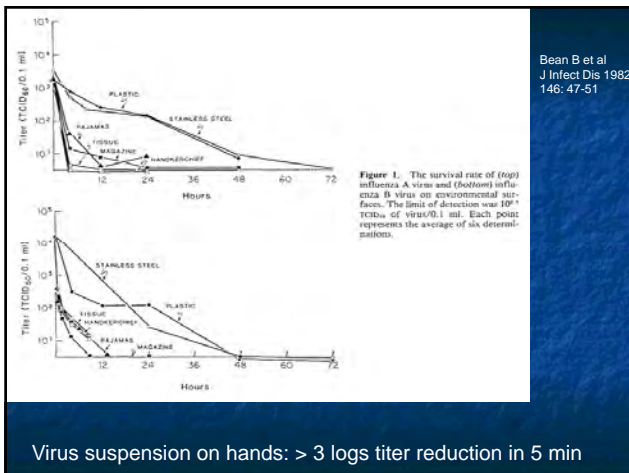


Transmission of Influenza

Raymond Tellier
 MD MSc FRCPC CSPQ FCCM D(ABMM)
 Associate Professor University of Calgary

Possible routes of transmission of influenza in humans

- Aerosols (droplets nuclei, airborne)
- Large droplets
- Fomites, direct contact



Schurman W, Eggers HG Antiviral Res 1983; 3: 25-41

TABLE 2
 Hand test: inactivation of eleven viruses by 'Desderman'

Virus	Sample titers (in log ₁₀ /0.2 ml)			Titer reduction control-test (in log ₁₀)
	Expected ^b	Control ^c	Test ^d	
FPV	6.2	2.5	<0	>2.5
Influenza A/WSN	5.5	2.5	<0	>2.5
Vaccinia MVA	4.4	1.4	<0	>1.4
Adeno 5	5.1	2.2	0.1	2.1
Polio 1	6.5	5.4	4.4	1.0
Polio 2	5.2	3.7	3.5	0.2
Coxsackie B3	6.0	4.3	3.2	1.1
Coxsackie B4	5.9	4.2	2.9	1.3
Echo 9 Hill	6.1	2.8	2.1	0.7
Echo 9 Barty	5.3	4.3	3.0	1.3
SV 40	5.9	3.9	3.0	0.9
Polio 1/5% FA ^a	6.8	5.7	<0	>5.7

^a 5% formaldehyde was included as a reference disinfectant.
^b Amount of virus applied to hands.
^c Amount of virus actually recovered from hands treated with water.
^d Amount of virus recovered from hands treated with disinfectant.

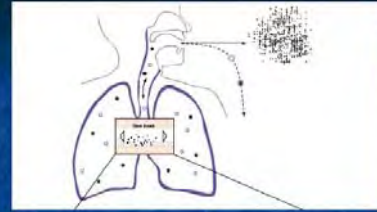
Hand test procedure required 12 min for completion
 Drop of 3 logs in 12 min

Grayson et al CID 2009; 48: 285-291

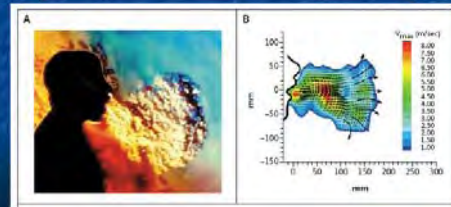
Table 2. Assessment, by PCR and culture, of the efficacy of various hand hygiene (HH) protocols against live H1N1 influenza virus on the hands of 14 human volunteers who were culture-positive at baseline.

HH product	Real-time RT-PCR, ^a mean Ct value ± SD (range)		Culture TCID ₅₀ /0.1 mL level, mean ± SD (range)	
	Palm	Glove juice	Palm	Glove juice
Control	24.0 ± 3.4 ^b (19.8–32.2)	24.3 ± 3.8 ^b (18.6–32.4)	3325 ± 8352 ^c (0–32,000)	1041 ± 1701 ^c (0–5600)
SW	37.6 ± 3.2 ^d (30.9–40.1)	39.4 ± 1.1 ^d (37.0–40.1)	0 (0–0)	0 (0–0)
ETOH only	34.9 ± 2.6 ^d (30.4–40.1)	33.3 ± 2.1 ^d (30.1–36.2)	0 (0–0)	0 (0–0)
ISOP-CHX	35.7 ± 2.2 ^d (32.8–40.1)	33.5 ± 2.5 ^d (30.5–39.9)	0 (0–0)	0 (0–0)
ETOH-CHX	34.4 ± 2.9 ^d (28.3–38.2)	33.3 ± 3.0 ^d (28.9–38.6)	0 (0–0)	0 (0–0)

Initial inoculum: 1.8×10^7 TCID₅₀ / 0.1 ml
Drop by 3-4 logs over 2 min



Pfeifer et al, Drug Discovery Today 2006; 1: 51-57



Tang JW, Smitson GS. New Engl J Med 2008; 359:15. e19

Aerosols

- Aerosols are dispersions in air (or a gas) of solid or liquid particles, small enough that they remain airborne for a long time because of their low settling velocity

