



HAL
open science

Correlation of mutations and recombination with growth kinetics of poliovirus vaccine strains

V. Pliaka, Z. Kyriakopoulou, D. Tsakogiannis, I. G. A. Ruether, C. Gartzonika, S. Levidiotou-Stefanou, A. Krikelis, P. Markoulatos

► To cite this version:

V. Pliaka, Z. Kyriakopoulou, D. Tsakogiannis, I. G. A. Ruether, C. Gartzonika, et al.. Correlation of mutations and recombination with growth kinetics of poliovirus vaccine strains. *European Journal of Clinical Microbiology and Infectious Diseases*, 2010, 29 (12), pp.1513-1523. 10.1007/s10096-010-1033-9 . hal-00618979

HAL Id: hal-00618979

<https://hal.science/hal-00618979>

Submitted on 5 Sep 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.

**Correlation of mutations and recombination with growth kinetics of
Poliovirus vaccine strains**

V. Pliaka^a, Z. Kyriakopoulou^a, D. Tsakogiannis^a, I.G.A. Ruether^a, C. Gartzonika^b, S. Levidiotou-Stefanou^b, A. Krikelis^c, P. Markoulatos^{a,*}

^aUniversity of Thessaly, School of Health Sciences, Department of Biochemistry & Biotechnology, Microbiology-Virology Laboratory, Larissa, Greece

^bUniversity of Ioannina, Medical School, Department of Microbiology, Ioannina, Greece

^cLUX Innovate Ltd , Edinburgh , United Kingdom

*Corresponding Author: Prof. P. Markoulatos
University of Thessaly, School of Health Sciences
Department of Biochemistry & Biotechnology
Ploutonos 26 & Aiolou
41221 Larissa, Greece.
Tel.: +302410 565274, Fax: +302410 565294
E-Mail: markoulatos@bio.uth.gr

Abstract

1 Attenuated strains of Sabin poliovirus vaccine replicate in the human gut
2
3 and in rare cases may cause vaccine-associated paralytic poliomyelitis
4 (VAPP). The genetic instability of Sabin strains constitutes one of the main
5
6 causes of VAPP, a disease that is most frequently associated with type 3 and
7
8 type 2 Sabin strains and more rarely with type 1 Sabin strains. In the present
9
10 study, the growth phenotype of eight OPV isolates (two non-recombinants and
11
12 six recombinants) as well as of Sabin vaccine strains was evaluated using two
13
14 different assays, Reproductive Capacity at different Temperatures test (RCT
15
16 test) and one-step growth curve test in Hep2 cells at two different
17
18 temperatures (37°C and 40°C). The growth phenotype of isolates was
19
20 correlated with genomic modifications in order to identify the determinants and
21
22 mechanisms of reversion towards neurovirulence. All the recombinant OPV
23
24 isolates showed a thermoresistant phenotype in RCT test. Moreover, both
25
26 recombinant Sabin-3 isolates showed significantly higher viral yield than Sabin
27
28 3 vaccine strain at 37°C and 40°C in one-step growth curve test. All the OPV
29
30 isolates displayed mutations at specific sites of the viral genome, which are
31
32 associated with the attenuated and temperature sensitive phenotype of Sabin
33
34 strains. The results showed that both mutations and recombination events
35
36 could affect the phenotype traits of Sabin derivatives and may lead to the
37
38 reversion of vaccinal strains to neurovirulent ones. The use of phenotypic
39
40 markers along with the genomic analysis may shed additional light in the
41
42 molecular determinants of the reversed neurovirulent phenotype of Sabin
43
44 derivatives.
45
46
47
48
49
50
51
52
53
54
55

56
57 *Keywords:* OPV isolates; mutations; recombination; temperature sensitivity;
58
59 growth kinetic analysis
60
61
62
63
64
65

Introduction

1
2 Poliovirus, the causal agent of poliomyelitis, is a member of the enterovirus
3
4 genus of the Picornaviridae family and exists as three immunologically defined
5
6 serotypes (P1, P2 and P3). The poliovirus is a nonenveloped virus composed
7
8 of a 7.5-Kb positive sense single-stranded polyadenylated RNA genome
9
10 surrounded by an icosahedral capsid [1]. The capsid is made of 60 protomers,
11
12 each containing a single copy of the four structural capsid proteins (VP1 to
13
14 VP4). The viral RNA contains a long open reading frame flanked by 5' and 3'
15
16 noncoding regions (5'-NCR and 3'-NCR, respectively) involved in viral
17
18 replication and translation. The predicted secondary structure of the 5'-NCR
19
20 defines six domains (numbered from I to VI) corresponding to highly
21
22 conserved structures formed by stems and loops [2, 3]. Domains II, IV, and V
23
24 and part of domain VI contribute to the internal ribosomal entry site which
25
26 allows cap-independent initiation of translation of the viral genome [4].
27
28 Structural and nonstructural viral proteins are coded by a single polypeptide
29
30 precursor which is cleaved by viral proteases.
31
32
33
34
35
36
37
38

39 Since 1960, poliomyelitis has been effectively controlled by the use of
40
41 inactivated or live attenuated vaccines. The Sabin live oral poliovirus vaccine
42
43 (OPV) is constituted of attenuated strains of each of the three serotypes
44
45 (Sabin 1, 2, and 3) which have been selected by numerous passages of wild-
46
47 type strains in monkey tissues in vivo and in vitro [5]. The Sabin strains are
48
49 temperature sensitive (ts) and the ts phenotype correlates with the attenuated
50
51 phenotype [6-8]. The molecular determinants of attenuation and temperature
52
53 sensitivity have been intensively studied to elucidate the mechanisms involved
54
55 and to improve the safety of Sabin vaccine strains. The genome of Sabin 1
56
57 differs from that of its parental virulent Mahoney virus by 54 point mutations.
58
59
60
61
62
63
64
65

1 Molecular determinants of the attenuation and temperature sensitivity of Sabin
2
3 1 have been found in the 5'-NCR and 3'-NCR of the genome, as well as in the
4 regions encoding VP1, VP3 and VP4 capsid proteins and the RNA
5 polymerase (3D) [9-15]. For Sabin types 2 and 3, the vaccine strains differ
6 from their progenitors by 23 and 11 mutations, respectively, and there is
7 evidence in both cases that attenuation is determined by just 2 or 3 of these
8 mutations. One substitution in the 5'-NCR of the genome and another that
9 changes an amino acid residue in a capsid protein (VP1 in Sabin 2 and VP3 in
10 Sabin 3) are the major determinants of attenuation and temperature sensitivity
11 [7, 8, 15-22].
12
13
14
15
16
17
18
19
20
21
22

23 Undoubtedly, the use of OPV in mass vaccinations has resulted in drastic
24 reductions in the number of poliomyelitis cases due to infections with wild-type
25 polioviruses from an estimated 350,000 cases in over 125 endemic countries
26 in 1988 to just 1,310 cases in four countries in 2007. However, in rare cases
27 (1 case per 750,000 primary vaccinees), OPV strains have been implicated in
28 vaccine-associated paralytic poliomyelitis (VAPP) [23]. The underlying cause
29 of VAPP is the genetic instability of OPV strains. During multiplication in the
30 human intestine, mutations at specific sites of the genome are associated with
31 the attenuated and temperature sensitive phenotype of Sabin strains and
32 recombination between Sabin strains may result in the loss of the attenuated
33 phenotype of OPV strains and the acquisition of traits characteristic of wild
34 polioviruses, such as increased neurovirulence and loss of temperature
35 sensitivity [24-26]. Moreover, changes are frequently observed in antigenic
36 properties of OPV derivatives representing a selection of viral variants that are
37 less prone to be neutralized by human antibodies [27].
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 The aim of the present study was to correlate the reverted phenotypic traits
2 of OPV isolates such as temperature sensitivity and growth kinetics with
3 mutations and recombination events on the viral genome.
4

5 **Materials and methods**

6 *Virus isolation*

7
8
9
10 Virus isolation was performed from faecal samples during 1978-1985 from
11 VAPP patients and healthy vaccinees in Hep-2 cells (Table 1) and the isolates
12 were re-cultured for the purpose of the present study in Hep-2 cells. Initial
13 isolation at that time occurred at the Hellenic Pasteur Institute and
14 identification/serotyping was done by sero-neutralization with rabbit polyclonal
15 antibodies, (National Institute for Public Health and Environment, RIVM, The
16 Netherlands), according to enclosed instructions. To avoid viral mixtures serial
17 ten-fold dilutions were performed; the last dilution presenting a complete
18 cytopathic effect (CPE) was passaged once again in Hep-2 cells, and the
19 serotype was retested as described above. Vaccine strains (Sabin types 1, 2,
20 3) used in this study were provided by the World Health Organization (WHO).
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

37 *Genotyping and identification of recombinant OPV isolates*

38
39
40 Primer pair UG52/UC53 was used for the identification of the vaccine origin
41 and for the genotyping of the isolates in the 5'-NCR genomic region [28].
42 Moreover, the genotype of isolates in VP1 capsid protein was determined.
43
44 The primer pairs used for complete VP1 gene sequencing were UG1/222 [29-
45 30] for the first half of the VP1 gene and serotype-specific primer pairs
46
47 S₁37/S₁688, S₂30/S₂688 and S₃26/S₃651 for Sabin 1, Sabin 2 and Sabin 3
48
49 isolates, respectively, for the second half of the VP1 gene [31].
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 The identification of recombination sites of isolates EP9, IF, ID, EP12,
2 EP16, EP23 and the sequencing from 2C-3D genomic region have been
3 previously published by our group [32-33].
4

