FEDERAL OFFICE OF PUBLIC HEALTH
BERNE - SWITZERLAND

HUG
Hôpitaux Universitaires de Genève

# Influenza surveillance in Switzerland
# Sentinella study

## Winter Season 2006 – 2007


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# 1. ACKNOWLEDGEMENTS

Werner Wunderli (see cover) retired as associate professor from the Central Laboratory of Virology (LCV), at the University Hospitals of Geneva, on 30 September 2007. He had held this title since 1992 and, as Quality Manager, he was also in charge of the accreditation of the laboratory. Werner supervised the serological and virological analyses for many years and lastly, participated in the installation of a P4D laboratory at the hospital. Among his numerous tasks, he was in charge of the National Centre of Influenza between 1992 and 2007. Werner's knowledge of cell culture, virology and, in particular, on influenza is tremendous. He was an active participant to the Commission for Pandemic Preparedness for Switzerland. He also served as publications officer on the committee of the European Society of Clinical Virology. Collaboration with Werner Wunderli was particularly pleasant since he is a very fair person and always open to discussion. We will miss him and wish him a very happy retirement.

Our special thanks go also to:

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- all those in charge of the Sentinella network at the Swiss Office of Public Health (OFSP), particularly Mark Witschi and Daniel Koch
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- the technicians of the Central Laboratory of Virology, and in particular Patricia Suter for her excellent work

# 2. RESUME-SUMMARY- ZUSAMMENFASSUNG

## 2.1. Résumé

L'épidémie grippale a été modérée cette année en Suisse comme en Europe. Les premiers virus influenza ont été détectés d'une manière sporadique à partir du mois de novembre 2006. L'épidémie a débutée seulement à la fin du mois de janvier 2007 pour atteindre un pic au cours du mois de février. Elle a durée 5 semaines. 948 échantillons ont été envoyés par les médecins sentinelle pendant la saison de surveillance dont 495 virus influenza ont été détectés par RT-PCR. 99,9 % étaient des virus influenza A, dont 317 (64 %) des virus influenza A (H3N2) et 39 (8 %) des virus influenza A (H1N1). 137 (28%) virus influenza A n'ont pas pu être sous-typés.
La culture des souches influenza s'est révélée très difficile cette année et seulement une partie des souches a pu être caractérisés par la méthode classique. La majorité des souches influenza A caractérisées par séquençage étaient antigéniquement et génétiquement proches des souches vaccinales influenza A/New-Caledonia/20/1999 (H1N1) et influenza A/Wisconsin/67/2005 (H3N2). Cependant, un nombre non négligeable de souches influenza A (H1N1) et A (H3N2) ont eu des modifications au niveau de l'hémagglutinine et se sont montré plus proche des souches détectées plus récemment comme influenza A/Nepal/921/2006 (H3N2) ou A/Solomon Island/3/2006 (H1N1). Une seule souche influenza B a été détectée cette année.
Les symptômes cliniques ainsi que la prévalence du virus influenza dans les différentes classes d'âge étaient très comparables à ceux observés l'année dernière.
La composition du vaccin pour la saison 2007-08 a été modifiée, la souche influenza A/New Caledonia/20/99 (H1N1) a été remplacée par la souche influenza A/Solomon Island/3/2006.

## 2.2. Summary

The influenza epidemic was of moderate intensity in Switzerland this year and similar to other European countries. The first sporadic cases were detected in November 2006. The epidemic phase began only at the end of January 2006 with an epidemic peak during the month of February which lasted five weeks. Nine hundred and forty-

eight samples were sent to the reference laboratory by the Sentinella practitioners during the surveillance period. From an analysis of these samples, 495 influenza viruses were detected by reverse-transcription polymerisation chain reaction (RT-PCR): 99.9 % were influenza A virus, of which 317 (64%) were influenza A (H3N2) virus and 39 (8%) influenza A (H1N1) virus. 137 influenza A viruses could not be further subtyped.

Cell culture of influenza strains revealed to be very difficult this year and only a part of these strains were able to be characterized by the classic method. The majority of influenza A strains characterized by sequencing were antigenically or genetically close to the vaccine strains influenza A/New-Caledonia/20/1999 (H1N1) and influenza A/Wisconsin/67/2005 (H3N2). However, a significant number of influenza A (H1N1) and A (H3N2) strains demonstrated modifications at the hemagglutinin (HA) level and showed a closer relationship to recently detected strains such as influenza A/Nepal/921/2006 (H3N2) or A/Solomon Island/3/2006 (H1N1). Only one influenza B strain was detected this year

Clinical symptoms as well as the prevalence of influenza virus in the different age categories were comparable with those observed during the past season.

The vaccine composition for the 20007-2008 season has been modified and the influenza A/New Caledonia/20/99 (H1N1) strain has been replaced by the influenza A/Solomon Island/3/2006 strain.

