

Influenza & Other Respiratory Viruses

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Influenza *Virus*

Epidemiology and investigation of influenza outbreaks in Pune city

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The National Influenza Center at NIV Pune is the main center in the country conducting continuous surveillance on influenza for the past 28 years.

Objectives

- Surveillance for influenza viruses
- Virus isolation and strain characterization.

Achievements

During the course of influenza surveillance in the year 2004, and between January and March 2005, a total of 737 respiratory specimens were collected from two hospitals and five dispensaries located in different areas of Pune. 156 samples were from age group 0-1 year, 340 from 1-9 years, 81 from 9-18 years, 113 from 18-60 and 47 from patients greater than 60 years. Specimens were processed in MDCK cell culture and 18 influenza virus isolates; 5 A (H3N2) and 13 type B were obtained. One isolate was from the age group 0-1 year, 15 from age group 1-9 years and 2 from the age group 9-18 years.

Influenza activity in the year 2004 was low and only one A (H3N2) isolate was obtained in the month of February; one in the month of March and two in the month of August. Increase in influenza activity was noted in the beginning of 2005, with one A (H3N2) strain isolated in the month of January and 3,5,4 isolates of type B in the months of January, February and March 2005 respectively (Figure-1). The type A (H3N2) isolates were identified as related to A/Panama/2007/99 (H3N2) strain and type B isolates as related to B/HongKong/1434/02 (B/Victoria lineage) strain in haemagglutination inhibition test.

Strains of influenza type B belonging to B/Victoria lineage were isolated at NIV, Pune between 1987 and 1989 and again in the year 2002. All the type B strains isolated in Pune between the years 1990 and 2001 and also in the year 2003 belonged to the B/Yamagata lineage. This indicates that reappearance of strains from B/Victoria and B/Yamagata lineage is occurring in alternate epidemics.

Influenza Virus

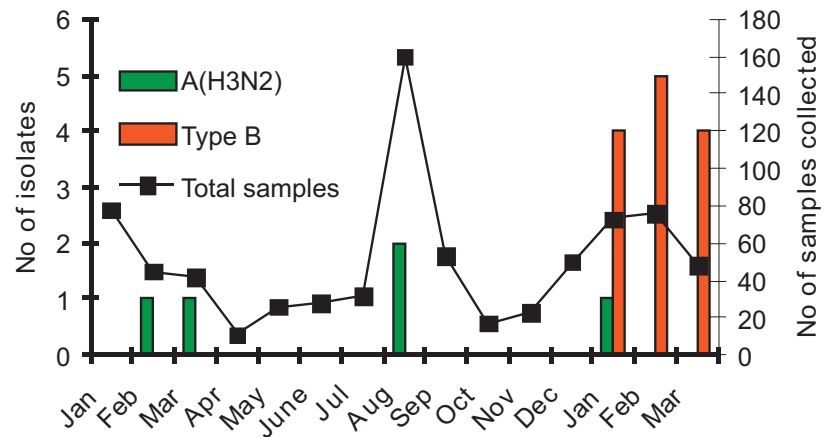


Figure1: Monthly data of specimen collection and influenza virus detection during the year 2004-05

Future plan

NIV being National Influenza Centre, year round surveillance for influenza will be continued.

Rapid detection and strain analyses of influenza viruses

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For rapid identification of new variant strains emerging in the community and to expand and upgrade the existing surveillance system, molecular and rapid diagnostic techniques for detection of newly emerging influenza virus strains are being developed.

Objectives

- Development of monoclonal antibodies for rapid detection using antigen capture ELISA.
- Standardization of molecular methodologies for genetic analyses of virus isolates and rapid identification of variant strains.

