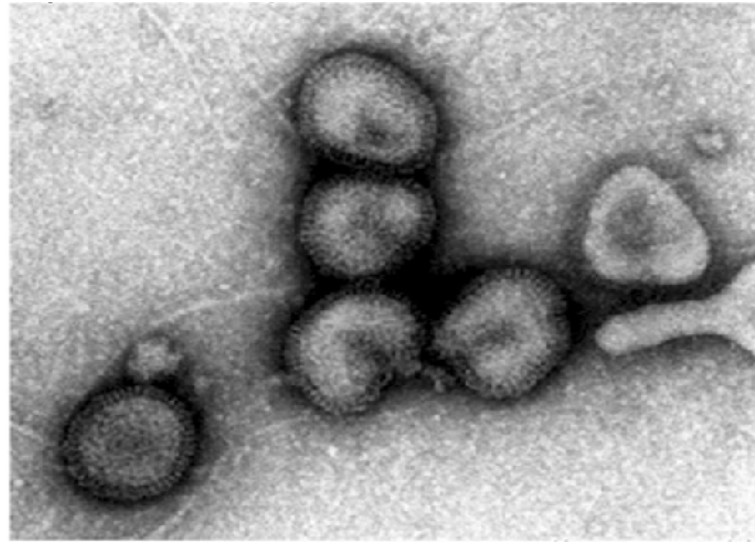


Flu Biochip: a Tool for Influenza A Virus Surveillance



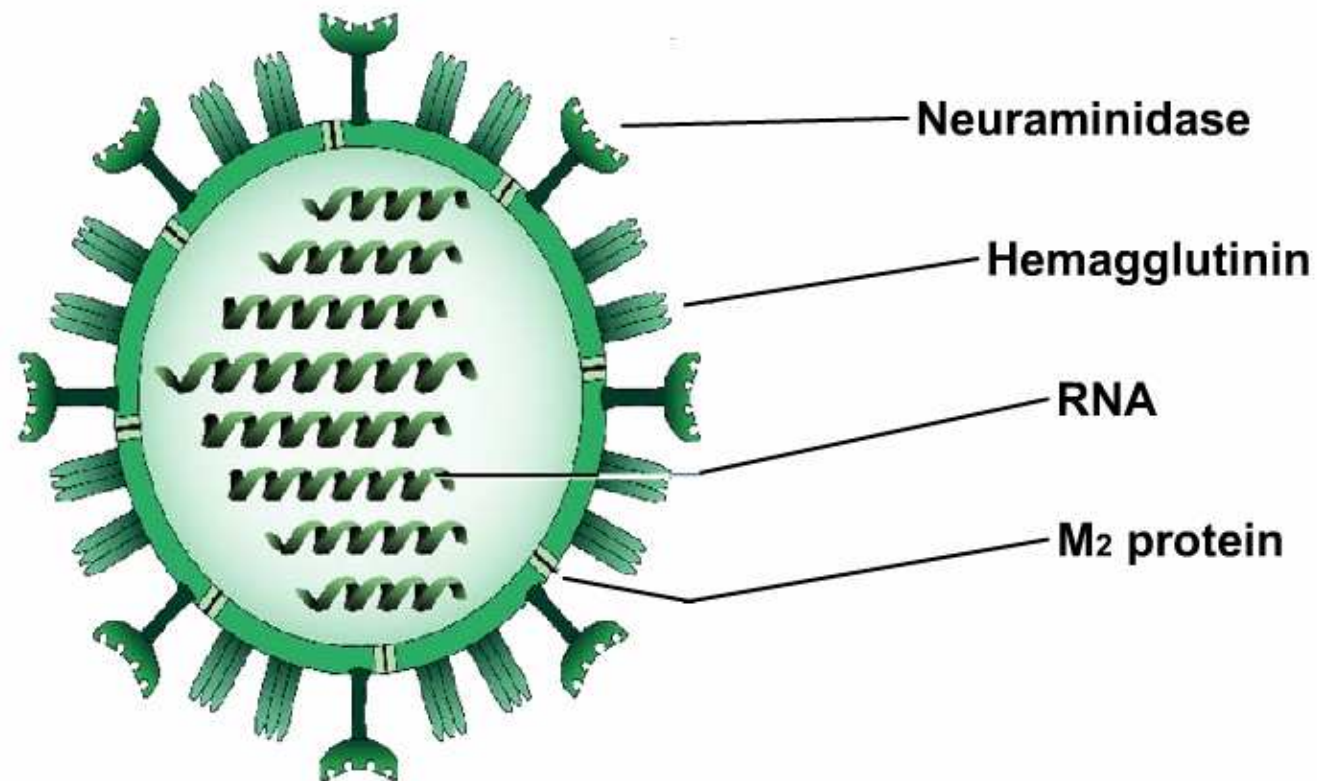
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T.V. Grebennikova^c, D. K. L'vov^c, A.S. Zasedatelev^a