### 2.3. Zusammenfassung

Die Intensität der Grippesaison war in der Schweiz wie auch in Europa moderat. Die ersten sporadischen Grippeviren wurden in der Schweiz im November nachgewiesen. Die eigentliche Epidemie begann aber erst Ende Januar 2007 um das Maximum im Februar zu erreichen. Diese dauerte etwa 5 Wochen. 948 Proben wurden von den Sentinella Ärzten während der Überwachungsperiode eingesandt. Daraus konnten 495 Influenzaviren mittels RT-PCR nachgewiesen werden. Davon

waren 99,9% Influenza A Viren wobei deren 317 (64%) Influenza A (H3N2) und 39 (8%) Influenza (H1N1) waren. 137 Influenza A konnten nicht typisiert werden.
Der Virusnachweis mittels Zellkultur gestaltete sich dieses Jahr sehr schwierig und nur ein Teil der Isolate konnte mit der klassischen Methode charakterisiert werden. Die Mehrzahl der Viren welche mittels Sequenzierung charakterisiert wurden, waren genetisch sowie auch antigenetisch mit den im Impfstoff enthaltenen Stämmen Influenza A/Neu Kaledonien/20/1999 (H1N1) oder mit Influenza A/Wisconsin/67/2005 (H3N2) verwandt.
Eine nicht vernachlässigbare Gruppe von Viren zeigten Veränderungen im Hämagglutinin und waren in der Folge mit erst kürzlich nachgewiesenen Varianten wie Influenza A/Nepal/921/2006 (H3N2) oder A/Solomon Island/3/2006 (H1N1) verwandt. Nur ein Influenza B Virus konnte nachgewiesen werden.
Die Art sowie auch die Häufigkeit der beobachteten Symptome in den verschiedenen Altersgruppen waren mit denjenigen der letzten Saison durchaus vergleichbar.
Die Zusammensetzung des Impfstoffes für den Winter 2007/2008 erfuhr eine Anpassung. Der Stamm Influenza A/Neu Kaledonien/20/1999 (H1N1) wurde durch den Stamm Influenza A/Solomon Island/3/2006 ersetzt.

# 3. INTRODUCTION

Influenza surveillance has been conducted in Switzerland for twenty-one consecutive years through the Sentinella surveillance network, founded and managed by the Swiss Office of Public Health (OFSP). It is comprised of an epidemiological surveillance part based on the weekly reports of the Sentinella network of practitioners, and a virological surveillance part based on the analysis of samples obtained from a representative number of patients seen by Sentinella practitioners. This year, with the aim of improving the coverage and the representativity of the virological surveillance of influenza, the sampling and the virological techniques used were significantly modified with respect to previous years. First, the list of participating practitioners was modified by increasing their number and geographical distribution. Second, the patient sampling procedure was adapted according to the different phases of the epidemic as follows: 1) all patients with suspected influenza before and after the epidemic phase and 2) a representative sample during the peak of the epidemic. Finally, the primary detection of the flu virus this year was conducted systematically by polymerase chain reaction after reverse transcription (RT-PCR), followed by cell culture in a second stage.

Another major change in surveillance was the introduction of the sequencing of a part of the hemagglutinin (HA) gene on a sample of positive cases. This method allows to observe with greater precision the modifications occurring on the virus surface which, in turn, allow to explain modifications of the virus antigenicity.

# 4. METHOD OF DETECTION FOR RESPIRATORY VIRUSES

## 4.1. Clinical identification of influenza cases

Clinical identification is based on a network of 203 practitioners in the community who record the total number of flu syndromes observed compared with the total number of consultations carried out during their daily consultations. The case definition used for a flu syndrome is the presence of fever >38° with or without an impression of sickness, myalgia, or a change of general state. In addition to fever, acute respiratory symptoms such as cough or rhinorhea must be present. These Sentinella

practitioners report the number of cases to the OFSP on a weekly basis, thus representing the medical consultations for influenza-like illness (MC-ILI). Among the cases which meet the clinical criteria definitions, a sub-group of 105 practitioners were asked to take a nasopharyngeal or pharyngeal swab from patients for despatch to the reference laboratory. Samples are placed in viral transport medium the same day and shipped to the reference centre. The geographical distribution of the Sentinella practitioners is shown in Figure 1. This year, two sampling procedures were introduced during the season according to epidemic activity. 1) Before and after the epidemic phase (MC-ILI <15%), a sample was taken from all patients consulting a Sentinella practitioner. 2) During the epidemic phase (MC-ILI >15%), only one sample from every 8$^{th}$ patient was taken.

