Intervirology *20:* 129–136 (1983)

0300-5526/83/0203-0129$2.75/0

Monoclonal Antibodies to Polioviruses

Comparison of Intratypic Strain Differentiation of Poliovirus Type 1 Using Monoclonal Antibodies versus Cross-Absorbed Antisera

Albert D. M. E. Osterhaus, Anton L. van Wezel, Ton G. Hazendonk, Fons G. C. M. UytdeHaag, Jack A. A. M. van Asten, Bert van Steenis

National Institute of Public Health, Bilthoven, The Netherlands

Key Words. Monoclonal antibodies · Intratypic serodifferentiation · Poliomyelitis virus

Summary. A panel of 10 monoclonal antibodies raised to 3 different poliovirus type 1 strains was tested in a micro-enzyme-linked immunosorbent assay and in a micro-neutralization test against 87 poliovirus type 1 strains. The results, evaluated in a newly developed system for intratypic strain characterization, were compared with the results obtained with the classical serodifferentiation system by using a small number of strain-specific, cross-absorbed antisera. The new system not only uses results obtained with strain-specific antibody preparations, but also uses the information obtained with monoclonal antibodies reacting with less unique antigenic determinants. In a theoretical pattern fitting computer program, each virus strain could be compared with all the other strains for which serological data were stored in the memory of the computer. The results obtained with the new system coincided well with those obtained with the classical system: all except one of the strains classified as Sabin-like or intermediate in the classical system scored 'perfect fit' or 'related' with the Sabin 1 vaccine strain in the new system. Likewise, all virus isolates classified as Kuwait-like in the classical system scored 'perfect fit' or 'related' with the Dutch Kuwait-like isolate strain 78-9030.

Although controlled well in developed countries, poliomyelitis remains a major problem in the developing world. In nonvaccinated populations, epidemics of poliomyelitis are mainly caused by poliovirus type 1, whereas the very few cases associated with the use of live attenuated vaccines are usually caused by poliovirus type 2 or 3 [1]. Precise methods of strain characterization of polioviruses are considered essential for understanding the epidemiology and for studies of vaccine efficacy. Apart from biochemical analysis of the viral genomes, which is restricted to specialized laboratories [2, 3], the most widely used method for this purpose is intratypic serodifferentiation with strain-specific antibody prepara-

Address inquiries to: Dr. A. D. M. E. Osterhaus, National Institute of Public Health, PO Box 1, 3720 BA Bilthoven (The Netherlands)

Received: February 7, 1983

tions obtained by cross-absorption of antisera with heterologous virus strains [4]. Recently, different groups have shown that, instead of cross-absorbed strain-specific antisera, monoclonal antibodies (MoAbs) produced by murine lymphocyte hybridomas can be used, indicating that high-titered homogenous standard preparations could become available for reference purposes [5–7]. Specific MoAbs which we had raised against the 3 Sabin vaccine strains proved to be highly specific for the reference strains and for vaccine-associated field isolates. However, a number of the strains which were recognized by a cross-absorbed anti-Sabin serum and therefore characterized as Sabin-like did not react significantly with the Sabin-specific MoAbs. This clearly showed the higher specificity of these MoAbs and indicated the need for panels of different MoAbs to perform the proper characterization on the basis of a more extensive antigenic mapping [7].

In the present paper the results obtained with 3 cross-absorbed antisera and 10 MoAbs tested in a micro-enzyme-linked immunosorbent assay (micro-ELISA) and a micro-neutralization test (micro-NT) against 87 different poliovirus type 1 strains are shown. The classical system of intratypic classification with a small number of cross-absorbed antisera is compared with a new classification system using the results obtained with panels of antibody preparations in a theoretical pattern fitting (TPF) computer program.