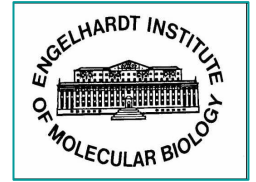
^aEngelhardt Institute of Molecular Biology

^bInstitute of Cell Biophysics

^cD. I. Ivanovsky Institute of Virology

Simplified Structure of Influenza Virus





Influenza Viruses

- Three types of influenza: A, B and C
- Influenza A:

Found in many animals – ducks, chickens, pigs, whales, horses and seals.

Wild birds are “natural” hosts for all flu subtypes.

Accounts for more than 80% of influenza infections in humans each year

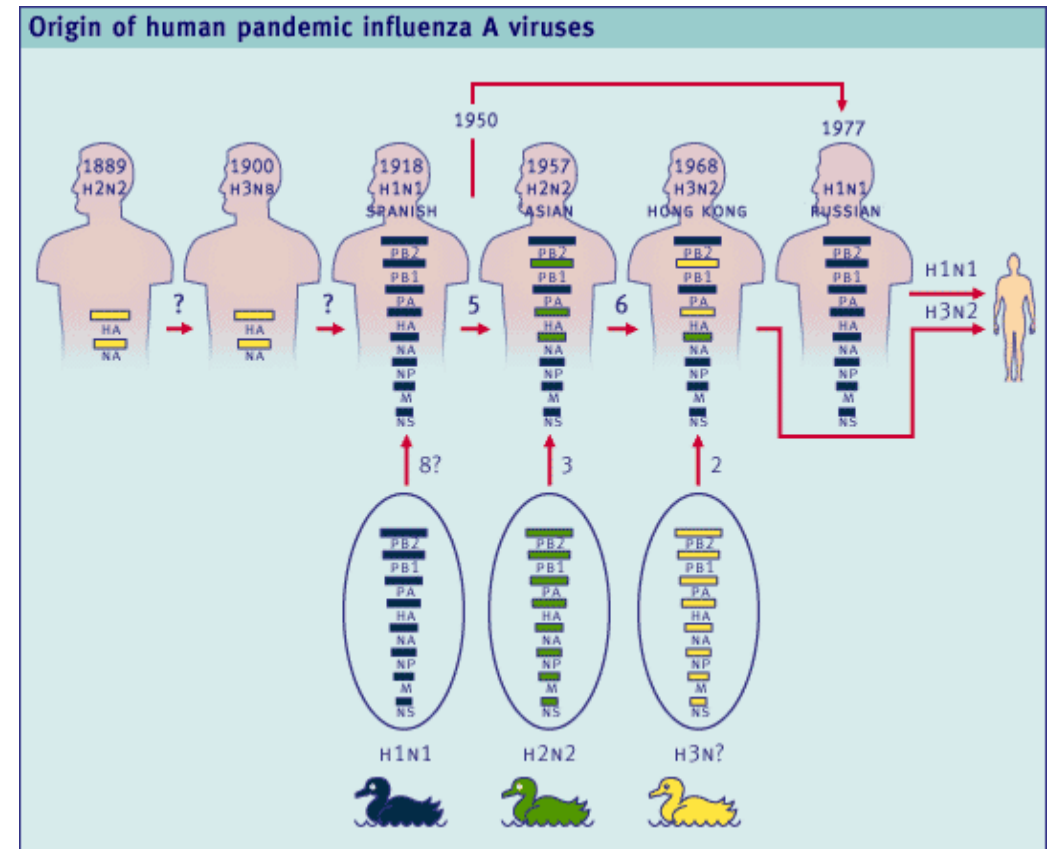
Type A has caused all pandemics.