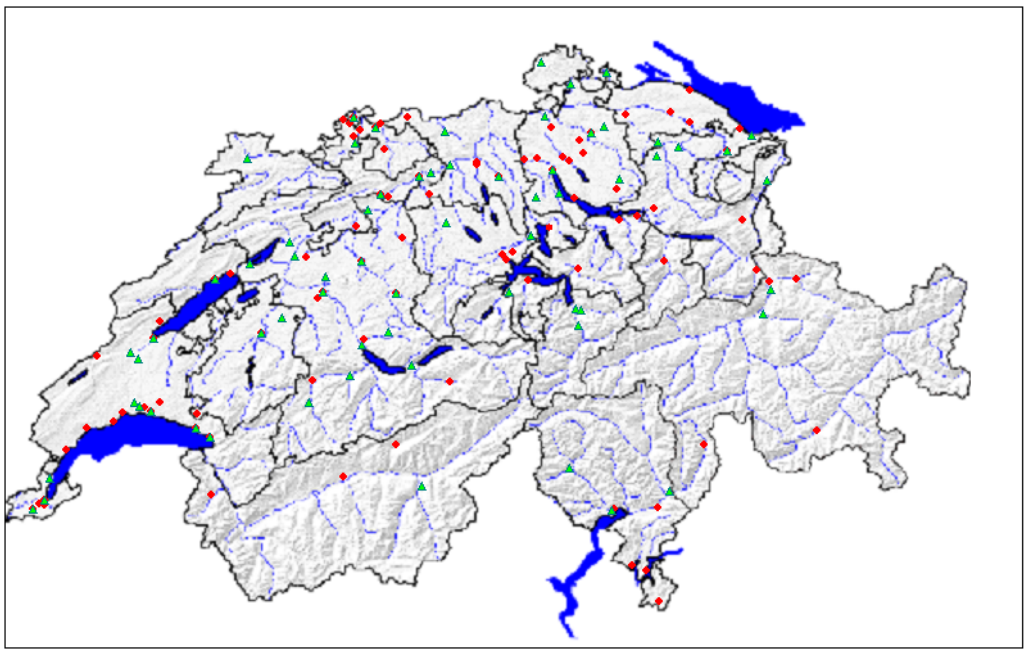


Figure 1: Geographical distribution of the 203 participants in the Sentinella network. Each participant is represented by a coloured dot: green = participants conducting sampling for virus detection; red = participants conducting no sampling

### 4.2. Detection of respiratory viruses

Influenza virus is detected in nasopharyngeal swabs taken and shipped by Sentinella participants (Figure 2). After viral genome extraction, the detection technique consists of two successive reactions leading to the amplification of the genome: reverse-transcription (RT) and polymerisation chain reaction (PCR). This molecular method was evaluated and compared with the traditional cell culture method and demonstrated real advantages over previous seasons such as greatly improved sensitivity and rapidity (Thomas et al, 2006). The RT-PCR assays used target and differentiate influenza A and B viruses (Van Elden, 2001). For influenza A viruses, a second RT-PCR is added in order to identify the nature of the neuraminidase (NA): N1 for influenza A (H1N1) viruses and N2 for influenza A (H3N2) and A (H1N2) viruses (Schweiger et al, 2000). All positive samples detected with RT-PCR were inoculated in cell culture for viral particle amplification and subsequent phenotypic analysis (subtyping). The goal of this analysis is to characterize the HA by the inhibition of the hemagglutination reaction with subtype-specific antibodies.

A selected number of negative samples was regularly inoculated on cells for virus culture. The goal of this strategy is to detect influenza strains that could escape RT-PCR detection through changes in the viral genome. After seven days of inoculation, cells were screened by the use of monoclonal antibodies for the presence of influenza A and B viruses.

### 4.3. Characterization of influenza viruses

If an influenza virus was detected on cell culture, the virus was characterized by an HA inhibition assay with subtype-specific antisera from immunised ferrets. Guinea pig red blood cells were used for this reaction. Results were interpreted according to an antigenic table adapted and established at the beginning of every season. The aim was to use antisera of strains which have a high probability to be circulating in the human population during the season under study. The choice of these antisera was essential in order to be able to differentiate the subtypes circulating and their antigenic relation with the strains included in the vaccine.