Achievements

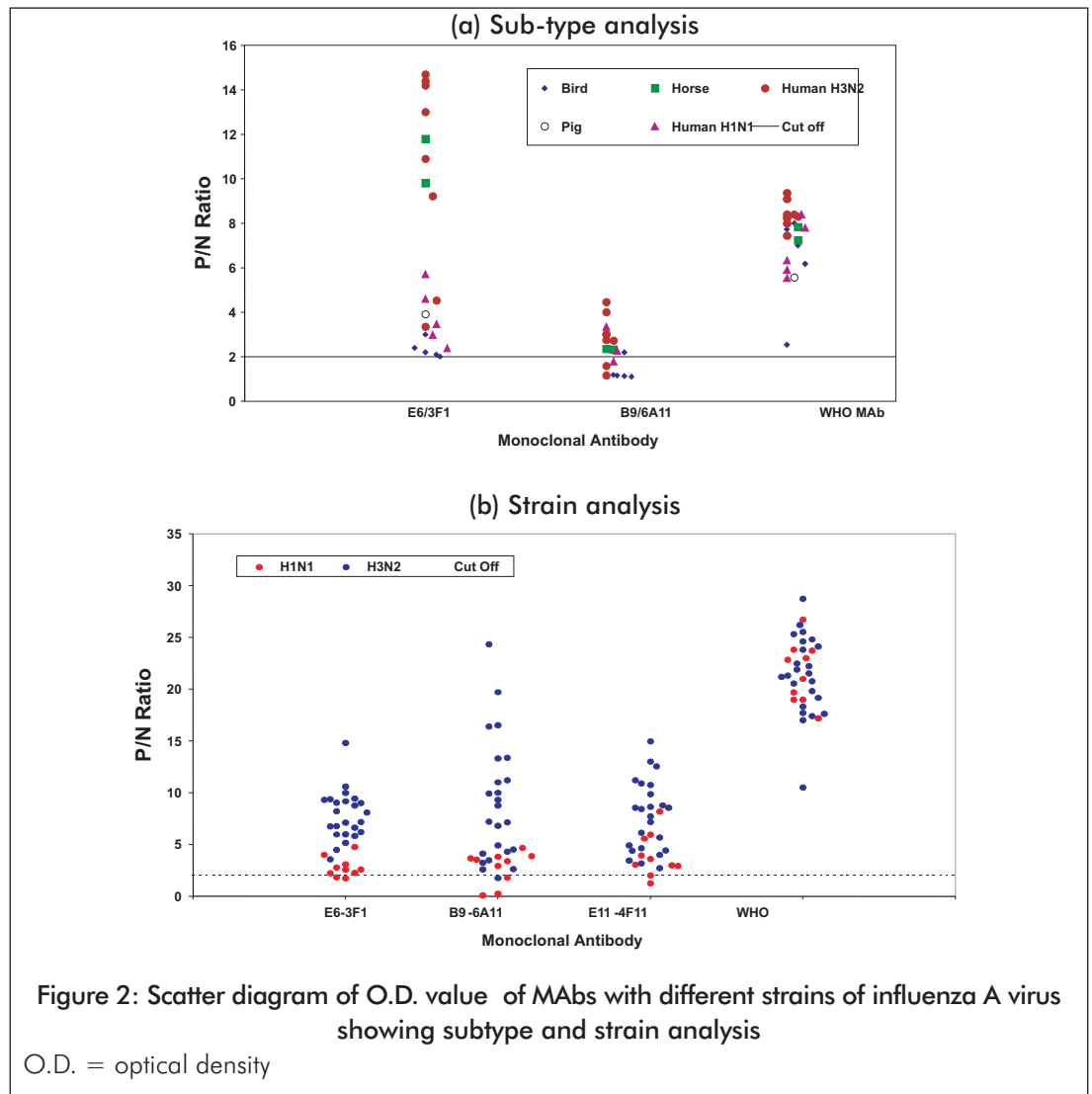
Development of monoclonal antibody based Antigen Capture ELISA (AC-ELISA) for detection of influenza A

Strain variation studies

NIV has undertaken the development of indigenous monoclonal antibody (MAG) based rapid diagnostic tests for influenza diagnosis. Preparation and characterization of MABs against an Indian influenza virus isolate, 8912370 A(H3N2), similar to A/Sichuan/68/89 was reported in the previous year. All MABs were found to be against N protein of influenza virus.