Sub-typed based on activity in surface glycoproteins:

- 1] hemagglutinin activity (H): 16 known varieties
 - 2] neuraminidase activity (N): 9 known varieties
- Shorthand names have an “H” and “N” number (**H3N2**)

Recent Influenza Pandemics

- 1918 H1N1 “Spanish” flu
20-50 million
- 1957 H2N2 “Asian” flu
>3 million
- 1968 H3N2 “Hong Kong” flu
>1 million



“Avian” flu H5N1

- cases 440
(August 2009)
- deaths 262
- no human to human
transmission

“Swine” flu H1N1

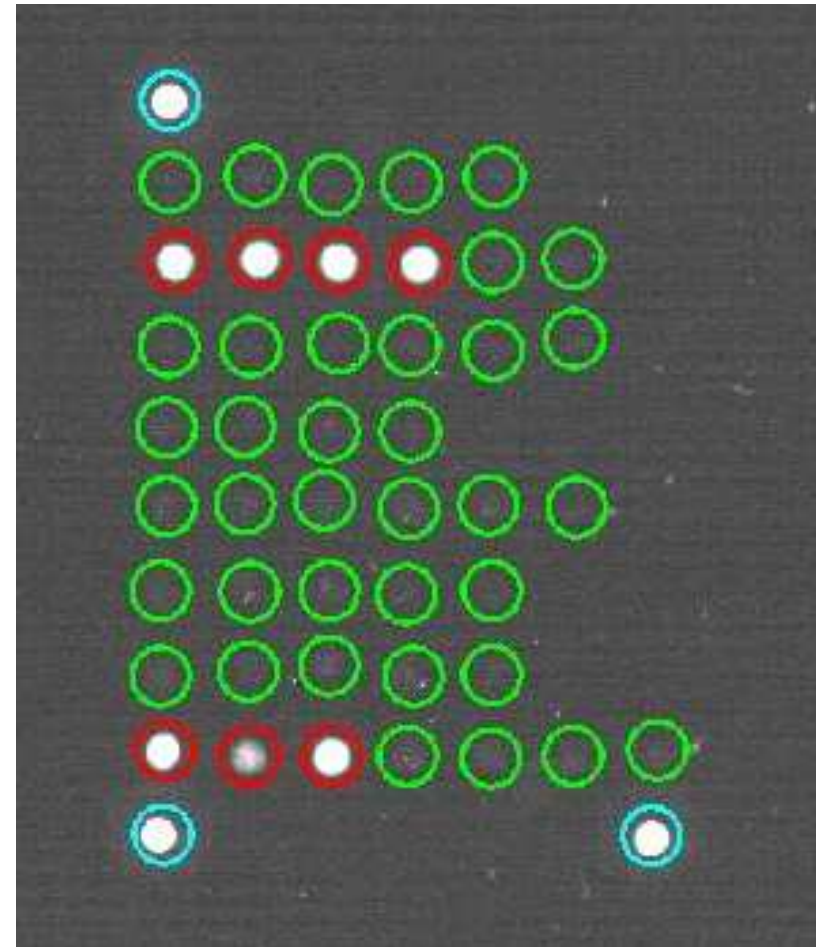
- cases >300 000
(September 2009)
- deaths >3 500
- adapted for
human to human
transmission

Influenza Subtyping

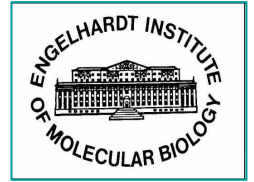
- **Virus isolation, culture, and characterization by immunoassay**
 - “gold standard”
 - 3 to 7 days to culture the virus prior to antigenic testing
 - reference antisera to HA subtypes is needed
- **PCR based methods**
 - 5 to 8 hours
 - limited number of subtypes detectable in a single assay