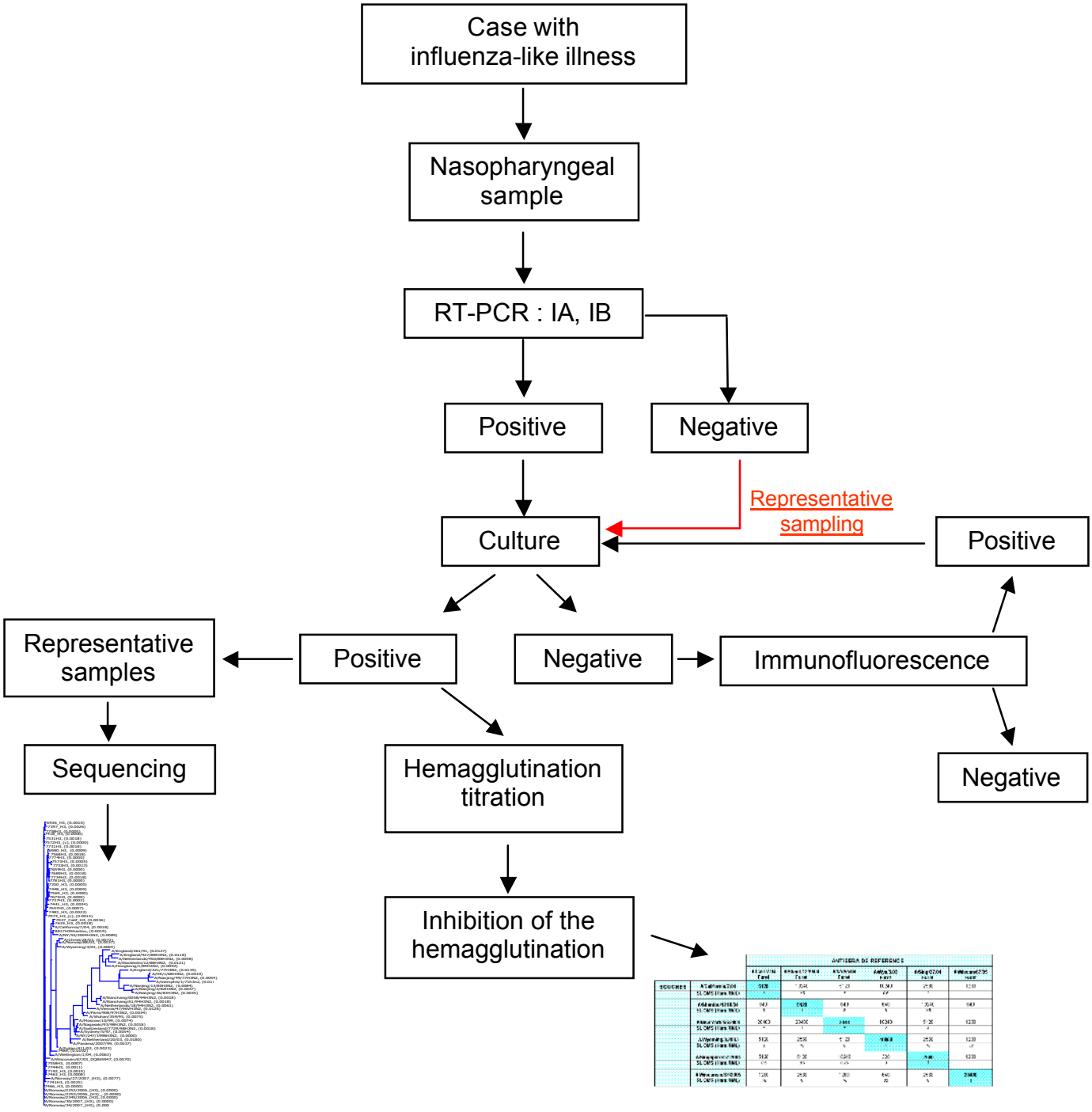



Figure 2 : Procedure used for the detection of respiratory viruses by cell culture

The titers obtained with each strain are identified and compared with standard antisera. This allows a precise identification of its antigenic characteristics. The criteria established for the 2006-2007 season are described in Table 1. As mentioned in the legend, the homologous titers allow a comparison with the titers obtained with the circulating influenza strain and to define its antigenic relation to the standard strains. In turn, this allows the detection of the antigenic variations present in the HA which is one of the major targets of the immune response.

Table 1 : Hemagglutination inhibition (IHA) titers of reference influenza strains incubated with the 2006/2007 reference antisera. The IHA titer obtained after incubation of a given strain with its corresponding antiserum is shown in bold type. This value is called the homologous titer (HT). The titer obtained with a strain isolated from a Sentinella sample (Sen) is then compared with the HT titer. If the ratio Sen/HT is ≤ 4, the strain is considered as antigenically related to the reference strain. If the ratio is > 4, the strain is considered as antigenically different from the reference strain.

a) Influenza A (H3N2)

| | | REFERENCE ANTIBODY | | | |
|---|---|---|---|---|---|
| | | A/Calif/7/04 Ferret | A/NY/55/04 Ferret | A/Wiscon/67/05 Ferret | A/Wyo/3/03 Ferret |
| Strains | A/California/7/04 | **512** | 1024 | 512 | 64 |
| | St. WHO (Hom./Het.) | ***1*** | *0.5* | *1* | *8* |
| | A/New York/55/2004 | 1024 | **1024** | 512 | 512 |
| | St. WHO (Hom./ Het.) | *1* | ***1*** | *2* | *2* |
| | A/Wisconsin/67/2005 | 512 | 256 | **4096** | 32 |
| | St. WHO (Hom./ Het.) | *8* | *16* | ***1*** | *128* |
| | A/Wyoming/3/2003 | 512 | 128 | 256 | **2048** |
| | St. WHO (Hom./ Het.) | *4* | *16* | *8* | ***1*** |

b) Influenza A (H1N1)

| | | REFERENCE ANTIBODY | | | | |
|---|---|---|---|---|---|---|
| | | A/N-Caledonia/ 20/99 Ferret | A/Neth./128/04 Ferret | A/Egypt/39/05 Ferret | A/Fukushima/9 7/06 Ferret | A/Hong-Kong/2652/06 Ferret |
| Strains | A/N-Caledonia/20/99 | **128** | 128 | 512 | 32 | 16 |
| | St. WHO (Hom./Het.) | ***1*** | *1* | *0.25* | *4* | *8* |
| | A/Netherland/128/2004 | 256 | **2048** | 2048 | 64 | 32 |
| | St. WHO (Hom./Het.) | *8* | ***1*** | *1* | *32* | *64* |
| | A/Egypt/39/05 | 64 | 256 | **1024** | 64 | 32 |
| | St. WHO (Hom./Het.) | *16* | *4* | ***1*** | *16* | *32* |
| | A/Fukushima/97/06 | 8 | 32 | 128 | **2048** | 1024 |
| | St. WHO (Hom./Het.) | *256* | *64* | *16* | ***1*** | *2* |
| | A/Hong-Kong/2652/06 | 8 | 32 | 64 | 256 | **1024** |
| | St. WHO (Hom./Het.) | *128* | *32* | *16* | *4* | ***1*** |

