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# DIFFERENTIATION OF INFLUENZA A VIRUS NUCLEOPROTEINS IN ENZYME-LINKED IMMUNOSORBENT ASSAY

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*Summary.* — The serum antibody titre to the nucleoprotein (NP) of the influenza virus recombinant MRC-11 was determined in virus strains A/USSA/5/80 (H3N2), A/Hong Kong/8/64 (H3N2), A/duck/Ukraine/63 (Hav7Neq2) and in a recombinant strain betwen A/tern/Frunse/334/78(Hav4Nav1) and A/PR/8/34(H0N1) using the enzyme-linked immunosorbent assay (ELISA). Significant differences between the NP of these strains were found proving the usefulness for ELISA for such investigations.

*Key words*: *influenza virus*; *nucleoproteins*; *antigenic determinants*; *enzyme linked immunosorbent assay*

## *Introduction*

In contrast to the high variability of haemagglutinin (HA) and neuraminidase (NA) of influenza virus, the antigenic structure of internal proteins i.e. of the nucleoprotein (NP) and of the matrix protein (M), belongs to the best conserved ones. However, sensitive biochemical approaches like polyacrylamide gel electrophoresis, peptide- and oligonucleotide mapping and hybridization techniques revealed mutational changes in these polypeptides or in their corresponding RNA segments (Palese and Schulman, 1976; Ritchey *et al.*, 1976, 1977, Scholtissek, 1977, Oxford *et al.*, 1978, Young *et al.*, 1979, Dimmock *et al.*, 1980, Sokolov, 1980). The first direct evidence of antigenic variations of NP was given by Schild *et al.* (1979) and Zakstelskaya 1979, Dimmock *et al.*, 1980, Sokolov, 1980). The first direct evidence of antigenic variations of NP was given by Schild *et al.* (1979) and Zakstelskaya *et al.* (1979). Using monoclonal antibodies Van Wyke *et al.* (1980) could divide the NP of human influenza virus strains isolated from 1933 to 1979 into 6 groups.

Herrmann (1978a) and Mohr *et al.* (1978) demonstrated several possibilities of the application of ELISA in the study of the antigenic structure of influenza viruses. In this communication we report the usefulness of ELISA for differentiation of various influenza A virus NP.

## *Materials and Methods*

Viruses and sera. The strains A/USSR/5/80 (H3N2), A/Hong Kong/8/64 (H3N2), and A/duck/ /Ukraine/63 (Hav7Neq2) were coming from the collection of the Ivanovsky Institute of Virology in Moscow. The strain A (H0Nav1) was prepared as a recombinant of A/tern/Frunse/334/78 (Hav4Nav1) and A/PR/8/34(H0N1). The viruses were concentrated from infected allantoic fluids by sedimentation (60,000 g, 90 min), purified in sucrose gradients (10—40%) and resuspended in ST-buffer solution (0.01 M Tris-HCl, 0.1 M NaCl, pH 7.4). Protein concentrations were determined in 1% SDS solution according to Kalb and Bernlohr (1977).

Immune serum against the NP of the virus recombinant MRS-11 (H3N2) was prepared in rats. The NP of this virus derived from A/PR/8/34 (Zakstelskaya *et al.*, 1979) and was isolated by the method of Zakstelska a *et al.* (1978). Immune serum to the NP of the strain A/USSR/90/77 and the antiserum against purified rat IgG were raised in rabbits.

ELISA was carried out as a four-layer assay using buffer solutions corresponding to those of Voller *et al.* (1976). Disposable polyvinyl chloride (PVC) trays were coated with rabbit anti-NP IgG prepared against strain A/USSR/90/77 (H1N1); 5 μg/ml IgG has been dissolved in sodium carbonate buffer (0.1 mol/l, pH 9.6) containing 0.2% sodium azide (100 μl per swell). After overnight at 4 °C the were washed 3 times with phosphate buffered saline (0.01 mol/l phosphate, 0.145 mol/l NaCl, pH 7.2) containing 0.5% Tween 20 (PBS/Tween 20). Then to each well 100 μl antigen was added containing 200 μl/ml virus splitted by 1% Triton X-100 and suspended in PBS/ /Tween 20. After incubation for 3 hr at 30 °C the wells were washed 5 times with PBS/Tween 20, then 100 μl of the rat anti-NP serum to the MRC-11 recombinant was added (diluted in the range from 1/500 to 1/6,000). After a further incubation for 3 hr at 30 °C and washing, each well received

