**The National Influenza Reference Centre**
**Swedish Institute for Infectious Disease Control**

**(SMI)**

# Annual Report

## September 2004-August 2005

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**Activities.**

1) Monitoring of influenza activity in Sweden

2) Reports from SMI on influenza activity in Sweden

3) Characterisation of influenza strains

4) Data from the 2004-2005 season

5) Quality control of laboratory diagnosis of influenza

6) Method Development and Research

## 1) MONITORING OF INFLUENZA ACTIVITY IN SWEDEN

### 1:1) The Sentinel System.

The Swedish influenza sentinel reporting system consists of 96 sentinel units recruited by the County Medical Officers of Communicable Disease Control. They include both individual GPs and larger health care centres of 2-5 GPs. Twenty out of twenty-one counties participated in the system during 2005 (Gotland county was not included). Date of visit, age and sex of the patients were reported, and the reports were sent to the county medical officers and to the National Influenza Centre at the Swedish Institute for Infectious Disease Control (SMI) by either fax or the web-based reporting system, SentiNet. Weekly reports were received from between 53–88 of the sentinel units, and the total weekly number of out-patient visits to these units ranged between 5325–14688.

### 1:2) Reports of laboratory verified influenza diagnoses.

Influenza isolation is performed at four virus laboratories, placed at University Hospitals and at SMI. The laboratories are relatively evenly distributed with regard to the population in different areas. The laboratories also perform influenza serology, antigen detection with immunoflourescense (IF) and genome detection by polymerase chain reaction (PCR). Another 19 microbiology laboratories diagnose influenza by IF assays, commercial ELISA KITs or nucleic acid amplification (NAA). During the influenza season, the 24 laboratories send weekly reports on the number of influenza cases, diagnosed by antigen detection, NAA and/or virus isolation. Serology results are not included in these reports.

### 1:3) Death rates.

At the end of the influenza season, information on the weekly death rate in Sweden is purchased from Statistics Sweden. Mean weakly death rate for influenza-free weeks between week 40 year 1993 and week 20 year 2005 is calculated, and used as reference for the demonstration of weekly excess mortality.

## 2) REPORTS FROM SMI ON THE INFLUENZA ACTIVITY IN SWEDEN

### 2:1) Weekly reports to the Swedish Collaborators.

Each Wednesday, national and international influenza information collected during the week, including the WHO and EISS reports, is summarised and made available at the SMI home-page (www.smittskyddsinstitutet.se). An electronic newsletter is also sent to all interested parties, including the County Medical Officers of Communicable Disease Control, departments of infectious diseases in Sweden, microbiological laboratories and to the National Board of Health and Welfare. A summary of the activity during the entire season is distributed towards the end of the summer, when all definitive data are available.

### 2:2) Other spread of information in Sweden.

The media are constantly interested in influenza, and usually contact SMI to get information. In most instances those contacts result in correct and informative articles. The institute has a journal ”Smittskydd” and an electronic newspaper “EpiAktuellt”, and during the influenza season reports on the situation are printed there, when appropriate. An information day for the persons who are active in the surveillance system was arranged in Stockholm in September 2004. The European Scientific Study Group on Influenza (ESWI) has supported a network in Sweden, working with the aim to increase the yearly influenza vaccination for risk groups in the country.

**2:3) Reports to WHO and other National Influenza centres.**
Sweden reports to WHO via Flunet, and starts reporting when the first laboratory verified case occurs. In 2000 Sweden joined the European Influenza Surveillance Scheme (EISS), and tries to provide weekly information to the EISS home-page.

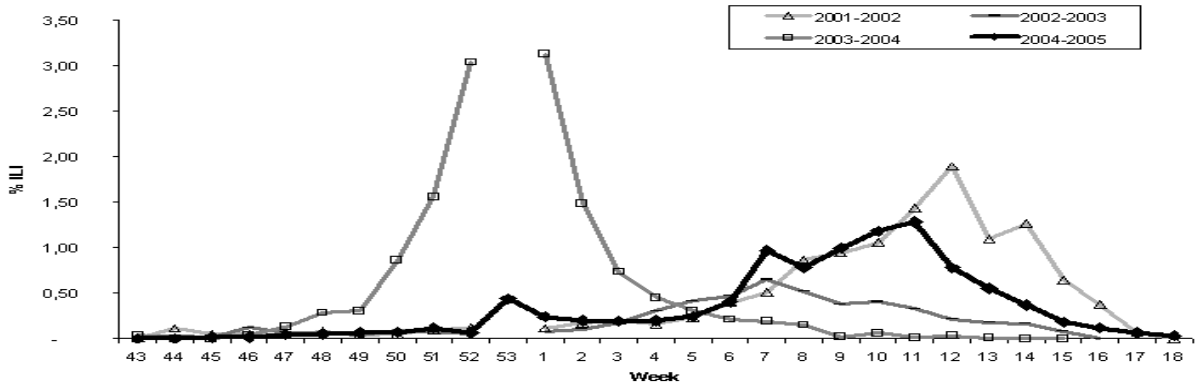
## 3) CHARACTERISATION OF INFLUENZA STRAINS

**3:1) Genotypic and fenotypic characterisation.**
Influenza strains are sent to SMI from the laboratories performing virus isolation. Isolated virus strains are examined for the type and subtype of virus by hemagglutination inhibition (HAI; reagents have been kindly donated from WHO and the Influenza Reference Centre in Rotterdam) and IF with monoclonal antibodies (from Laboratories de Virologie, Lyon). HA and NA-sequencing is also performed. For further characterisation with ferret sera, these strains are also sent to Mill Hill in London.

