*Acta virol.* 27 : 407—411, 1983

# THE INFLUENCE OF pH AND IONIC STRENGTH ON THE SINGLE RADIAL IMMUNODIFFUSION TEST IN QUALITATIVE ASSAY OF INFLUENZA VIRUS HAEMAGGLUTININ

H. WILLKOMMEN, S. PLATEN, H. STÄBER

State Control Institute for Sera and Vaccines, DDR-1100 Berlin, G.D.R.

*Received August 16, 1982*

*Summary.* — A significant dependence between different ionic strength and pH value of virus suspension on one hand and the haemagglutinin (HA) content as determined by single-radial-immunodiffusion (SRD) test on the other hand was observed after cleavage of influenza virus recombinant NIB-6 (H1N1) with sodium lauroyl sarcosinate (SLS). In contrast, no such relationship was found when the HA of NIB-4 (H3N2) recombinant strain was determined.

*Key words*: *influenza virus*; *haemagglutinin*; *SRD test standardization*

## *Introduction*

In the WHO requirements for inactivated influenza virus vaccines the SRD test is recommended for determination of the HA content (WHO, Technical Report, 1979). An essential step in applying this test for the assay of whole virus preparations is the virus cleavage with detergents. This is to liberate the influenza virus HA for quantitative agar diffusion and for immunological reaction with corresponding incorporated antibodies.

In our studies results of the HA content of influenza strain A/USSR/90/77 (H1N1), varied according to the changes of suspending medium before virus cleavage. We aimed at answering the question whether the measurable HA content determined by the SRD test depends on different ionic strength and pH-value of the solution used for suspending the tested material. The amount of detectable HA of the recombinants NIB-4 (A/England/391/77 × × A/PR/8/34) and NIB-6 (A/USSR/90/77 × A/PR/8/34) was determ ned after cleavage with SLS in the SRD test.

## *Materials and Methods*

*Virus strains.* The lyophilized influenza A/1/79 (H3N2) reference strain, the recombinant strains NIB-4 (A/England/231/77 × A/PR/8/34) with a HA content of 29 ± 0.8 μg per vial (Willkommen *et al.*, 1981*a*) and NIB-6 (A/USSR/90/77 × A/PR/8/34) were used. The latter, a H1N1 strain, was prepared by VEB Sächsisches Serumwerk, Dresden. It was purified, stabilized by gelatine and lyophilized in 0.5 ml aliquotes. Its HA content was calibrated in SRD

test by comparison with the working reagent 78/508 of the National Institute for Biological Standards and Control (NIBSC), London (containing 40 μg HA per vial). When the H1N1-strain was reconstituted in 1.0 ml PBS, its HA content was 57 ± 4 μg per vial.

*Antisera.* Anti-HA sera against the recombinants NIB-4 (Willkommen *et al.*, 1981*a*) and NIB-6 were obtained from Prof. Döhner, Institut für Medizinische Mikrobiologie und Epidemiologie der Universität Greifswald. They were used in the SRD test in a final dilution of 1 : 350 and 1 : 400, respectively.

*Detergent for virus cleavage.* Sodium lauroyl sarcosinate (SLS), 35 w/v was supplied from Fa. FERAK, Berlin-West.

*Single-radial-immunodiffusion (SRD) test.* The test was performed as previously described (Willkommen *et al.*, 1981*b*). Briefly, the lyophilized virus preparations were reconstituted either in distilled water or in 0.15, 0.30, 0.40, 0.50 and 1.0 mol/l NaCl solutions prepared in 0.01 mol/l phosphate buffer (PB), pH 7.2—7.4. Adding the amount of NaCl contained in the lyophilisate, the final NaCl concentrations in the suspensions were 0.075, 0.15, 0.30, 0.45, 0.55, 0.65 and 1.15 mol/l. In addition, 0.1 mol/l PB pH 6.0, 7.0 and 8.0 were used for resuspending the lyophilisates. To ensure complete suspending, the suspensions were incubated for about 15 min at room temperature and subsequently treated with SLS (final concentration 1%) for virus cleavage. After 30 min incubation at room temperature the virus antigens were tested in the SRD test.

## *Results*

The SRD test for determination of the influenza virus HA is to be performed in buffered 0.15 mol/l NaCl solution (Wood *et al.*, 1977, Willkommen *et al.*, 1981*b*); no other ions should be introduced. Therefore, the tests are limited either to evaluate the influence of different saline concentrations when pH-values are constant or to evaluate the pH-values at constant salt concentration (ionic strength). Great differences were observed in HA content of the two viral strains. Namely, when the ionic concentration was reduced to 0.075 mol/l NaCl and the pH value was adjusted to 6.0, no HA