Aerodynamic diameter of particle	Settling time in still air (3 m fall)
100 μm	10 sec
40 μm	1 min
20 μm	4 min
10 μm	17 min
5 μm	67 min
≤3 μm	Essentially do not settle

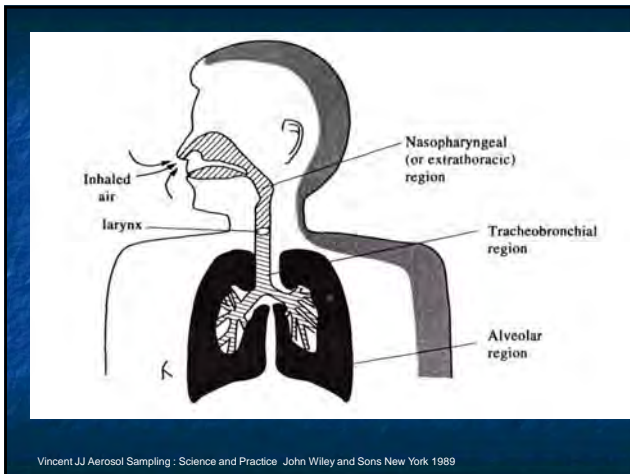
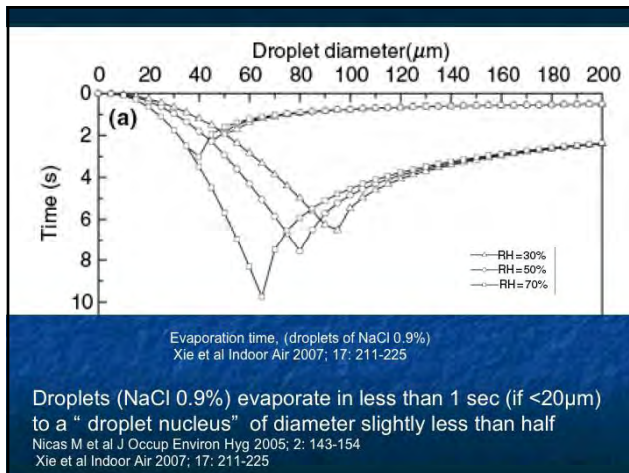
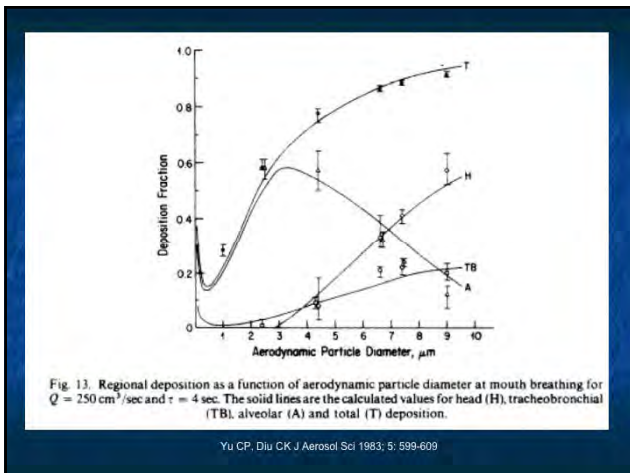


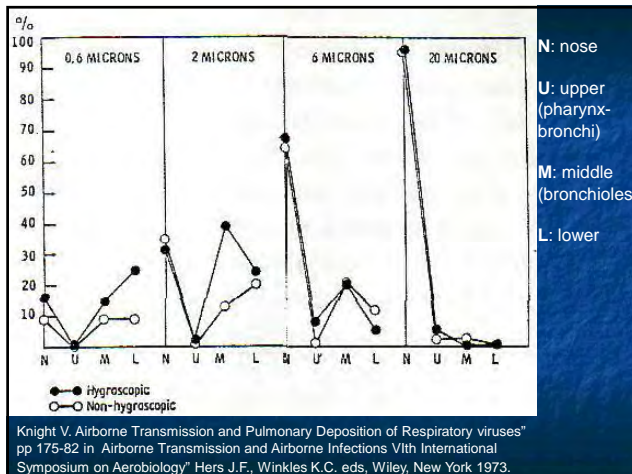
TABLE 11.5 Inhalable, Thoracic, and Respirable Fractions*

Aerodynamic Diameter (μm)	Inhalable Fraction	Thoracic Fraction	Respirable Fraction
0	1.00	1.00	1.00
1	0.97	0.97	0.97
2	0.94	0.94	0.91
3	0.92	0.92	0.74
4	0.89	0.89	0.50
5	0.87	0.85	0.30
6	0.85	0.81	0.17
8	0.81	0.67	0.05
10	0.77	0.50	0.01
15	0.70	0.19	0.00
20	0.65	0.06	0.00
25	0.61	0.02	0.00
30	0.58	0.01	0.00
35	0.56	0.00	0.00
40	0.55	0.00	0.00
50	0.52	0.00	0.00
60	0.51	0.00	0.00
80	0.50	0.00	0.00
100	0.50	0.00	0.00

*ACGIH (1997).

Hinds WC
Aerosol Technology 2nd ed
John Wiley & Sons
New York 1999





Size cut-off of aerosols?

