

## History of Sabin attenuated poliovirus oral live vaccine strains\*

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The full data concerning the history of attenuated poliovirus strains developed by one of us (Sabin, 1965) for vaccine production do not appear in a single journal. Over the past few years we have had frequent requests for the details such as isolation and attenuation and accordingly we felt that bringing the data together in the report below would be both helpful and informative to those involved in the production and control of poliovirus vaccine (oral) prepared from these strains.

### Type 1 LS-c, 2ab/KP<sub>2</sub> (Sabin Original Virus = SO)

The Mahoney virus was isolated in 1941 by Drs Fancis and Mack from the pooled faeces of three healthy children in Cleveland.

Drs Li and Schaeffer received the strain from Dr Salk after it had undergone 14 monkey *in-vivo* and two monkey *in-vitro* testicular tissue culture passages. Li & Schaeffer (1954) subjected this strain, Monk14 T2 (Mahoney strain), to a further nine similar *in-vitro* passages. From Monk14 T11 they established four separate virus lines by further passages in monkey testicular tissue and kidney cell cultures, by passages in the central nervous systems of white mice usually by the intraspinal route of injection and by alternate passages in the skin of rhesus or cynomolgus monkeys and tissue cultures. For the intradermal injections, ten 0.1 ml amounts of undiluted culture fluid were introduced adjacent to each other into the shaved skin of the abdomen. The four passaged strains were designated LS, LS-a, LS-b and LS-c and as they continued to grow in cell culture

\* Received for publication 4 December 1972.

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they were differentiated by their host reactions. LS and LS-a reacted similarly in that they were mouse and monkey spinal cord variants. LS-b acted as a mouse cerebral strain, but LS-c was a 'non-neurotropic' strain for mice and monkeys by either route. It was derived from the thirty-third consecutive *in-vitro* passage of Monk14 T2 (the first 15 in testicular tissue and the subsequent 18 in kidney cells), then by alternate passage in monkey skin (the first two of these were *in-vivo* rhesus passages and the later ones were performed in cynomolgus monkeys) and monkey kidney cell cultures. The LS-c strain is Monk14 MS10 T43 level.

The LS-c strain underwent five passages in cynomolgus monkey kidney cell cultures, including three terminal dilution passages, prior to submission to a series of three consecutive single plaque passages (Sabin, 1956). The progeny of ten selected individual plaques were tested for neurovirulence in cynomolgus monkeys inoculated intraspinally with  $10^6$ ,  $10^5$  and  $10^4$  tissue culture infective virus doses, and the LS-c, 2ab strain was selected because it possessed the optimum properties. The original type 1 virus (SO) was prepared by two further passages in cynomolgus monkey kidney cell cultures and designated LS-c, 2ab/KP<sub>2</sub> of 10/10/56. Its volume was 100 ml, the pH was 8.2, the titre was  $7.9 \log_{10}$  TCID<sub>50</sub> per ml (Tissue Culture Infectious Dose 50%) and only one cynomolgus monkey out of five receiving undiluted material intraspinally exhibited slight paralysis. The two groups of animals inoculated with suspension diluted 1/10 and 1/100 showed no paralysis. At the end of 1956 Merck, Sharp & Dohme Research Laboratories prepared a Lot of 25 l by one passage of the original virus in rhesus monkey kidney cell cultures. This material was designated LS-c, 2ab/KP<sub>3</sub> (MSD, SOM or SO + 1), and aliquots were used for the world-wide field trials before it was licensed as the Sabin original vaccine, and as the Sabin seed virus for the production of vaccine.

#### Type 2 P712, Ch, 2ab/KP<sub>2</sub> (Sabin Original Virus = SO)

The original P712 virus was a naturally occurring strain of poliovirus possessing low neurovirulence for cynomolgus monkeys by the intraspinal route (Sabin, 1956). The faeces from a number of healthy children in Louisiana were sent by Drs Fox and Gelfand to Dr Sabin, who isolated the P712 strain from one of these specimens.

Because of its low initial neurovirulence for monkeys it was passaged four times in cynomolgus monkey kidney cell cultures, three of which were terminal dilution ones. The progeny from a number of plaques were obtained, and nine were submitted to three consecutive plaque passages (Sabin, 1957). The purified plaque progeny with the least neurovirulence for cynomolgus monkeys, inoculated intraspinally as with type 1 progeny, was fed to chimpanzees and the excreted strain possessing the least residual neurovirulence (P712, Ch) was further purified by three consecutive passages from single plaques, and the strain designated P712, Ch, 2ab selected as the vaccine virus. The original type 2 virus (SO) was prepared by two further passages in cynomolgus monkey kidney cell cultures and named P712, Ch, 2ab/KP<sub>2</sub> of 10/10/56. Its volume was 100 ml, the pH was 8.2, the titre was  $7.3 \log_{10}$  TCID<sub>50</sub> per ml and none of the three groups of five cynomolgus monkeys each inoculated with 0.1 ml amounts of undiluted virus suspension and suspension diluted tenfold and hundredfold showed any degree of paralysis. As with the type 1 attenuated poliovirus, Merck, Sharp and Dohme Research Laboratories made a 23 l Lot by one passage of the original type 2 virus in rhesus cultures. This is the P712, Ch, 2ab/KP<sub>3</sub> (MSD, SOM or SO + 1) and aliquots were used for the field trials before it was licensed as the Sabin original vaccine and as the Sabin seed virus.

