch et al., 2010; Lau et al., 2008; Sharp et al., 2010]. Interestingly, these studies demonstrated high prevalence values (up to 40%) for these PARV4-related animal viruses.

Another member of *Parvoviridae* family identified recently, human Bocavirus (HBoV) [Allander et al., 2005], had been more investigated and is suspected to be associated with wheezing and respiratory disease, mainly in young children [Brown, 2010].

Despite these advances, the natural history and the clinical role of these new viruses, and more particularly that of PARV4, remain unknown to a large extent.

1
2
3 73 Aim of this study consisted in the evaluation of PARV4 DNA prevalence in two
4
5 74 different cohorts of haemodialysis patients and lung transplant recipients, combined
6
7
8 75 with the analysis of available serological and biological markers. An additional
9
10 76 investigation of HBoV DNA in lung transplant patients was also performed.
11
12
13 77
14
15 78

17 79 **MATERIALS AND METHODS**

20 80 21 22 81 **Clinical samples**

23
24 82 Two hundred and eighty-nine plasma samples originating from two cohorts of
25
26
27 83 in-patients from south-eastern France were analyzed retrospectively:

28
29 84 i) 185 haemodialysis patients (mean age 64 years; sex ratio 1.37 (men:women),
30
31 85 mean duration of dialysis 30 ± 28 months) from the Nephrology and Renal Transplant
32
33 86 Centre, Conception Hospital (Marseille) (date of sampling: 2007);

34
35
36 87 ii) 104 adult lung transplant recipients (mean age 42 years, sex ratio 0.73)
37
38 88 transplanted between October 2005 and August 2008, and followed at the Lung
39
40 89 Transplant Centre of the University Hospital of Marseille (date of sampling: from
41
42 90 June 2007 to December 2008); blood samples were not available before
43
44 91 transplantation.
45
46
47

48 92 Information relating to the presence of specific antibodies to HCV, HBV, HIV, and
49
50 93 HBs antigen in the plasma samples tested was collected. Of the 185 haemodialysis
51
52 94 patients, 22 tested positive for HCV, 75 for HBV, 4 for HIV, and 5 for HBsAg. Of the
53
54 95 104 lung transplant recipients, only 2 tested positive for a serological marker of HCV.
55
56

57 96 Blood samples were collected in vacuum tubes (Vacutainer, SST, Becton
58
59 97 Dickinson, Meylan, France), centrifuged, and plasma aliquots were stored at -80°C

1
2
3 98 prior to DNA extraction. Total nucleic acids were extracted from 1 ml of plasma by
4
5 99 using the MagNA Pure LC instrument (Roche Diagnostics, Meylan, France), as
6
7
8 100 described previously [Biagini et al., 2008; Touinssi et al., 2010].
9

101

102 **PARV4 PCR assay**

103 Samples were screened for the presence of PARV4 DNA by real-time PCR
104 (StepOne Plus, Applied Biosystems, Courtaboeuf, France) using a TaqMan detection
105 system located on the ORF2 of the viral genome. Primers used were PARV4Fwd (5'-
106 CTAAGGAAACTGTTGGTGATATTGCT-3') and PARV4Rev (5'-
107 GGCTCTCCTGCGGAATAAGC-3'), in combination with fluorogenic probe PARV4-N
108 (5'-FAM-TCCTACYGCCSCTCCTCCTTCTT-TAMRA-3') [Touinssi et al., 2010].

109 Amplification reactions were performed using 5 µl of extracted nucleic acids in
110 a 20 µl reaction volume (TaqMan Fast Universal PCR kit, Applied Biosystems)
111 according to manufacturer's instructions. The amplification conditions were 95°C for
112 20 seconds, followed by 50 cycles of 95°C for 1 second and 60°C for 20 seconds.

113 The sensitivity of the TaqMan assay was estimated at 10 copies of PARV4
114 DNA per reaction using dilutions of a synthetic template corresponding to the target
115 sequence (103 nt) [Biagini et al., 2008; Touinssi et al., 2010].
116

117 **HBoV PCR assay**

118 A real-time PCR assay for HBoV DNA located on the non-structural protein
119 (NP-1) gene was further applied to samples belonging to the lung transplant cohort.
120 Primers used were STBoNP-1f (5'-AGCATCGCTCCTACAAAAGAAAAG-3') and
121 STBoNP-1r (5'-TCTTCATCACTTGGTCTGAGGTCT-3'), in combination with
122

1
2
3 122 fluorogenic probe STBoNP-1pr (5'-FAM-AGGCTCGGGCTCATATCATCAGGAACA-
4
5 123 TAMRA-3') [Tozer et al., 2009].
6
7