- Virtually everyone agrees that $\leq 5\mu\text{m}$ are aerosols
- Virtually everyone agrees that $> 20\mu\text{m}$ are large droplets
- Most agree that $\leq 10\mu\text{m}$ are aerosols
- Some include 10-20 μm

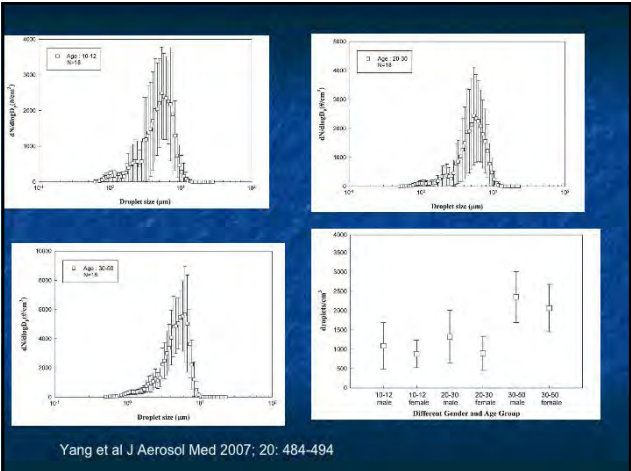
TABLE II. Numbers of Particles in Different Initial Diameter Ranges Emitted in One Cough and One Sneeze According to Duguid

(Nicas M et al J Occup Environ Hyg 2005; 2: 143-54)

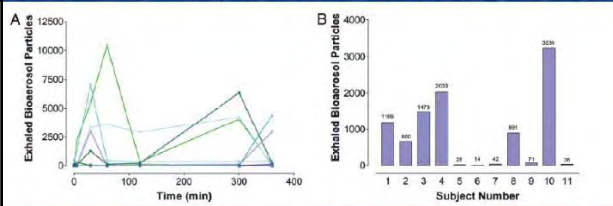
Diameter Range (μm)	Number of Particles in a Cough	Number of Particles in a Sneeze
1-2	50	26,000
2-4	290	160,000
4-8	970	350,000
8-16	1600	280,000
16-24	870	97,000
24-32	420	37,000
32-40	240	17,000
40-50	110	9,000
50-75	140	10,000
75-100	85	4,500
100-125	48	2,500
125-150	38	1,800
150-200	35	2,000
200-250	29	1,400
250-500	34	2,100
500-1000	12	1,000
1000-2000	2	

99.9% of volume is contained in particles $> 8\mu\text{m}$

Source: Data from Duguid, "The Size and Duration of Air-Carriage of Respiratory Droplets and Droplet-Nuclei." *Journal of Hygiene* 4:471-480, Table 3 (1946).



Aerosols exhaled during normal breathing
($> 150 \text{ nm}$; $< 1 \mu\text{m}$)



Edwards DA et al Proc Natl Acad Sci USA 2004; 101:17383-17388

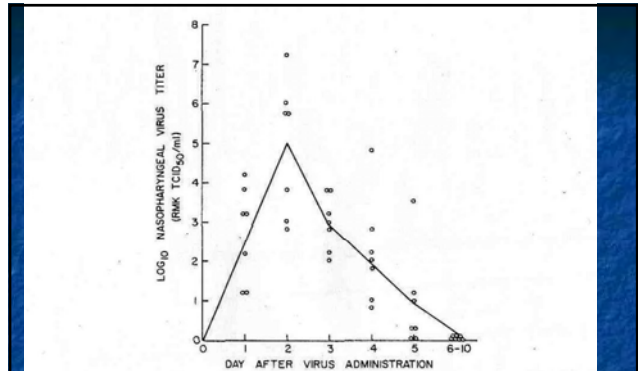


Fig. 13.9. Quantities of influenza A (H3N2) recovered from NW specimens obtained daily after inoculation from 7 infected volunteers. RMK, rhesus monkey kidney. From Murphy et al. (1973b). *J. Infect. Dis.* 128, 479-487. Copyright 1973.

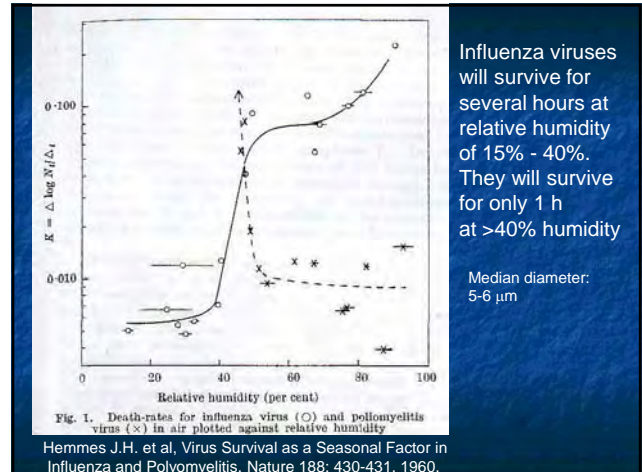
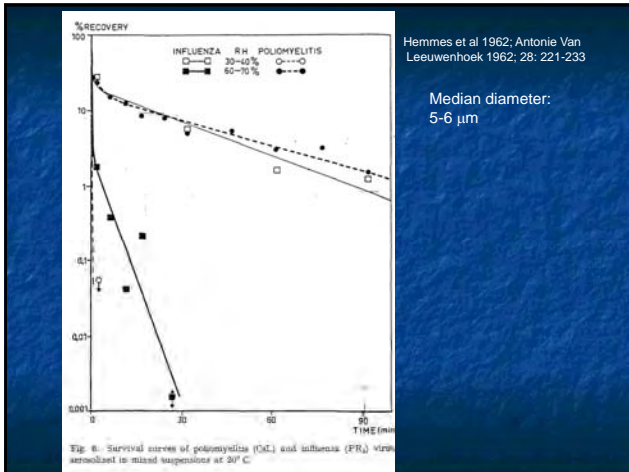
(Douglas R.G. Influenza in Man, Pp 375-447 in The Influenza Viruses and Influenza, Kilbourne E.D. ed, Academic Press, New York 1975.)