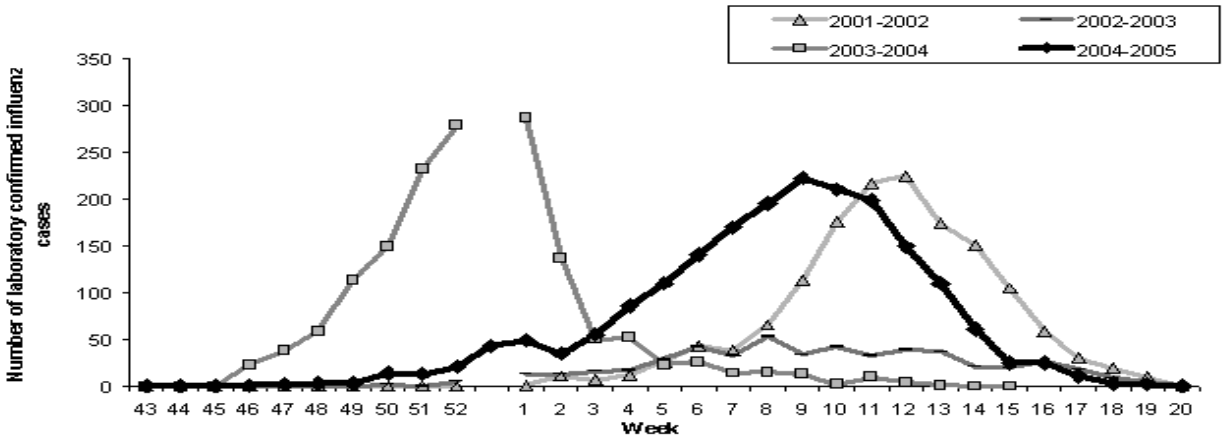
## 4) DATA FROM THE 2004-2005 SEASON
This season, the influenza activity started around Christmas and peaked around week 10 (Fig 4:1 and 2). Overall, the activity was widespread, and of medium intensity. The majority of the reported cases were adults and elderly, with cases aged 65 or older dominating in the laboratory system, while adults between 16–64 years were in majority in the sentinel system (Fig 4:3). The increasing proportion of young children reported in the laboratory system during the previous seasons was not seen in 2004-2005 (Fig 4:4). The total number of laboratory diagnoses was higher in relation to the previous seasons and was 2016, compared to 1590 the previous season. 1802 were influenza A and 214 were influenza B. Of strains sent to SMI for further subtyping 72% were influenza A H3 (Table). There were one H1N1 and 13 B strains. The activity continued until the end of April. The dominating circulation of H3 probably explains the relatively higher rate of laboratory verified cases in comparison to those reported in the sentinel system, since H3 causes more severe disease, prompting more patients to seek hospital care and thereby increasing the likelihood to have a sample sent to the laboratory.

This seasons late peak of influenza activity is reflected in the excess mortality seen between weeks 7 - 15 (Fig 4:6).

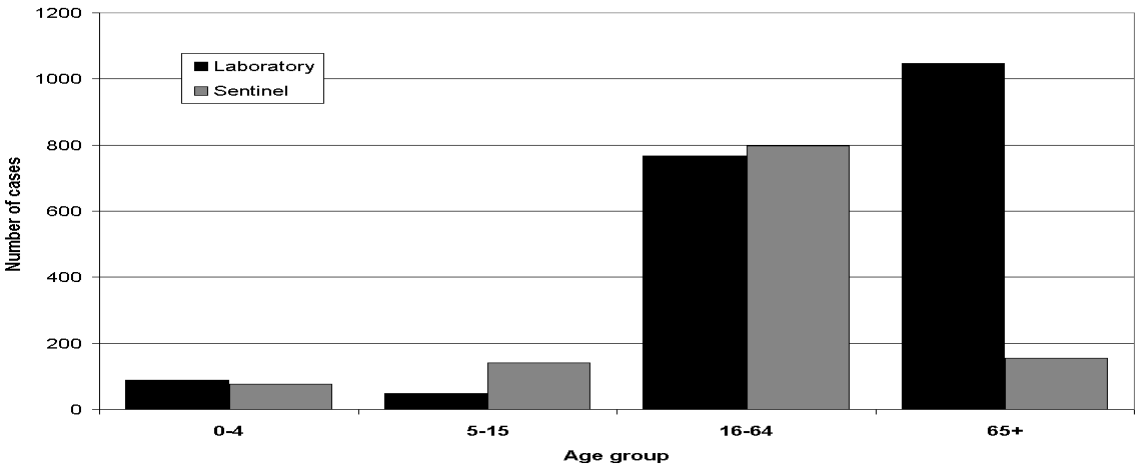
All the influenza strains further characterised were very similar to the strains prevalent in Europe (Fig 4:7-10. No mutations know to induce resistance against neuraminidase inhibitors were identified in the sequenced NA-genes.