Sub-type analysis

AC-ELISA was developed using indigenous MAB as a capturing antibody and anti-influenza type A rabbit polyclonal serum as detector antibody. Two clones (E6/3F1, B9/6A11) and WHO type A pool MABs were used for sub-type analysis of 21 strains of influenza A comprising of 11 subtypes: Human A(H1N1), A(H2N2), A(H3N2); equine A(H7N7), A(H3N8); swine A(H1N1); avian A(H4N2), A(H5), A(H6), A(H7N1), A(H9N2). The amount of virus antigen was equalized by adjusting the antigen to four hemagglutination units (Figure 2 a & b) MAB E6/3F1 and WHO MAB detected all the eleven subtypes, were as B9/6A11 MAB detected five subtypes. E6/3F1 MAB showed two groups based on difference in optical density(O.D.) in AC-ELISA (figure a); one group comprising of some strains of A(H3N2) and equine strains of subtypes A(H3N8), A(H7N7); second group comprising of human A(H1N1) strains, some A(H3N2) strains, strains of bird and pig subtypes. WHO MAB and B9/6A11 MAB did not show such grouping.



Strain analysis

Three clones E6/3F1, E11/4F11, B9/6A11 and WHO MAbs were used for strain analysis of 26 influenza A(H3N2), 10 influenza A (H1N1) strains isolated from 1976-2004 in Pune and three A(H3N2) and one A(H1N1) reference strains in AC ELISA. The amount of virus antigen was equalized by adjusting the antigen to four hemagglutination units.

E11/4F11, E6/3F1 and WHO MAbs detected all the A(H3N2) strains. B9/6A11 MAb detected 28/29 A(H3N2) strains. Of the ten A(H1N1) strains, WHO MAb detected all ten A(H1N1) strains, E11/4F11 MAb detected 9/10, E6/3F1 MAb detected 8/10, and B9/6A11 detected 7/10 strains.

All three MAbs showed variation on reactivity with different A(H1N1) and A(H3N2) strains. Interestingly, E6/3F1 MAb could differentiate between A(H1N1) and A(H3N2) strains based on O.D. values, while WHO MAb did not show O.D. difference.

The N protein is one of the type specific antigens of influenza viruses that distinguish between the influenza type A, B and C viruses. This study on sub-type and strain variation using indigenous N protein MAbs showed that;

- (1) N protein of human A(H3N2) strains and equine subtypes A(H3N8), A(H7N7) share a similar epitope.
- (2) Human A(H1N1), A(H2N2), swine A(H1N1), bird subtypes A(H4N2), A(H5), A(H6), A(H7N1), A(H9N2), and some human A (H3N2) strains share similar epitope.
- (3) The epitopes of N protein of human A (H1N1) and A (H3N2) strains show significant variation.

Comparison of sensitivity of AC-ELISA with Hemagglutination (HA) test

Purified influenza A virus was tested simultaneously in HA and in AC-ELISA by using E6/3F1, G9/6A11 and WHO type A MAbs. HA test using Guinea pig red blood cells (RBCs) showed positivity till 1:320 dilution and 1:160 using fowl RBCs. E6/3F1 MAb showed positivity till 1:1280 dilution, G9/6A11 MAb showed positivity till 1:2560 dilution, while WHO type A MAb showed positivity till 1:10,240 dilution. Thus AC-ELISA was more sensitive than HA test. A minimum of 60 ng of homologous purified antigen was detected in AC-ELISA, using E6/3F1 MAb, while WHO MAb detected a minimum of 7.8 ng of purified antigen.

Detection of influenza A in MDCK cell supernatant

A total of 179 specimens collected during the year 2003-2004 were processed in MDCK cell culture and supernatant tissue culture fluid (TCF) were tested in AC-ELISA, by using E6/3F1 MAb and hemagglutination (HA) test. All fifteen HA positive TCFs (identified as H3N2 by HI test) were positive for influenza A in AC-ELISA. Sensitivity and specificity of the AC-ELISA compared to HA test for detection influenza A from infected TCFs was 100 % (Table 1).