Aerosols and long range transmission

- Aerosols can be carried over long range by air currents/turbulences
- Long range infection risk modulated by dilution, removal by ventilation, amount of infectious agents at the source, biological decay, infectious dose
- Low frequency of long range infection is difficult to demonstrate or rule out, especially if the disease is present in the community



Gelfand HM, Posch J. Am J Epidemiol 1971; 93: 234-237



Experimental infection with influenza (human volunteers)

- Experimental infection studies permit to separate clearly the aerosol route of transmission from transmission by large droplets
- Homogeneous small particle aerosols without large droplets
- Large droplets transmission is by intranasal drops (no accompanying aerosols)

TABLE I. Aerosol Administration of Influenza A2/Bethesda/10/63 to Volunteers.

Inhaled virus (TCID ₅₀)	Vol #	Illness	Virus recovery (days after inoc)	Neutralization antibody	
				Before inoculation	28 days after
126	1	*	*	1280	3200
	2			2560	2560
	3			640	1280
78	4			160	160
	5			320	320
	6			320	320
50	7			40	1280
	8			80	80
	9			80	80
1	10	+	3-7	<5	80
	11			<5	<5
	12			<5	<5
	13			<5	<5
5	14		4-7	<5	320
	15			40	40
	16			80	80
	17			40	40
2	18			10	1280
	19	+		20	1280
	20	+	2-6	5	5120
	21			<5	<5
	22	+	2-6	<5	640
	23			<5	<5
	24			<5	<5

* Blank spaces indicate no response.

Alford RH et al Proc Soc Exp Biol Med 1966; 122: 800-804 1 to 3 μ m

Comparison of human infectious dose of influenza virus by aerosol or intranasal route

- Aerosol (airborne):
HID₅₀ = 0.6 to 3 TCID₅₀
- Intranasal (large droplet)
HID₅₀ = 127 to 320 TCID₅₀

(Douglas R.G. Influenza in Man. Pp 375-447 in The Influenza Viruses and Influenza, Kilbourne E.D. ed, Academic Press, New York 1975.)

Table 2. Fifty percent human infectious dose in units of 50% tissue culture infectious doses (TCID₅₀) for 4 respiratory viruses by 1.5 micron diameter aerosol.

site	(1) nose	(2) pharynx, bronchi	(3) bronchioles	(4) alveolar ducts	(5) total ret. estabed	(7) total inhaled	(8) nasal drops
percent of inhaled dose	37.0	1.0	25.0	21.0	84.0	16.0	HID ₅₀
rhinovirus type 13	0.24	0.007	0.170	0.14	0.56	0.12	0.68* (0.3-2.0)
coronavirius A type 21	10.10	0.200	7.000	5.000	23.20	4.80	28.00 (15-49)
adenovirus type 4	0.18	0.005	0.125	0.11	0.42	0.08	0.50 (0.2-1.4)
influenza A2/hongkong/1063	1.08	0.030	0.750	0.63	2.49	0.51	3.00 (estimate)

Knight V,pp. 175-182 in: Hers JF, Winkles KC, eds. Airborne Transmission and Airborne Infections Vith International Symposium on Aerobiology. New York: Wiley, 1973

"When the infectious dose deposited by aerosol in the nose is smaller than the infectious dose by nasal drops, it is probable that the lower respiratory tract is the site of initiation of infection" (V. Knight)

Diseases caused by experimental infections

- by the aerosol route: disease is very similar to that seen in natural infections.
- by intranasal drops (large droplets): disease is milder, with a longer incubation period and usually no involvement of the lower respiratory tract.