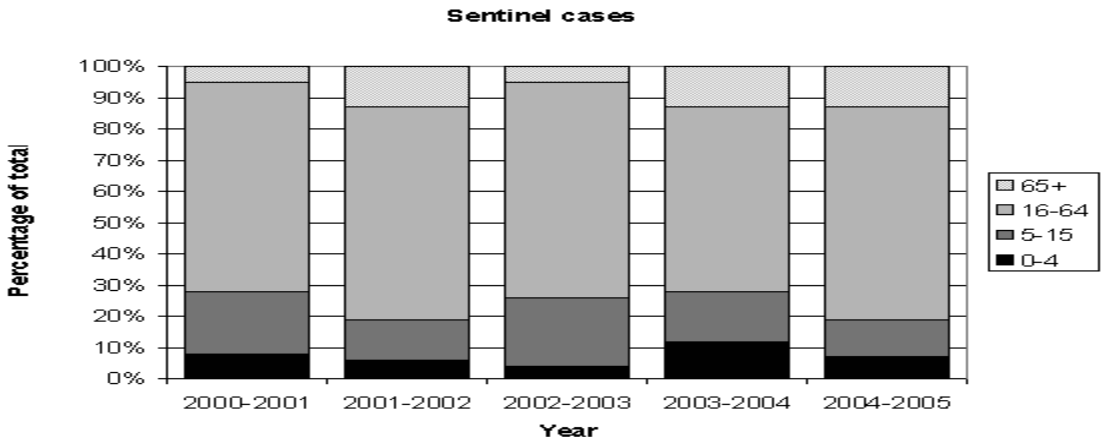

***4:1)*** Proportion (%) cases with influenza-like illness (ILI) out of total number of patient visits in the sentinel system