5 *Rct marker test*

6
7
8 Reproductive capacities at different temperatures (Rct marker) were
9 evaluated by Rct test. The Rct value is defined as the difference between the
10 \log_{10} virus titer of a viral stock measured at the optimal temperature (37°C)
11 and that of the supraoptimal temperature (40°C). Briefly, each virus stock was
12 decimally diluted through 10^{-7} . A 0.1-ml of medium containing 4×10^4 Hep-2
13 cells was added into each well of two 96-well plastic plates. A 0.1-ml volume
14 of each virus dilution was inoculated into four wells of each of the two plates.
15 One plate was incubated at 37°C and the other at 40°C. After 5 days of
16 incubation at the appropriate temperatures, the titers were determined by an
17 endpoint micromethod [34] and were expressed in \log_{10} 50% tissue culture
18 infective dose units (TCID₅₀) per ml. Viruses were considered thermosensitive
19 (ts) if the RCT value (between 37°C and 40°C) was greater or equal to 2.00
20 and thermo resistant (not-ts) when the RCT value was inferior to 2.00.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 *One-step growth curve experiment*

41
42 One-step growth curve experiments were performed in Hep-2 cells at 37
43 and 40°C, with a multiplicity of infection of 10 as determined by titration of
44 virus stocks on Hep-2 cells, as has been previously described [35]. Briefly,
45 100 µl of Hep-2 cells (10^4 cells/100 µl) were added into each well of twelve 96-
46 well plastic plates. After the cells were attached to the plates, the isolates and
47 the reference Sabin vaccine strains (Sabin 1, Sabin 2 and Sabin 3) were
48 inoculated each into five replica wells of 96-well plastic plates. After the plates
49 were rocked for 2 hours at 37°C, the cells were thoroughly washed two times
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 each with 300 µl of Eagle MEM to remove unbound virus. Then, 100 µl of
2 Eagle MEM was added into each well and the plates were incubated at 37°C
3 or 40°C for 0, 1, 2, 4, 8 and 16 hours. The plates were subjected to three
4 consecutive freeze-thaw cycles and the viral titers of the supernatants were
5 determined by the TCID₅₀ assay on Hep-2 cells at 37°C for each of the above
6 mentioned hours post-infection. The titration was repeated five times for each
7 of the post-infection hours.
8
9

10 *Computational analysis*

11 ClustalW was used for alignment of the sequences (nucleotide and amino
12 acid) of 5'-NCR, VP1, 2C, 3A, 3B, 3C and 3D genomic regions of all virus
13 isolates with those of the reference strains cited in GenBank (Sabin type 1:
14 AY184219, Sabin type 2: AY184220 and Sabin type 3: AY184221). Amino
15 acid sequences of VP1, 2C, 3A, 3B, 3C and 3D genomic regions of all virus
16 isolates were obtained by using Gene-Runner V 3.05.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

32 **Results**

33 *Genotypes and recombination types of OPV isolates*

34 The 5'-NCR and VP1 genotypes of isolates were identified. One strain was
35 genotyped as Sabin 1, four strains as Sabin 2 and three strains as Sabin 3
36 (Table 1). Among the eight isolates, two (II and 729) were identified as non-
37 recombinant and six (EP9, IF, ID, EP12, EP16 and EP23) were identified as
38 recombinant in one site. The recombination type was S2/S1 for four isolates
39 (EP9, IF, ID, EP12) and the recombination site was located in the 3D region
40 for isolates IF and EP12, in the 3C region for isolate ID and in the 3A region
41 for isolate EP9. Isolates EP16 and EP23 were S3/S2 and S3/S1 recombinant
42 respectively and the recombination site was located in the 2C region (Table
43 1).
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Temperature sensitivity of OPV isolates

1 The Rct marker assay is based on a comparison of the temperature-
2 sensitive multiplication of poliovirus Sabin strains and the non-temperature-
3 sensitive multiplication of virulent strains.
4
5
6

7
8 The non-recombinant Sabin-1 isolate II showed a partial reversion to non-ts
9 phenotype with a Rct value of 2.0 units. Three of recombinant Sabin-2 isolates
10 (EP9, IF, ID: S2/S1) showed a non-ts phenotype with Rct values ranging from
11 0.0 to 0.75 units while one (EP12: S2/S1) showed partial reversion to non-ts
12 phenotype with Rct value of 1.75 units. Both recombinant Sabin-3 isolates,
13 (EP16: S3/S2) and (EP23: S3/S1), showed partial reversion to non-ts
14 phenotype with Rct values of 1.0 and 1.5 units, respectively. The non-
15 recombinant Sabin-3 isolate 729 showed a ts phenotype with Rct value of 3.5
16 units (Table 2). The Sabin vaccine strains (Sabin1, Sabin2, Sabin3) exhibited,
17 as expected, Rct values greater to 2.00 units.
18
19
20
21
22
23
24
25
26
27
28
29
30
31

One-step growth curve experiment

32
33 To determine whether the ts phenotype, as determined by Rct assay, was
34 indicative of growth defects, one-step growth curve experiments were carried
35 out for each isolate. We compared the growth rates and virus yields at 37°C
36 and 40°C of each isolate with that of the corresponding genotype of Sabin
37 vaccine strain (Sabin 1, Sabin 2 or Sabin 3) in one-step growth experiments in
38 Hep-2 cells.
39
40
41
42
43
44
45
46
47
48

49 The non-recombinant Sabin-1 isolate II replicated with kinetics similar to
50 that of its progenitor Sabin 1 vaccine strain showing that the viral yield rises
51 after 2 hours post-infection at 37°C (Fig. 1) while at 40°C its growth was
52 blocked as was also the case for Sabin 1 vaccine strain. However, the
53 isolate II showed 1.0 log₁₀ unit lower viral yield than Sabin 1 vaccine strain at
54
55
56
57
58
59
60
61
62
63
64
65

1 the initial stages of the infectious life cycle (0-2 hours post-infection) resulting
2 in a difference of 2.5 log₁₀ units at 4 hours post infection.

3 The recombinant Sabin-2 isolates EP9, EP12 and ID displayed similar
4 growth kinetics to that of their progenitor Sabin 2 vaccine strain (Fig. 2). More
5 specifically, they showed the same or lower viral yield than Sabin 2 vaccine
6 strain during the infectious life cycle at both 37 and 40°C. The recombinant
7 Sabin-2 isolate IF also displayed similar growth kinetics to that of its
8 progenitor Sabin 2 vaccine strain (Fig. 2). However, it showed higher viral
9 yield than Sabin 2 vaccine strain at 4 and 8 h post-infection at 37°C.
10 Moreover, it showed 1.0-1.5 log₁₀ units higher viral yield than Sabin 2 vaccine
11 strain at 4, 8 and 16 h post-infection at 40°C.
12
13
14
15
16
17
18
19
20
21
22
23
24

25 Both recombinant Sabin-3 isolates EP16 and EP23 showed different
26 growth kinetics from that of their progenitor Sabin 3 vaccine strain (Fig. 3).
27 Isolates EP16 and EP23 showed 2.0 and 1.7 log₁₀ units, respectively, higher
28 viral yield than the Sabin 3 vaccine strain at 0 and 1 hour post-infection.
29 Moreover, the viral yield of isolates EP16 and EP23 rises after 1 hour post-
30 infection at 37°C while that of Sabin 3 vaccine rises after 2 hours post-
31 infection. The isolates EP16 and EP23 replicated efficiently at both 37 and
32 40°C. More specifically, the isolates EP16 and EP23 showed 2.0-3.5 and 1.0-
33 2.0 log₁₀ units, respectively, higher viral yield than Sabin 3 vaccine strain at
34 both 37 and 40°C during the infectious life cycle. The non-recombinant Sabin-
35 3 isolate 729 replicated with kinetics similar to that of Sabin 3 vaccine strain at
36 both 37 and 40°C (Fig. 3).
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53

54 *Nucleotide and amino acid sequence analysis*

55 In an attempt to correlate the phenotypic markers of the isolates with some
56 molecular markers, we investigated the nucleotide and amino acid positions in
57
58
59
60
61
62
63
64
65

1 the genomes of the isolates that have been involved in the attenuated and
2 thermo sensitive phenotype of Sabin vaccine strains.

3 The nucleotide sequences of 5'-NCR, VP1, 2C, 3A, 3B, 3C and 3D
4 genomic regions were investigated in order to establish their respective
5 predicted amino acid sequences. Tables 2 and 3 show the sequence
6 differences with respect to Sabin vaccine strains. Mutations that result in loss
7 of the attenuated and thermo sensitive phenotype of Sabin vaccine strains
8 have been identified in the genomes of all isolates.