c) Influenza B

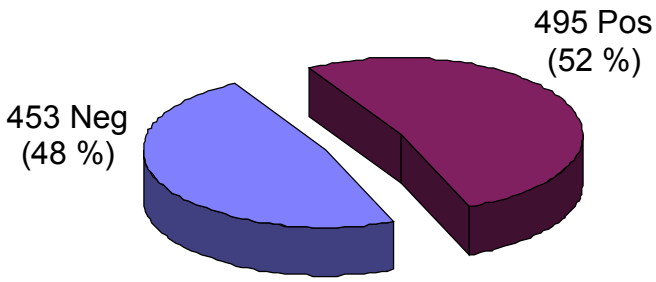
| | | ANTISERA DE REFERENCE | | | | | |
|---|---|---|---|---|---|---|---|
| | | B/HK/335/01 Furet | B/Shand/7/97 Mouton | B/Malaysia/ 2506/04 Furet | B/Shang./361 /02Furet | B/Egypt/144/05 Furet | B/Florida/7/05 Furet |
| Strains | B/Hong Kong/335/2001 | **64** | 1024 | 512 | < 8 | < 8 | < 8 |
| | St. OMS (Hom./Hét.) | ***1*** | *0.0625* | *0.125* | - | - | - |
| | B/Shandong/7/97 | 32 | **1024** | 512 | < 8 | < 8 | < 8 |
| | St. OMS (Hom./Hét.) | *32* | ***1*** | *2* | - | - | - |
| | B/Malaysia/2506/2004 | 32 | 512 | **1024** | < 8 | < 8 | < 8 |
| | St. OMS (Hom./Hét.) | *32* | *2* | ***1*** | - | - | - |
| | B/Shanghai/361/2002 | < 8 | 8 | 64 | **1024** | 1024 | 512 |
| | St. OMS (Hom./Hét.) | - | *128* | *16* | ***1*** | *2* | *2* |
| | B/Egypt/144/05 | < 8 | < 8 | 64 | 512 | **512** | 512 |
| | St. OMS (Hom./Hét.) | - | - | *8* | *1* | ***1*** | *1* |
| | B/Florida/7/05 | < 8 | 16 | 64 | 1024 | 512 | **1024** |
| | St. OMS (Hom./Hét.) | - | *64* | *16* | *1* | *2* | ***1*** |

# 5. RESULTS FROM THE 2006-2007 SEASON

## 5.1. Detection of influenza virus in nasopharyngeal specimens

Surveillance began on 25 September 2006 and ended on 20 April 2007 after a period of 21 weeks. Nine hundred and forty-eight samples were obtained from 105 Sentinella practitioners. Of these, 495 influenza viruses were detected by RT-PCR, representing a mean positive rate of 52% for the whole season (Figure 3a).

a)




b)

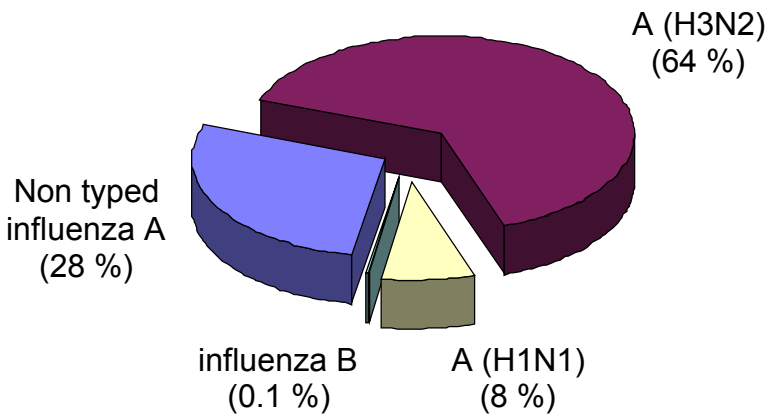
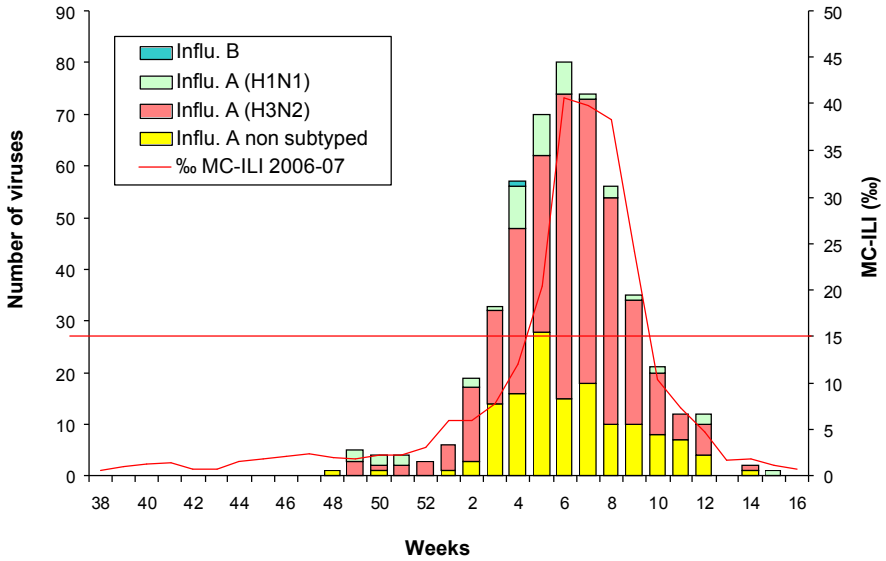



Figure 3: Number and percentage of nasopharyngeal samples positive for any influenza virus during the 2006-2007 season (n = 948). a) Number of RT-PCR-positive and -negative samples received during the season. b) Number and percentage of different types and subtypes of influenza viruses detected during the season. Pos: positive samples; Neg:negative samples

Among these 494 influenza viruses, all except one were influenza A viruses. Of the latter, 318 influenza A (H3N2) viruses and 39 influenza A (H1N1) viruses were detected (Figure 3b). One hundred and thirty-seven influenza A viruses were not able to be subtyped.