8 124 Amplification conditions were identical as those adopted for PARV4 detection.
9
10 125 The detection limit of the real-time PCR assay was 10 copies of HBoV DNA per
11
12 126 reaction [Tozer et al., 2009].
13
14

15 127

17 128 **Sequence analysis**

19
20 129 Real-time PARV4 and HBoV amplification products (from negative or positive
21
22 130 samples, n=15 when available) were chosen at random in both cohorts tested, and
23
24 131 analysed by agarose gel electrophoresis. Positive PCR products were excised and
25
26 132 purified from agarose (Qiaquick Gel extraction kit, Qiagen, Courtaboeuf, France),
27
28 133 cloned into the pGEM-T Easy Vector System (Promega, Charbonnières les Bains,
29
30 134 France) and further sequenced on both strands with M13 universal primers (BigDye
31
32 135 Terminator v1.1 Sequencing Kit, Applied Biosystems) on an ABI PRISM 3130XL
33
34 136 genetic analyzer (Applied Biosystem, Courtaboeuf, France).
35
36
37

38 137 Sequences were compared with those deposited in the GenBank database
39
40 138 using NCBI's online BLAST2 program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).
41
42

43 139

44 140

48 141 **RESULTS**

49 142

53 143 **Haemodialysis patients**

54
55 144 Among the 185 samples tested, 30.8% (n=57) were positive for PARV4 DNA
56
57 145 using the real-time approach described. Analysis of serological markers revealed that
58
59 146 PARV4 DNA was detectable in 27.3% (6/22) and 38.7% (29/75) of patients positive
60

1
2
3 147 for HCV and HBV, respectively. PARV4 DNA was further identified in 60% (3/5) of
4
5 148 patients positive for HBsAg, but not in any of the samples positive for HIV (n=4). The
6
7 149 titre of PARV4 DNA in the positive samples was low (<500 copies/ml of plasma).

8
9
10 150 No specific correlation was found between PARV4 viraemia and duration of dialysis
11
12 151 or other available biological/clinical markers.

13
14
15 152

16 17 153 **Lung transplant patients**

18
19
20 154 PARV4 DNA was detected in 13.5% plasma samples (14/104), including one
21
22 155 of the two patients with HCV positive pretransplant viraemia who received 24 weeks
23
24 156 treatment with pegylated interferon alfa plus ribavirin, with a rapid virological
25
26 157 response and a negative viraemia before transplantation. A low titre of PARV4 DNA
27
28 158 (<500 copies/ml) was also noted. Patients with positive sera for PARV4 DNA were
29
30 159 transplanted for cystic fibrosis (n= 8), emphysema (n=1), alpha 1 antitrypsin
31
32 160 deficiency (n=1), pulmonary fibrosis (n= 3), sarcoidosis (n=1).

33
34
35
36 161 Dating of positive samples ranged from 1 (day of transplant procedure) to 1097 days
37
38 162 after the allograft with a median of 206 days. No other serological marker was
39
40 163 identified in the cohort studied. No difference in clinical outcome and survival was
41
42 164 noted between recipients positive for PARV4 and those who were negative.

43
44
45
46 165 In order to analyse tentatively the time-course of infection in relation to patients
47
48 166 positive for PARV4 DNA, additional serial plasma samples obtained at ± 6 months,
49
50 167 with respect to the date of initial positive detection, were investigated when available.
51
52 168 In each case (n=3, on average), attempts to detect PARV4 DNA in these samples
53
54 169 remained unsuccessful.

55
56
57
58 170 Real-time PCR approach directed toward HBoV DNA gave no positive signal
59
60 171 in any of the samples tested.

1
2
3 1724
5 1736
7 1748
9
10 175 **Genetic variability**

11
12
13 176 Positive real-time PARV4 amplification products selected at random (n=15 and
14
15 177 n=14 for haemodialysis patients and lung transplant recipients cohorts, respectively)
16
17 178 were confirmed by analysis on agarose gel electrophoresis, whereas negative
18
19
20 179 samples did not shown any amplification pattern. Amplification products confirmed
21
22 180 their PARV4 origin following cloning and sequencing. Most of them exhibited 100%
23
24 181 nucleotide identity with the PARV4 prototype isolate (GenBank accession no.
25
26
27 182 AY622943) in this region, while three samples exhibited a point mutation (Fig. 1).

28
29 183 Absence of amplification was confirmed for each of the negative real-time
30
31 184 HBoV amplification products analyzed.

