Memoranda
Mémorandums

Memoranda are statements concerning the conclusions or recommendations of certain WHO scientific meetings; they are signed by the participants in the meeting.

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Reconsideration of influenza A virus nomenclature: a WHO Memorandum*

The system of nomenclature for influenza A viruses recommended by WHO in 1971 provided a basis for the designation of these viruses into types based on their nucleoprotein antigens. Influenza A viruses were further divided into subtypes based on the antigenic character of their haemagglutinin and neuraminidase components. To review the relevance to influenza virus nomenclature of new information on the antigenic and molecular characterization of influenza A viruses a meeting was held in Atlanta, GA, USA, in November 1978 under the auspices of WHO. Although the 1971 system of nomenclature has worked well, new information on relationships between haemagglutinin and neuraminidase subtypes indicates that the number of subtypes could be reduced. However, for the present, the participants in the meeting recommend that the 1971 system should still be used, without modification, at least until a further meeting is held in 1980. In the meantime, WHO wishes to encourage studies that will further define these relationships and solicits comments relevant to the proposals outlined in this Memorandum.

The present system for the nomenclature of influenza viruses is based on recommendations by the participants in a World Health Organization meeting in 1971 (*1*). The influenza viruses were divided into types A, B, and C on the basis of the antigenic character of the internal nucleoprotein (NP) antigen. The other elements of the nomenclature included the host from which the strain was isolated, the geographical location, the strain number, and the year of isolation. Influenza A viruses were further divided into subtypes on the basis of the character of the haemagglutinin (H) and neuraminidase (N) antigens. A uniform system of nomenclature was recommended for influenza viruses from human and non-human (swine, equine, avian) sources.

The H antigen subtypes of human influenza A viruses were designated: H0, H1, H2, and H3; the one H antigen subtype of swine influenza viruses: Hsw1; the two H antigen subtypes of equine viruses: Heq1 and Heq2; and the eight H antigen subtypes of avian influenza viruses: Hav1–Hav8. The N antigens were similarly divided into subtypes (*1*). Among human influenza A viruses there were two N antigen subtypes (N1 and N2); among swine influenza viruses there was one subtype (N1), closely related to the human N1 subtype. Equine influenza viruses were divided into two N antigen subtypes, designated Neq1 and Neq2. For avian influenza A strains there were eight subtypes of N antigen. Two of these (N1 and N2) were shared with human influenza A viruses, two (Neq1 and Neq2) were shared with equine viruses, and four subtypes (Nav1–Nav4) were unique to viruses of avian origin.

Since 1971 much more has been learned about the epidemiology and ecology of influenza viruses, further information has become available on the antigenic and biological characteristics of viruses, and newer biochemical methods have made possible better characterization of viral proteins and nucleic acids. To review the relevance of these findings to the 1971 system of nomenclature of influenza viruses, a meeting was held in November 1978 in

* This Memorandum was drafted by the signatories listed on page 232 on the occasion of an informal meeting held in Atlanta, GA, USA, in November 1978. A French translation will appear in a future edition of the *Bulletin*.

Atlanta, GA, USA, under the auspices of WHO. This Memorandum describes the conclusions of this meeting.

There has not been any new evidence since 1971 indicating a need to reconsider the nomenclature of influenza B and C viruses, and thus the taxonomy of these viruses was not considered at this meeting.

IMMUNOLOGICAL RELATIONSHIPS

In discussions of the antigenic analysis of influenza viruses, the following types of reaction were considered:

(1) reactions involving inhibition of biological activities, including haemagglutination-inhibition (HI), neuraminidase-inhibition (NI), neutralization of virus infectivity, and inhibition of virus growth;

(2) immunodiffusion reactions, including single radial diffusion (SRD) and double immunodiffusion (DID);

(3) techniques such as radioimmunoassay (RIA) and enzyme-linked immunoadsorption (ELISA); and

(4) more complex immunological reactions such as *in vitro* assays of cytotoxicity mediated by antibody and/or effector cells, and *in vivo* immunological priming and cross-protection studies.

HI was considered to provide the most important single test system for antigenic analysis of influenza viruses. Provided that appropriate precautions are taken to ensure the specificity of reactions (the use of antisera prepared to antigenic hybrid viruses with irrelevant neuraminidase, and elimination of antibodies directed at host components), reliable measurements of the degree of cross-relationship between influenza virus haemagglutinins can be obtained by HI tests. The use of antisera prepared by hyperimmunization makes it possible to identify related H antigens within a subtype, while postinfection sera are valuable in distinguishing between strains showing minor degrees of antigenic variation within a subtype. Cross-adsorption of hyperimmune sera can be used to provide sera of high strain specificity for use in detecting variation within a subtype and cross-reactive sera reacting broadly within a subtype.