The epidemic began at the end of January 2007, but the first sporadic cases were detected at the end of November 2006. During week 2, a significant increase was observed in the number of viruses detected until week 6 (Figure 4, annex 1). The number of detected viruses peaked during the following week and only decreased to the beginning of the season baseline level during week 13. The epidemic phase, corresponding to the weeks reporting a rate of MCI-ILI >15% began during week 5 and ended from week 10 onwards. Maximum activity was observed during week 6 with the highest rate of consultations for flu symptoms reported, as well as the highest number of viruses detected.

a)

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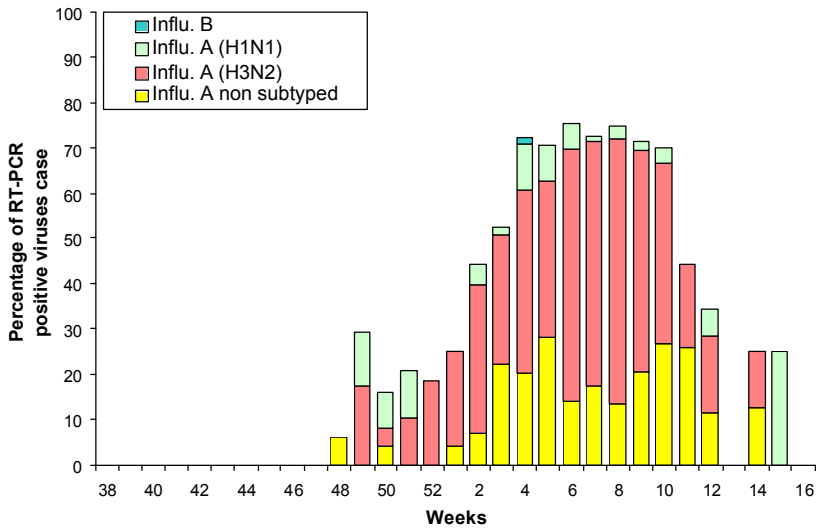



Figure 4: Number of influenza viruses detected per week.
a) Number of viruses detected per week.
b) Percentage of influenza viruses detected per week.
MC-ILI: Medical consultations for influenza-like illness

## 5.2. Antigenic and genetic characterization of influenza viruses

### 5.2.1. Influenza A (H3N2)

Influenza viruses detected this year in the Sentinella samples were almost exclusively of type A (99.9 %). Among these, 64% have been subtyped as influenza A (H3N2) by RT-PCR. Cell culture of viral strains proved to be painstaking work. Thus, the majority of viruses were only detected by RT-PCR, even when cell culture showed negative. When culture-positive was demonstrated, several passages were necessary to obtain sufficient virus titer levels. This was the case for 42 influenza A strains (37, A (H3N2); 5, A (H1N1)). Among the influenza A strains (H3N2), 18 (43%) were antigenically close to the vaccine strain influenza A/Wisconsin/67/05 (H3N2), three (7%) to influenza A/California/7/04 (H3N2), two (5%) to influenza A/New York/55/04, and four (10%) were close to the newly-appeared strain, influenza A/Nepal/921/06 (H3N2). Five (12%) viruses were close to the influenza A strain (H1N1). These results are given in more detail in the section 5.2.2. Finally, 10 (24%) influenza A strains (H3N2) showed weakened IHA titers with antisera of A/Wisconsin/67/05 and A/California/7/04 strains. Only five of these strains could be tested with the antiserum A/Nepal/921/06; however, no important affinity with this antiserum was demonstrated.

This year, titers obtained after culture of the majority of positive samples detected by RT-PCR were insufficient to determine their subtype by IHA reaction and only the HA sequence reaction allowed to characterize certain strains. This well illustrates the potential limits of cell culture which depend on the characteristics of the circulating strains and their possibilities to grow in culture.

Sequences were compared with each other, as well as with the reference strains. Influenza strains from the Sentinella samples are identified (Figure 5) by the four-digit registration number of the patients attributed by the laboratory, followed by the two letters of the canton of origin of the sample and, finally, the month and year of the sample. On the basis of these data, a phylogenetic tree has been constructed taking into consideration the distance or the similarities between the HA sequences of these influenza viruses (Figure 5). Overall, the influenza A (H3N2) strains detected among

patients during this season were genetically very close to the vaccine strain influenza A/Wisconsin/67/2005. However, the phylogenetic tree allowed us to detect two subgroups of influenza A (H3N2) strains which, although very similar, demonstrated differences which allowed to suppose a phylogenetic evolution from two different lineages, both descendants of a common ancestor. The first group, identified by a red frame, is comprised of strains which are the most closely related to the strain A/Wisconsin/67/2005. They present a particular signature characteristic of this vaccine strain and not found in A/H3N2 strains circulating during past seasons. This concerns a modified amino acid, a lysine, which has replaced an arginine in position 173 (R173K).