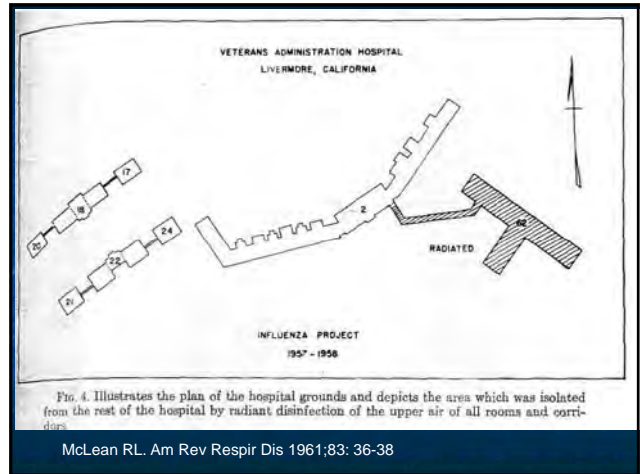
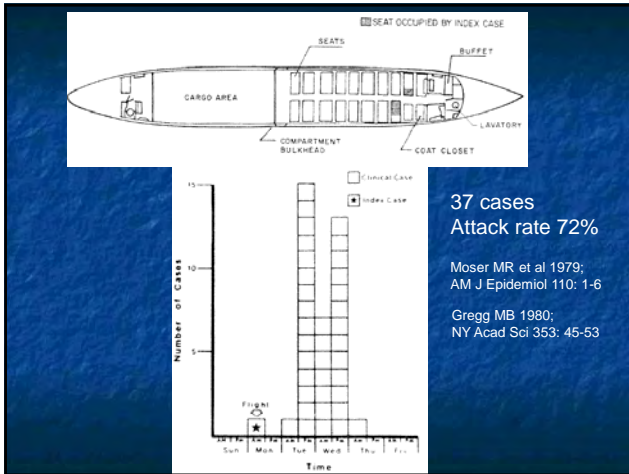
1) Douglas R.G. Influenza in Man. Pp 375-447 in The Influenza Viruses and Influenza, Kilbourne E.D. ed, Academic Press, New York 1975
 2) Little J.W. et al. J Med Virol 3: 177-188, 1979.
 3) Knight V,pp. 175-182 in: Hers JF, Winkles KC, eds. Airborne Transmission and Airborne Infections Vith International Symposium on Aerobiology. New York: Wiley, 1973

TABLE I. Virus, Subjects and Virus Characterization

	Experimental influenza			Naturally acquired influenza		
	A/England/42/72	A/Sydney/74	A/Victoria/75	A/Port Chalmers/74	A/Port Chalmers/74	A/Victoria/75
Number of subjects	8	8	8	15	8	21
Age (mean)	22.3	22.5	22.9	20.0	21.1	21.3
Sexes	3	2	3	0	2	2
Passage history	Human	Human	Human	-	-	-
Dose	10 ^{5.0} TCID ₅₀ ^a	10 ^{5.0} TCID ₅₀ ^a	10 ^{5.0} TCID ₅₀ ^a	-	-	-
Route	Nasal drops	Nasal drops	Nasal drops	-	-	-
Virus isolation	8	8	7	10	7	20
> 4-fold antibody response	7	6	7	9	4	16
Signs and symptoms	8	8	8	15	8	21
Virus isolation and/or antibody response	8	8	8	15	8	21
Ill subjects	8	8	6	13	8	21
Febrile illness (> 100° F)	7	5	4	12	8	21
Cough	2	5	5	12	8	21

^a 100% infectious efficiency.
^b 100% infectious efficiency.
^c 100% infectious efficiency.

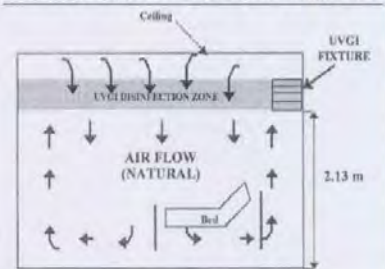
Little JW et al; J Med Virol 1979; 3: 177-188



UV irradiation

- Very effective in inactivating viruses in small particle aerosols
- Poor inactivation of viruses in large droplets: inhibited by high humidity
- Poor inactivation on surfaces: poor penetration
- Only upper air of rooms irradiated: hence only aerosols (which rise by thermal mixing) will be exposed.

Figure 3. Section view of wall-mounted UVGI fixture irradiating the upper room space over a hospital bed



Brickner et al Public Health Reports 2003; 118: 99-114

TABLE 9
NUMBER OF PATIENTS WITH ACUTE RESPIRATORY SYMPTOMS
Phase 2, November 16, 1957-March 16, 1958

Week of	Radiated		Nonradiated	
	Influenza	Other	Influenza	Other
12/15	0	0	2	0
12/22	0	1	1	5
12/29	0	0	0	8
1/5	0	2	7	4
1/12	0	0	18	6
1/19	0	0	10	4
1/26	0	1	1	1

TABLE 10
SEROLOGIC DATA FOR ENTIRE PERIOD OF STUDY

	Initial Negative	Fourfold Rise	Fw Cent Positive
Patients:			
Radiated.....	209	4	2
Nonradiated...	396	75	19
Personnel.....	511	92	18

Antiviral therapy 4: 143-149

Safety and efficacy of once daily intranasal zanamivir in preventing experimental human influenza A infection

David P. Calfee¹, Amy W. Peng², Elizabeth K. Hussey², Monica Lobo¹ and Frederick G. Hayden^{1*}

¹Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, Va, USA
²Glaxo Wellcome Inc., Research Triangle Park, N.C., USA

Table 4. Prevention of experimental infection with influenza A/Texas/91 (H1N1) with various intranasal zanamivir regimens

Study group	Number of subjects	Number (%) shedding virus	Number (%) with seroconversion	Number (%) with infection
Zanamivir 48 h prior to inoculation	12	6 (50)	7 (58)	8 (67)
Zanamivir 4 h prior to inoculation	12	1 (8)*	4 (33)	4 (33)
Zanamivir daily for 3 days	10	1 (10)*	0 (0)†	1 (10)*
Placebo	9	7 (78)	7 (78)	7 (78)

*P<0.05 compared to placebo.
 †P<0.05 compared to placebo.

