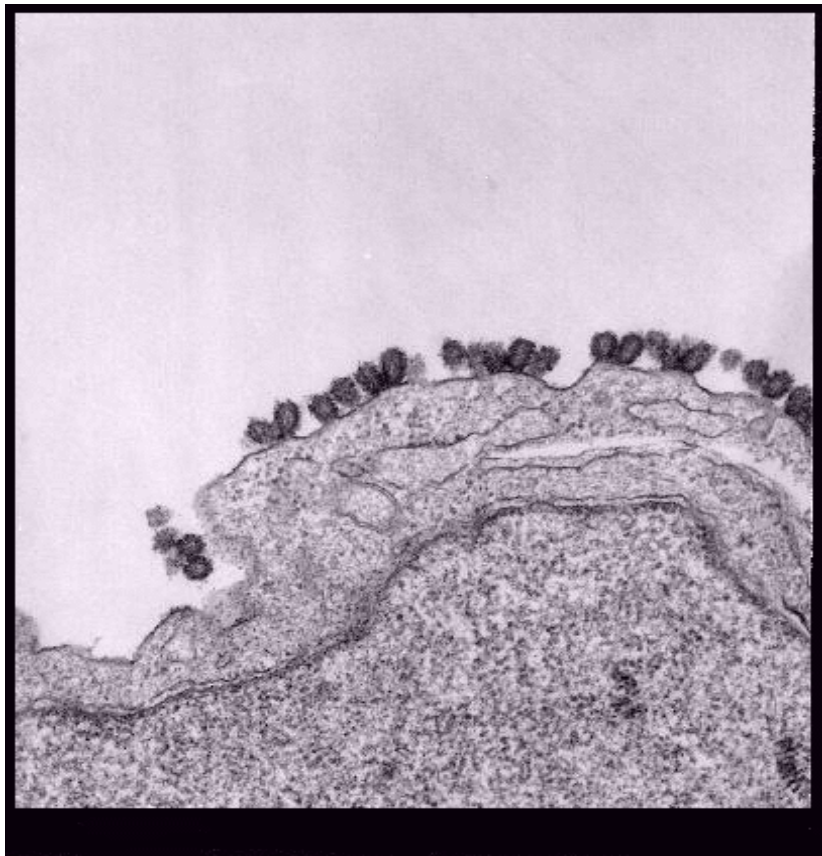


NIAID RESOURCES
FOR
INFLUENZA RESEARCH



National Institute of Allergy and Infectious Diseases, NIH

January, 1998

Antisera To Isolated Antigens of Influenza Viruses

Production and testing of goat antisera to isolated surface glycoproteins of reference strains of influenza A and B viruses has been completed by the St. Jude Children's Research Hospital a number of years ago. (Dr. Robert G. Webster, Principal Investigator). In addition to the antisera against the various hemagglutinins and neuraminidases, specific antisera were prepared to isolated matrix (M) and ribonucleoprotein (RNP) subunits of A and B viruses.

Methods for virus production, purification, disruption, and antigen isolations are available upon request. Antisera to the isolated antigens were prepared in goats with antigen emulsified in Freund's complete antigen.

Serological assays (hemagglutination, HA, and hemagglutination inhibition, HI) were performed as described by Fazekas and Webster (1966). In HI tests the dilutions of antisera were allowed to interact with antigen for 60 minutes at 20°C before addition of chicken erythrocytes. Neuraminidase (NA) was assayed by the method Warren (1959) except that the color was extracted into n-butanol containing 5% (v/v) concentrated hydrochloric acid (Aminoff, 1961). Neuraminidase-inhibition (NI) tests were done as described (Webster and Periera, 1968). To preclude steric inhibition in NI tests an antigenic hybrid possessing an irrelevant HA subunit was used. Immunodiffusion tests were done in Hyland double immunodiffusion plates after disruption of purified virus (HA 6.0 log₁₀/ml) with SDS as described by Schild and Periera (1969).

All antisera are freeze-dried in 1.0 ml volumes. Samples of each freeze-dried serum were retested in Dr. Webster's laboratory by HI, NI, and immunodiffusion tests. Tests were also done in collaboration with Dr. G. C. Schild to check the specificity of antisera against reference strains and to test the antisera against exotic viruses that cannot be worked within the United States such as A/Fowl/Plague/Dutch/27 (H7N7). Some of the antisera are monospecific and react with the desired subunits. Others, however, react with more than the desired subunit. These reactions do not negate the value of these reference reagents.