Antigenic relationships between N antigens may be established by means of NI tests, although the meeting emphasized the importance of ensuring the specificity of the test by the use of antisera to antigenic hybrid strains. Hyperimmune anti-neuraminidase sera are of value in distinguishing between N antigen subtypes, but with such sera it may not be possible to detect minor antigenic drift within a subtype. Sera from infected animals can be used to detect antigenic variation within a subtype but are often of low potency. In certain cases, the NI test cannot be performed because of the low enzymatic activity of a particular virus. In these circumstances, other tests are available for the characterization of N antigens, including elution-inhibition, plaque-size reduction, or inhibition of virus growth by specific anti-neuraminidase sera.

Virus neutralization tests have not offered advantages over HI tests for the serological classification of influenza viruses, and these tests suffer from their complexity and the difficulty of interpreting neutralization kinetics, as well as other practical drawbacks such as the restricted host range of influenza viruses.

The SRD test is considered to be a valuable procedure for quantifying antibodies or antigens. This test can be used to measure antibody to H or N antigens when intact virus particles are used as antigen, and antibody to the internal nucleoprotein and/or M antigens of the virus when disrupted virus particles are used. In general, the use of SRD tests has not provided new information about interrelationships between strains that has not also been detected in inhibition tests or DID reactions. Nevertheless, these tests may be useful for confirmation of results obtained in other test systems.

The DID test has proved to be a valuable method for comparing antigenic relationships among both H and N antigens using hyperimmune sera specific for one or the other of these antigens. Similarities between antigens are detected as lines of common precipitin, whereas the existence of variation between antigens is revealed by spurs of precipitin when different antigens are permitted to diffuse radially inwards toward a single serum. These tests were recommended (*1*) for the antigenic characterization of influenza viruses into H and N subtypes.

Collaborative studies (*8*) employing DID tests have confirmed many of the H and N subtype designations described in the previous recommendations (*1*) on influenza virus nomenclature. Evidence had been obtained that Hsw1, H0, and H1 antigens share some antigenic determinants. DID tests have also indicated that Heq1 and Hav1 antigens are related, that Nav2 and Nav3 are related, and that the latter antigens are related to Nav6, an antigen described in 1971. Confirmation of relationships between H3, Heq2, and Hav7 has also been obtained by means of DID tests.

Studies in animals and man support the views obtained by inhibition and immunodiffusion reactions that:

(*a*) H0, H1, and Hsw1 haemagglutinins can be included in a single H subtype. The practical significance of this was clearly demonstrated in vaccine trials in man in 1976 when priming by natural infection with H0 or H1 virus potentiated the immune response to Hsw1N1 vaccine (*7*).

(*b*) Heq1 and Hav1 haemagglutinins can be included in a single subtype. Evidence to support this is the demonstration that, in the absence of detectable HI antibody to Hav1, animals primed with Heq1 were protected against fatal A/FPV/Rostock/34 (Hav1N1) virus infection.

(*c*) H3, Heq2, and Hav7 haemagglutinins can be included in a single subtype. For example, experiments have shown that immunization of mice with Heq2 virus protected against death from subsequent challenge with mouse-adapted H3N2 virus.

Minor cross-reactions between distinct influenza A subtypes have been demonstrated.

Further developments in cellular immunology (*2, 12*) and studies of the cellular basis of antibody synthesis (*11*) may lead to explanations of the heterotypic and/or synergistic antibody and cross-protection responses that have been observed. However, antigenic analyses by several methods have provided consistent results which indicate that some subtypes previously classified separately show a significant degree of relationship in terms of laboratory tests, ecology, and public health, and can be reclassified into a more limited number of subtypes.

Concerning minor degrees of antigenic variation, it was proposed in the 1971 system of nomenclature that minor degrees of antigenic variation in H and N antigens (antigenic drift) be reflected in the designation of representative reference viruses. The participants in the present meeting recommended that this method of designating minor antigenic changes be retained.

On the basis of the results of immunological tests, the H antigens of influenza viruses of human and non-human origin could be arranged into 11 antigenically distinct subtypes, while the N antigens could be divided into 8 subtypes. Tables 1 and 2 show the relationships between the subtypes designated in the 1971 nomenclature system (*1*) and certain subtypes suggested since 1971, and the proposed new groupings of H and N antigens, based on current information.