Short-Term Treatment with Zanamivir to Prevent Influenza: Results of a Placebo-Controlled Study

Laurent Kaiser,¹ Dan Henry,² Nancy P. Flack,³ Oliver Keene,⁴ and Frederick G. Hayden¹

From the ¹Division of Epidemiology and Virology, Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville; ²Foothill Family Clinic, Salt Lake City, Utah; ³Glaxo Wellcome, Research Triangle Park, North Carolina; and ⁴Glaxo Wellcome Research and Development, Greenford, Middlesex, United Kingdom.

Table 2. Incidence of symptomatic influenza (S) or asymptomatic influenza (AS) after initiation of prophylaxis, by treatment group.

Proven influenza	Placebo (n = 144)	Zanamivir			Total no. of subjects
		Intranasal (n = 141)	Inhaled (n = 144)	Intranasal and inhaled (n = 146)	
S or AS during 21 d after initiation	27 (19)	28 (20) ←	16 (11) ←	21 (14)	92
S during 10 d after initiation	11 (8)	9 (6)	4 (3)	6 (4)	30
OR (95% CI)		0.81 (0.30-2.22)	0.31 (0.09-1.04)	0.51 (0.17-1.49)	
S during 5 d of prophylaxis	9 (6)	8 (6) ←	3 (2) ←	5 (3)	25
OR (95% CI)		0.9 (0.30-2.72)	0.27 (0.07-1.05)	0.52 (0.17-1.58)	

NOTE. Data are no. (%) of subjects, except as indicated. ORs and 95% CIs stratified by center were calculated by use of Mantel-Haenszel estimates with test-based CIs.

Table 3. Selected Trials of Prophylaxis with the Use of Neuraminidase Inhibitors.

Study and Drug	No. of Patients	Characteristics of Patients	Setting of Prophylaxis	Reduction in Incidence of Influenza*
Zanamivir				
Monto et al. ¹³	1107	Healthy adults	Seasonal prophylaxis in the community	69% (laboratory-confirmed influenza)
Cooper et al. ¹⁴	Pooled number	Healthy adults	Prophylaxis after exposure in household	81%
Oseltamivir				
Hayden et al. ¹⁵	1559	Healthy adults	Seasonal prophylaxis in the community	87% (laboratory-confirmed influenza); 74% (influenza-like illness)
Welliver et al. ¹⁶	955	Teenagers and adults (>12 yr)	Prophylaxis after exposure in household	89% (laboratory-confirmed influenza); 84% (disease in the household)
Hayden et al. ¹⁹	812	All ages (including children >1 yr)	Prophylaxis after exposure in household	68% (laboratory-confirmed influenza) (85% excluding patients who tested positive at start of prophylaxis); children, 55% (80% excluding patients who tested positive at start of prophylaxis)†
Peters et al. ¹⁸	548	Elderly persons (>80% vaccinated against influenza)	Seasonal prophylaxis in institutional setting	92% (laboratory-confirmed influenza)

*Influenza was defined as both laboratory-confirmed influenza and influenza-like illness, unless otherwise indicated.
 †Results were compared with the treatment of index cases.

N Engl J Med 2005;353:1363-73.

Table 2. Influenza virus type, results for each qPCR replicate, and exhalation rate.

Subject ID	Influenza virus type (sub-type)	qPCR of Filter Extract ^a			Influenza virus RNA exhalation rate ^b
		Replicate 1	Replicate 2	Replicate 3	
A-05	A (H3)	47	21	44	20
A-07	A (H3)	ND	ND	<5	<3.2
A-08	B	ND	ND	ND	ND
A-11	B	ND	ND	ND	ND
A-21	A (H3)	ND	ND	ND	ND
A-23	A (H3)	ND	ND	<6	<3.2
A-24	B	ND	7	ND	<3.2
A-25	B	ND	ND	ND	ND
A-34	B	ND	ND	ND	ND
B-01	A (H3)	ND	ND	ND	ND
B-09	B	ND	ND	ND	ND
B-25	B	ND	ND	ND	ND
A-37 (control)	ND	ND	ND	ND	ND
A-38 (control)	ND	ND	ND	ND	ND

^aNumber of influenza RNA copies detected per well (5 µl cDNA per well).
^bInfluenza virus RNA copies/mixture
 ND = not detected by qPCR; limit of quantification was 6 influenza virus RNA copies per qPCR well when all three replicates were detected.
 doi:10.1371/journal.pone.0002691.t002

Fabian P et al PLoS One 2008; 3: e2691

60% of patients with Influenza A have detectable viral RNA
 14% of patients with Influenza B have detectable viral RNA

< 0.1% of particles were > 5µm;
 87% were < 1 µm

Table 1. Clinical investigation of airborne influenza in a hospital emergency department.