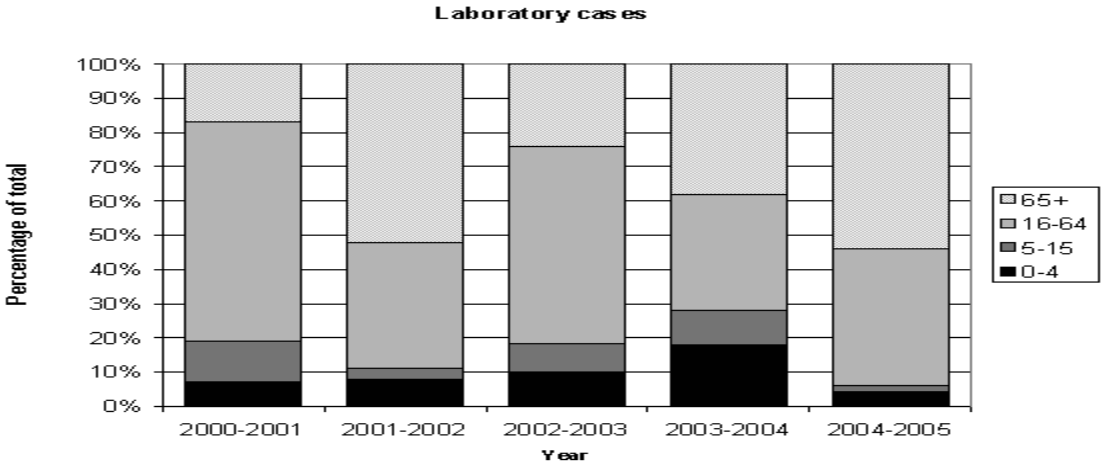

***4:2)*** Number of laboratory verified influenza cases



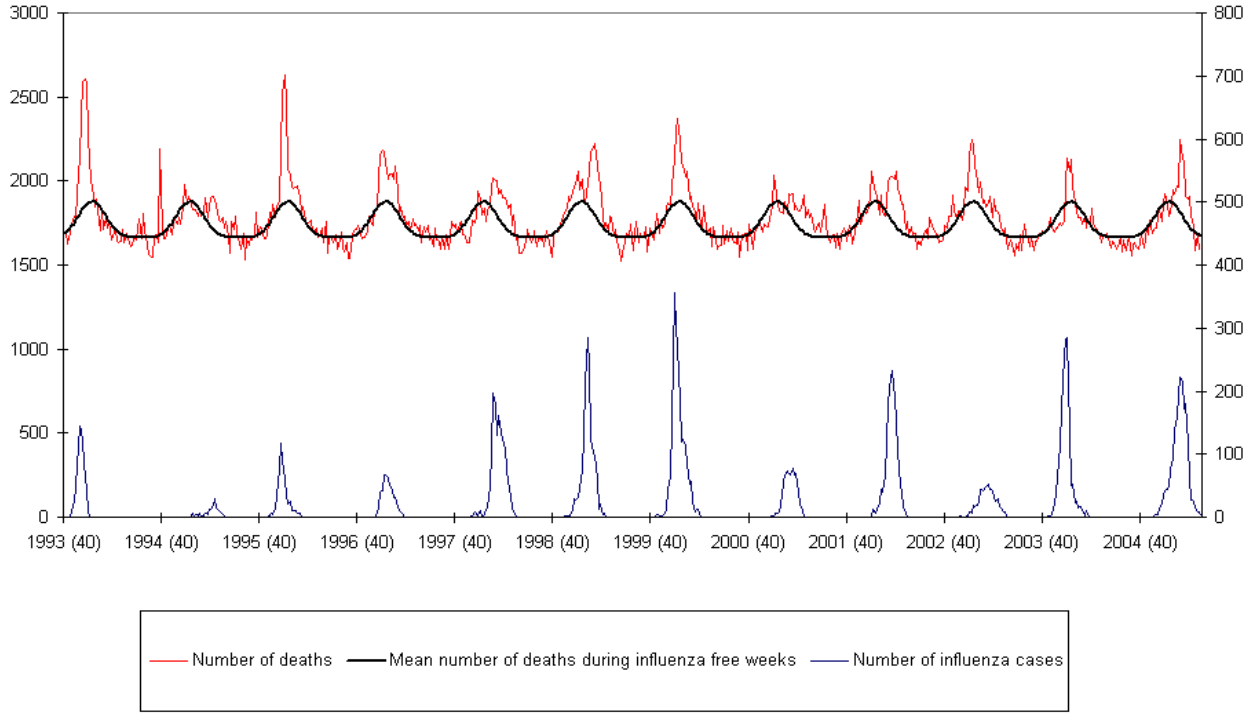

**4:3)** Age distribution of sentinel ILI cases and laboratory verified cases 2004-2005




***4:4*)** Age distribution during the last five seasons for sentinel cases