Table 1: ELISA results

AC-ELISA	Isolation (Gold standard)		Total
	Positive	Negative	
Positive	15	0	15
Negative	0	164	164
Total	15	164	179

Sensitivity: 100% Specificity: 100%

Immunofluorescence antibody (IF) test using type A influenza MAbs:

In earlier report, we have reported that MAbs react with homologous A(H3N2) antigen in IF test. Further, all the 18 clones were tested for their reactivity with the three human influenza subtypes A(H1N1), A(H2N2), A(H3N2) in IF test. We found that only three MAbs viz. E6/3F1, E11/4F11 and B10/6A11 reacted with all three subtypes (Table 2).

We could obtain three groups of MAbs based on their reactivity in IF test. (a) MAbs reacting with all the tested subtypes (b) MAbs reacting with only A(H3N2) subtype (c) MAbs not reacting with any subtype.

Table 2: Reactivity of MAbs in IF test with Type A and Type B influenza viruses.

No.	MAb.	H1N1	H2N2	H3N2	Type B	Normal MDCK
1	E6/3F1	+ F	+ F	+++ P	-	-
2	G7/3F1	-	-	+++ M	-	-
3	A2/3F1	-	-	+++ P	-	-
4	E2/3F1	-	-	+++ F	-	-
5	D8/3F1	-	-	+++ P	-	-
6	D10/3F1	-	-	++ F	-	-
7	E11/4F11	+ F	+ F	+ F	-	-
8	B7/4F11	-	-	++ P	-	-
9	H6/4F11	-	-	-	-	-
10	C3/4F11	-	-	-	-	-
11	E1/4F11	-	-	-	-	-
12	G9/6A11	-	-	++ P	-	-
13	B9/6A11	-	-	+ M	-	-
14	G2/6A11	-	-	-	-	-
15	B7/6A11	-	-	++ P	-	-
16	B8/6A11	-	-	+ P	-	-
17	D7/6A11	-	-	++ F	-	-
18	B10/6A11	+++ P	++ F	+++ P	-	-
19	WHO MAb	++++ P	++++ P	++++ P	-	-
20	Chemicon kit MAB.	++++ P	++++ P	++++ P	-	-
21	Normal PF.	-	-	-	-	-

Key: Number of + indicates grade of fluorescence, Number of cells: P- Plenty, M-Medium, F- Few.

As three MAbs viz. E6/3F1, E11/4F11 and B10/6A11 were reacting with all three human subtypes, these MAbs were short listed for further studies. These MAbs were tested with equine subtypes A(H3N8)-A/equine/Hissar., A(H7N7)-A/equine/Prague/1/56, and avian sub-type A(H4N2)-NIV# 7811114 (Table 3).

Table 3: Reactivity of MAbs in IF test with influenza A subtypes

No.	MAB	A(H3N8)	A(H7N7)	A(H4N2)
1	E6/3F1	+++ M	+++ M	+++ M
2	E11/4F11	++ F	++ F	++ F
3	B10/6A11	-	-	-
4	WHO type A MAbs.	+++ M	+++ M	+++ M
5	Chemicon Kit Type A MAbs	+++ P	+++P	+++ P

Key: Number of + indicates grade of fluorescence, Number of cells: P- Plenty, M-Medium, F- Few.

MAbs E6/3F1 and E11/4F11 reacted with all the three sub-types where as MAb B10/6A11 did not react with any.

Future plan

Molecular techniques will be used to screen clinical samples and characterization of influenza isolates.

Respiratory Syncytial virus (RSV), influenza, Parainfluenza and Adenoviruses have been incriminated as major causes of acute respiratory infection in pediatric cases. RSV is the single most important cause of hospitalization for serious respiratory tract viral disease (bronchiolitis & pneumonia) in infants and young children worldwide. It is also suspected as possible cause of childhood asthma. The need to develop rapid and specific test for RSV detection is need-of-hour issue.

Monoclonal antibody (Mab) based IF test and ELISA for detection of Respiratory Syncytial Virus (RSV) infection

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Objectives

- Diagnostic development and sero epidemiology of RSV infections.

Achievements

RSV detection using NIV ELISA

Development of MAb based antigen capture ELISA for the detection of RSV infection has been reported in the previous years. A total of 199 nasopharyngeal aspirates collected during the years 2003 (114) and 2004 (85) were tested in NIV ELISA for the detection of RSV antigen.