Table 1-4 contain pertinent information on specificity, titer, and identity of these reagents. Please note that the current nomenclature for H and N of influenza A are given in parenthesis in these tables.

References

AMINOFF, D. (1961). Methods for the quantitative estimation of N-acetyl-neuraminic acid and their application to hydrolysates of sialomucoids. *Biochem. J.* 81, 384-392.

FAZEKAS, de ST. GROTH, S. and WEBSTER, R. G. (1966). Disquisitions on original antigenic sin. I., Evidence in Man. *J. Exp. Med.* 124, 331-345.

SCHILD, G. C. and PERIERA, H. G. (1969). Characterization of the ribonucleoprotein and neuraminidase of influenza A viruses by immunodiffusion. *J. Gen. Virol.* 4, 355-363.

WARREN, L. (1959). The thiobarbitruic acid assay of sialic acids. *J. Biol. Chem.* 234, 1971-1975.

WEBSTER, R. G. and PERIERA, H. G. (1968). A common surface antigen in influenza viruses of human and avian sources. *J. Gen. Virol.* 3, 201-208.

Table 1

Antisera to Influenza A Virus Antigens Prepared in Goats
Summary of Reactions in Immunodiffusion Test

RRB Catalog Number	Antisera	Reference Strain of Virus	Antibodies to Matrix Protein Single Radial Diffusion	Antibodies to RNP Double-Immunodiffusion	Reactions in Double-Immunodiffusion tests
V-314-511-157	H0 (H1)*	A/PR/8/34	-	-	H0 (H1)
V-314-521-157	H1	A/FM/147	-	-	H1
V-314-541-157	H2	A/Singapore/1/57	-	-	H2
V-314-591-157	H3	A/Hong Kong/1/68 (Labeled A/Aichi/2/68)	-	-	H3, Heq2, (H3) Hav7 ^c (H3)
V-317-501-157	Hsw1 (H1)	A/Swine/Iowa/15/30 (labeled A/Swine/Wisconsin/15/30)	-	-	Hsw1
V-316-561-157	Heq1 (H7)	A/Equine/Prague/1/56	-	-	Heq1, (H7), Hav1 ^c (H7)
V-316-571-157	Heq2 (H3)	A/Equine/Miami/63	-	-	Heq2, (H3), Hav7 ^c (H3)
V-315-501-157	Hav1 (H7)	A/FPV/Dutch/27	-	±	Hav1 (H7), Heq1 ^c (H7)
V-315-511-157	Hav2 (H10)	A/Chick/Germ/"N"/49	+	-	Hav2 (H10)
V-315-521-157	Hav3 (H11)	A/Duck/Eng/56	-	-	Hav3 (H11)
V-315-531-157	Hav4 (H4)	A/Duck/Czech/56	-	-	Hav4 (H4) very weak
V-315-541-157	Hav5 (H5)	A/Tern/S. Africa/61	-	+	Hav5, H5 H0 (H1), H1 ^c
V-315-552-157	Hav6 (H6)	A/Turkey/Mass/65	-	-	Hav6 (H6)
V-315-561-157	Hav7 (H3)	A/Duck/Ukr/1/63	-	-	Hav7 (H3) H3, Heq2 ^c (H3)
V-315-571-157	Hav8 (H8)	A/Turkey/Ont/6118/68	-	+	Hav8 (H8)
V-308-513-157	N1	[A/New Jersey/8/76] ^c (Labeled A/Eql/Prague/1/56-NJ/8/76)	+	+	N1 [Heq1] ^d (H7)
V-308-541-157	N2	A/Singapore/1/57	-	-	N2
V-310-561-157	Neq1 (N7)	A/Equine/Prague/1/56	±	-	Neq1 (N7)
V-310-571-157	Neq2 (N8)	A/Equine/Miami/1/63	-	±	Neq2 (N8)j[H0] ^d
V-309-521-157	Nav1 (N6)	A/Duck/England/56	+	-	Nav1 (N6)
V-309-541-157	Nav2 (N3)	A/Tern/S. Africa/61	+	+	Nav2 (N3), Nav3 ^c