Day	No. of patients reporting influenza-like symptoms	Total no. of stationary samplers	Total no. of personal samplers	Samplers showing results positive for influenza virus	No. of TCID ₅₀ -equivalent RNA particles detected in the sampler			
					First stage	Second stage	Filter	
1	4	9	4	Waiting room (lower sampler)	480	0	0	480
				Waiting room (upper sampler)	0	13,428	2862	16,278
				Reception and triage room	0	1941	0	1941
				Personal sampler (physician 1)	3160	0	0	3160
2	0	13	0	Personal sampler (physician 2)	309	0	0	309
				Personal sampler (physician 3)	0	4623	0	4623
				Waiting room (upper sampler)	1114	0	0	1114
3	5	13	1	None	
4	3	13	0	Children's waiting room (lower sampler)	4026	11,040	0	15,066
				Children's waiting room (upper sampler)	6762	<100	0	6762
				Waiting room (lower sampler)	15,532	0	0	15,532
				Waiting room (lower sampler)	0	1367	0	1367

Blachere et al CID 2009; 48: 438-440

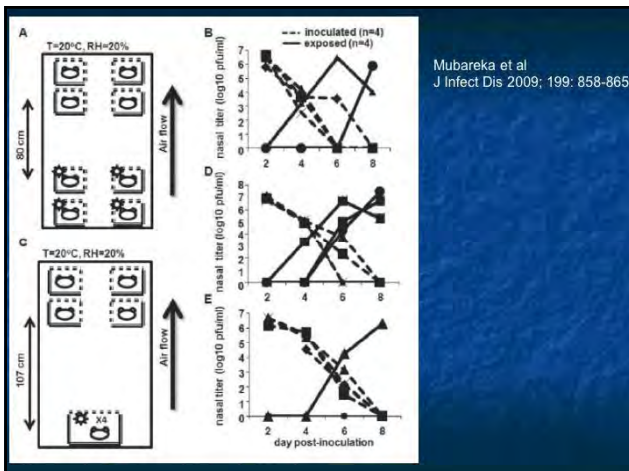
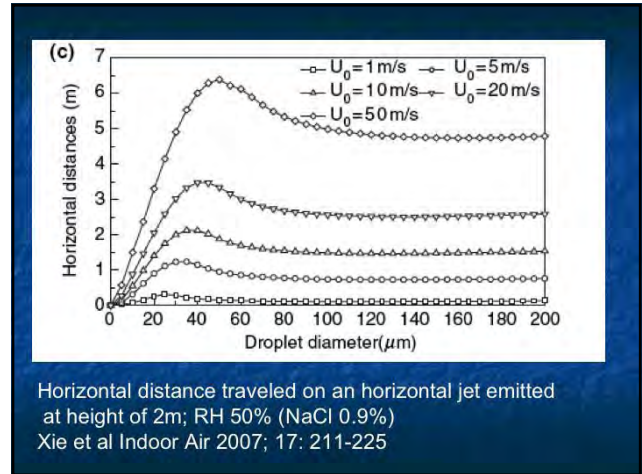
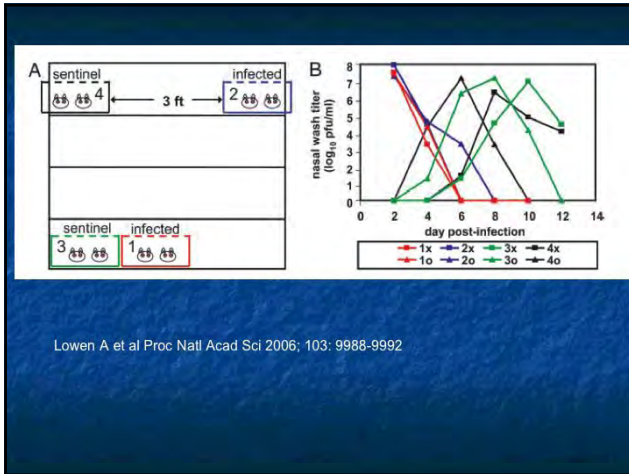
First stage: > 4µm
 2nd stage: 1-4 µm
 Filter: < 1 µm

Transmission of influenza A virus by aerosol to:

- Mice (human influenza; adapted strains)
- Squirrel monkeys (human influenza)

Transmission of Influenza A by aerosols to AND between

- Ferrets (human influenza)
- Horses (equine influenza)
- Quails (HPAI A(H5N1))
- Guinea pigs (human influenza)



JAMA[®] Surgical Mask vs N95 Respirator for Preventing Influenza Among Health Care Workers: A Randomized Trial

Online article and related content current as of July 20, 2010.