Table 1. Proposed regrouping of the haemagglutinin subtypes of influenza A viruses on the basis of serological and biochemical data

Previous subtypes (1971 system)	Proposed groups
H0, H1, Hsw1	H1
H2	H2
H3, Heq2, Hav7	H3
Hav4	H4
Hav5	H5
Hav6	H6
Heq1, Hav1	H7
Hav8	H8
Hav9	H9
Hav2	H10
Hav3	H11

Table 2. Proposed regrouping of the neuraminidase subtypes of influenza A viruses on the basis of serological data

Previous subtypes (1971 system)	Proposed groups
N1	N1
N2	N2
Nav2, Nav3, Nav6	N3
Nav4	N4
Nav5	N5
Nav1	N6
Neq1	N7
Neq2	N8

Recent studies (*9*) have suggested that some antigenic subgrouping of nucleoprotein antigens of influenza A viruses may be possible based on precipitin tests. So far, DID reactions have not detected any antigenic differences among M proteins of influenza A viruses.

The advent of procedures for obtaining monoclonal antibody preparations (*3*), together with the ability to detect antibody–antigen reactions by more sensitive techniques (such as RIA or ELISA), indicate that more definitive quantitative estimates of the degree of relationship between influenza antigens may be possible in the future. The significance of this to the problem of nomenclature is not clear,

but it should be borne in mind that it may eventually be possible to define subgroups of nucleoprotein and M protein antigens, as well as other influenza antigens, including polymerase proteins and non-structural protein.

BIOCHEMICAL CONSIDERATIONS

Protein analyses

Polyacrylamide gel electrophoresis and peptide mapping have been used for analysis of the proteins of influenza A viruses (*6*). Polyacrylamide gel electrophoresis has not provided data useful for distinguishing between subtypes, but the results of peptide mapping experiments on the glycoproteins, on the other hand, are compatible with the immunological grouping given in the above section.

RNA analysis

Three different methods have been used for the characterization of viral RNA: (*a*) comparison of the migration patterns of RNA segments on polyacrylamide gels, (*b*) virion RNA-complementary RNA hybridization (*10*) (this method can be rendered very sensitive by determining the melting profiles of the hybrid RNA molecules in the presence of formaldehyde), and (*c*) oligonucleotide fingerprint analysis after digestion by T_1 ribonuclease of individual virion RNA segments (*13*).

The results of hybridization studies on RNA have supported the immunological grouping of the haemagglutinins of the influenza A viruses because there was considerable base-sequence homology between the haemagglutinin genes of viruses of the following H subtypes: H0, H1, and Hsw1; H3, Heq2, and Hav7; and Hav1 and Heq1 (*10*).

Similar studies of the neuraminidase genes have not shown any disagreement with N antigen subtype designations based on the results of serological tests.

The influenza A viruses so far tested can be divided into two subgroups according to the genetic relatedness of genes coding for the non-structural (NS) protein (Table 3). Within one group, the base-sequence homology was between 85% and 100%, whereas among members of the different groups the base-sequence homology was about 40%. Serological data relating to the NS proteins are not yet available.

The genes coding for viral components other than H, N, and NS were found to be more highly conserved. However, it remains to be established whether or not the properties of these other genes can be used in the classification of influenza viruses.

Table 3. Grouping of influenza subtypes according to the genetic relatedness of RNA segment 8 (NS gene)

Influenza virus strain	Subtype	Group
A/fowl plague virus/Rostock/34	(Hav1N1)	1
A/chicken!Germany/N/49	(Hav2Neq1)	2
A/turkey/Canada/63	(Hav6Neq2)	2
A/turkey/Oregon/71	(Hav1Nav2)	2
A/duck/Ukraine/1/63	(Hav7Neq2)	1
A/turkey/England/63	(Hav1Nav3)	1
A/Puerto Rico/8/34	(H0N1)	1
A/Fort Monmouth/1/47	(H1N1)	1
A/Singapore/1/57	(H2N2)	1
A/Hong Kong/1/68	(H3N2)	1
A/swine/1976/31	(Hsw1N1)	1
A/equine/Miami/1/63	(Heq2Neq2)	1
A/equine/Prague/1/56	(Heq1Neq1)	1
A/duck/England/56	(Hav3Nav1)	1
A/duck/Czechoslovakia/63	(Hav4Nav1)	1
A/turkey/Ontario/7732/66	(Hav5Nav6)	1
A/duck/Germany/1868/68	(Hav6N1)	1
A/turkey/Ontario/6118/68	(Hav8Nav4)	1
A/chicken/Scotland/59	(Hav5N1)	1
A/duck/Memphis/546/74	(Hav3Nav6)	1
A/fowl plague virus/Dutch/27	(Hav1Neq1)	1
A/heron/Chabarovsk/700/73	(Hav7Neq2)	2
A/duck/Chabarovsk/698/73	(Hav7Neq2)	2
A/duck/Chabarovsk/1610/72	(Hav7Neq2)	2

BIOLOGICAL MARKERS

Biological markers in influenza viruses are of two types: (*a*) markers not dependent on viral replication for their demonstration, and (*b*) markers dependent on viral replication for their exhibition. For the most part, markers not dependent on virus replication involve the surface proteins—haemagglutinin and neuraminidase—while markers expressed through replication may reflect changes in other viral polypeptides.