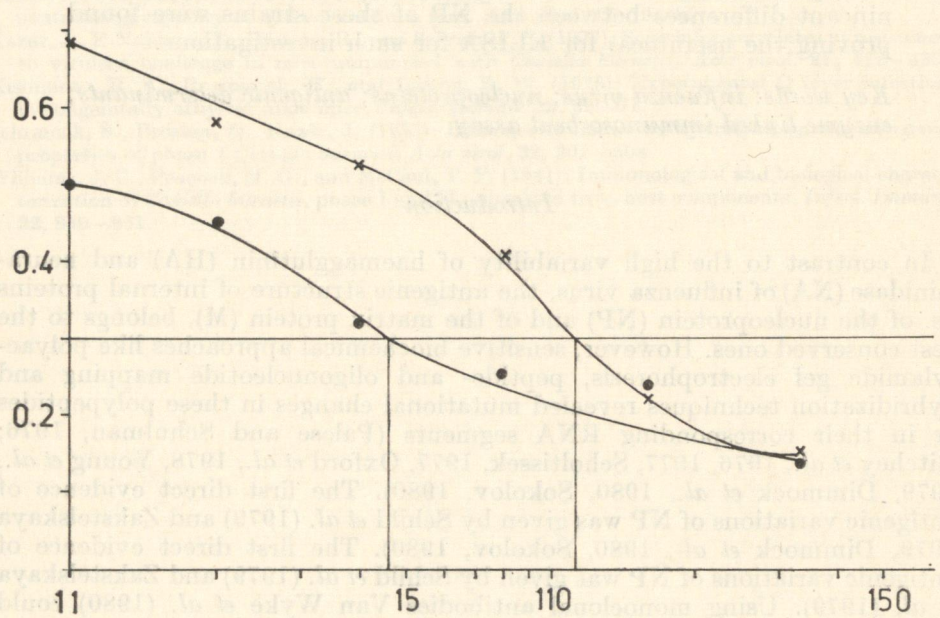


**Fig. 1.**

Antigenic differences between the nucleoprotein (NP) of influenza virus strains Strains A/USSR/5/80 (H3N2) (×——×) and A/duck/Ukraine/63 (Hav7Neq2 (●——●) differ by titration of the anti-NP serum with MRC-11 NP antigens. The endpoint titre is defined as extinction $E_{400nm} = 0.300$.
Absicssa: extinction; ordinate: serum dilution reciprocals (in thousands).

**Table 1. Demonstration of antigenic differences between the NP of various influenza A virus strains**

| Antigen | Titre* (M ± SD) |
|---|---|
| A/USSR/5/80 (H3N2) (stock I) | 11 000 ± 1 300 |
| A/USSR/5/80 (H3N2) (stock II) | 13 600 ± 5 200 |
| A/Hong Kong/8/69 (H3N2) | 8 100 ± 100 |
| A/duck/Ukraine/63 (Hav7Neq2) | 4 600 ± 750 |
| Recombinant virus (H0Nav1)** | 6 800 ± 350 |

* Anti-NP serum dilution reciprocals (means of at least 3 determinations).
** A/tern/Frunse/334/78 (Hav4Nav1) × A/PR/8/34 (H0N1).

100 µl of anti-rat globulin labelled with alkaline phosphatase (incubated overnight at 30 °C). The enzyme-labelled antibodies bound to antigen were estimated after addition of 300 µl 4-nitro--phenylphosphate in diethanolamine buffer solution (pH 9.6) after incubation for 6 min at 30 °C. The extinction at 400 nm was recorded using the photometer "Spekol" complemented with the measuring device EKA (VEB Carl Zeiss Jena, GDR).

## *Results and Discussion*

To find out whether there are antigenic differences between the NP of various influenza virus strains, the rat anti-NP serum (MRC-11) was tested with the NP of the above mentioned strains. The antibody titres were defined as those serum dilutions which gave an extinction ($E_{400nm}$) of 0.300 (Hermann, 1978b). A typical result can be seen in Fig. 1 showing clear cut differences in the anti-NP serum titres as well as in the maximal extinctions using the strain A/USSR/5/80 and A/duck/Ukraine/63. No significant differences in the titres were observed with two different strain A/USSR/5/80 (I and II) preparations (Table 1). The titres with all other strains significantly ($P \leq 0.05$) differed from each other. This means that the NP of the tested strains posess distinct differences of their antigenic structure.