Scheme of the Flu Biochip

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| a | M | | | | | | |
| b | H1 ₁ | H1 ₂ | H1 ₃ | H2 ₁ | H2 ₂ | | |
| c | H3 ₁ | H3 ₂ | H3 ₃ | H3 ₄ | H3/H4 | H4 | |
| d | H5 ₁ | H5 ₂ | H5 ₃ | H5 ₄ | H5 ₅ | H6 | |
| e | H7 ₁ | H7 ₂ | H7/H10 /H15 | H8 | | | |
| f | H9 ₁ | H9 ₂ | H10 | Neg | Neg | Neg | |
| g | H11 | H12 | H13 | H14 | H15 | | |
| h | N1 ₁ | N1 ₂ | N1 ₃ | N1 ₄ | N1 ₅ | | |
| i | N2 ₁ | N2 ₂ | N2 ₃ | N2 ₄ | N2 ₅ | N2 ₆ | N2 ₇ |
| j | M | | | | | | M |



Scheme of Analysis



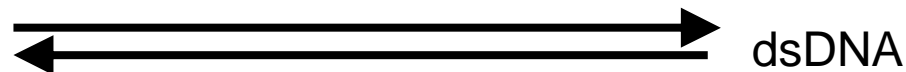
I st.



Reverse transcription of HA and NA segments



Symmetric PCR reaction



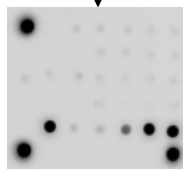
II st.

Asymmetric PCR reaction with fluorescently labeled dUTP

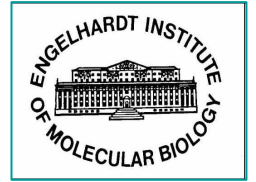


III st.

Hybridization



Steps of Analysis



Two-stage RT
multiplex
amplification

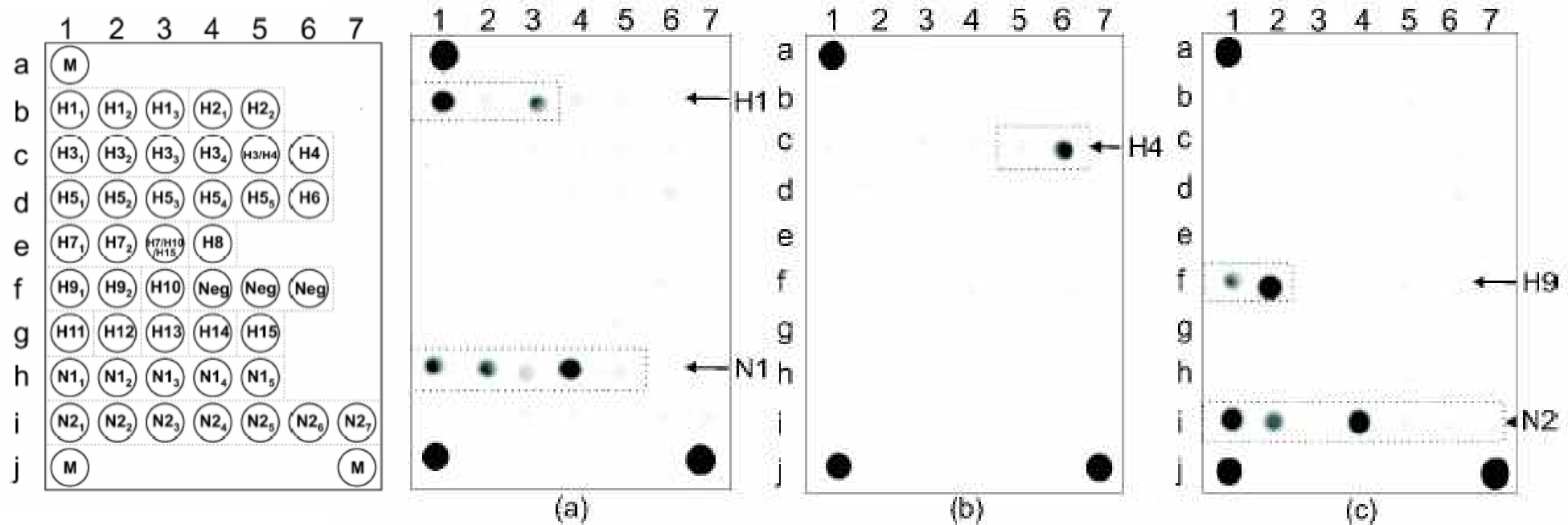
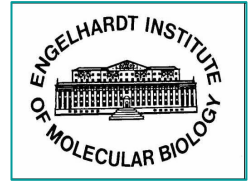