32
33
34 18535
36 18637
38 187 **DISCUSSION**39
40
41 188

42
43 189 The real distribution and implication of PARV4 in humans is still under
44
45 190 investigation. Using the same molecular approach, the detection of PARV4 in about
46
47
48 191 20% of individuals without proven pathology like blood donors was reported recently,
49
50 192 suggesting a non-negligible dispersion of the virus in the population [Touinssi et al.,
51
52 193 2010]. This study demonstrates that about 31% of a cohort of patients in
53
54 194 maintenance haemodialysis is infected with PARV4. Analysis of serological markers
55
56
57 195 characterising the cohort tends to confirm that the prevalence of the virus would be
58
59 196 higher in individuals showing, at least for HBV, hepatitis-related markers of infection.

1
2
3 197 Such findings would be in accordance with the lower prevalence (13.5%) identified
4
5 198 here in the cohort of lung transplanted patients, in which HCV, HBV and HIV markers
6
7
8 199 were hardly absent due to the preliminary selection of patients undergoing lung
9
10 200 allograft. However, the fact that this prevalence value was largely lower than that
11
12 201 identified in the normal population was unexpected and possibly due to undiscovered
13
14
15 202 aspects of PARV4 infection with respect to the immunosuppressive treatment of
16
17 203 patients.

18
19 204 HBoV DNA was not identified in the cohort of adult lung transplant recipients.
20
21
22 205 This would confirm its preferential implication in respiratory diseases of young
23
24 206 children, even if many aspects of its natural history remain to be clarified [Schildgen
25
26
27 207 et al., 2008].

28
29 208 A low titre of PARV4 viral particles (<500 copies/ml) was identified in all
30
31 209 positive samples identified in both cohorts. This is an agreement with previous
32
33 210 studies relating generally mild PARV4 levels in blood samples, although values
34
35 211 reaching up to 10^6 copies/ml were reported as well [Fryer et al., 2006; Fryer et al.,
36
37 212 2007; Biagini et al., 2008; Touinssi et al., 2010]. In addition, the presence of
38
39 213 serological markers of HCV or HBV did not modify significantly the titre of viral
40
41 214 particles of the positive samples in the study. It is conceivable, however, that
42
43 215 fluctuations in viraemia would occur according to the immune status of the host, and
44
45
46 216 that additional serial samples tested negative here in the case of lung transplant
47
48 217 patients may represent periods of lowest replication of the virus, leading to
49
50 218 undetectable levels in blood.

51
52
53 219 It was also shown that point mutations may be identifiable in the short genomic
54
55 220 zone dedicated to PARV4 detection. This is in agreement with a recent study
56
57
58 221 demonstrating notable differences in the detection of the virus, even if two slightly
59
60

1
2
3 222 different real-time approaches were used [Touinssi et al., 2010]. It is obvious that
4
5 223 detection strategies correlate directly with the progressive estimation of the real
6
7
8 224 genetic diversity of the virus. The fact that new, divergent, human and animal
9
10 225 variants were identified recently highlights the need to optimize detection methods
11
12
13 226 consistently.

14
15 227 This study demonstrates that haemodialysis and lung transplant patients could
16
17 228 be infected by PARV4, extending the spectrum of viral infections identifiable in these
18
19 229 cohorts [Alpers and Kowalewska, 2007; Fischer, 2008]. Many aspects characterizing
20
21 230 PARV4 are still largely unknown: both natural history and implication in host health,
22
23 231 along with its genetic diversity and real dispersion in human and animal species need
24
25 232 to be address in a near future. Interestingly, some aspects of this virus would be
26
27 233 close to those identified in another viral family identified thirteen years ago,
28
29 234 *Anelloviridae* [Biagini, 2009], which biology remains largely misunderstood.

30
31
32
33
34 235
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

236 **REFERENCES**

- 237
- 238 Adlhoch C, Kaiser M, Ellerbrok H, Pauli G. 2010. High prevalence of porcine
239 Hokovirus in German wild boar populations. *Virol J* 7:171.
- 240 Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B.
241 2005. Cloning of a human parvovirus by molecular screening of respiratory tract
242 samples. *Proc Natl Acad Sci USA* 102:12891-12896.
- 243 Alpers CE, Kowalewska J. 2007. Emerging paradigms in the renal pathology of viral
244 diseases. *Clin J Am Soc Nephrol* 2:S6-S12.
- 245 Biagini P, Dussol B, Touinssi M, Brunet P, Picard C, Moal V, Belhouchet M, Gallian
246 P, Cantaloube JF, Attoui H, Berland Y, de Micco P. 2008. Human parvovirus 4
247 in kidney transplant patients, France. *Emerg Infect Dis* 14:1811-1812.
- 248 Biagini P. 2009. Classification of TTV and related viruses (anelloviruses). *Curr Top*
249 *Microbiol Immunol* 331:21-33.
- 250 Brown KE. 2010. The expanding range of parvoviruses which infect humans. *Rev*
251 *Med Virol* 20:231-244.
- 252 Fischer SA. 2008. Emerging viruses in transplantation: there is more to infection after
253 transplant than CMV and EBV. *Transplantation* 86:1327-1339.
- 254 Fryer JF, Kapoor A, Minor PD, Delwart E, Baylis SA. 2006. Novel parvovirus and
255 related variant in human plasma. *Emerg Infect Dis* 12:151-154.
- 256 Fryer JF, Delwart E, Hecht FM, Bernardin F, Jones MS, Shah N, Baylis SA. 2007.
257 Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from
258 blood donors and symptomatic individuals. *Transfusion* 47:1054-1061.