***4:5)*** Age distribution during the last five seasons for laboratory cases




**4:6)** Diagram of the weekly number of deaths in Sweden from week 40 1993 to week 20 2005, and the number of laboratory verified influenza cases during the same period. (The peak of mortality in September 1994 reflects the Estonia ferry catastrophe). Adjusted mean for corresponding influenza-free weeks is also included.
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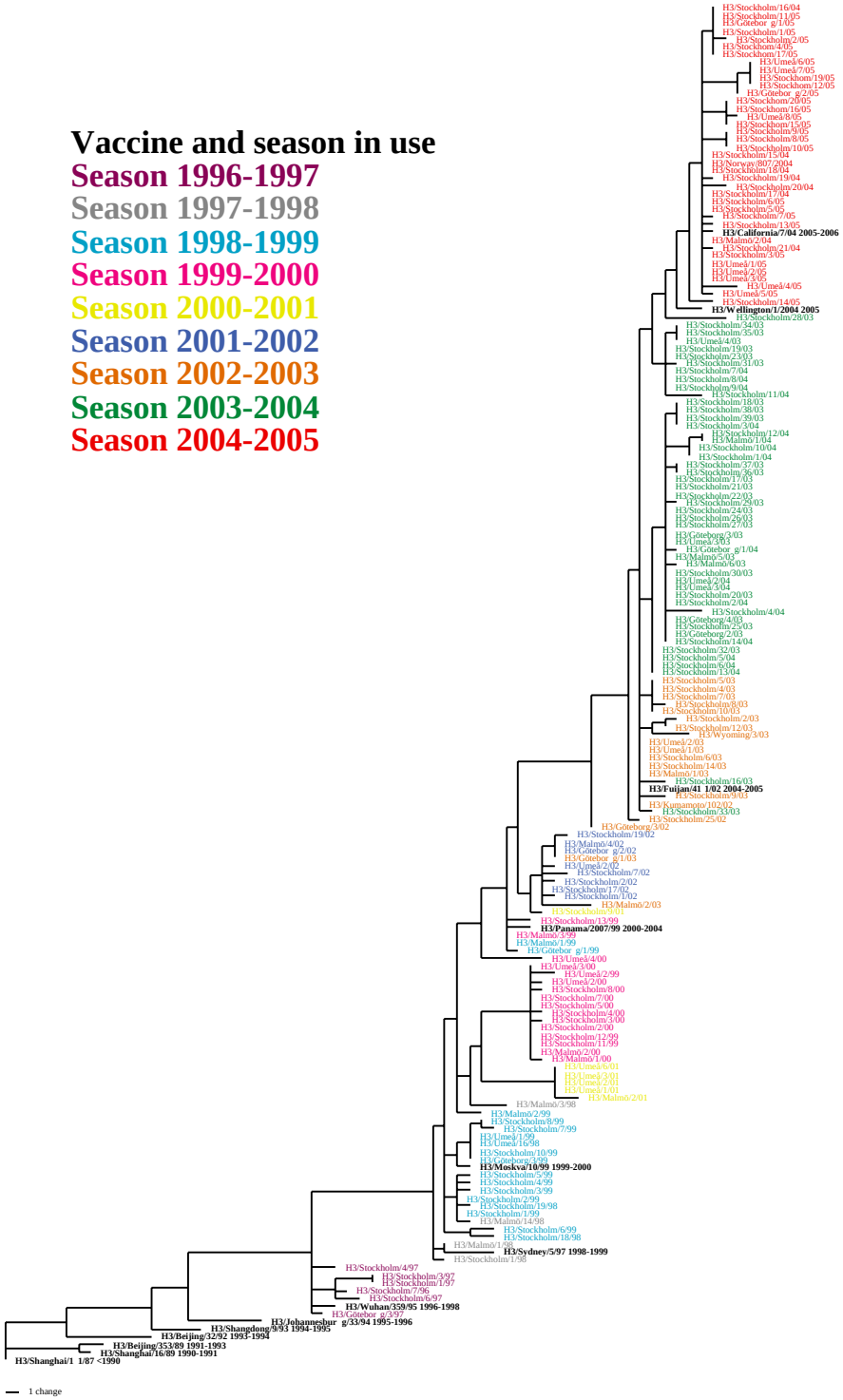
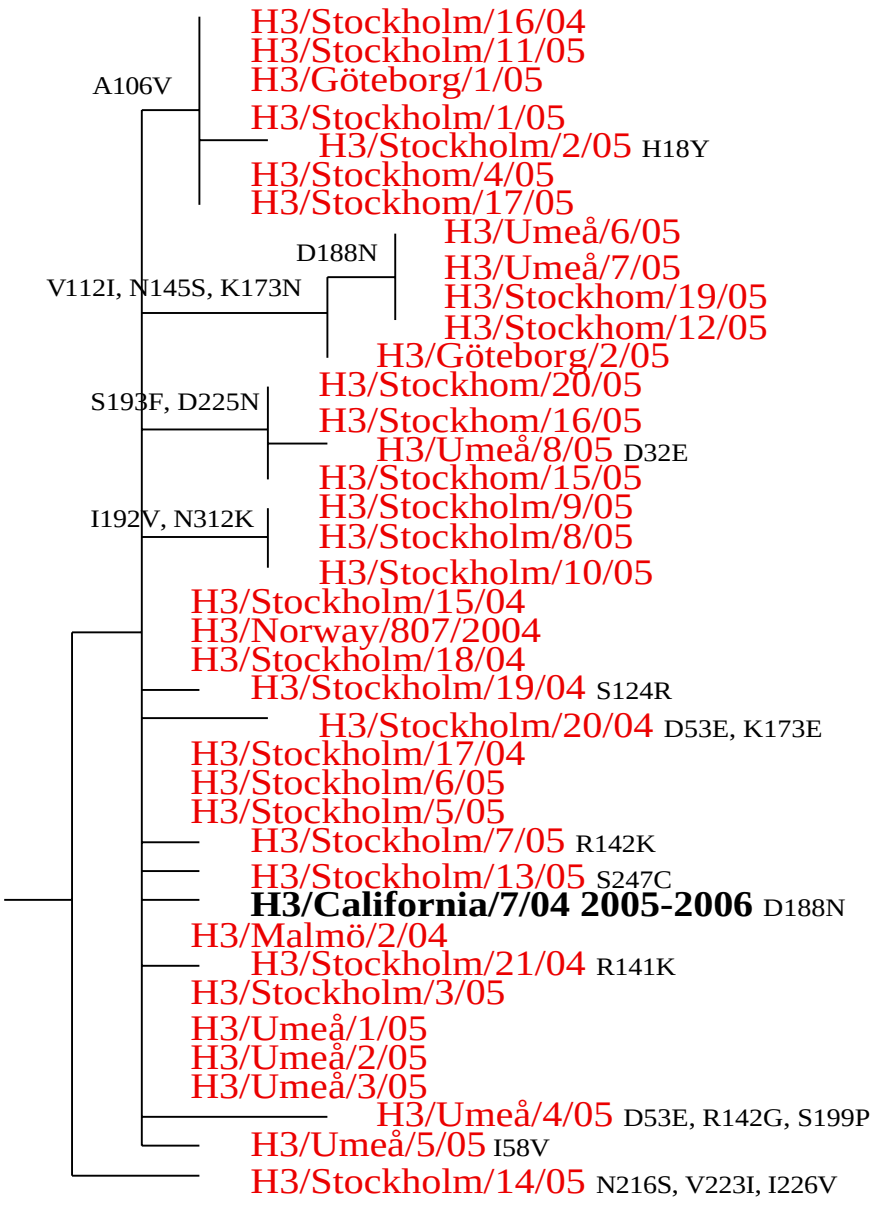
**Table 4:1)** Isolates for which extended feno- and genotypings were performed.