All the 199 specimens were screened for detection of RSV using commercial IF test (Chemicon). Of the specimens collected, 136 were negative and 63 positive for RSV antigen. NIV ELISA could detect RSV from only 36 samples of the 63 samples that were positive for RSV by Chemicon test. All other specimens were negative indicating 57.1% sensitivity and 100% specificity.

Table 4: Results of Chemicon IF test

NIV AC-ELISA	Chemicon IF test (Gold standard)		Total
	Positive	Negative	
Positive	36	0	36
Negative	27	136	163
	63	136	199

Respiratory viral infection among pediatric cases 2002-2004

This study examined the association of specific virus infection with acute respiratory tract condition among hospitalised and outpatient children in Pune (Figure 3). Nasopharyngeal aspirates collected year around between 2002 and 2004 from KEM hospital, Pune were subjected to IF test using Commercial Kit (Chemicon) for the detection of influenza A, B, RSV, parainfluenza 1,2,3 and adenoviruses.

Of the 383 samples screened, viruses were detected in 143 (37.0%) cases including infections caused by RSV (25.6%), influenza (5.7%), parainfluenza (2.1%) and adenovirus (0.8%). Eleven (2.8%) samples were positive for respiratory virus panel pool but did not have sufficient cells for further identification.

Viruses were detected among 29.4% of outpatients and 45.2% of hospitalised cases. RSV was the most prevalent virus detected among hospitalised as well as outpatient children