9
10 In non-recombinant Sabin-1 isolate II, the mutation U525C was identified
11 (Table 2). The suppressive mutation U525C restores the stability of a base
12 pair between nt 480 and 525 (AU in Mahoney, GU in Sabin 1, and GC in
13 isolate II) in domain V of 5'-NCR and has been correlated with reversion to
14 neurovirulence [6, 36]. The mutation C583G is situated in a double stranded
15 region of domain VI of IRES and leads to a G-G mismatch. In VP1 coding
16 region, the reversion at nt 2795 (A→G [Thr-106-Ala]), a well-known
17 determinant of attenuation [6, 9] was also identified. Amino acid residue 106 is
18 located at the exterior of the capsid, specifically in VP1 BC loop, and is
19 involved in hydrophobic interactions with residue in position 90 [37]. Moreover,
20 substitutions Ala-96-Thr and Lys-99-Asn were identified in isolate II. Both of
21 them are highly exposed in the exterior of the capsid and are located in N-Agl
22 site as well as in VP1 BC loop, which is participating in the formation of the
23 north rim of the canyon and is involved in the binding of receptor CD155 to the
24 virus [38-40]. Amino acid substitution Glu-168-Gly is exposed at the outer
25 surface of the virion and is located in EF loop which is implicated in
26 hydrophobic interactions with receptor CD155 [39-40]. Amino acid substitution
27 Gln-220-Arg is situated in the exterior of the virion, in N-AgIIa site [38].

All Sabin-2 isolates displayed the mutation A481G in 5'-NCR (Table 2).

1 This substitution is a reversion to the wild-type genotype and has been
2 associated with increased neurovirulence and thermo resistance [8, 10, 41-
3 45], probably by preventing a new base pair to form between residues 481
4 and 511, which would weaken the structure of 5'-NCR. Additionally, the
5 mutation U398C was identified in two isolates (EP9 and IF) and the mutation
6 U437C was identified in one isolate (EP9). These substitutions have also
7 some effect in attenuation and thermo sensitivity of Sabin 2 vaccine strain
8 even though minor [46]. Both of these mutations are situated in a double
9 stranded region of domain IV of IRES and in particular they convert a base
10 pair GU into GC. It is noteworthy the presence of mutation C584G in three
11 Sabin-2 isolates (EP9, IF and EP12) which leads to a G-G mismatch. In VP1
12 coding region, amino acid substitutions in residue 143 were identified in two
13 isolates (EP9 and EP12). Residue 143 is exposed on the external surface of
14 the virion in the DE loop and the substitution of the attenuating Ile by Val, Thr
15 or Asn has been correlated with increased neurovirulence [8, 10, 41, 42, 45,
16 47]. In isolate EP12, the substitution Phe-136-Cys was also identified. The
17 residue 136 is situated in the hydrocarbon-binding pocket of VP1 interacting
18 with a sphingosine molecule.

All Sabin-3 isolates displayed the mutation U472C in 5'-NCR (Table 2).

1 This substitution is a reversion to the wild-type genotype and has been
2 associated with increased neurovirulence and thermo resistance [7, 17, 48-
3 50]. The nt 472 and 537 pair in the 5'-NCR in a predicted secondary structure.
4 The attenuating U at nt 472 of 5'-NCR of Sabin 3 vaccine strain resulted in the
5 alteration of a C-G base pair (nt 472-537) found in the neurovirulent wild strain
6 P3/Leon/37 to a U-G base pair found in Sabin 3 vaccine strain, suggesting

1 that the attenuating phenotype is partially associated with a weakening of a
2 base pairing in a highly conserved structure. It is noteworthy the presence of
3 mutation C586G in all three Sabin-3 isolates. This substitution is situated in a
4 double stranded region of domain VI of IRES and leads to a G-G mismatch as
5
6
7
8 the mutations C583G in Sabin-1 isolate (II) and C584G in three Sabin-2
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
61
62
63
64
65

that the attenuating phenotype is partially associated with a weakening of a base pairing in a highly conserved structure. It is noteworthy the presence of mutation C586G in all three Sabin-3 isolates. This substitution is situated in a double stranded region of domain VI of IRES and leads to a G-G mismatch as the mutations C583G in Sabin-1 isolate (II) and C584G in three Sabin-2 isolates (EP9, IF, EP12). These mutations may confer a selective advantage in OPV isolates as they have been also observed in isolates of previous studies [51-52]. In VP1 coding region, all three Sabin-3 isolates displayed the reversion at nt 2493 (C→U [Thr-6-Ile]) which is a determinant of attenuation [53-55]. Residue 6 is situated at the N-terminus of VP1 in the interior of the capsid and the substitution of the attenuating Thr by Ile probably strengthens the hydrophobic interactions between the VP1 amino terminus and endosomal cell membranes during virus cell entry [54, 56]. In isolate EP23, the substitution Ile-258-Val, partially exposed at the outer surface of the virion and near to N-Agl, was also identified.

In the 2C-3D non-structural genomic region of the isolates, nucleotide and amino acid substitutions were also identified (Table 3). The reversion at nt 6203 (C→U [His-73-Tyr]) of 3D polymerase coding region has been correlated with the attenuated and thermo sensitive phenotype of Sabin 1 vaccine strain [6, 10, 13, 25, 57, 58]. The C6203U mutation was identified in two recombinant Sabin-2 isolates (EP9: S2/S1 and ID: S2/S1) in a Sabin 1 background but was not identified in the recombinant isolate EP23 (S3/S1) which has also a Sabin 1 background in 3D region. The 2C coding region is known to contain a conserved stem-loop structure (nt 4443-4504 in Poliovirus type 2) serving as an essential cis-acting replicative element (CRE) involved in the uridylation of VPg [59-60]. The substitution of either of the three A of a

1 conserved AAACA sequence (in Poliovirus type 1 and type 3) in the loop of
2 the CRE reduces both VPg uridylation and virus yield [60]. The substitution
3 G4473A in isolate EP9 (S2/S1), converts the conserved AAGCA sequence of
4 Sabin 2 vaccine strain to the conserved AAACA sequence of Sabin 1 vaccine
5 strain. It has been proposed that the presence of G at nt 4473 is implicated in
6 the attenuated phenotype of Sabin 2 vaccine strain [60].
7
8
9
10
11
12

13 **Discussion**

14
15 In this study, eight OPV isolates which were isolated from healthy
16 vaccinees and VAPP cases during the time period 1978-1985, were
17 investigated. More specifically, we determined the phenotypic traits such as
18 thermo sensitivity and growth kinetics of each isolate and attempted to
19 correlate them with genomic modifications.
20
21
22
23
24
25
26

27 The Sabin vaccine strains differ from their progenitors in biological
28 properties other than neurovirulence, some of which have been used as in
29 vitro markers to monitor the quality of new batches of vaccine prior to monkey
30 neurovirulence assays. Temperature sensitivity and plaque size have been
31 used as in vitro phenotypic markers in previous studies and were showed to
32 correlate with the neurovirulence of poliovirus [6-10, 13, 25, 45, 58, 61].
33 MAPREC test which is based on the presence of mutations at known major
34 determinants of attenuation has also been correlated with the neurovirulence
35 of poliovirus [62]. One-step growth curve experiment was also used as an
36 indication of viral multiplication ability that might correlate with viral virulence
37 [9, 19, 63-65].
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53

54 In the present study, the growth phenotype of eight OPV isolates (two non-
55 recombinants and six recombinants) as well as of Sabin vaccine strains in
56 Hep2 cells at two different temperatures (37°C and 40°C) was evaluated using
57
58
59
60
61
62
63
64
65

1 two different assays, RCT test and one-step growth curve analysis. In non-
2 recombinant Sabin-1 isolate (II) the presence of mutations at known
3 determinants of attenuation and thermo sensitivity or in other positions of 5'-
4 NCR and VP1 regions involved in virus replication, does not favor the
5 reversion of its growth phenotype at both 37°C and 40°C. It is noteworthy the
6 lower virus titre (1 log₁₀ unit) of isolate II than Sabin 1 vaccine strain at the first
7 stages of the infectious life cycle (0 and 1 h post-infection). This initial
8 difference leads to a lower virus titre of isolate II than Sabin 1 vaccine strain
9 throughout the infectious life cycle and may originate from the different
10 adsorption and/or penetration properties of isolate II in comparison to that of
11 Sabin 1 vaccine strain. However, isolate II showed partial reversion to non-ts
12 phenotype (Rct value=2.0). The presence of many other determinants of
13 thermo sensitivity (nt 935, 1944, 2438 and 2741) in the 5' end of the genome
14 of Sabin 1 vaccine strain makes difficult the reversion of Sabin-1 isolates to a
15 non-ts phenotype [9].
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34

35 All Sabin-2 isolates were S2/S1 recombinants. Isolates EP9, IF and ID
36 showed a non-ts phenotype while isolate EP12 showed partial reversion to
37 non-ts phenotype as it was proved by RCT test values. The different
38 phenotypes of Sabin-2 isolates may correlate with the presence of mutations
39 at nt 398 (isolates EP9 and IF) and at nt 6203 (isolates EP9 and ID). The
40 presence of mutations at nt 481, 584 and 2909 in isolate EP12 seems to have
41 minor contribution to the reversion to a non-ts phenotype. In the one-step
42 growth curve test, all Sabin-2 isolates displayed similar growth kinetics to
43 Sabin 2 vaccine strain. However, isolates EP9, ID and EP12 displayed lower
44 virus titre (≤ 1 log₁₀ unit) than Sabin 2 vaccine strain at the first stages of the
45 infectious life cycle (0 and 1 h post-infection) as in the case of Sabin-1 isolate
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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
61
62
63
64
65

II. Moreover, isolate IF showed higher viral yield than Sabin 2 vaccine strain at 4, 8 and 16 h post-infection at 40°C. The discrepancy of the results of the two assays concerning the reversion of thermosensitivity may correlate with the fact that they show the viral multiplication for different time periods.