| Name | Antigenically similar to: | Comments |
|---|---|---|
| **Influenza B** | | |
| B/Umeå/1/05 | B/Jiangsu/10/03 | |
| B/Stockholm/1/05 | B/Shanghai/361/02 | |
| B/Stockholm/2/05 | B/Shanghai/361/02 | |
| B/Stockholm/3/05 | B/Shanghai/361/02 | |
| B/Stockholm/4/05 | B/Shanghai/361/02 | |
| B/Umeå/2/05 | B/Shanghai/361/02 | |
| B/Stockholm/5/05 | B/Shanghai/361/02 | |
| B/Stockholm/6/05 | B/Shanghai/361/02 | |
| B/Stockholm/7/05 | | |
| B/Stockholm/8/05 | | |
| B/Stockholm/9/05 | | |
| B/Stockholm/10/05 | | |
| **Influenza H1N1** | | |
| A/Umeå/1/04 | A/New Caledonia/20/99 | |

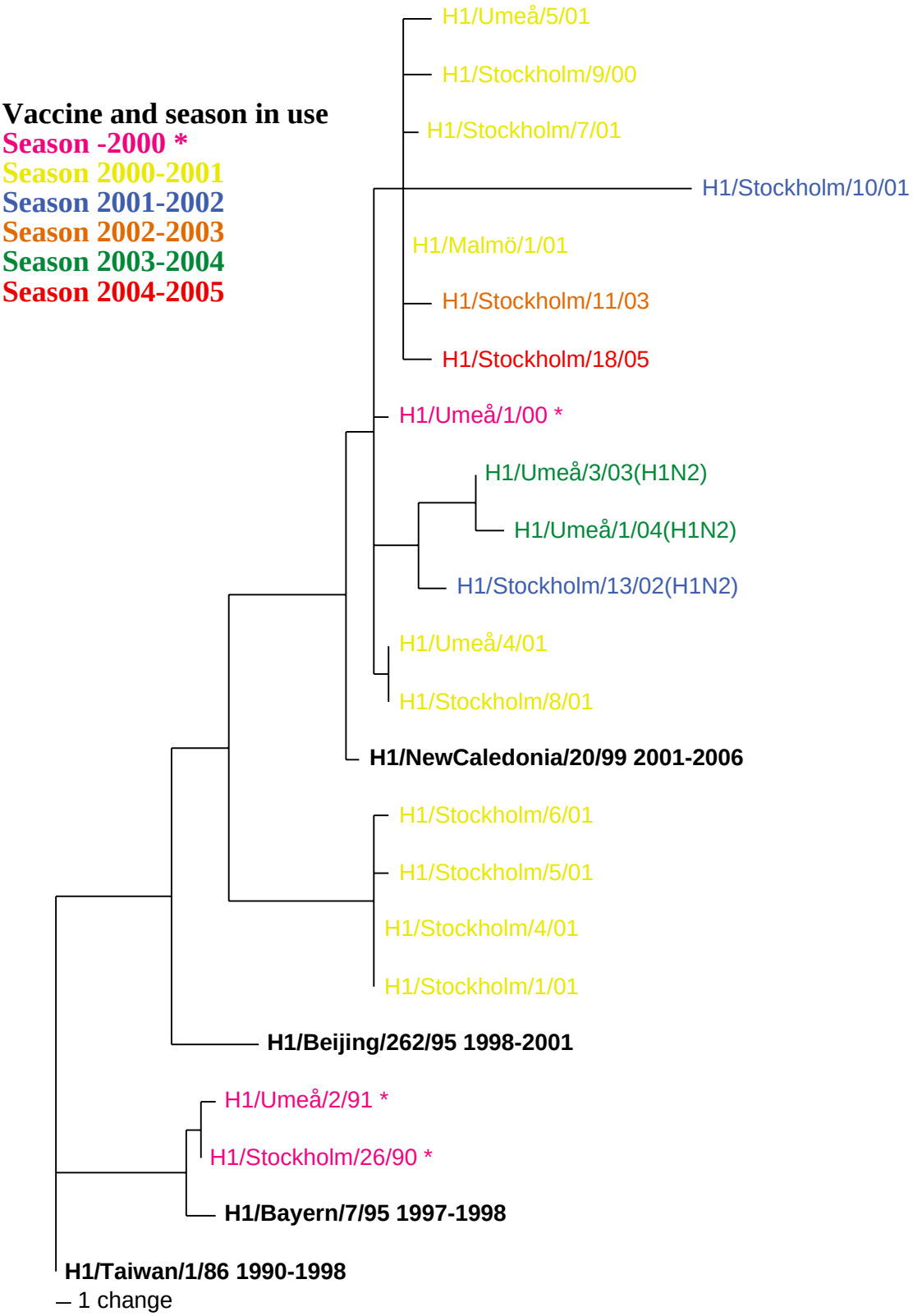
| **Influenza H3N2** | | |
|---|---|---|
| A/Stockholm/15/04 | A/California/7/04 | Traveller from Thailand |
| A/Stockholm/16/04 | A/California/7/04 | |
| A/Stockholm/17/04 | A/California/7/04 | |
| A/Stockholm/18/04 | A/California/7/04 | |
| A/Stockholm/19/04 | A/California/7/04 | |
| A/Stockholm/20/04 | A/California/7/04 | |
| A/Stockholm/21/04 | A/California/7/04 | |
| A/Malmö/2/04 | A/California/7/04 | |
| A/Stockholm/1/05 | A/California/7/04 | |
| A/Stockholm/2/05 | A/California/7/04 | |
| A/Umeå/1/05 | A/California/7/04 | |
| A/Umeå/2/05 | A/California/7/04 | |
| A/Umeå/3/05 | A/California/7/04 | |
| A/Umeå/4/05 | A/California/7/04 | |
| A/Göteborg/1/05 | A/California/7/04 | |
| A/Stockholm/3/05 | A/California/7/04 | |
| A/Stockholm/4/05 | A/California/7/04 | |
| A/Stockholm/5/05 | A/California/7/04 | |
| A/Stockholm/6/05 | A/California/7/04 | |
| A/Stockholm/7/05 | A/California/7/04 | |
| A/Stockholm/8/05 | A/California/7/04 | |
| A/Stockholm/9/05 | A/California/7/04 | |
| A/Stockholm/10/05 | A/California/7/04 | |
| A/Stockholm/11/05 | A/California/7/04 | |
| A/Göteborg/2/05 | A/California/7/04 | |
| A/Stockholm/12/05 | A/California/7/04 | |
| A/Stockholm/13/05 | A/California/7/04 | |
| A/Stockholm/14/05 | A/California/7/04 | |
| A/Umeå/5/05 | A/California/7/04 | |
| A/Umeå/6/05 | A/California/7/04 | |
| A/Umeå/7/05 | A/California/7/04 | |
| A/Stockholm/15/05 | A/California/7/04 | |
| A/Stockholm/16/05 | A/California/7/04 | |
| A/Stockholm/17/05 | A/California/7/04 | |
| A/Stockholm/18/05 | A/California/7/04 | |
| A/Stockholm/19/05 | A/California/7/04 | |
| A/Umeå/8/05 | A/California/7/04 | Traveller from China |
| A/Stockholm/20/05 | | Traveller from China |