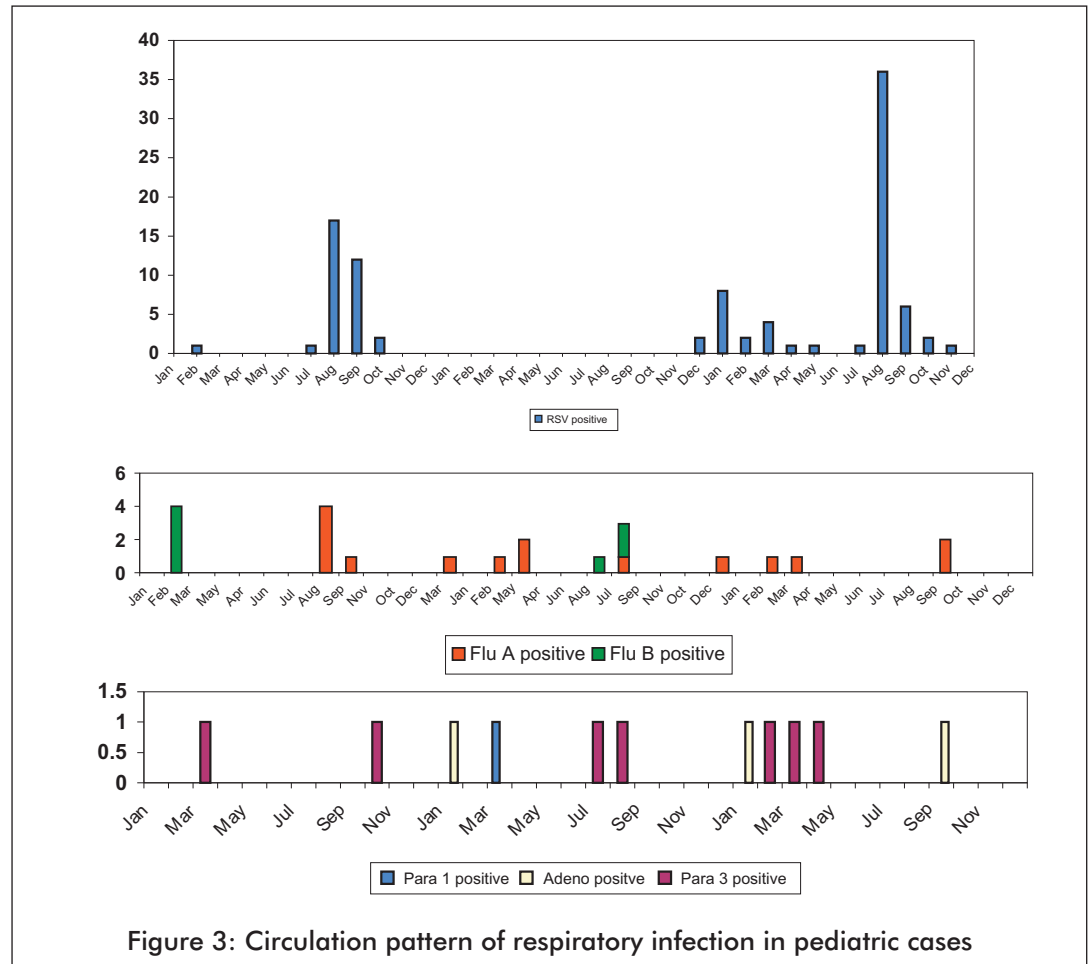


Figure 3: Circulation pattern of respiratory infection in pediatric cases

RSV

whereas influenza was detected mostly in the outpatient cases. Sporadic cases of parainfluenza and adenovirus were detected only in hospitalised cases. RSV showed marked activity in the month of August of the years 2002 and 2004 with 78% of the hospitalised cases positive for RSV in this month and 42.8-47.8% cases positive in outpatients. Lack of RSV activity was observed in the year 2003. Figure shows month wise pattern of respiratory virus circulation in pediatric cases.

Future plan

Epidemiological investigation of RSV infection in Pune will be continued employing MAb based immunodiagnosis tests developed at NIV.

Standardization of 'in vitro' protocols for screening of compounds for anti-viral activity

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Objective

- To standardize protocols and screen compounds for antiviral activity.

Achievements

One compound submitted by a commercial company was tested for its antiviral activity against influenza A, HSV-1 and CoxB3. No anti-viral activity was detected in the 'in vitro' tests.

Amantadine sensitivity of influenza A viruses

A study to check naturally emerging amantadine resistant influenza A virus strains in Pune has been undertaken. Influenza A virus strains isolated from 2000-2004 are being screened for amantadine resistance in MDCK cells. A total of 24 strains have been screened for amantadine sensitivity (Table 5). All the strains were found to be sensitive to amantadine with varying degrees of sensitivity. Screening of other strains and RT-PCR for confirmation is in progress.

Table 5: Influenza A virus strains tested for Amantadine sensitivity

Year of Isolation	Strain No.	Log titer	Log s sensitivity*	Strain No.	Log titer	Log sensitivity*
2000	003150	5.5	4.0	003783	4.0	3.0
	003180	6.0	5.0	003827	5.0	2.5
	003589	5.0	2.0			
2002	025502	4.0	2.5	025506	5.0	2.5
2003	03287	5.0	2.0	035403	5.5	4.5
	031744	3.0	2.5	036393	5.0	4.0
	031896	6.0	3.0	036689	5.0	5.0
	032261	6.0	5.0	036800	6.0	6.0
	032699	6.0	5.0	033990	6.0	2.0
	032969	7.0	4.0	038072	7.0	3.0
	035402	5.0	2.0			
2004	04600	5.0	1.0	045007	5.0	1.0-1.5
	042350	5.0	1.0	045058	4.0	1.0-1.5

*Log difference in virus titres in absence and presence of amantadine

Future plan

Testing of activity of candidate antiviral compounds against influenza A, HSV-1 and Cox B3 will be undertaken as a service project.

Adenovirus

Development of ELISA for the diagnosis of Adenovirus infection

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Adenoviruses cause respiratory illness outbreaks in humans specially among military recruits with high morbidity. It is imperative to develop a sensitive and rapid diagnostic test for Adenoviral infections.

Objective

Development of an ELISA based rapid diagnostic test for the detection of adenovirus infection.

Achievements

Mice were immunized with a human adenovirus isolate available at NIV and ascitic fluid induced for harvesting peritoneal fluid (PF). Titration of rabbit immune serum and mouse PF in indirect ELISA was performed and titers found to be 10^3 in mouse PF, 10^6 in rabbit serum. Standardization of the antigen capture ELISA in progress.