- Hybridization and
washing



- Imaging and
conclusion

Hybridization Patterns of Reference Flu Strains



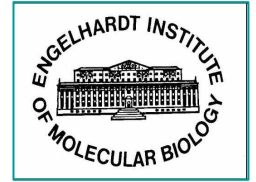
- A/USSR/90/77(H1N1) (a)

- A/Duck/Czechoslovakia/56(H4N6) (b)

- A/Swine/Hong Kong/9/98(H9N2) (c)

The analysis of 21 reference strains never resulted in cross-hybridization of the amplified fragments with elements belonging to different groups within HA- or NA-clusters.

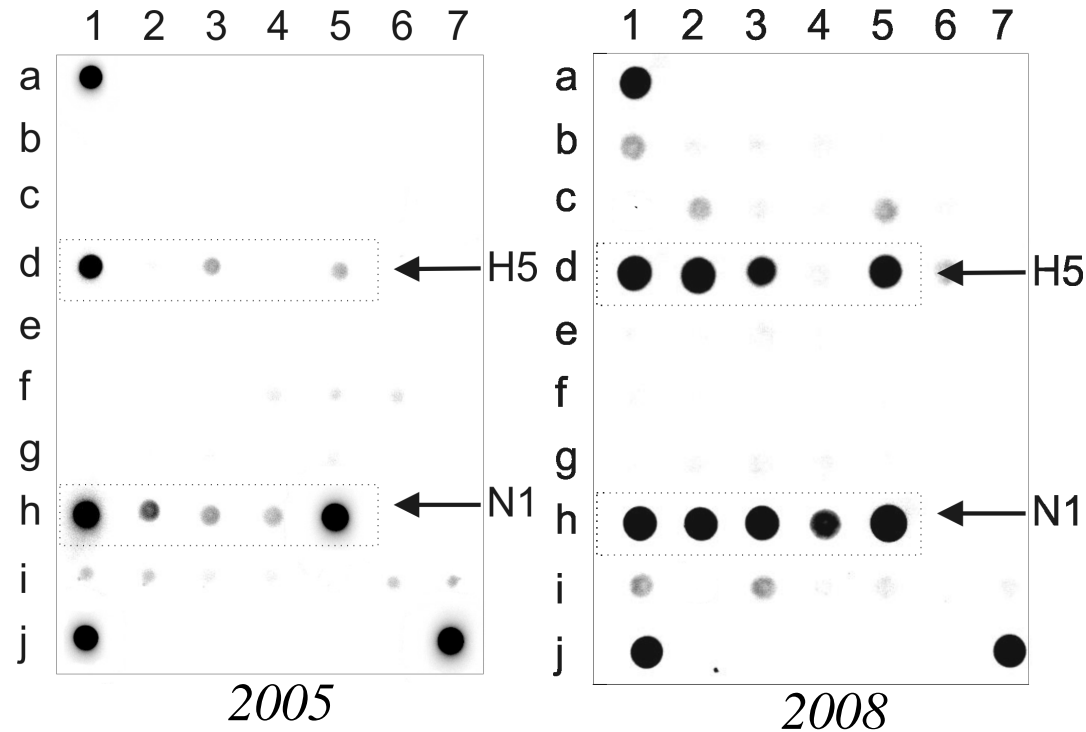
Identification of the Causative Agent of Local Epizooties in Russia