Materials and Methods

Viruses and Absorbed Antisera

87 different poliovirus type 1 strains were used in these studies. They were selected and clustered partly on the basis of their reactivities with strain-specific absorbed antisera and partly on the basis of geographical distribution. 7 of these strains were isolated by Dr. *Magrath* (National Institute for Biological Standards and Control, London) from an immunodeficient child after oral polio vaccination during the period 1961–1963 [8]. For use as antigens in the micro-ELISA the respective virus strains were propagated in 75-cm^2 monolayer cultures of secondary cynomolgus (*Macaca fascicularis*) kidney cells. After complete CPE had developed, the cultures were frozen and thawed twice and the supernatants were used as antigens after low-speed centrifugation. These preparations were also used in micro-NT after dilution to 100 $TCID_{50}$ per inoculum.

Specific rabbit antisera – anti-Sabin-1-like (anti-SL), anti-Kuwait-like (anti-KL) and specific anti-poliovirus type 1 nonreactive with SL strains (NSL) – were prepared by absorption with heterologous virus strains as previously described [4]. In brief, specific anti-SL was prepared by absorption of anti-Sabin 1 serum with Mahoney virus; anti-KL by absorption of anti-Kuwait serum with Sabin 1 virus and Mahoney virus; and anti-NSL by absorption of a mixture of anti-Mahoney and anti-Kuwait sera with Sabin 1 virus.

Monoclonal Antibodies

A panel of 10 MoAbs which we generated in the course of previous studies [7] was selected. The MoAbs were produced by lymphocyte hybridomas which had been cloned at least twice by limiting dilutions and were propagated either in syngeneic Balb/c mice by passaging them as solid or ascitic tumors or in cell cultures. Ascitic fluids or cell culture media were used in dilutions as antibody preparations. MoAbs 14D2 and 17C5 were generated against the Mahoney laboratory strain; MoAbs 7D7, 5D9, 7D5 and 7E8 against the Dutch isolate strain 78-9030; and MoAbs 2F4, 3D8, 4E6 and 1D4 against the Sabin LSc2ab vaccine strain. MoAbs raised against strain 78-9030 were used as cell culture media; the others were used as ascitic fluids.

Serological Assays

All MoAbs and absorbed antisera were tested in a micro-ELISA and a micro-NT with all 87 poliovirus type 1 strains, essentially as previously described [7]. In brief, the micro-ELISA was carried out as follows. Microtiter trays were coated with a standard dilution of a bovine anti-poliovirus type 1 IgG preparation as a catching antibody for the poliovirus antigen, which was used in excess. The test sample was added and a horseradish peroxidase-conjugated sheep anti-mouse Ig preparation was used with 5-aminosalicylic acid and H_2O_2 as a substrate. For testing a MoAb or absorbed

antiserum with heterologous virus strains, the highest antibody dilution showing a maximal extinction value ($E_{450\ nm}$) in the homologous system was determined. This dilution was also used in heterologous assays. Extinction values ($E_{450\ nm}$) $\times 10^3$ were used to compare the results of different tests. For testing a MoAb or a cross-absorbed antiserum with heterologous virus strains in the micro-NT, the dilution of the antibody preparation was determined which showed a neutralization titer of 512 in the homologous system using 100 $TCID_{50}$. This dilution was used to test heterologous virus strains. Micro-NTs carried out with MoAbs were read after 3 days instead of 5 days to prevent problems with incomplete neutralization due to possible low affinity of the antibodies.

Classical System of Intratypic Strain Classification

Strains were classified into 4 groups on the basis of results with absorbed antisera in micro-ELISA and micro-NT: (i) Strains with a positive reaction with the specific anti-NSL and a negative reaction with the anti-SL were classified as NSL strains. (ii) Strains with a positive reaction with the specific anti-SL and a negative reaction with the anti-NSL were classified as SL strains. (iii) Strains with a positive reaction with both the anti-NSL and the anti-SL were classified as SL(IM) strains and were considered to be related to Sabin 1 virus. (iv) Strains with a positive reaction to the specific anti-KL were classified as NSL-KL strains.