The recombinant Sabin-3 isolates EP16 (S3/S2) and EP23 (S3/S1) reverted partial to non-ts phenotype while the non-recombinant Sabin-3 isolate 729 displayed a ts phenotype as it seems from Rct test value. Isolates EP16 and EP23 replicated efficiently at both 37 and 40°C in one-step growth curve test. More specifically, isolates EP16 and EP23 showed 2.0 and 1.7 log₁₀ units, respectively, higher viral yield than the Sabin 3 vaccine strain at 0 and 1 hour post-infection. The presence of specific mutations (U472C, C586G in 5'-NCR and C2493U in VP1 coding region) and the recombination events may lead to different properties of isolates in comparison to Sabin 3 vaccine strain. These different properties may lead to the higher viral yield of isolates EP16 and EP23 at the initial stages of the infectious life cycle and to the earlier raise of virus titre than the Sabin 3 vaccine strain. The non-recombinant isolate 729 replicated with kinetics similar to that of Sabin 3 vaccine strain at both 37 and 40°C. However, it showed higher viral yield (<1 log₁₀ unit) than Sabin 3 vaccine strain at 0 and 1 h post-infection as isolates EP16 and EP23. The reversion at nt 2493 may be responsible for the higher viral yield of all three Sabin-3 isolates than the Sabin 3 vaccine strain at the initial stages of the infectious life cycle. Isolates EP16 and EP23 showed mutations at the same determinants of attenuation and thermosensitivity (nt 472 of 5'-NCR and 2493 of VP1) as the isolate 729. Consequently, the different phenotypes of isolates EP16 and EP23 in comparison with that of 729 are probably associated with the recombination event. This would be in

1 disagreement with a previous report by Macadam et al. 1989 [7] showing that
2 similar type 3 recombinant viruses were slightly more ts than their non-
3 recombinant counterparts containing the same reverted mutations.
4

5
6 Consequently, in non-recombinant Sabin-1 isolate the presence of
7 mutations at known determinants of attenuation and thermo sensitivity does
8 not affect its growth phenotype. Sabin 1 vaccine strain is genetically more
9 stable than Sabin 2 and Sabin 3 vaccine strains and this fact may explain why
10 Sabin 3 and Sabin 2 are more frequently isolated from VAPP cases than are
11 Sabin 1 (only about 12% of cases) [66-67]. Moreover, the presence of S1/SX
12 (SX: S2 or S3) recombinants is extremely rare. The sequences of Sabin type
13 1 origin are regularly found in SX/S1 recombinant strains [7, 45, 68-71]. The
14 above may correlate with the increased fitness of SX/S1 recombinants than
15 that of the S1/SX recombinants. The determinants of attenuation and thermo
16 sensitivity of Sabin 1 vaccine strain are scattered along the whole genome but
17 most of them are situated in the 5'-NCR and capsid region [9]. Sabin 1
18 vaccine strain may exchange the 5' end of its genome with that of Sabin 2 or
19 Sabin 3 vaccine strains in order to get rid of these fitness-decreasing
20 mutations.
21
22

23
24 In Sabin-2 isolates the presence of mutations at nt 398 of 5'-NCR and 6203
25 of 3D coding region favours considerably the reversion to non-ts phenotype
26 and may lead to the reversion towards neurovirulence. The mutation at nt 398
27 of 5'-NCR has been frequently observed in Sabin-2 isolates from VAPP cases
28 (8, 41, 72). The mutation at nt 6203 of 3D coding region has been also
29 observed in Sabin-1 isolates from VAPP cases (14, 24, 45).
30
31

32
33 In Sabin-3 isolates, the recombination of Sabin 3 vaccine strain with Sabin
34 1 or Sabin 2 and the acquisition of genome structure S3/S1 or S3/S2 favour
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
61
62
63
64
65

1 the growth phenotype and the reversion towards neurovirulence. This
2 observation is in accordance with the fact that S3/SX recombinants have been
3 frequently isolated from VAPP cases [28, 32, 33, 68, 73-75].
4

5 Thus, both mutations and recombination could affect the phenotype traits of
6 Sabin derivatives and may lead to neurovirulent viral strains. Moreover, the
7 recombination allows either the congregation of reversed determinants of
8 attenuation and thermo sensitivity in the viral genome or the elimination of
9 some determinants of attenuation and thermo sensitivity from the viral
10 genome.
11

12 In conclusion, both Rct assay and one-step growth curve analysis are
13 effective markers for the estimation of neurovirulence of Sabin derivatives.
14 Moreover the use of phenotypic markers such as the one-step growth curve
15 analysis and the Rct assay along with the genomic analysis may shed
16 additional light in the molecular determinants of the reversed neurovirulent
17 phenotype of Sabin derivatives.
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 **Acknowledgements**

48 The work was supported by research grants of the Postgraduate Program
49 “Applications of Molecular Biology-Genetics. Diagnostic Biomarkers”, code
50 3817, of the University of Thessaly, School of Health Sciences, Department of
51 Biochemistry & Biotechnology.
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

- 1 [1] Wimmer E, Hellen CUT, Cao X (1993) Genetics of poliovirus. *Annu Rev*
2
3 *Genet* 27:353-436.
4
- 5 [2] Pilipenko EV, Blinov VM, Romanova LI, Sinyakov AN, Maslova SV, Agol VI
6
7 (1989) Conserved structural domains in the 5'-untranslated region of
8
9 picornaviral genomes: an analysis of the segment controlling translation
10
11 and neurovirulence. *Virology* 168:201-209.
12
13
- 14 [3] Skinner MA, Racaniello VR, Dunn G, Cooper J, Minor PD, Almond JW
15
16 (1989) New model for the secondary structure of the 5' non-coding RNA of
17
18 poliovirus is supported by biochemical and genetic data that also show that
19
20 RNA secondary structure is important in neurovirulence. *J Mol Biol*
21
22 207:379-392.
23
24
- 25 [4] Pelletier J, Kaplan G, Racaniello VR, Sonenberg N (1988) Cap-
26
27 independent translation of poliovirus mRNA is conferred by sequence
28
29 elements within the 5' noncoding region. *Mol Cell Biol* 8:1103-1112.
30
31
32
33
- 34 [5] Sabin AB, Boulger LR (1973) History of Sabin attenuated poliovirus oral
35
36 live vaccine strains. *J Biol Stand* 1:115-118.
37
38
- 39 [6] Christodoulou C, Colbere-Garapin F, Macadam A, Taffs LF, Marsden S,
40
41 Minor P, Horaud F (1990) Mapping of mutations associated with
42
43 neurovirulence in monkeys infected with Sabin 1 poliovirus revertants
44
45 selected at high temperature. *J Virol* 64:4922-4929.
46
47
- 48 [7] Macadam AJ, Arnold C, Howlett J, John A, Marsden S, Taffs F, Reeve P,
49
50 Hamada N, Wareham K, Almond J, Cammack N, Minor PD (1989)
51
52 Reversion of the attenuated and temperature-sensitive phenotypes of the
53
54 Sabin type 3 strain of poliovirus in vaccinees. *Virology* 172:408-414.
55
56
57
58
59
60
61
62
63
64
65

- 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
61
62
63
64
65
- [8] Macadam AJ, Pollard SR, Ferguson G, Dunn G, Skuce R, Almond JW, Minor PD (1991) The 5' noncoding region of the type 2 poliovirus vaccine strain contains determinants of attenuation and temperature sensitivity. *Virology* 81:451-8.
- [9] Bouchard MJ, Lam DH, Racaniello VR (1995) Determinants of attenuation and temperature sensitivity in the type 1 poliovirus Sabin vaccine. *J Virol* 69:4972-8.
- [10] Georgescu MM, Tardy-Panit M, Guillot S, Crainic R, Delpeyroux F (1995) Mapping of mutations contributing to the temperature sensitivity of the Sabin 1 vaccine strain of poliovirus. *J Virol* 69:5278-5286.
- [11] Kawamura N, Kohara M, Abe S, Komatsu T, Tago K, Arita M, Nomoto A (1989) Determinants in the 5' noncoding region of poliovirus Sabin 1 RNA that influence the attenuation phenotype. *J Virol* 63:1302-1309.
- [12] Omata T, Kohara M, Kuge S, Komatsu T, Abe S, Semler BL, Kameda A, Itoh H, Arita M, Wimmer E, Nomoto A (1986) Genetic analysis of the attenuation phenotype of poliovirus type 1. *J Virol* 58:348-358.
- [13] Tardy-Panit M, Blondel B, Martin A, Tekaiia F, Horaud F, Delpeyroux F (1993) A mutation in the RNA polymerase of poliovirus type 1 contributes to attenuation in mice. *J Virol* 67:4630-4638.
- [14] Otelea D., Guillot S., Furione M., Combiescu AA, Balanant J, Candrea A, Crainic R (1993) Genomic modifications in naturally occurring neurovirulent revertants of Sabin 1 polioviruses. *Dev Biol Stand* 78:33-38.
- [15] Ogra PL, Faden HS, Abraham R, Duffy LC, Sun M, Minor PD (1991) Effect of prior immunity on the shedding of virulent revertant virus in feces after oral immunization with live attenuated poliovirus vaccines. *J Infect Dis* 164(1):191-4.