- 1
2
3 259 Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E. 2005. New
4
5 260 DNA viruses identified in patients with acute viral infection syndrome. *J Virol*
6
7 261 79:8230-8236.
- 8
9
10 262 Lau SK, Woo PC, Tse H, Fu CT, Au WK, Chen XC, Tsoi HW, Tsang TH, Chan JS,
11
12 263 Tsang DN, Li KS, Tse CW, Ng TK, Tsang OT, Zheng BJ, Tam S, Chan KH,
13
14 264 Zhou B, Yuen KY. 2008. Identification of novel porcine and bovine parvoviruses
15
16 265 closely related to human parvovirus 4. *J Gen Virol* 89:1840-1848.
- 17
18
19 266 Lurcharchaiwong W, Chieochansin T, Payungporn S, Theamboonlers A,
20
21 267 Poovorawan Y. 2008. Parvovirus 4 (PARV4) in serum of intravenous drug users
22
23 268 and blood donors. *Infection* 36: 488-491.
- 24
25
26 269 Schildgen O, Müller A, Allander T, Mackay IM, Völz S, Kupfer B, Simon A. 2008.
27
28 270 Human bocavirus: passenger or pathogen in acute respiratory tract infections?
29
30 271 *Clin Microbiol Rev* 21:291-304.
- 31
32
33 272 Sharp CP, LeBreton M, Kantola K, Nana A, Diffo JL, Djoko CF, Tamoufe U, Kiyang
34
35 273 JA, Babila TG, Ngole EM, Pybus OG, Delwart E, Delaporte E, Peeters M,
36
37 274 Soderlund-Venermo M, Hedman K, Wolfe ND, Simmonds P. 2010. Widespread
38
39 275 infection of chimpanzees and gorillas with homologues of human parvoviruses
40
41 276 B19, PARV4 and human bocavirus in the wild. *J Virol* 84:10289-10296.
- 42
43
44 277 Simmonds P, Douglas J, Bestetti G, Longhi E, Antinori S, Parravicini C, Corbellino M.
45
46 278 2008. A third genotype of the human parvovirus PARV4 in sub-Saharan Africa.
47
48 279 *J Gen Virol* 89:2299-2302.
- 49
50
51 280 Touinssi M, Brisbarre N, Picard C, Frassati C, Dussol B, Uch R, Gallian P,
52
53 281 Cantaloube JF, de Micco P, Biagini P. 2010. Parvovirus 4 in blood donors,
54
55 282 France. *Emerg Infect Dis* 16:165-166.
- 56
57
58
59
60

- 1
2
3 283 Tozer SJ, Lambert SB, Whiley DM, Bialasiewicz S, Lyon MJ, Nissen MD, Sloots TP.
4
5 284 2009. Detection of human bocavirus in respiratory, fecal, and blood samples by
6
7
8 285 real-time PCR. *J Med Virol* 81:488-493.
9
10 286 Vallerini D, Barozzi P, Quadrelli C, Bosco R, Potenza L, Riva G, Gregorini G,
11
12 287 Sandrini S, Tironi A, Montagnani G, De Palma M, Torelli G, Delwart E, Luppi M.
13
14 288 2008. Parvoviruses in blood donors and transplant patients, Italy. *Emerg Infect*
15
16
17 289 *Dis* 14:185-186.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3 290 **LEGENDS**
4

5
6 291

7
8 292 **Fig. 1.** Alignment of PARV4 partial sequences showing location of point mutations in
9
10 293 the zone of real-time detection. HDx and LTx : sequences identified from
11
12 294 haemodialysis and lung transplant patients, respectively (GenBank accession nos
13
14 295 HM802325-27). Grey area : nucleotide sequence targeted by the real-time probe.
15
16
17 296 Lowercase letters : 5'/3' ends of real-time primers.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review