Comparison of Serological Test Results by Means of TPF

To detect similarities in serological reaction patterns between two virus strains a TPF computer program was developed. The results obtained in either the micro-ELISA or the micro-NT with the panel of 10 MoAbs or the panel of 3 absorbed antisera for a certain virus strain were matched with those obtained by testing a reference virus strain with the same panels in the same way. The difference obtained with each antibody preparation between reference virus and test virus was quadrated and summated with the differences obtained with other antibody preparations. This resulted in a panel of 4 nonfitting values (nfv) per virus strain tested against 1 reference strain: (i) a nfv based on the summation of the differences in micro-ELISA obtained with the 10 MoAbs = E_{10}; (ii) a nfv based on the summation of the differences in micro-NT obtained with the 10 MoAbs = N_{10}; (iii) a nfv based on the summation of the differences in micro-ELISA obtained with the 3 absorbed antisera = E_3; and (iv) a nfv based on the summation of the differences in micro-NT obtained with the 3 absorbed antisera = N_3.

Based on test results, nfv limits were chosen to classify each of the virus strains tested into one of the three following categories of fitting with the reference strain used: perfect fit, related, or nonrelated. For example, the following limits were chosen when the Sabin 1 vaccine strain was used as a reference:

Perfect fit:	$E_{10}<10$	$N_{10}<100$	$E_3<1$	and	$N_3<10$
Related:	$10\leq E_{10}\leq 50$	$100\leq N_{10}\leq 200$	$1\leq E_3\leq 10$	and	$10\leq N_3\leq 50$
Nonrelated:	$E_{10}>50$	$N_{10}>200$	$E_3>10$	and	$N_3>50$