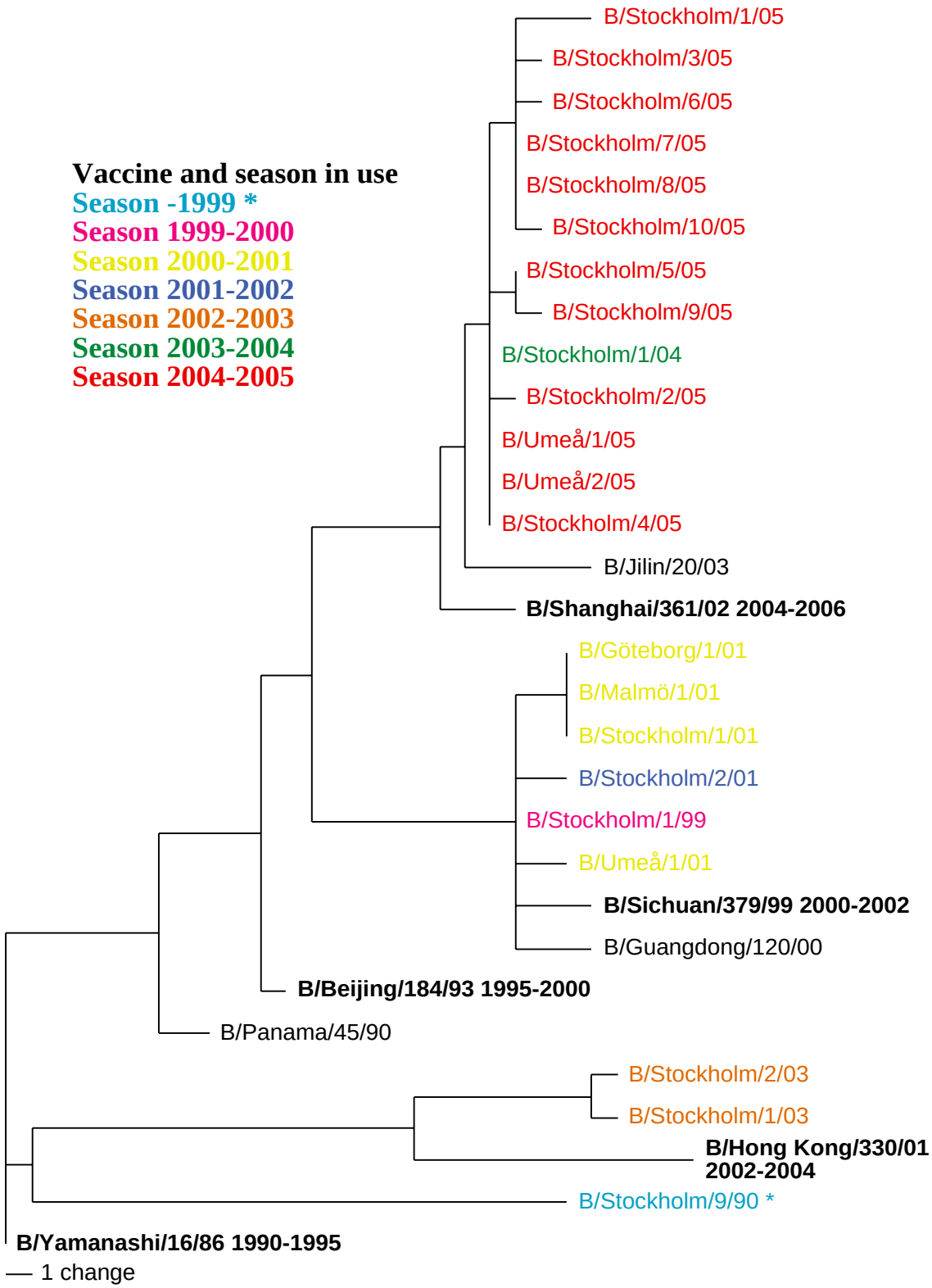

**4:7)** The phylogenetic tree of the amino acid sequences of HA of influenza A/H3 strains isolated in Sweden 1996-2005 compared to the vaccine strains.




**4:8)** The phylogenetic tree of the amino acid sequences of HA of influenza A/H3 strains isolated in Sweden 2004-2005 and their alterations compared to the vaccine strain.




**4:9)** The phylogenetic tree of the amino acid sequences of HA of influenza A/H1 strains isolated in Sweden compared to the vaccine strains.




**4:10)** The phylogenetic tree of the amino acid sequences of HA of influenza B strains isolated in Sweden compared to the vaccine strains.

## 5) QUALITY CONTROL OF LABORATORY DIAGNOSIS OF INFLUENZA

In collaboration with the organisation for External Quality Assessment in Sweden (Equalis), panels for quality control of antigen detection with IFA or ELISA and PCR and for virus isolation, were sent to laboratories performing this types of diagnostic assays in Sweden. The influenza panel for IF consisted of 8 different acetone fixed preparations of the influenza strains expected for the season, grown in MDCK cells, and mixed with different proportions of uninfected cells from a lymphoblastoid cell line. Twenty-three laboratories, reporting altogether 27 data sheets participated. Most of the participating laboratories answered the panel correctly (183/216 analyses). The results of the External Quality control from 1994-2004 related to methods is presented (Table 5:1)

**Table 5:1)** External Quality Control Assessment in Sweden (Equalis).
Results of panels for influenza antigen detection from 1994-2003. The number (%) of reported correct results related to total number of examinations performed with the methods.

| | **Influenza A/H1** | **Influenza A/H3** | Influenza B |
|---|---|---|---|
| **Imagen** | 196/230 (85%) | 168/198 (85%) | 157/216 (73%) |
| **Chemicon indirekt IF** | 91/92 (99%) | 78/83 (94%) | 83/87 (95%) |
| **WHO** | 28/29 (97%) | 24/25 (96%) | 25/26 (96%) |
| **Biosoft/Argene** (not 2002, 2003, 2004) | 5/6 (83%) | 6/6 (100%) | 5/7 (71%) |
| **Biotrin** | 12/13 (92%) | 12/13 (92%) | 10/10 (100%) |
| **DPC-PathoDx** | 79/93 (85%) | 73/80 (91%) | 56/66 (85%) |
| **Chemicon direkt IF** (2002, 2003, not 2004) | 15/18 (83%) | 11/12 (92%) | 9/10 (90%) |
| **Becton-Dickinson** (2002, 2003, not 2004) | 8/10 (80%) | 8/8 (100%) | 2/6 (33%) |
| **Realtids PCR** (2003, 2004) | 5/5 (100%) | 6/6 (100%) | 4/4 (100%) |

## 6) METHOD DEVELOPMENT AND RESEARCH

-A realtime PCR for detection of influenza A matrix has been developed. It has been used for evaluation of clinical nose swabs and cloak samples from birds.
-A sequencing system for influenza A M2 gene has been established, (human and avian strains), with the aim to determine amatadine resistance. We are now developing a H5 specific realtime PCR system, we have previous developed systems for H1 and H3 (Mia Brytting, Asa Wiman).
-Studies of nasal vaccination of mice with subunit influenza vaccine, and a lipid adjuvant (Eurocine®) have been completed. Both general and local production of IgG and IgA antibodies, and protection measured by quantitative, real time PCR were enhanced by the adjuvant (Pernilla Peterson et al). –
-Changes in the HA gene, occurring during passage of influenza strains in e human epithelial cell-line and in MDCK have been mapped
-A telephone survey was conducted during week 7 to identify the true burden of influenza illness in the society (Lara Payne et al)

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