- 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
61
62
63
64
65
- [16] Minor PD, Dunn G, Evans DM, Magrath DI, John A, Howlett J, Phillips A, Westrop G, Wareham K, Almond JW, Hogle JM (1989) The temperature sensitivity of the Sabin type 3 vaccine strain of poliovirus: molecular and structural effects of a mutation in the capsid protein VP3. *J Gen Virol* 70:1117-1123.
- [17] Westrop GD, Wareham KA, Evans DM, Dunn G, Minor PD, Magrath DI, Taffs F, Marsden S, Skinner MA, Schild GC, et al (1989) Genetic basis of attenuation of the Sabin type 3 oral poliovirus vaccine. *J Virol* 63:1338-1344.
- [18] Guest S, Pilipenko E, Sharma K, Chumakov K, Roos RP (2004) Molecular mechanisms of attenuation of the Sabin strain of poliovirus type 3. *J Virol* 78:11097-11107.
- [19] Gutiérrez AL, Denova-Ocampo M, Racaniello VR, del Angel RM (1997) Attenuating mutations in the poliovirus 5' untranslated region alter its interaction with polypyrimidine tract-binding protein. *J Virol* 71:3826-3833.
- [20] Evans DM, Dunn G, Minor PD, Schild GC, Cann AJ, Stanway G, Almond JW, Currey K, Maizel JV Jr. Evans DM, Dunn G, Minor PD, Schild GC, Cann AJ, Stanway G, Almond JW, Currey K, Maizel JV Jr. (1985) *Nature*. 314(6011):548-50.
- [21] Gnanashanmugam D, Falkovitz-Halpern MS, Dodge A, Fang M, Wong LJ, Esparza M, Hammon R, Rivas-Merelles EE, Santos JI, Maldonado Y (2007) Shedding and reversion of oral polio vaccine type 3 in Mexican vaccinees: comparison of mutant analysis by PCR and enzyme cleavage to a real-time PCR assay. *J Clin Microbiol* 45(8):2419-25.
- [22] Martinez CV, Old MO, Kwock DK, Khan SS, Garcia JJ, Chan CS, Webster R, Falkovitz-Halpern MS, Maldonado YA (2004) Shedding of

sabin poliovirus Type 3 containing the nucleotide 472 uracil-to-cytosine point mutation after administration of oral poliovirus vaccine. *J Infect Dis* 190(2):409-16.

[23] Centers for Disease Control and Prevention (1997) Poliomyelitis prevention in the United States: introduction of a sequential vaccination schedule of inactivated poliovirus vaccine followed by oral poliovirus vaccine. *Recommendations of the Advisory Committee on Immunization Practices (ACIP)*. *Morb Mortal Wkly Rep* 46:1–25.

[24] Furione M, Guillot S, Otelea D, Balanant J, Candrea A, Crainic R (1993) Polioviruses with natural recombinant genomes isolated from vaccine-associated paralytic poliomyelitis. *Virology* 196:199-208.

[25] Georgescu MM, Balanant J, Macadam A, Otelea D, Combiescu M, Combiescu AA, Crainic R, Delpeyroux F (1997) Evolution of the Sabin type 1 poliovirus in humans: characterization of strains isolated from patients with vaccine-associated paralytic poliomyelitis. *J Virol* 71:7758-7768.

[26] Cherkasova EA, Yakovenko ML, Rezapkin GV, Korotkova EA, Ivanova OE, Ereemeeva TP, Krasnoproshina LI, Romanenkova NI, Rozaeva NR, Sirota L, Agol VI, Chumakov KM (2005) Spread of vaccine-derived poliovirus from a paralytic case in an immunodeficient child: an insight into the natural evolution of oral polio vaccine. *J Virol* 79:1062-70.

[27] Yakovenko ML, Korotkova EA, Ivanova OE, Ereemeeva TP, Samoilovich E, Uhova I, Gavrilin GV, Agol VI (2009) Evolution of the Sabin vaccine into pathogenic derivatives without appreciable changes in antigenic properties: need for improvement of current poliovirus surveillance. *J Virol* 83 (7):3402-6.

- 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
61
62
63
64
65
- [28] Georgopoulou A, Markoulatos P, Spyrou N, Vamvakopoulos NC (2000) Improved genotyping vaccine and wild-type poliovirus strains by restriction fragment length polymorphism analysis: clinical diagnostic implications. *J Clin Microbiol* 38:4337-4342.
- [29] Balanant J, Guillot S, Candrea A, Delpeyroux F, Crainic R (1991) The natural genomic variability of poliovirus analyzed by a restriction fragment length polymorphism assay. *Virology* 184:645-654.
- [30] Mulders MN, Reimerink JH, Stenvik M, Alaeddinoglu I, van der Avoort HG, Hovi T, Koopmans MP (1999) A Sabin vaccine-derived field isolate of poliovirus type 1 displaying aberrant phenotypic and genetic features, including a deletion in antigenic site 1. *J Gen Virol* 80:907-916.
- [31] Dedepsidis E, Karakasiliotis I, Paximadi E, Kyriakopoulou Z, Komiotis D, Markoulatos P (2006) Detection of unusual mutation within the VP1 region of different re-isolates of poliovirus Sabin vaccine. *Virus Genes* 33:183-191.
- [32] Karakasiliotis I, Markoulatos P, Katsorchis T (2004) Site analysis of recombinant and mutant poliovirus isolates of Sabin origin from patients and from vaccinees. *Mol Cell Probes* 18:103-109.
- [33] Paximadi E, Karakasiliotis I, Mamuris Z, Stathopoulos C, Krikelis V, Markoulatos P (2006) Genomic analysis of recombinant sabin clinical isolates. *Virus Genes* 32:203-210.
- [34] Nakano JH, Hatch MH, Thieme W, Nottay B (1978) Parameters for differentiating vaccine-derived and wild poliovirus strains. *Prog Med Virol* 24:178-206.

- 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
61
62
63
64
65
- [35] Malnou CE, Pöyry TA, Jackson RJ, Kean KM (2002) Poliovirus internal ribosome entry segment structure alterations that specifically affect function in neuronal cells: molecular genetic analysis. *J Virol* 76(21):10617-26.
- [36] Rezapkin GV, Chumakov KM, Lu Z, Ran Y, Dragunsky EM, Levenbook IS (1994) Microevolution of Sabin 1 strain in vitro and genetic stability of oral poliovirus vaccine. *Virology* 202:370-378.
- [37] Cherkasova EA, Korotkova EA, Yakovenko ML, Ivanova OE, Ereemeeva TP, Chumakov KM, Agol VI (2002) Long-term circulation of vaccine-derived poliovirus that causes paralytic disease. *J Virol* 76:6791-9.
- [38] Minor PD, Ferguson M, Evans DM, Almond JW, Icenogle JP. (1986) Antigenic structure of polioviruses of serotypes 1, 2 and 3. *J Gen Virol* 67:1283-1291.
- [39] Harber J, Bernhardt G, Lu HH, Sgro JY, Wimmer E. (1995) Canyon rim residues, including antigenic determinants, modulate serotype-specific binding of polioviruses to mutants of the poliovirus receptor. *Virology* 214:559-570.
- [40] He Y, Bowman VD, Mueller S, Bator CM, Bella J, Peng X, Baker TS, Wimmer E, Kuhn RJ, Rossmann MG. (2000) Interaction of the poliovirus receptor with poliovirus. *Proc Natl Acad Sci USA* 97:79-84.
- [41] Muzychenko AR, Lipskaya GYu, Maslova SV, Svitkin YV, Pilipenko EV, Nottay BK, Kew OM, Agol VI (1991) Coupled mutations in the 5'-untranslated region of the Sabin poliovirus strains during in vivo passages: structural and functional implications. *Virus Res* 21:111-122.
- [42] Macadam AJ, Pollard SR, Ferguson G, Skuce R, Wood D, Almond JW, Minor PD (1993) Genetic basis of attenuation of the Sabin type 2 vaccine strain of poliovirus in primates. *Virology* 192:18-26.