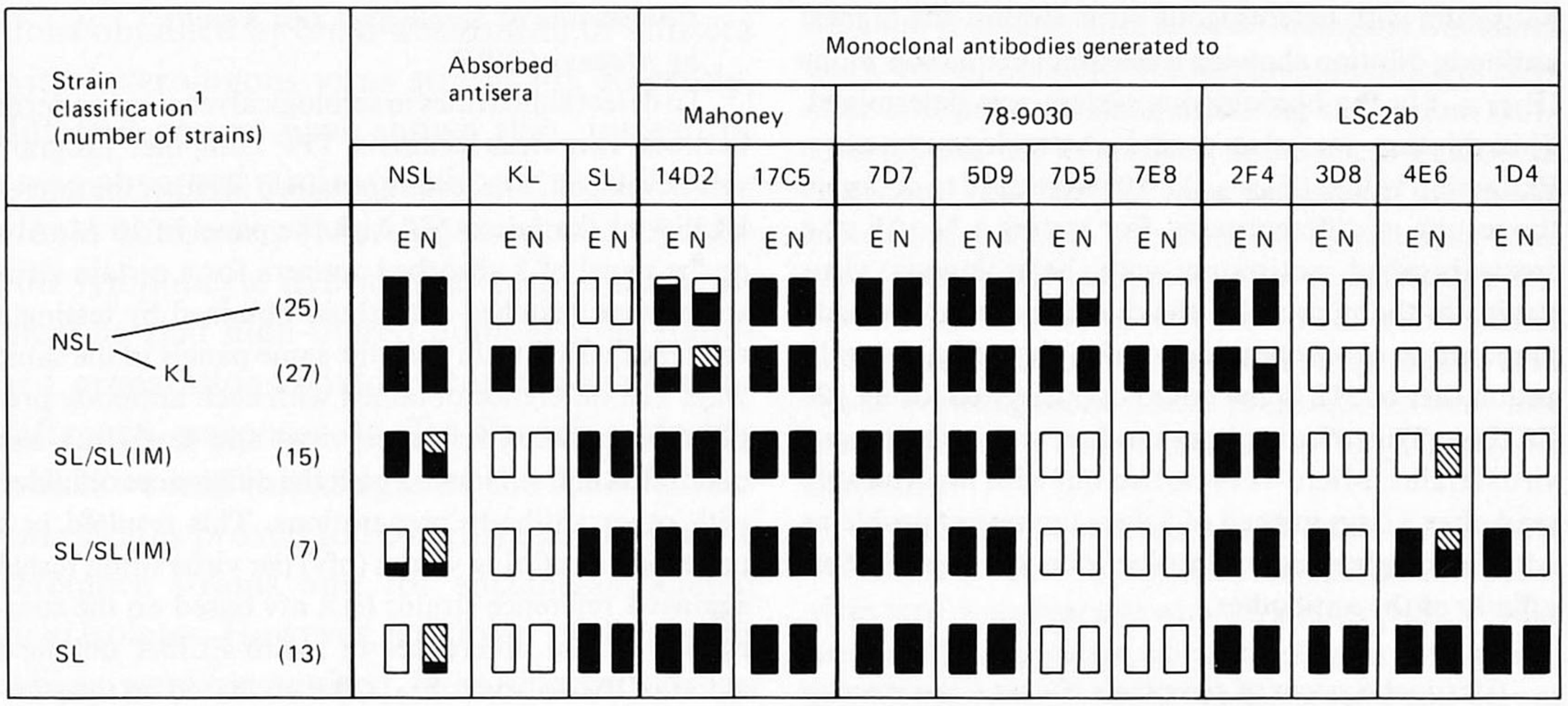
Results

Strain Characterization with Specific Antibody Preparations

To assess the specificities of the 3 absorbed antisera and the 10 MoAbs for the 87 different poliovirus type 1 strains, they were tested by both micro-ELISA and micro-NT against all the virus strains. The results of these tests are summarized in figure 1.

With the specific cross-absorbed SL serum a clear distinction was made between SL and SL(IM) strains on the one hand and NSL strains on the other hand. Both SL and SL(IM) strains are considered to be related to the Sabin 1 virus. The specificity of the NSL serum was not absolute for NSL strains since it reacted with some SL(IM) strains in the micro-ELISA and/or micro-NT and also with SL strains in the micro-NT. With the anti-KL serum, KL strains were clearly identified.

MoAbs 14D2 and 17C5, raised to the Mahoney strain, were nonspecific in both test systems. Also, MoAbs 7D7 and 5D9 raised to



[1]Based upon reactivity with absorbed antisera.

Fig. 1. Intratypic differentiation of poliovirus type 1 strains with absorbed antisera and MoAbs. ■ = Strains positive in micro-ELISA (E) or micro-NT (N). ◪ = Strains positive (black) and negative (white) in micro-ELISA or micro-NT. ▨ = Strains positive in micro-NT after 3 days, negative after 5 days. □ = Strains negative in micro-ELISA or micro-NT. NSL = Non-Sabin-like; KL = Kuwait-like; SL = Sabin-like; IM = intermediate.

KL strain 78-9030 from The Netherlands and MoAb 2F4 raised to the Sabin 1 strain reacted with almost all 87 virus strains in both test systems. In contrast, MoAb 7D5 raised to strain 78-9030 reacted exclusively with the KL and some of the other strains of the NSL cluster. MoAb 7E8, which was also raised to strain 78-9030, was even more specific for KL strains and proved to possess the same specificity as the anti-KL specific absorbed antiserum: both preparations recognized the same NSL strains. The higher specificity obtained with the 3 anti-Sabin 1 MoAbs 3D8, 4E6 and 1D4, as compared with the specific anti-SL absorbed antiserum, is also shown in figure 1 and has been reported previously [7]. On the basis of these specificities, the specific MoAb 7E8 could replace the absorbed specific anti-KL serum for classification of KL strains and the 3 specific anti-Sabin 1 MoAbs could replace the specific anti-SL serum for classification of SL and some of the SL(IM) strains.