RRB Catalog Number	Antisera	Reference Strain of Virus	Antibodies to Matrix Protein Single Radial Diffusion	Antibodies to RNP Double- Immunodiffusion	Reactions in Double- Immunodiffusion tests
					(N3) [H0] ^d
V-309-581-157	Nav3 (N3)	A/Turkey/England/63	±	-	Nav3 (N3), Nav2 ^c (N3) [H0] ^d
V-309-571-157	Nav4 (N4)	A/Turkey/Ontario/6118/ 68	-	+	Nav4 (N4)
V-309-592-157	Nav5 (N5)	A/Shearwater/Australia/ 72	-	-	Nav5 (N5)
V-304-501-157	A-RNP	A/Scotland/74	±	+	RNP
V-306-501-157	A-matrix	A.NWS/Eq1	+	-	M

^aAgarose contained boiled A/Chick/Germany/N/49

^bWith reference antisera to influenza A-RNP (Schild)

^c [Old nomenclature] e.g. Hav7 = H3. Antisera showing cross reactions

^dAntisera with [] contain antibodies to an unrelated hemagglutinin antigen

^eAntisera to other than a reference strain

+ = strong reaction

± = trace reaction

*Current nomenclature for influenza A viruses is given in parentheses

NOTE: Since these antisera were prepared, additional HA and NA subtypes have been described including H9, H12 through H15, and N9.

Table 2

Results in Microtiter HI Tests of "Specific" antisera to the Isolated Subunits of Influenza "A" Viruses
Antigens

RB Catalog #	Goat antiserum	Reference Strain of Influenza Virus	HON1 (PR8)	HON1 (Bei)	H1N1(FM1)(H1N1)	H2N2 (Asian)	H3N2 (Hong Kong)	Hsw1N1 (Swine)	Heq1Neq1 (Equine 1)	Heq2Neq2 (Equine 2)	Hav1 Nav2 (Turkey/Oregon/71)	Hav2 Neq2 (Chick/Germany/49)	Hav3 Nav1 (Duck/Eng/56)	Hav4 Nav1 (Duck/Czech/56)	Hav5 Nav2 (Tem/So. Africa/61)	Hav6 N2 (Turkey/Mass/65)	Hav7 Neq2 (Duck/Ukr/63)	Hav8 Nav4 (Turkey/Ontario/4118)
V-314-511-157	H0 (H1)*	A/PR/8/34	10,240	80	80	<	80	40	40	40	<	40	20	<	<	40	<	<
V-314-501-157	H0 (H1)	A/Bel/42	320	5,120	40	<	<	20	20	<	<	<	<	<	<	<	<	<
V-314-521-157	(H1)	A/FM/1/47	640	80	10,240	<	<	320	20	<	<	<	<	<	<	80	<	<
V-314-541-157	H2	A/Singapore/1/57	20	20	40	2,560	20	20	<	20	<	<	<	<	<	<	<	<
V-314-591-157	H3	A/Hong Kong/1/68	160	<	320	<	8,000	20	20	160	<	40	<	<	<	40	80	<
V-317-501-157	Hsw1 (H1)	A/Swine/Iowa/15/30	80	<	80	<	20	1,280	<	<	<	<	<	<	<	<	<	<
V-316-561-157	Heq1 (H7)	A/Eq/Prague/1/56	80	<	80	<	<	<	8,000	<	320	20	<	<	<	<	<	<
V-316-571-157	Heq2 (H3)	A/Eq/Miami/1/63	40	<	80	<	1,000	<	80	5,120	<	<	<	<	<	<	20	<
V-315-501-157	Hav1 (H7)	A/FPV/Dutch/27	80	<	80	<	<	<	1,000	<	320	<	<	<	<	<	<	<
V-315-511-157	Hav2 (H10)	A/Chick/Germ/N/49	<	<	<	<	<	<	<	<	<	1,280	<	<	<	<	<	<
V-315-521-157	Hav3 (H11)	A/Duck/Eng/56	<	<	<	<	20	<	<	<	<	<	640	<	<	<	<	<
V-315-531-157	Hav4 (H4)	A/Duck/Czech/56	20	<	20	<	<	<	<	<	<	<	<	160	<	<	<	<
V-315-541-157	Hav5 (H5)	A/Tem/S. Africa/61	<	20	<	<	<	<	<	<	<	<	<	<	640	<	<	<
V-315-552-157	Hav6 (H6)	A/Turkey/Mass/65	160	20	20	<	20	<	<	<	<	40	<	<	<	1,280	<	<
V-315-561-157	Hav7 (H3)	A/Duck/Ukr/1/63	80	20	20	<	4,000	<	<	160	<	20	<	<	<	<	1,280	<
V-315-571-157	Hav8 (H8)	A/Turkey/Ont/6118/68	160	20	20	<	20	<	<	<	<	20	<	<	<	<	<	320