The second group of strains, identified by the green frame, is comprised of strains presenting with additional modified amino acids: an Isoleucine instead of an asparagine in position 6 (N6I); an alanine instead of a threonine in position 128 (T128A); a glycine instead of an arginine in position 142 (R142G); a serine instead of a leucine in position 157 (L157S); and a glutamic acid instead of an arginine in position 173 (R173E). These same modifications are also found in a strain which appeared more recently in 2006, influenza A/Nepal/921/06 strain (not shown in Figure 5). Thus, we were able to determine that a strain antigenically close to the vaccine strain influenza A/Wisconsin/67/2005 appeared during the 2006-2007 season, but which presented at least three modified amino acids in the surface protein, i.e., the HA. A three-dimensional model of the HA was constructed (Wilson, 1981 ; Bullough, 1994). These modified amino acids are shown in yellow on the peptide chain of the HA in the 3D structure (Figure 6). The majority of these modifications concern amino acids exposed on the surface of the HA, with the exception of the N61 modification which concerns a modification of the protein found inside the viral membrane. The question which now arises is to discover the extent of the influence of these differences in the amino acids on the virus antigenicity, i.e., on the efficiency of recognition of this surface protein by the patient antibodies after vaccination. This property could have an important impact on the efficiency of the vaccination.

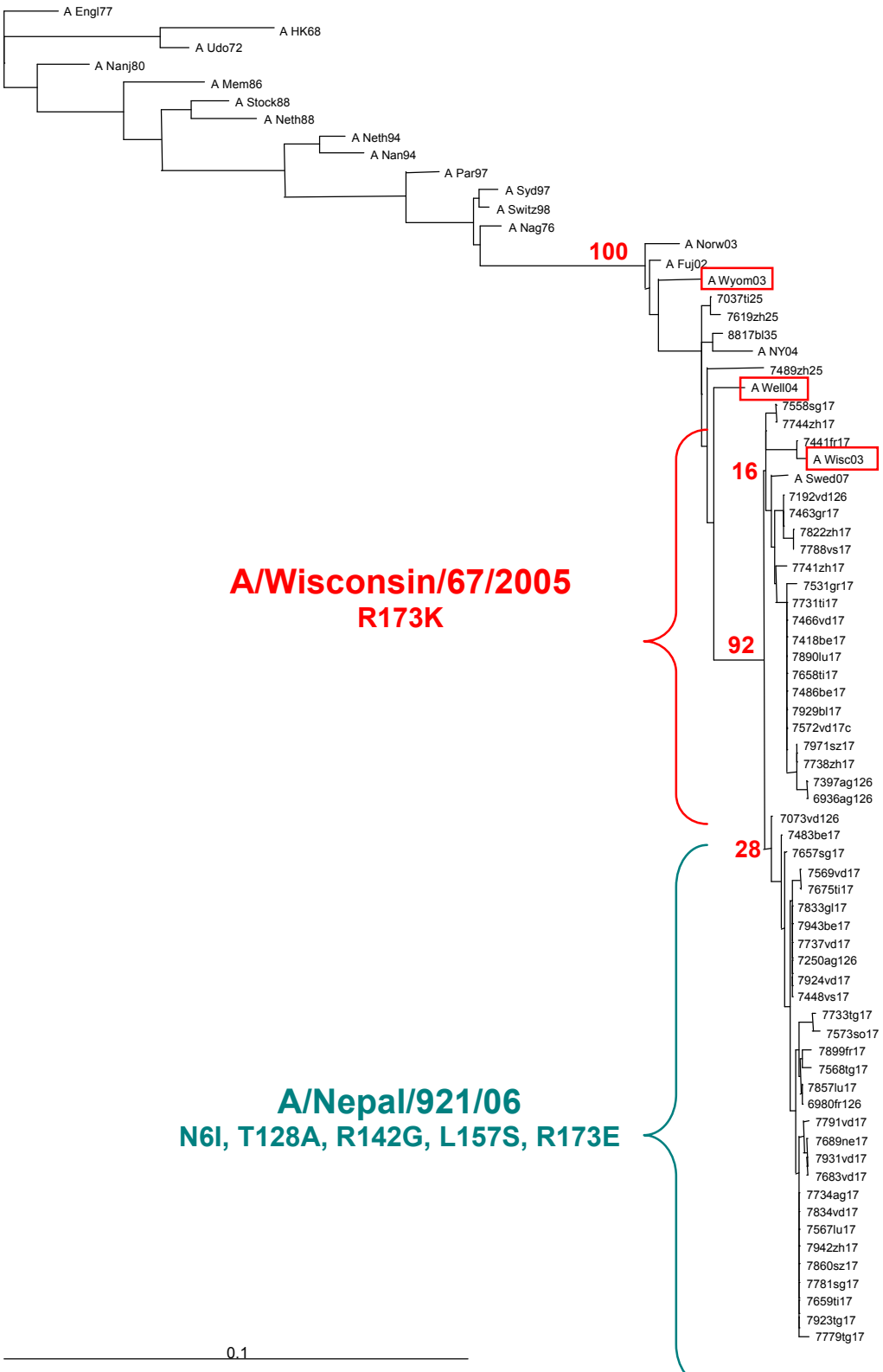



Figure 5: Phylogenetic comparison sequences of influenza A hemagglutinin

The virus name is composed of the four-digit registration number of the patient attributed by the laboratory, followed by the two letters of the canton and then the month and the year; reference strains are framed in red. Red numbers represent the bootstrap values validating the statistical significant of the phylogenetic tree (values >80% indicate a significant difference).