Strain Characterization with Panels of Antibody Preparations

We wanted to determine the value of panels of MoAbs (and/or absorbed antisera) as an alternative for the use of strain-specific antibody preparations for strain characterization. Therefore, the serological test results obtained with the different poliovirus type 1 strains were compared with those obtained with the Sabin 1 vaccine strain and with the Dutch KL isolate strain 78-9030, using the TPF computer program. A selection of the results is shown in table I. Most of the strains which had been classified SL with the absorbed antisera in the classical system scored 'perfect fit' with the

Table I. Intratypic characterization of a selection[1] of the 87 poliovirus type 1 strains with the classical system and with the TPF computer program[2]

Virus code	Classical characterization with absorbed antisera		nfv-Sabin 1 reference				nfv 78-9030 reference			
	E	N	E_{10}	N_{10}	E_3	N_3	E_{10}	N_{10}	E_3	N_3
LSc2ab (Sabin)	SL	SL	×	×	×	×	−	−	−	−
881	SL	SL	×	×	×	×	−	−	−	−
I2141	SL	SL	×	×	×	×	−	−	−	−
I2140	SL	SL	+	+	+	×	−	−	−	−
I2136	SL(IM)	SL(IM)	+	+	+	×	−	−	−	−
Staphorst 22	SL(IM)	SL(IM)	+	+	+	+	−	−	−	−
9770	SL	SL(IM)	−	−	+	+	−	+	−	−
Mahoney	NSL	NSL	−	−	−	−	−	+	−	−
78-9030	NSL(KL)	NSL(KL)	−	−	−	−	×	×	×	×
576/69/3	NSL(KL)	NSL(KL)	−	−	−	−	×	×	×	×
LSO-4958	NSL(KL)	NSL(KL)	−	−	−	−	×	×	×	×
104324	NSL(KL)	NSL(KL)	−	−	−	−	×	×	+	×
D268	NSL(KL)	NSL(KL)	−	−	−	−	×	+	×	×
LSO-3868	NSL(KL)	NSL(KL)	−	−	−	−	×	×	+	+
301/66/3	NSL(KL)	NSL	−	−	−	−	+	+	−	+
314/66/3	NSL(KL)	NSL	−	−	−	−	+	+	−	+

[1] Readers wishing full details on all 87 strains tested should request this information from the authors.

[2] Results of computer comparisons of the serological data obtained with the 87 strains in micro-ELISA (E) and micro-NT (N) using the panel of 10 MoAbs (E_{10}, N_{10}) or the 3 absorbed antisera (E_3, N_3) and those obtained with 2 reference strains (Sabin 1 strain and Dutch isolate strain 78-9030) are expressed in the following nfv: − = 'nonrelated'; + = 'related'; and × = 'perfect fit'. For limits chosen, see 'Materials and Methods'.

Table II. Intratypic characterization of 7 poliovirus isolates from an immunodeficient child with the classical system and with the TPF computer program[1]

Virus code	Classical characterization with absorbed antisera		nfv-Sabin 1 reference				nfv-1357 (NSL) reference			
	E	N	E_{10}	N_{10}	E_3	N_3	E_{10}	N_{10}	E_3	N_3
Aspinall 18XI61	SL	SL	×	+	×	×	−	−	−	−
Aspinall 3XII61	SL	SL	+	+	×	×	−	−	−	−
Aspinall 1I62	SL	SL	×	×	×	×	−	−	−	−
Aspinall 18II62	SL(IM)	SL(IM)	+	+	+	×	+	−	+	−
Aspinall 7III62	NSL	NSL	−	−	−	−	×	×	×	×
Aspinall 22XI62	NSL	NSL	+	+	−	−	×	+	×	×
Aspinall 12VI63	NSL	NSL	−	−	−	−	×	×	×	×

[1] Results of computer comparisons of the serological data obtained with the 7 isolates and those obtained with 2 other strains serving as references (Sabin 1 strain and NSL strain 1357) are expressed in nfv as in table I. Different nfv limits were chosen for each reference strain.