- 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
61
62
63
64
65
- [43] Minor PD, Macadam AJ, Stone DM, Almond JW (1993) Genetic basis of attenuation of the Sabin oral poliovirus vaccines. *Biologicals* 21:357-363.
- [44] Guillot S, Otelea D, Delpeyroux F, Crainic R (1994) Point mutations involved in the attenuation/neurovirulence alternation in type 1 and 2 oral polio vaccine strains detected by site-specific polymerase chain reaction. *Vaccine* 12:503-507.
- [45] Georgescu MM, Delpeyroux F, Tardy-Panit M, Balanant J, Combiescu M, Combiescu AA, Guillot S, Crainic R (1994) High diversity of poliovirus strains isolated from the central nervous system from patients with vaccine-associated paralytic poliomyelitis. *J Virol* 68:8089-8101.
- [46] Friedrich F (1996) Genomic modifications in Sabin vaccine strains isolated from vaccination-associated cases, healthy contacts and healthy vaccinees. *Acta Virol* 40:157-70.
- [47] Equestre M, Genovese D, Cavalieri F, Fiore L, Santoro R, Perez Bercoff R (1991) Identification of a consistent pattern of mutations in neurovirulent variants derived from the sabin vaccine strain of poliovirus type 2. *J Virol* 65:2707-10.
- [48] La Monica N, Almond JW, Racaniello VR (1987) A mouse model for poliovirus neurovirulence identifies mutations that attenuate the virus for humans. *J Virol* 61:2917-20.
- [49] La Monica N, Racaniello VR (1989) Differences in replication of attenuated and neurovirulent polioviruses in human neuroblastoma cell line SH-SY5Y. *J Virol* 63:2357-60.
- [50] Chumakov KM, Powers LB, Noonan KE, Roninson IB, Levenbook IS (1991) Correlation between amount of virus with altered nucleotide

sequence and the monkey test for acceptability of oral poliovirus vaccine.

Proc Natl Acad Sci U S A 88:199-203.

[51] Pliaka V , Dedepsidis E, Kyriakopoulou Z, Papadopoulou I, Levidiotou S, Markoulatos P (2007) Use of mutational pattern in 5'-NCR and VP1 regions of polioviruses for molecular diagnosis. Mol Cell Probes 21:267-275.

[52] Pliaka V, Dedepsidis E, Kyriakopoulou Z, Papadi G, Tsakogiannis D, Pratti A, Levidiotou-Stefanou S, Markoulatos P (2010) Growth kinetic analysis of bi-recombinant poliovirus vaccine strains. Virus Genes 40(2):200-11.

[53] Weeks-Levy C, Tatem JM, DiMichele SJ, Waterfield W, Georgiu AF, Mento SJ (1991) Identification and characterization of a new base substitution in the vaccine strain of Sabin 3 poliovirus. Virology 185:934-7.

[54] Tatem JM, Weeks-Levy C, Georgiu A, DiMichele SJ, Gorgacz EJ, Racaniello VR, Cano FR, Mento SJ (1992) A mutation present in the amino terminus of Sabin 3 poliovirus VP1 protein is attenuating. J Virol 66:3194-3197.

[55] Mento SJ, Weeks-Levy C, Tatem JM, Gorgacz EJ, Waterfield WF (1993) Significance of a newly identified attenuating mutation in Sabin 3 oral poliovirus vaccine. Dev Biol Stand 78:93-100.

[56] Fricks CE, Hogle JM (1990) Cell-induced conformational change in poliovirus: externalization of the amino terminus of VP1 is responsible for liposome binding. J Virol 64:1934-45.

[57] Martin A, Benichou D, Couderc T, Hogle JM, Wychowski C, Van der Werf S, Girard M (1991) Use of type 1/type 2 chimeric polioviruses to study determinants of poliovirus type 1 neurovirulence in a mouse model. Virology 180:648-658.

- 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
61
62
63
64
65
- [58] McGoldrick A, Macadam AJ, Dunn G, Rowe A, Burlison J, Minor PD, Meredith J, Evans DJ, Almond JW (1995) Role of mutations G-480 and C-6203 in the attenuation phenotype of Sabin type 1 poliovirus. *J Virol* 69:7601-7605.
- [59] Paul AV, Rieder E, Kim DW, van Boom JH, Wimmer E (2000) Identification of an RNA hairpin in poliovirus RNA that serves as the primary template in the in vitro uridylylation of VPg. *J Virol* 74:10359-10370.
- [60] Rieder E, Paul AV, Kim DW, van Boom JH, Wimmer E (2000) Genetic and biochemical studies of poliovirus cis-acting replication element cre in relation to VPg uridylylation. *J Virol* 74:10371-10380.
- [61] Martín J, Odoom K, Tuite G, Dunn G, Hopewell N, Cooper G, Fitzharris C, Butler K, Hall WW, Minor PD (2004) Long-term excretion of vaccine-derived poliovirus by a healthy child. *J Virol* 78:13839-13847.
- [62] Horie H, Yoshida H, Matsuura K, Miyazawa M, Wakabayashi K, Nomoto A, Hashizume S (2002) Isolation of vaccine-derived type 1 polioviruses displaying similar properties to virulent wild strain Mahoney from sewage in Japan. *J Med Virol* 68:445-451.
- [63] Cello J, Toyoda H, Dejesus N, Dobrikova EY, Gromeier M, Wimmer E (2008) Growth phenotypes and biosafety profiles in poliovirus-receptor transgenic mice of recombinant oncolytic polio/human rhinoviruses. *J Med Virol* 80:352-9.
- [64] Malnou CE, Werner A, Borman AM, Westhof E, Kean KM (2004) Effects of vaccine strain mutations in domain V of the internal ribosome entry segment compared in the wild type poliovirus type 1 context. *J Biol Chem* 279:10261-10269.

- 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
61
62
63
64
65
- [65] De Jesus N, Franco D, Paul A, Wimmer E, Cello J (2005) Mutation of a single conserved nucleotide between the cloverleaf and internal ribosome entry site attenuates poliovirus neurovirulence. *J Virol* 79:14235-43.
- [66] Strebel PM, Sutter RW, Cochi SL, Biellik RJ, Brink EW, Kew OM, Pallansch MA, Orenstein WA, Hinman AR (1992) Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus-associated disease. *Clin Infect Dis* 14:568-579.
- [67] Strebel PM, Aubert-Combiescu A, Ion-Nedelcu N, Biberi-Moroeanu S, Combiescu M, Sutter RW, Kew OM, Pallansch MA, Patriarca PA, Cochi SL (1994) Paralytic poliomyelitis in Romania, 1984-1992. Evidence for a high risk of vaccine-associated disease and reintroduction of wild-virus infection. *Am J Epidemiol* 140:1111-1124.
- [68] Cuervo NS, Guillot S, Romanenkova N, Combiescu M, Aubert-Combiescu A, Seghier M, Caro V, Crainic R, Delpeyroux F (2001) Genomic features of intertypic recombinant sabin poliovirus strains excreted by primary vaccinees. *J Virol* 75:5740-51.
- [69] Driesel G, Diedrich S, Kunkel U, Schreier E (1995) Vaccine-associated cases of poliomyelitis over a 30 year period in East Germany. *Eur J Epidemiol* 11:647-654.
- [70] Gavrilin GV, Cherkasova EA, Lipskaya GY, Kew OM, Agol VI (2000) Evolution of circulating wild poliovirus and of vaccine-derived poliovirus in an immunodeficient patient: a unifying model. *J Virol* 74:7381-90.
- [71] Savolainen-Kopra C., Samoilovich E., Kahelin H., Hiekka AK, Hovi T., Roivainen M (2009) Comparison of poliovirus recombinants: accumulation of point mutations provides further advantages. *J Gen Virol* 90 (Pt 8):1859-68.

- 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
61
62
63
64
65
- [72] Friedrich F, Filippis AM, Ferreira FC, Schatzmayr HG, Da-Silva EE (1995) Genomic characterization of type 2 polioviruses isolated from vaccine-associated cases in Brazil. *Braz J Med Biol Res* 28(7):733-42.
- [73] Blomqvist S, Bruu AL, Stenvik M, Hovi T (2003) Characterization of a recombinant type 3/type 2 poliovirus isolated from a healthy vaccinee and containing a chimeric capsid protein VP1. *J Gen Virol* 84:573-80.
- [74] Martín J, Samoilovich E, Dunn G, Lackenby A, Feldman E, Heath A, Svirchevskaya E, Cooper G, Yermalovich M, Minor PD (2002) Isolation of an intertypic poliovirus capsid recombinant from a child with vaccine-associated paralytic poliomyelitis. *J Virol* 76:10921-10928.
- [75] Paximadi E, Karakasiliotis I, Bolanaki E, Krikelis A, Markoulatos P (2007) Vaccine derived bi- and multi-recombinant Sabin strains. *Virus Genes* 35:541-548.