Markers not dependent on virus replication

(*a*) *Haemagglutinin markers and non-specific (non-antibody) inhibitors.* Inhibitor-susceptibility markers, like binding-affinity and adsorption markers and differences in red-blood-cell (RBC) species agglutinated, are often different manifestations of the same haemagglutinin property.

All non-specific inhibitors of viral haemagglutinins are glycoproteins containing neuraminic acid and are associated with serum α_2 macroglobulin. Distinctions among them (α' β' γ) may be more dependent on the test virus used than on differences in their chemical nature. The biological significance of inhibitor sensitivity or resistance remains unknown.

A correlation between sensitivity to different inhibitors and antigenic variation has often been suggested. For example, antigenic shift from H1N1 to H2N2 was accompanied by a loss of sensitivity to β inhibitor and a gain of sensitivity to γ inhibitor. However, inhibitor markers are not sufficiently well defined to make a significant contribution to viral classification. For future studies, well-characterized (preferably purified) inhibitor preparations and cloned virus strains should be used.

(i) *Binding-affinity or adsorption markers* (expressed by haemagglutination–disagglutination or elution, or variation in RBC species agglutinated) may be concordant with inhibitor resistance. None of these markers is strikingly associated with any given viral subtype, although A and B prototype strains appear to differ with respect to adsorption on aluminium phosphate or hexadecylamine.

(ii) *Stability markers.* Influenza virus haemagglutinins vary with respect to their stability to physical and chemical agents, including proteases. Too few viruses have been systematically examined to establish whether or not subtype relationships will emerge from such analyses. Preliminary evidence suggests that stability at low pH may characterize some avian viruses that replicate in the intestinal tract.

Haemagglutinin cleavage appears to be influenced by both the virus and the host cell and might be relevant to host range and virulence.

(*b*) *Neuraminidase markers.* Genetic dimorphism with respect to the amount of neuraminidase per particle is well documented, but its genetic basis is unknown. It is not yet clear whether such dimorphism occurs with enzymes other than N2 neuraminidase. This enzyme appears to stand apart from the other neuraminidase subtypes of human influenza A viruses in stability and activity.

(*c*) *Other markers.* Predominant particle morphology (spherical or filamentous) distinguishes early and late passage strains and is transferable as a genetic marker, but it is not characteristic for viral type or subtype.

Although there are hints that virion transcriptase activity may differ among strains and subtypes, no systematic comparison of enzymes of different subtypes has been made.

Markers dependent on viral replication

Not surprisingly, the complex events involved in viral replication create difficulties in identifying components of the process useful in strain or subtype differentiation. Clearly, marked differences exist in host range both *in vivo* and *in vitro,* defined ultimately by the permissiveness of target cells for one virus or another.

Plaque markers appear to have little value in establishing viral relationships because they can be markedly influenced by minor changes in the viral genome, or because on the contrary, viral subtypes with marked polygenic differences may have the same plaque phenotype.

The temperature optima for replication or viral yield in conventional laboratory hosts do not differentiate clearly among subtypes or types, although influenza B and C viruses do propagate better in the laboratory at 33–35°C.

Naturally occurring temperature-sensitive strains of H1N1 viruses have been isolated since 1977. Neither the pathogenic nor taxonomic significance of such strains is clear.

Amantadine sensitivity requires further exploration as a possible method of differentiating between influenza A and C viruses, as well as among A virus subtypes.

Host range

The present taxonomic system was not designed to provide information on the host range or virulence of influenza viruses. The isolation of antigenically similar influenza A viruses from different hosts is now well established. Isolates from different hosts may show similarities in both surface glycoproteins or they may show antigenic similarities in either the haemagglutinin or the neuraminidase molecules.

Examples in which one subtype of the surface antigens has been found in influenza viruses from different species are numerous (*4*) and counterparts of each of the neuraminidase antigens of influenza viruses from man, pigs, and horses have been isolated in avian species. Similarly, counterparts of many (but not all) of the haemagglutinin subtypes of man, pigs, and horses have been isolated from avian species.