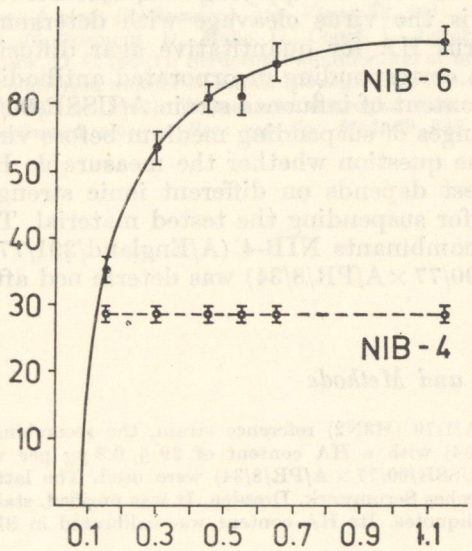


**Fig. 1.**

Influence of NaCl concentration on the HA activity of NIB-4 (H3N2) and NIB-6 (H1N1) influenza A recombinant strains as determined by SRD test

Abscissa: mol/l NaCl; ordinate: μg HA/ml (means and their standard deviations)

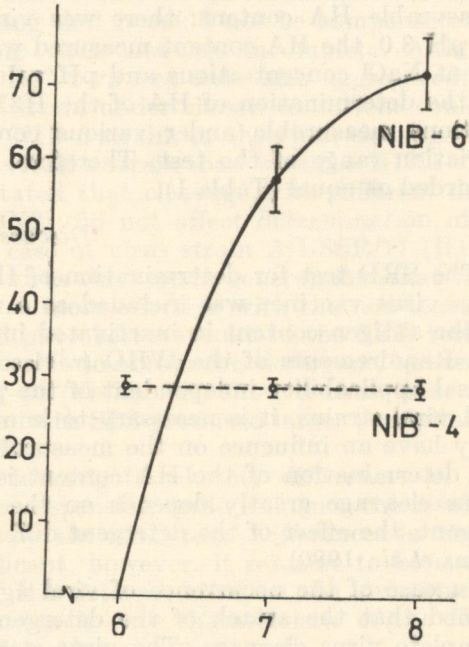


**Fig. 2.**
Influence of pH value on HA activity of NIB-4 (H3N2) and NIB-6 (H1N1) influenza A recombinant strains in SRD test
Abscissa: pH values; ordinate: μg HA/ml (means and their standard deviations)

could be detected in the recombinant NIB-6 (H1N1). On the other hand, higher NaCl concentrations led to a significant increase in the HA content determined by the SRD test. It was shown that only about 50% of the maximum amount of HA detected by the SRD test was measurable in the range of physiological ionic concentration, the maximum amount being reached only with an NaCl concentration of about 1 mol/l (Fig. 1).

**Table 1. Influence of different NaCl concentrations and pH values of the suspension medium on the HA activity of NIB-4 (H3N2) viral strain in the SRD test**

| Suspension medium | HA concentration μg/ml | | | |
|---|---|---|---|---|
| | 1* | 2* | 3* | m ± s |
| 0.15 mol/l NaCl | 29.0 | 29.0 | 29.0 | |
| 0.30 mol/l NaCl | 27.7 | 29.0 | 27.7 | |
| 0.40 mol/l NaCl | 26.8 | 28.5 | 27.9 | |
| 0.50 mol/l NaCl | 26.8 | 28.6 | 27.1 | 28.3 ± 0.8 |
| pH 6.1 | 28.1 | 29.4 | 28.8 | |
| pH 7.0 | 28.0 | 28.9 | 28.6 | |
| pH 7.9 | 28.0 | 29.8 | 28.5 | |

* Number of the trial.

A similar relationship could be observed between the pH value and the measurable HA content: there was virtually no precipitation at pH 6.0. At pH 8.0, the HA content measured was 72 ± 5.0 µg HA/ml (Fig. 2). Different NaCl concentrations and pH values of the solution had no influence on the determination of HA of the H3N2 strain (NIB-4). The HA concentrations measurable under various conditions were within the "normal" variation range of the test. Therefore, the different HA values are to be regarded as equal (Table 1).

## *Discussion*

The SRD test for determination of the HA content in inactivated influenza virus vaccines was included as a test system for the standardization of the antigen content in inactivated influenza virus vaccines, according to the Requirements of the WHO (revised in 1979). In order to get an universal applicability independent of the production technology of the vacine and viral strains, it is necessary to know and to consider all factors which may have an influence on the measurable HA content. A significant factor for determination of the HA content is the degree of virus cleavage. The virus cleavage greatly depends on the type and concentration of the detergent, the effect of the detergent differing with various viral strains (Williams *et al.*, 1980).

In case of the occurrence of viral aggregates, however, it could be presumed that the attack of the detergent was impaired resulting in an incomplete virus cleavage. The virus state in the suspension, i.e. the occurrence of single particles or aggregates can be influenced by the ionic strength, the pH value or the ionic composition of the solution (Dunlap *et al.*, 1975; Floyd and Sharp, 1977, 1978, 1979). The efficiency of these factors depends on specific characteristics of the virus, and as expected differences between various viruses could be observed (Floyd and Sharp, 1979). As seen by electron microscope, influenza viruses inclined to aggregation and formed quite a lot aggregates at low ionic strength (Dunlap *et al.*, 1975).