Type 3 Leon 12a<sub>1</sub>b/KP3 (Sabin Original Virus = SO)

The Leon virus was obtained from the brain-stem and spinal cord of an 11-year-old boy, who had died of bulbo-spinal poliomyelitis in Los Angeles in 1937. It was isolated by Drs Kessel and Stimpert in rhesus monkeys and maintained in the same species by the intracerebral route for 20 subsequent passages in 1951. It underwent eight further passages in rhesus monkey testicular tissue culture before the strain was sent by Dr Melnick to Dr Sabin (Sabin *et al.*, 1954). After three passages in cynomolgus monkey kidney cell cultures the virus produced prostrating paralysis within 4-6 days in each of four intracerebrally inoculated cynomolgus monkeys. Thirty rapid passages at *c.* 24-h intervals, using large inocula ( $10^5$  to  $10^6$  TCID<sub>50</sub>) were carried out in cynomolgus kidney cultures. These were succeeded by three terminal dilution passages, followed by one passage using a large inoculum of the progeny of the third terminal dilution. This strain, Leon KP34, exhibited a marked reduction in its neurovirulence in that none of the 28 cynomolgus monkeys inoculated intracerebrally with  $7.2 \log_{10}$  TCID<sub>50</sub> per ml developed either clinical or histological poliomyelitis. The progeny from nine selected plaques, after purification by three consecutive plaque passages, was subjected to the neurovirulence test in three groups of cynomolgus monkeys inoculated intraspinally with 6.0, 5.0 and  $4.0 \log_{10}$  TCID<sub>50</sub> of virus. The progeny designated as 12a<sub>1</sub>b showed the least neurovirulence and was selected for the production of vaccine (Sabin, 1956). This strain was passaged three times in cynomolgus monkey kidney cell cultures to give the original type 3 virus (SO) named Leon 12a<sub>1</sub>b/KP<sub>3</sub> of 10/10/56 (Sabin 1957). The volume was 10 ml, the pH was 6.8, the titre was  $6.5 \log_{10}$  TCID<sub>50</sub> per ml and three groups of five cynomolgus monkeys were each inoculated intraspinally with 0.1 ml amounts of undiluted virus suspension as well as  $10^{-1}$  and  $10^{-2}$  dilutions. The monkeys receiving the undiluted material and those inoculated with suspension diluted one hundredfold remained symptomless, whereas one of the five animals which had the tenfold dilution showed minimal clinical signs and focal histological poliomyelitis adjacent to the site of injection in the lumbar cord. Merck, Sharp and Dohme prepared a Lot of 251 by one passage (as with types 1 and 2) using the original type 3 virus (SO). This Lot is Leon 12a<sub>1</sub>b/KP<sub>4</sub> (MSD, SOM or SO+1) and was used in the field trials before being licensed as the Sabin original vaccine and as the Sabin seed virus.

The three types of the large Lots produced by Merck, Sharp and Dohme in rhesus monkey kidney cell cultures contained SV40 (W.H.O., 1969).

*Alternative Leon 12a<sub>1</sub>b vaccine strains*

The original SO type 3 virus which was free of SV40 was supplied to Lederle Laboratories, who prepared their seed Lot (No. 45B-85) by one passage of the original virus, previously mixed with SV40 antiserum, in cercopithecus monkey kidney cell cultures; this was then used to make a larger Lot, designated No. 3-393. This material was approved by the Division of Biologics Standards, United States Public Health, and it represents passage level SO+2 (W.H.O., 1969). Aliquots have been used by some manufacturers for the production of working seed, i.e. SO+3 so that their vaccines represented SO+4

The original SV40-free type 3 virus (SO) was also given to other manufacturers, of whom two prepared working seed in one passage, i.e. SO+1, so that their vaccines represented the second cell culture passage level or SO+2. The other producers made a further cell culture passage before preparing the working seed, i.e. SO+2 meaning their vaccines were third passage level or SO+3. Another manufacturer prepared their working seed

by two consecutive cell culture passages of the SOM virus, i.e. SO+3 so that their vaccines were fourth passage level or SO+4.

The majority of the manufacturers receiving the Sabin seed virus (SOM or SO+1) types 1, 2 and 3 made their working seeds by one passage to free SOM from SV40. Hence their vaccines were third passage level or SO+3 (W.H.O., 1969). One producer (Chumakov, Dzagurov, Lashkevich, Grachov, Mironova, Ralph & Elbert, 1964) freed SOM+2 from SV40 by 2 heat treatments at 34°C in the presence of 1 M MgCl<sub>2</sub>, after which six plaques were selected and grown on vervet monkey cell cultures and pooled to form working seed. This was then subjected to one more heat treatment at 34°C in the presence of magnesium chloride. The seed virus represents SOM+5 or SO+6 so that the vaccines were SOM+6, i.e. SO+7. Finally, another manufacturer (Stones, MacDonald, McDougall & Ramsbottom, 1964) grew SOM type 3 in vervet monkey cells in the presence of SV40 antiserum. This material was then subjected to phenol extraction and plaque purification to form an RNA working seed equivalent to SOM+4 and RNA vaccines to SOM+5, i.e. SO+6.

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