Mark Loeb; Nancy Dafoe; James Mahony; et al.
JAMA. 2009;302(17):1865-1871 (doi:10.1001/jama.2009.1466)
<http://jama.ama-assn.org/cgi/content/full/302/17/1865>

Loeb et al Jama Oct 2009

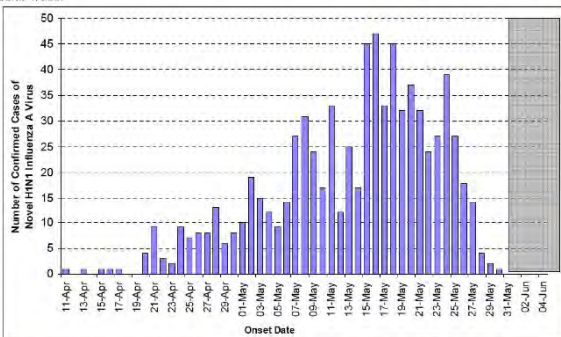
	Surgical Mask	N95	Stats
vaccinated	30.2%	28%	
infected	23.6% (2.8%)	22.9% (1.9%)	P=0.86
compliance	100%	85.7%	
ILI (cough, fever)	4.2%	1.0%	P=0.06
fever	5.66%	0.9%	P=0.007

Table 2. Comparison of Laboratory-Confirmed Influenza Between the Surgical Mask and N95 Respirator Groups

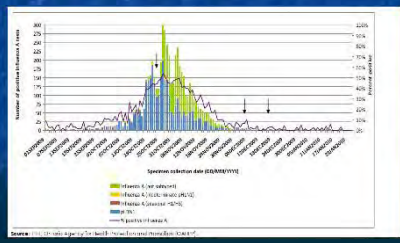
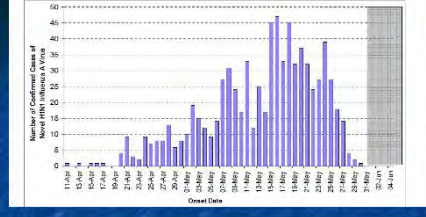
	No. (%)		Absolute Risk Difference, % (95% CI)	P Value
	Surgical Mask (n = 212)	N95 Respirator (n = 210)		
Laboratory-confirmed influenza ^a	50 (23.6)	48 (22.9)	-0.73 (-8.8 to 7.3)	.86
RT-PCR influenza A	5 (2.4)	1 (0.5)	-1.88 (-4.13 to 0.36)	.22
RT-PCR influenza B	1 (0.5)	3 (1.4)	0.96 (-0.89 to 2.81)	.37
≥4-Fold rise in serum titers A/Brisbane/59/2007 (H1N1) ^b	25 (11.8)	21 (10)	-1.79 (-7.73 to 4.15)	.55
≥4-Fold rise in serum titers A/Brisbane/10/2007 (H3N2) ^b	42 (19.8)	49 (23.3)	3.52 (-4.32 to 11.36)	.38
≥4-Fold rise in serum titers B/Florida/4/2006 ^b	15 (7.1)	19 (9.0)	2.0 (-3.0 to 7.17)	.46
≥4-Fold rise in serum titers A/TN/1560/09 (H1N1) ^b	17 (8.0)	25 (11.9)	3.89 (-1.82 to 9.59)	.18

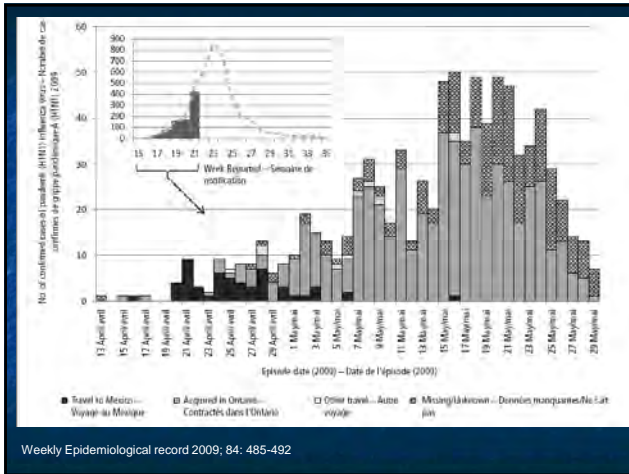
Abbreviations: CI, confidence interval; RT-PCR, reverse-transcriptase polymerase chain reaction.
^aInfluenza detected by 1 or more of the following: RT-PCR A, RT-PCR B, and ≥4-fold rise in serum titers to A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Florida/4/2006. Serology includes only nonvaccinated nurses.
^bIncludes both vaccinated and nonvaccinated nurses. Two hundred ninety-four nurses were not vaccinated (147 in each group).

Figure 3: Confirmed cases of Novel H1N1 Influenza A Virus by Symptom Onset Date in Ontario, Apr 11 - June 4, 2009



Source: Ontario Ministry of Health and Long-Term Care, Integrated Public Health Information System (PHIS) database, extracted at 8:30 am [14/06/2009]





Conclusions

- Several lines of scientific investigations strongly support a role for aerosols in influenza transmission
- In the presence of proper ventilation long range transmission seems to occur at low frequency (if at all)
- Short range aerosol transmission is not merely an academic distinction. It has profound implications for pathogenesis and infection control