The above antigenic relationships among influenza A viruses do not correlate with host range or virulence or with other genetic properties of the virus and the only direct evidence for transmission of influenza viruses between species comes from the isolation of genetically and immunologically indistinguishable Hsw1N1 viruses from pigs and man on the same farm (*5*). There is epidemiological and serological evidence to suggest that the H3N2 influenza viruses isolated from swine spread to this species from man.

Since the results obtained so far represent only a beginning in the analysis of genetic control of the virulence of influenza viruses, it is premature to attempt to include information about the host range or virulence in the nomenclature for influenza viruses.

In summary, few non-antigenic biological markers are at present useful in influenza virus taxonomy.

CONCLUSIONS

It was the consensus of the participants in the meeting that the system of nomenclature recommended in 1971 has provided a valuable framework for the antigenic description of influenza A viruses and they confirm their support for the general principles of that system. However, it was agreed that recent findings on the subtyping of haemagglutinins and neuraminidases of influenza A viruses by immunological and biochemical methods suggest modifications in the designation of subtypes. There is now evidence of 11 distinct subtypes of H antigen and 8 distinct subtypes of N antigen. Although a novel H (Hav9) and a novel N (Nav5) subtype have been identified since 1971, the total number of subtypes could be reduced by merging some subtypes previously given distinct designations.

Subtype designations indicating species of origin of the virus are thought to be unnecessary since viruses of the same subtype may be isolated from several species and the strain designation already specifies the host of origin. As far as biochemical evidence is available, it supports the immunological evidence for subtype designation. Concerning other components of the virus, RNA hybridization studies suggest some differences between genes coding for proteins other than H and N, particularly the non-structural (NS) protein. Antigenic characterization of the nucleoprotein (NP) antigens among influenza A viruses has revealed differences among virus strains, particularly those obtained from different host species. It is too early to evaluate the significance for virus nomenclature of the findings with NS and NP. In contrast, it seems unlikely that biological characteristics such as virulence, inhibitor sensitivity, and disease impact will prove useful as criteria for classification.

In order for a system of nomenclature to be useful, it must reflect as accurately as possible known virus relationships. WHO wishes to encourage studies that might further define these relationships and solicits comments that bear on the changes proposed in the present system.[a] The meeting recommended that WHO convene a meeting in 1980 to review additional information and to institute appropriate changes. In the meantime, it is proposed that the system of nomenclature recommended in 1971 (*1*) should be used without modification.

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REFERENCES

1. A revised system of nomenclature for influenza viruses. *Bulletin of the World Health Organization,* **45**: 119-124 (1971).
2. BIDDISON, W. E. ET AL. Antibody to influenza virus. II. The antigenicity of the viral hemagglutinin. *Journal of experimental medicine,* **146**: 690-697 (1977).
3. GERHARD, W. The analysis of the monoclonal immune response to influenza virus. II. The antigenicity of the viral hemagglutinin. *Journal of experimental medicine,* **144**: 985-995 (1976).
4. HINSHAW, V. S. ET AL. Influenza A viruses: combinations of hemagglutinin and neuraminidase subtypes isolated from lower animals and birds. *Archives of virology* (in press).
5. HINSHAW, V. S. ET AL. The prevalence of influenza viruses in swine and the antigenic and genetic relatedness of influenza viruses from man and swine. *Virology,* **84**: 51-62 (1978).
6. LAVER, W. G. & WEBSTER, R. G. Studies on the origin of pandemic influenza. III. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain. *Virology,* **51**: 383-391 (1973).
7. PARKMAN, P. D. ET AL. Summary of clinical trials of influenza vaccines. *Journal of infectious diseases,* **134**: 100-107 (1976).
8. SCHILD, G. C. ET AL. Considerations for the nomenclature of influenza viruses. *Archives of virology* (in press).
9. SCHILD, G. C. ET AL. Antigenic variation in the nucleoprotein antigens of influenza A viruses. *Virology* (in press).
10. SCHOLTISSEK, C. The genome of the influenza virus. *Current topics in microbiology and immunology,* **80**: 139-169 (1978).
11. VIRELIZIER, J. L. Antibody responses to antigenic determinants of influenza virus hemagglutinin. I. Thymus dependence of antibody formation and thymus independence of immunological memory. *Journal of experimental medicine,* **140**: 1559-1570 (1974).
12. ZWEERINK, H. J. ET AL. Cytotoxic T cells to type A influenza virus: viral hemagglutinin induces A-strain specificity while infected cells confer cross-reactive cytotoxicity. *European journal of immunology,* **7**: 630-635 (1977).
13. NAKAJIMA, K. ET AL. Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. *Nature (London),* **274**: 334-339 (1978).