| Geography | Number of specimens | Influenza subtyping | |
|--------------------------|---------------------|---------------------|------------------|
| | | Flu Biochip | Reference method |
| Novosibirsk region, 2005 | 8 | H5N1 8/8 | H5N1 8/8 |
| Astrakhan region, 2005 | 10 | H5N1 10/10 | H5N1 10/10 |
| Republic of Tuva, 2006 | 4 | H5N1 4/4 | H5N1 4/4 |
| Primorye territory, 2008 | 4 | H5N1 4/4 | H5N1 4/4 |

- RNA samples were isolated from pools of internal organs of dead birds

Genotyping of H5N1 Flu



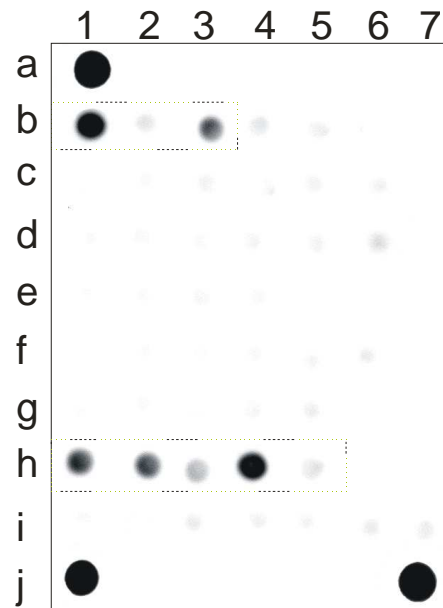
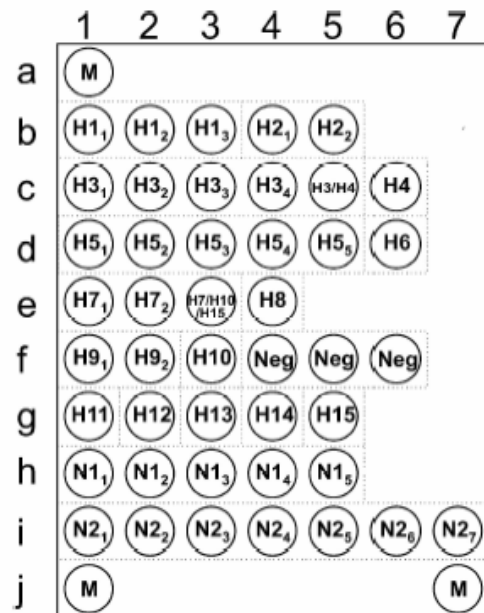
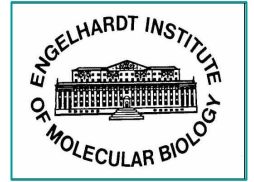
nucleotide homology:

- HA 94,1 %
- NA 94,7 %.

2005 - A/chicken/Novosibirsk/64/05 (H5N1)

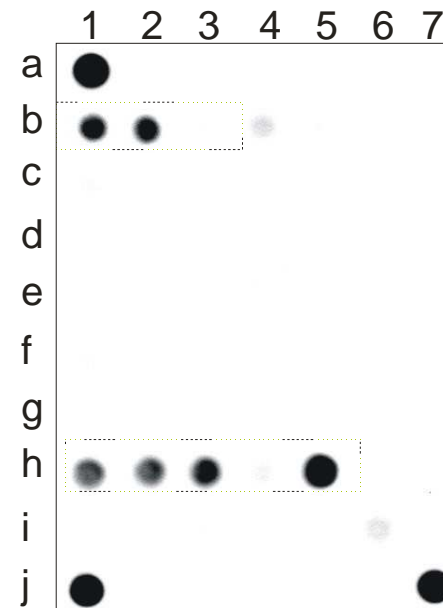
2008 - A/chicken/Primorje/1/08 (H5N1)