Sabin 1 vaccine strain in the micro-ELISA and the micro-NT with both MoAbs (E_{10}, N_{10}) and absorbed antisera (E_3, N_3). Strains classified SL(IM) scored 'related' with this strain with MoAbs and absorbed antisera in most cases. One of the 87 strains (strain 9770) which had been classified SL/SL(IM) with absorbed antisera did not show this relationship in E_{10} or N_{10}, although it did score 'related' in E_3 and N_3. A limited number of NSL strains which did not show a relationship with the Sabin 1 vaccine strain in N_{10}, E_3 or N_3 did score 'related' in E_{10}. The analysis of the relationship of all virus strains with the Dutch virus isolate 78-9030 showed a clustering of 'perfect fit' scores with MoAbs (E_{10}, N_{10}) and absorbed antisera (E_3, N_3) in both serological test systems among strains previously characterized NSL(KL). In the micro-NT a number of other strains scored 'related' to this Dutch isolate strain with MoAbs (N_{10}) and absorbed antisera (N_3). The results of the comparison of the virus isolates from the immunodeficient child with the Sabin 1 vaccine strain and an NSL isolate from a paralytic case of poliomyelitis (strain 1357) are shown in table II. The phenomenon of changing gradually from SL via SL(IM) towards NSL, as was shown with the classical system [8], was also observed with the TPF computer program. The virus isolates initially scored 'perfect fit' or 'related' with the vaccine strain and 'nonrelated' with the NSL strain. The later virus isolates gave essentially the opposite score: 'nonrelated' with the vaccine strain and 'related' or 'perfect fit' with the NSL strain. Minor discrepancies were found between scores obtained with MoAbs and absorbed antisera.

In the same way, each of the strains could be checked for its antigenic relationship with any of the other strains. Also each newly isolated strain may, after serological testing, be introduced into the computer system and immediately checked for antigenic relationships with the other strains.

The nfv limits may also be used to determine relative fitting values (rfv), which are defined as follows: rfv = 3 nfv/nonrelated limit. The computer program enables us to produce a 3-dimensional plot of the rfv of a certain strain to 3 reference strains. This gives a rapid visual impression of the relatedness of a strain to relevant reference strains. An example is given in figure 2 which, on the basis of rfv obtained with the micro-ELISA results, shows that the Dutch isolate strain 78-9030 is closely related to the Kuwait strain LSO 4958 and not to the Sabin 1 or the Mahoney strain.

Discussion

For the intratypic strain characterization of poliovirus type 1, some of the strain-specific MoAbs proved to be valuable tools that may replace cross-absorbed, strain-specific antisera in the classical system, offering the advantages mentioned above. MoAb 7E8 generated against the Dutch isolate strain 78-9030 showed the same specificity for KL strains as a cross-absorbed antiserum developed for this purpose. With the 3 SL-specific MoAbs (3D8, 4E6 and 1D4), SL and SL(IM) strains were specifically recognized. However, a number of the strains designated SL or SL(IM) on the basis of their reaction patterns with specific anti-NSL and anti-SL cross-absorbed antisera in the micro-ELISA were not recognized by these MoAbs (fig. 1), although most of these strains were recognized by MoAb 4E6 in the micro-NT.

MoAbs offer a new opportunity to characterize strains not only on a qualitative basis – the presence of specific antigenic determinants