1 **Table 1**

2
3 Data for the OPV isolates.
4
5

6

7 Isolate	8 5'-NCR and VP1 9 genotype	Source	Recombination type	Crossover point	Accession numbers 5'-NCR/VP1 /2C-3'-NCR	Reference
10 II	S1	Cord tumor	nonrecombinant	-	EU598476/FJ609769/-	[32, 52]
11						
12						
13 EP9	S2	Healthy vaccinee	S2/S1	5314-5318/S2 ^a	EU598464/EU598478 /AY736178	[33]
14						
15 IF	S2	Healthy vaccinee	S2/S1	6247-6281/S2	FJ609757/FJ609772 /AY297764 (2C), AY297760 (3D)	[32]
16						
17						
18						
19 ID	S2	VAPP	S2/S1	5521-5526/S2	FJ609758/FJ609773 /EU715814	[32]
20						
21						
22 EP12	S2	Healthy vaccinee	S2/S1	6337-6362/S2	EU598465/EU598479 /AY736179	[33]
23						
24 EP16	S3	Healthy vaccinee	S3/S2	4892-4914/S3	EU598466/FJ609775 /AY736180	[33]
25						
26						
27 EP23	S3	VAPP	S3/S1	4880-4887/S3	EU598468/EU598480 /AY736181	[33]
28						
29 729	S3	Healthy vaccinee	nonrecombinant	-	EU598477/FJ609778/-	[52]
30						

31

32 ^aNumbering according to Sabin 2 (AY184220) or Sabin 3 (AY184221) vaccine
33 strains.
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
61
62
63
64
65

Table 2

Location of mutations in 5'-NCR and VP1 sequences of OPV isolates and comparison with the corresponding nucleotide and amino acid positions in Sabin vaccine strains. Numbering according to vaccine strains Sabin 1 (AY184219), Sabin 2 (AY184220) and Sabin 3 (AY184221). Mutations identified in more than one isolates are underlined. Reproductive capacity values of OPV isolates and Sabin vaccine strains at different temperatures (Rct marker) are also indicated.

Isolate	Recombination type	Mutations		$\Delta \log R_{ct} 40$
		5'-NCR	VP1	
II	S1 nonrecombinant	<u>525</u> U-C <u>583</u> C-G	2605 G-A - 2765 G-A 96-Ala-Thr 2776 G-U 99-Lys-Asn 2795 A-G 106-Thr-Ala 2917 C-U - 2982 A-G 168-Glu-Gly 3138 A-G 220-Gln-Arg	2.0
EP9	S2/S1	<u>398</u> U-C <u>437</u> U-C <u>481</u> A-G <u>584</u> C-G <u>576</u> C-U	<u>2909</u> U-A 143-Ile-Asn 3252 C-U -	0.75
IF	S2/S1	<u>398</u> U-C <u>481</u> A-G <u>584</u> C-G	-	0.0
ID	S2/S1	<u>481</u> A-G <u>567</u> U-A	-	0.0
EP12	S2/S1	<u>481</u> A-G <u>597</u> U-A <u>584</u> C-G	2859 A-G - 2888 U-G 136-Phe-Cys <u>2909</u> U-C 143-Ile-Thr	1.75
EP16	S3/S2	<u>472</u> U-C <u>586</u> C-G	<u>2493</u> C-U 6-Thr-Ile 2608 A-G - 3259 A-G -	1.0
EP23	S3/S1	<u>472</u> U-C <u>586</u> C-G	<u>2493</u> C-U 6-Thr-Ile 3248 A-G 258-Ile-Val 3352 A-G -	1.5
729	S3 nonrecombinant	248 G-A <u>472</u> U-C <u>586</u> C-G	<u>2493</u> C-U 6-Thr-Ile 2659 G-A - 2716 G-A -	3.5
Sabin 1	-			3.5
Sabin 2	-			3.0
Sabin 3	-			3.5

Table 3

Location of mutations in 2C-3D genomic region of isolates EP9, IF, ID, EP12, EP16, EP23 and comparison with the corresponding nucleotide and amino acid positions in Sabin vaccine strains. The genomic region from 2C-3D of non-recombinant isolates II and 729 was not sequenced. Numbering according to vaccine strains Sabin 1 (AY184219), Sabin 2 (AY184220) and Sabin 3 (AY184221). Mutations identified in more than one isolates are underlined.

Isolate	Region	Origin	Mutations
EP9	2C	S2	4473 G-A -
IF		S2	4842 G-A -
ID		S2	4456 G-A 112-Val-Ile
EP12		S2	4773 C-U -
			4174 C-G 18-Leu-Val
			4992 U-A -
EP16		S3/S2	-
EP23		S3/S1	-
EP9	3A	S2/S1	5130 U-C ^a -
IF		S2	-
ID		S2	-
EP12		S2	-
EP16		S2	-
EP23		S1	5191 A-G -
EP9	3B	S1	5389 A-U -
IF		S2	-
ID		S2	-
EP12		S2	-
EP16		S2	-
EP23		S1	-
EP9	3C	S1	5665 U-C -
			5698 A-C 87-Arg-Ser
			5720 A-C 95-Thr-Pro
			5760 A-G 108-Lys-Arg
			5959 G-A -
IF		S2	-
ID		S2/S1	-
EP12		S2	-
EP16		S2	-
EP23		S1	5791 U-C -
EP9		S1	<u>6203</u> C-U 73-His-Tyr
			6217 A-G -
IF		S2/S1	-
ID		S1	<u>6203</u> C-U 73-His-Tyr
			6373 U-C -
EP12		S2/S1	6781 C-U ^a -
EP16		S2	6219 G-A -
			6327 U-C -
EP23		S1	6474 G-A 163-Arg-Lys

^aNumbering according to Sabin 2 (AY184220) or Sabin 1 (AY184219) vaccine strains.

- 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
- 61
- 62
- 63
- 64
- 65

1 **Fig.1.** One-step growth curve analysis of Sabin-1 isolate II in comparison with
2 Sabin 1 vaccine strain in Hep2 cells. Cells were infected at an MOI of 10 and
3 incubated at 37°C or 40°C. Total virus production at different times (0-16
4 hours) post-infection was determined by TCID₅₀ assay on Hep2 cells. Each
5 point represents the mean (±standard deviation) of virus titers from five
6 different experiments.
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
61
62
63
64
65

II

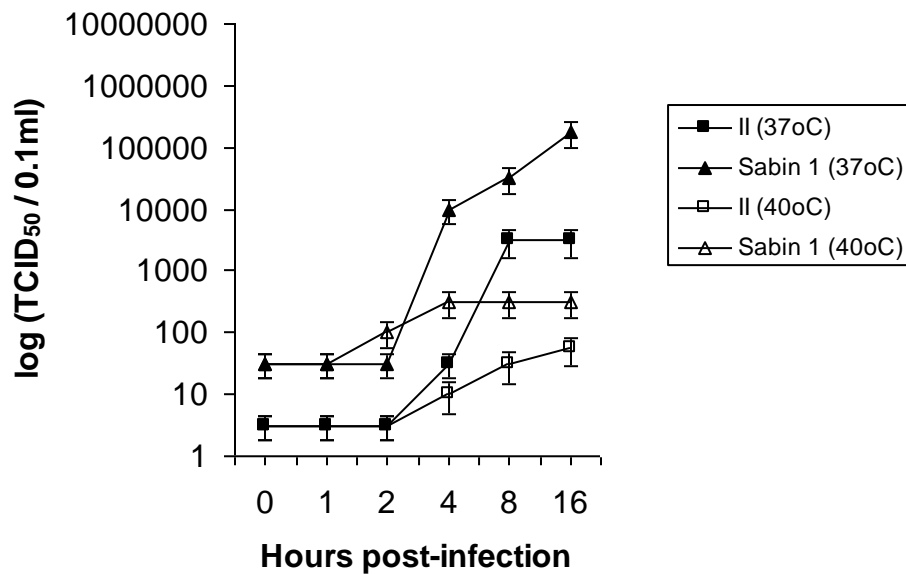
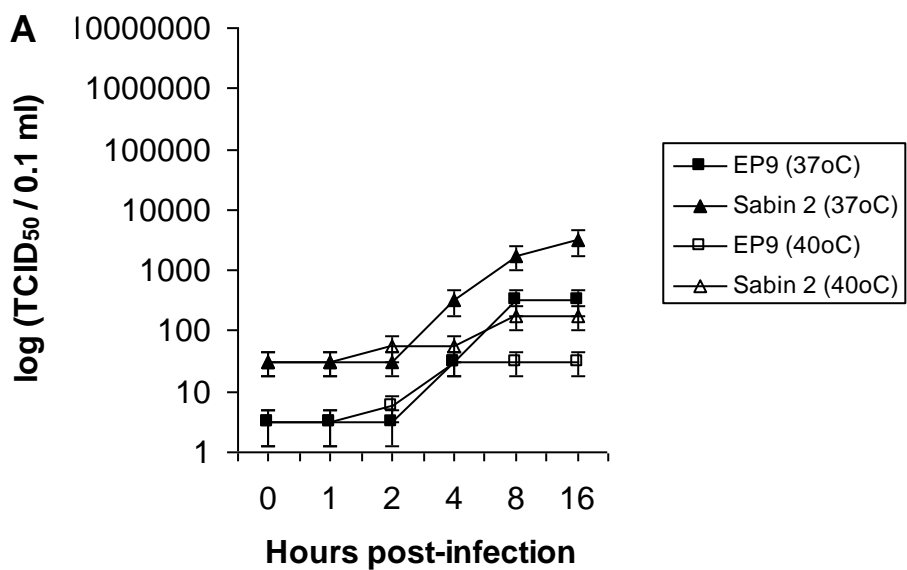
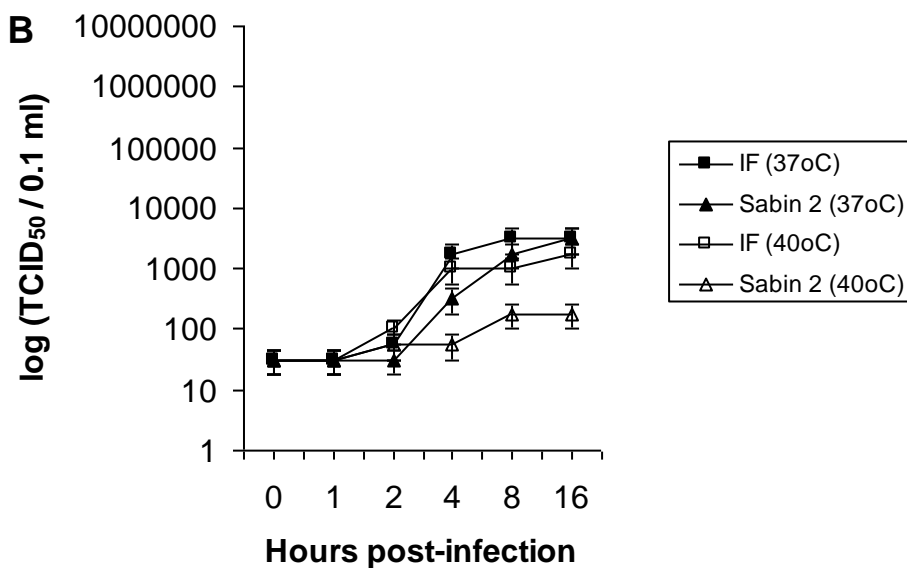