Our results are in accordance with those of Zakstelskaya *et al.* (1979), Schild *et al.* (1979), and Van Wyke *et al.* (1980) who clearly showed that the antigenic structure of the influenza A virus NP undergoes some changes. The remarkable difference between the NP of the strains A/USSR/5/80 and A/duck/Ukraine/63 remains to be further investigated to test whether it does not indicate a more common difference between human and avian influenza virus strains. ELISA makes such investigations rather easy. In order to get reliable results, however, some basic conditions have be fullfilled. The first antibody layer which is attached to the PVC blisters should contain antibodies mainly to NP. The antigen should be used after disruption by a suitable detergent and in a concentration sufficient for a complete saturation of the antibodies of the first layer. As to the third layer it is essential, that the anti-NP serum does not contain any antibodies against other viral proteins except the NP and in the case that it is not labelled directly with an enzyme, it should differ in species specificity from that of the first layer.

## References

Dimmock, N. J., Carver, A. S., and Webster, R. G. (1980): Categorization of nucleoproteins and matrix proteins from type A influenza virus by peptide mapping. *Virology* **103**, 350—356.

Herrmann, H. (1978*a*): Differenzierung von Influenzavirus-Antigenen mit dem Enzym-Immunoassay. *Arch. exp. Vet.-Med.* **32**, 489—494.

Herrmann, H. (1978*b*: Enzym-Immunoassay mit PVC-Tiefzieh-Blistern als Träger. *Z. med. Labor.-Diagn.* **19**, 152—156.

Kalb, V. F., and Bernlohr, R. W. (1977): A new spectrophotometric assay for protein in cell extracts. *Anatyt. Biochem.* **82**, 362—271.

Mohr, C., Döhner, L., Herrmann, B., and Herrmann, H. (1978): Untersuchungen zur Differenzierung der Matrix-Proteine von Influenza A-Viren mit dem Enzymimmunoassay. *Arch. exp. Vet.-Med.* **32**, 495—500.

Oxford, J. S., McGeoch, D. J., Schild, G. C., and Beare, A. S. (1978): Analysis of virion RNA segments and polypeptides of influenza A virus recombinants of defined virulence. *Nature (Lond.)* **273**, 778—779.

Palese, P., and Schulman, J. L. (1976): Differences in RNA patterns of influenza A virus. *J. Virol.* **17**, 876—884.

Ritchey, M. B., Palese, P., and Schulman, J. L. (1976): Mapping of the influenza virus genome. III. Identification of genes coding for nucleoprotein, membrane protein and non-structural protein. *J. Virol.* **20**, 307—313.

Ritchey, M. B., Palese, P., and Schulman, J. L. (1977): Differences in protein patterns of influenza A viruses. *Virology* **76**, 122—128.

Schild, A. C., Oxford, J. S., and Newman, R. W. (1979): Evidence for antigenic variation in influenza A nucleoprotein. *Virology* **93**, 569—573.

Scholtissek, C. (1978): The genome of the influenza virus. *Curr. Top. Microbiol. Immunol.* **80**, 139—169.

Sokolov, V. M. (1980): Comparative study of influenza A virus nucleoproteins (in Russian). *Vop. Virus.* **25**, 553—554.

Van Wyke, K. L., Hinshaw, V. S., Bean, W. I., and Webster, R. G. (1980): Antigenic variantion of influenza A virus nucleoprotein. *J. Virol.* **35**, 24—30.

Voller, A., Bidwell, D. E., and Bartlett, A. (1976): Enzyme immunoassays in diagnostic medicine. Theory and practice. *Bull. Wld. Hlth Org.* **53**, 55—56.

Young, J. F., Desselberger, U., and Palese, P. (1979): Evolution of human influenza A viruses in nature: Sequential mutations in the genomes of new H1N1 isolates. *Cell* **18**, 73—83.

Zakstelskaya, L. Ya., Ivanova, V. T., Manykin, A. A., and Klimenko, S. N. (1978): Method of influenzavirus nucleoprotein preparation (in Russian). Avtorskoe svidetelstvo No. 650625.

Zakstelskaya, L. Ya., Ivanova, V. T., Yamnikova, S. S., and Zhdanov, V. M. (1979): Group:- and strain-specific determinants of the influenza virus nucleoproteins (in Russian). *Vop. Virus.* **24**, 105—108.