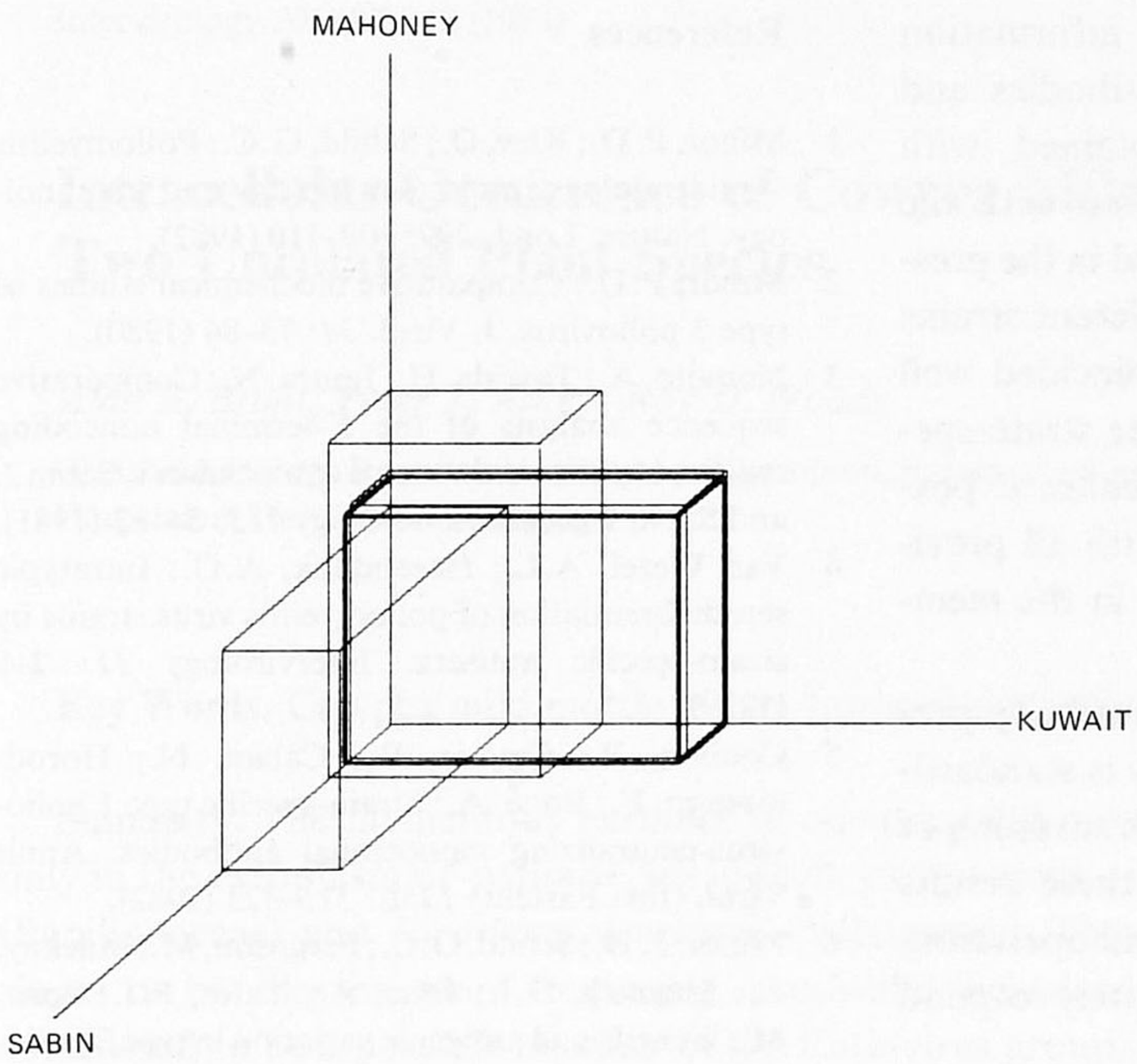


Fig. 2. Visual impression of the relatedness between poliovirus type 1 strain 78-9030 and 3 type 1 reference strains (Mahoney, Kuwait and Sabin) obtained by a 3-dimensional computer plot of rfv obtained with the micro-ELISA results. Dark box is formed by rfv of strain 78-9030, light boxes by rfv of the respective reference strains.

– but also on a quantitative basis, by providing information about the presence of other less unique antigenic determinants. For this purpose we designed a comparison system, in which five main criteria were taken into account: (i) Observed differences should not be leveled out by the comparison method. (ii) A large difference must be considered of greater importance than a number of relatively small differences. (iii) There should be a variable indicating the extent to which virus strain and reference strain match. (iv) Slight differences in titer of antibody or virus preparation do occur, which makes a real 'perfect fit' very unlikely to occur. (v) The method should be readily applicable to large numbers of samples using a cumulating number of reference preparations.