We observed a strong influence of different ionic strengths and pH values of the suspension on the virus cleavage of the H1N1-recombinant and thus on the amount of HA measured by the SRD test. Thus, no HA could be detected with a NaCl concentration of the 0.075 mol/l in the suspension, whereas the HA content measured with a NaCl concentration of 1.15 mol/l was 70 ± 2 µg HA per vial (Fig. 1). In the same way, a different pH value of the suspension resulted in great differences in the HA content measured for the H1N1-strain. A reduction of the pH value as low as to pH 6.0 made it impossible to measure the HA content in the SRD test, whereas the elevation to pH 8.0 resulted in a HA content of 72 ± 5 µg per vial (Fig. 2). It has to be noted that the HA concentrations measurable under the respective conditions were reproducible. Unlike recombinant NIB-6 (H1N1), recombinant NIB-4 (H3N2) showed no different results when suspending the virus in suspensions of greater ionic strength of different pH values (Figs. 1 and 2).

The reason for different behaviour of the two viral strains investigated

could be the aggregation of the viruses. This would mean that the aggregation of H1N1-viruses was so strong that it could not be compensated by the effect of the detergent leaving virus cleavage incomplete. Although Dunlap *et al.* (1975) showed that H3N2-viruses were also aggregated, the aggregation tendency of the NIB-4 strain under chosen conditions could be far less expressed than that of the NIB-6 strain, or the virus cleavage with SLS was far more effective with the NIB-4 strain than with the H1N1-virus. Similarly, Williams *et al.* (1980) stated that cleavage with different detergents of virus strain A/Texas (H3N2) did not affect determination of the HA content by SRD as it did in case of virus strain A/USSR/77 (H1N1). Apart from that they showed that virus cleavage in general, and that of the A/USSR/77 strain in particular, was more effective with the non-ionogenic detergent Emulphogen BC 720 and gave better results in the SRD test. At the time of our studies, we were not able to obtain this detergent, but results with Triton N-101 and Nonidet P-40 (Fluka AG) showed that these non-ionogenic detergents in comparison with SLS produced larger precipitations in the SRD test (unpublished results).

The aim of this study was to investigate whether differences in ionic strength and pH value of the virus suspension could influence the results of determination of the HA content by the SRD test. Differences in the behaviour of two strains were significant, however, it remains to be studied whether the strong dependence of HA determination on the ionic strength and pH value of the virus suspension is only characteristic of the NIB-6 recombinant or can be observed with other strains as well.

This finding suggests that such influences on the SRD test have to be considered and should be verified when using other viral strains and detergents, respectively.

## References

Dunlap, R. C., Brown, E. R., and Berry, D. W. (1975): Determination of the viral particle content of influenza vaccines by electron microscopy. *J. biol. Standard.* **3**, 281–289.

Floyd, R., and Sharp, D. G. (1977): Aggregation of poliovirus and reovirus by dilution in water. *Appl. Environ. Microbiol.* **33**, 159–167.

Floyd, R., and Sharp, D. G. (1978): Viral aggregation: Quantitation and kinetics of the aggregation of poliovirus and reovirus. *Appl. Environ. Microbiol.* **35**, 1079–1083.

Floyd, R., and Sharp, D. G. (1979): Viral aggregation: Buffer effects in the aggregation of poliovirus and reovirus at low and high pH. *Appl. Environ. Microbiol.* **38**, 395–401.

Williams, M. S., Mayner, R. E., Daniel, N. J., Phelan, M. A., Rastogi, S. C., Bozeman, F. M., and Ennis, F. A. (1980): New developments in the measurements of the hemagglutinin content of influenza virus vaccines by single-radial-immunodiffusion. *J. biol. Standard.* **8**, 289–296.

Willkommen, H., Welke, G., Döhner, L., and Hehme, N. (1981*a*): Herstellung von Arbeitsreagenzien für die quantitative Bestimmung des Influenzahämagglutinins mit dem SRD-Test. *Z. ges. Hyg.* **27**, 610–611.

Willkommen, H., Welke, G., Hehme, N., Paschek, C., Hirsch, U., Petzold, D., Döhner, L., and Herrmann, B. (1981*b*): Ergebnisse eines Ringversuches zur Hämagglutininbestimmung mit dem radialen Immunodiffusionstest. *Z. ges. Hyg.* **27**, 832–835.

Wood, J. M., Schild, G. C., Newman, R. W., and Seagroatt, V. (1977): An improved single-radial-immunodiffusion technique for the assay of influenza hemagglutinin antigen: application for potency determination of inactivated whole virus and subunit vaccines. *J. biol. Standard.* **5**, 237–247.

*Wld Hlth Org. techn. Rep. Ser.* (1979), pp. 638.