<<<20 Figures are the reciprocals of the dilution at the end points as given in Advanced Laboratory Techniques for Influenza Diagnosis

*Current nomenclature for influenza A viruses is given in parentheses.

Table 3

Neuraminidase. Titers of "Specific" Antisera to the Isolated
Subunits of Influenza "A" Viruses
Antigens

Research Resources Branch Catalog Number	Goat antiserum to:	Reference Strain of Influenza Virus	HON1 (A/NJ/8/76)	N2 (A/Singapore/57)	Heq1 Neq1 (A/Equine/Prague /1/56) (H7N7)	Heq2 Neq2 (A/Equine/Miami/1/63)	Hav3 Nav1 (Duck/Eng/56)	Hav5 Nav2 (Tern/ So. Africa/61)(H5N 3)	H0 Nav3 (NWS Nav3)	Hav8 Nav4 (TyOnt/6118)	H0 Nav5 (A/Shearwater /Australia/72)	HI Tests
V-308-513-157	Heq1N1 (H7N1)	A/Equine/Prague/1/56 NJ/76 (N) (Heq1)	300	<	10	<	<	<	<	<	<	HI vs Eq1 3,200
V-308-541-157	N2	A/Singapore/1/57	<	2,000	<	<	<	<	<	<	<	HI vs NWS: 40
V-310-561-157	Neq1 (N7)	A/Equine/Prague/1/56	<	<	800	<	<	<	<	<	<	HI vs NWS: 20
V-310-571-157	Neq2 (N8)	A/Equine/Miami/1/63	<	<	<	1,000	<	<	<	<	<	HI vs NWS: 6,000
V-309-521-157	Nav1 (N6)	A/Duck/England/56	<	<	<	<	600	<	<	<	<	HI vs NWS: 20
V-309-541-157	Nav2 (N3)	A/Tern/S. Africa/61	<	<	<	<	<	1,000	600	<	<	HI vs NWS: <20
V-309-581-157	Nav3 (N3)	A/Turkey/England/63	<	<	<	<	<	150	300	<	<	HI vs NWS: 1,200
V-309-571-157	Nav4 (N4)	A/Turkey/Ontario/ 6118/68	<	<	<	<	<	<	<	1,000	<	HI vs NWS: 320
V-309-592-157	Nav5 (N5)	A/Shearwater/ Australia/72	<	<	<	<	<	<	<	<	2,000	HI vs NWS: 2,560

<= Less than 20

Figures are the reciprocals of the dilution at the end points as given in Advanced Laboratory Techniques for Influenza Diagnosis.
*Current nomenclature for influenza A viruses is given in parentheses.

Table 4
Antisera to Influenza B Viruses
Summary of Reactions

RRB Catalog Number	Antisera	Hemagglutinin Inhibition (Micro)			Neuraminidase Inhibition		Double Immunodiffusion		
		B/Lee/1940	B/HK/8/73	A/NWS/34	B/Lee/1940	B/HK/8/73	B/Lee/1940	B/HK/8/73	A/NWS
V-318-501-157	B/Lee/1940 (HA)	10,240	80	<	<	<	+	±	-
V-318-511-157	B/HK/8/73 (HA)	160	20,480	<	<	10	±	+	-
V-312-501-157	B/Lee/1940 (NA)	80	160	<	1,000	150	+	±	-
V-312-511-157	B/HK/8/73 (NA)	160	40	<	700	100	±	+	-
V-307-501-157	B/HK/8/73 (M)	<	<	<	<	<	+	+	-

+ = Strong line of precipitation.

± = Weak line of precipitation.

< = less than 20.

Figures are the reciprocals of the dilution at the end points as given in Advanced Laboratory Techniques for Influenza Diagnosis.