Fig.2. One-step growth curve analysis of Sabin-2 isolates EP9 (A), IF (B), ID (C) and EP12 (D) in comparison with Sabin 2 vaccine strain in Hep2 cells. Cells were infected at an MOI of 10 and incubated at 37°C or 40°C. Total virus production at different times (0-16 hours) post-infection was determined by TCID₅₀ assay on Hep2 cells. Each point represents the mean (±standard deviation) of virus titers from five different experiments.

EP9

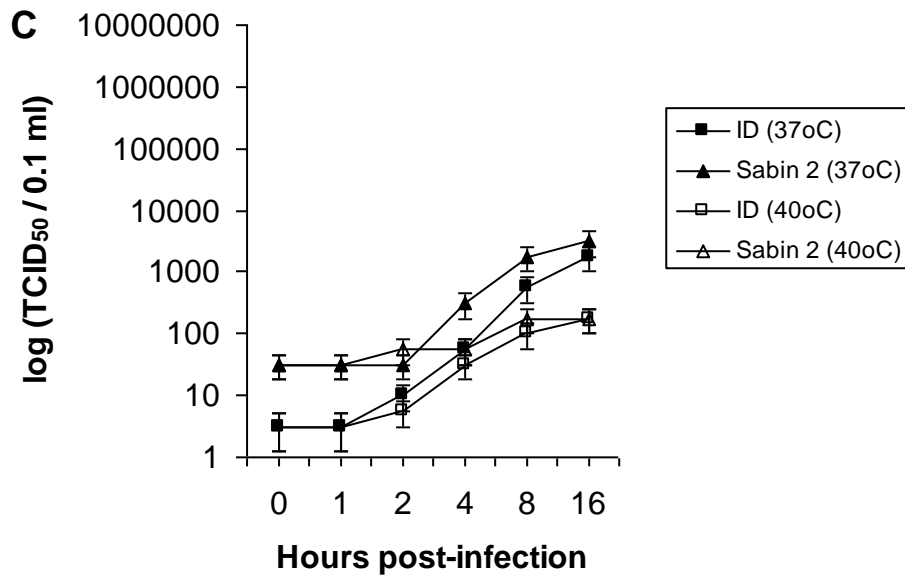


IF



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
61
62
63
64
65

ID



EP12

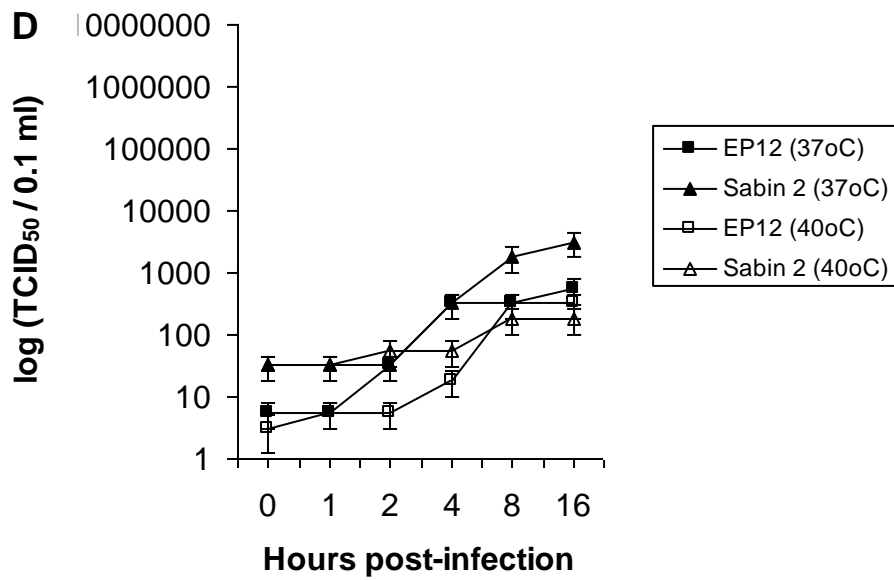
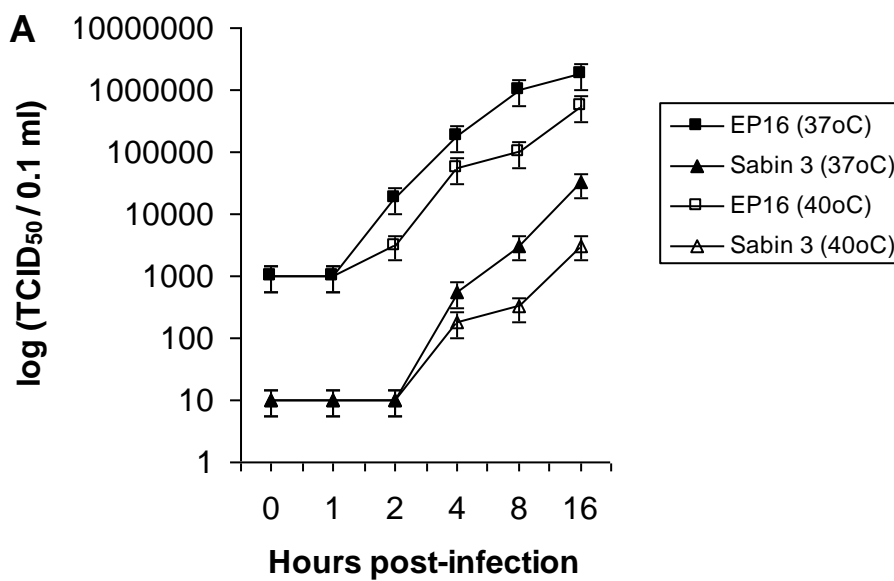
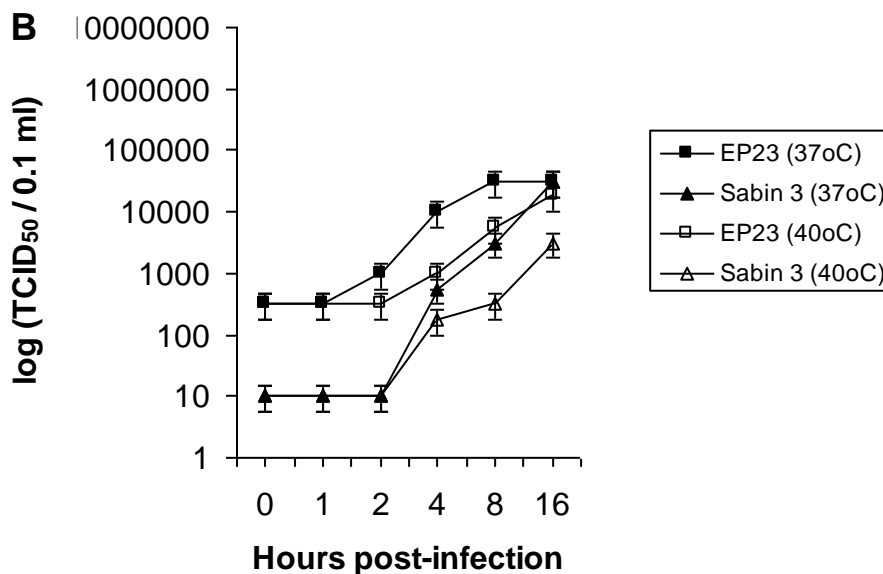


Fig.3. One-step growth curve analysis of Sabin-3 isolates EP16 (A), EP23 (B) and 729 (C) in comparison with Sabin 3 vaccine strain in Hep2 cells. Cells were infected at an MOI of 10 and incubated at 37°C or 40°C. Total virus production at different times (0-16 hours) post-infection was determined by TCID₅₀ assay on Hep2 cells. Each point represents the mean (±standard deviation) of virus titers from five different experiments.

EP16



EP23



729