By defining the nfv as the sum of the quadrates of the differences in test results, the first 3 demands are met. The 4th demand is met by setting limits other than nfv = 0 to the 'perfect fit' classification. Adequate programming and modern computer systems easily take care of the 5th demand. The TPF program developed was tested in more than 45,000 nfv calculations. The nfv limits resulting from a comparison of the results obtained with the MoAbs in both serological systems with previous classifications made with cross-absorbed antisera were then set (see 'Materials and Methods').

It should be emphasized that this computerized system, using the results of serological tests obtained with MoAbs, depends entirely on the quality and the number of the MoAbs used. We do not pretend to have offered an optimal panel of MoAbs for this purpose but have shown that in principle the system may offer a better alternative for strain characterization with a small number of specific antisera.

This is because it uses both the information obtained with strain-specific antibodies and the additional information obtained with MoAbs of other specificities. Even with the suboptimally selected MoAbs used in the present study, comparison of the different strains with the Sabin vaccine strain coincided well with the classical system using the strain-specific antisera. The system also makes it possible to compare a new strain with all previously tested strains accumulated in the memory of the computer.

Once the distribution of the antibody preparations over the microtiter tray is standardized, it will be possible to combine scanning of the results and comparison of these results with stored data to a one-command operation, which will run a scan and immediately respond with the best-fitting references.

This system may also be used for intratypic strain characterization of other viruses where slight antigenic changes rapidly occur, e.g., influenza viruses.

Acknowledgments

The authors thank Mr. *G. Drost*, Mr. *J. van der Lee*, Mr. *C. Siebelink* and Mr. *M. van Olderen* for skillful technical assistance and Ms. *C. Kruyssen* for preparation of the manuscript.

References

1 Minor, P.D.; Kew, O.; Schild, G.C.: Poliomyelitis – epidemiology, molecular biology and immunology. Nature, Lond. *299:* 109–110 (1982).

2 Minor, P.D.: Comparative biochemical studies of type 3 poliovirus. J. Virol. *34:* 73–84 (1980).

3 Nomoto, A.; Toyoda, H.; Imura, N.: Comparative sequence analysis of the 5′-terminal noncoding regions of poliovirus vaccine strain Sabin 1, Sabin 2, and Sabin 3 genomes. Virology *113:* 54–63 (1981).

4 Van Wezel, A.L.; Hazendonk, A.G.: Intratypic serodifferentiation of poliomyelitis virus strains by strain-specific antisera. Intervirology *11:* 2–8 (1979).

5 Couillin, P.; Crainic, R.; Cabau, N.; Horodniceanu, F.; Boué, A.: Strain-specific type 1 poliovirus-neutralizing monoclonal antibodies. Annls Virol. (Inst.Pasteur) *133E:* 315–323 (1982).

6 Minor, P.D.; Schild, G.C.; Ferguson, M.; Mackay, A.; Magrath, D.I.; John, A.; Yates, P.J.; Spitz, M.: Genetic and antigenic variation in type 3 polioviruses: characterization of strains by monoclonal antibodies and T1 oligonucleotide mapping. J. gen. Virol. *61:* 167–176 (1982).

7 Osterhaus, A.D.M.E.; van Wezel, A.L.; van Steenis, B.; Drost, G.A.; Hazendonk, T.G.: Monoclonal antibodies to polioviruses: production of specific monoclonal antibodies to the Sabin vaccine strains. Intervirology *16:* 218–244 (1981).

8 Kapsenberg, J.G.; Coutinho, R.A.; Hazendonk, A.G.; Ran, A.B.R.; van Wezel, A.L.: Epidemiological implications of the isolations and intratypic serodifferentiation of poliovirus strains in the Netherlands. Dev. biol. Standard., vol. 47, pp. 293–301 (Karger, Basel 1981).