Hybridization Patterns of H1N1 Strains



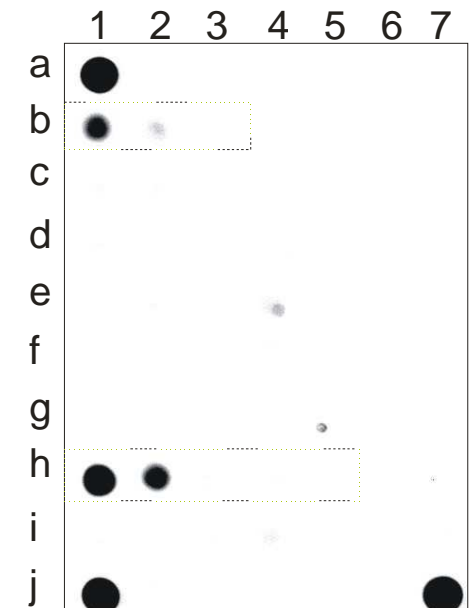
(#1,#2, H1N1)

Seasonal strain



(#8,#9,#10, H1N1)

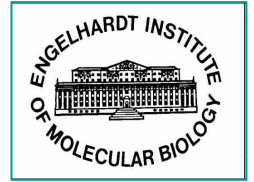
Pandemic strain



(#7, H1N1)

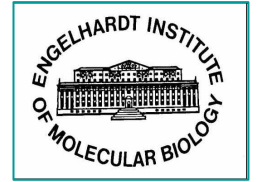
Swine strain

Conclusions



- We developed a gel-based biochip for molecular subtyping of Influenza A virus. The whole procedure takes less than 10 hours and enables one to identify 15 varieties of HA and 2 varieties of NA.
- The diagnostic technique is based on two-stage multiplex amplification of the HA and NA gene fragments followed by on-chip hybridization of the fluorescently labeled PCR-products.
- The assay was validated using a panel of known standard strains of Influenza virus
- The initial experiments on field samples and clinical samples were carried.
- Hybridization pattern enables one to differentiate flu strains within one subtype.

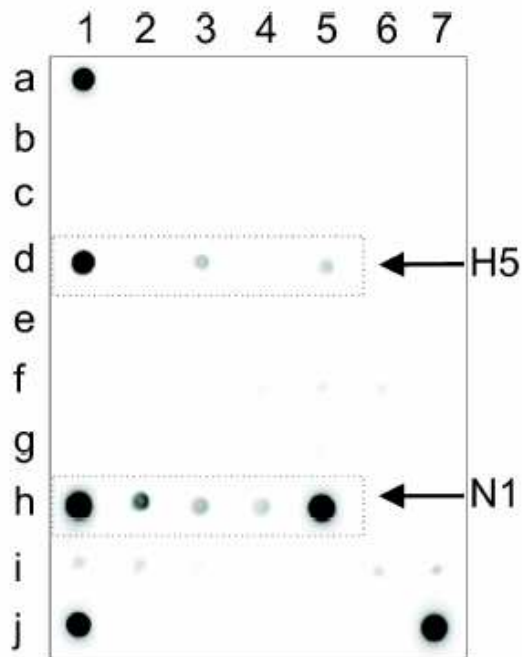
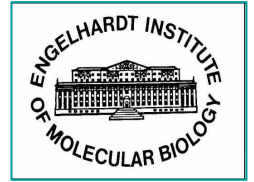
Introduction of the developed method for influenza subtyping in laboratory practice may substantially improve influenza A epidemiological surveillance.



“The late 20th century will be witness to the virtual elimination of infectious disease. To write about infectious disease is almost to write of something which has passed into history”

Sir Frank Macfarlane Burnet
Virologist, 1960 Nobel Prize Winner

Biochip Analysis of 41 Field Samples



- Source of RNA

- 1]pools of internal organs of died birds
- 2]cloacal swabs of sick birds and birds without clinical symptoms in infected area

- 100% specificity

- 76% sensitivity

- The results were compared with “gold-standard” virus isolation with subsequent antigenic testing