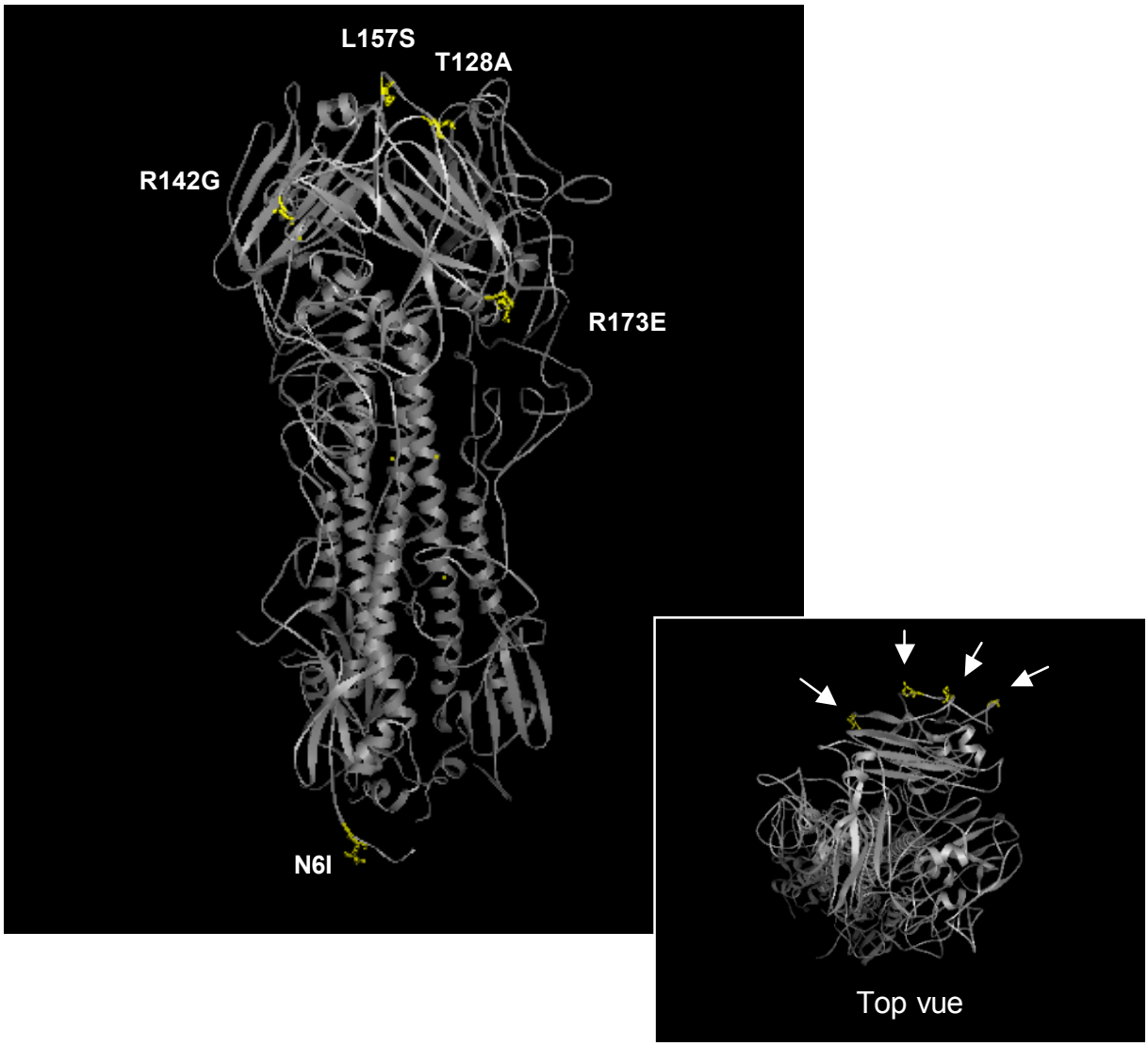



Figure 6: 3D Structure Scheme of the hemagglutinin (Rasmol, OpenGL 1.4.0.)

### *5.2.2. Influenza A (H1N1)*

Thirty-nine influenza A (H1N1) viruses were detected by RT-PCR. Only five viruses yielded a sufficient titer after culture to be analysed by IHA (annex 3). All five were antigenically close to the influenza A/New Caledonia/20/99 strain. However, two of these viruses showed a better affinity for more recent antisera: the first was close to the influenza A/Netherlands/128/04 strain, and the second to the influenza A/Egypt/39/05 strain (annex 3).

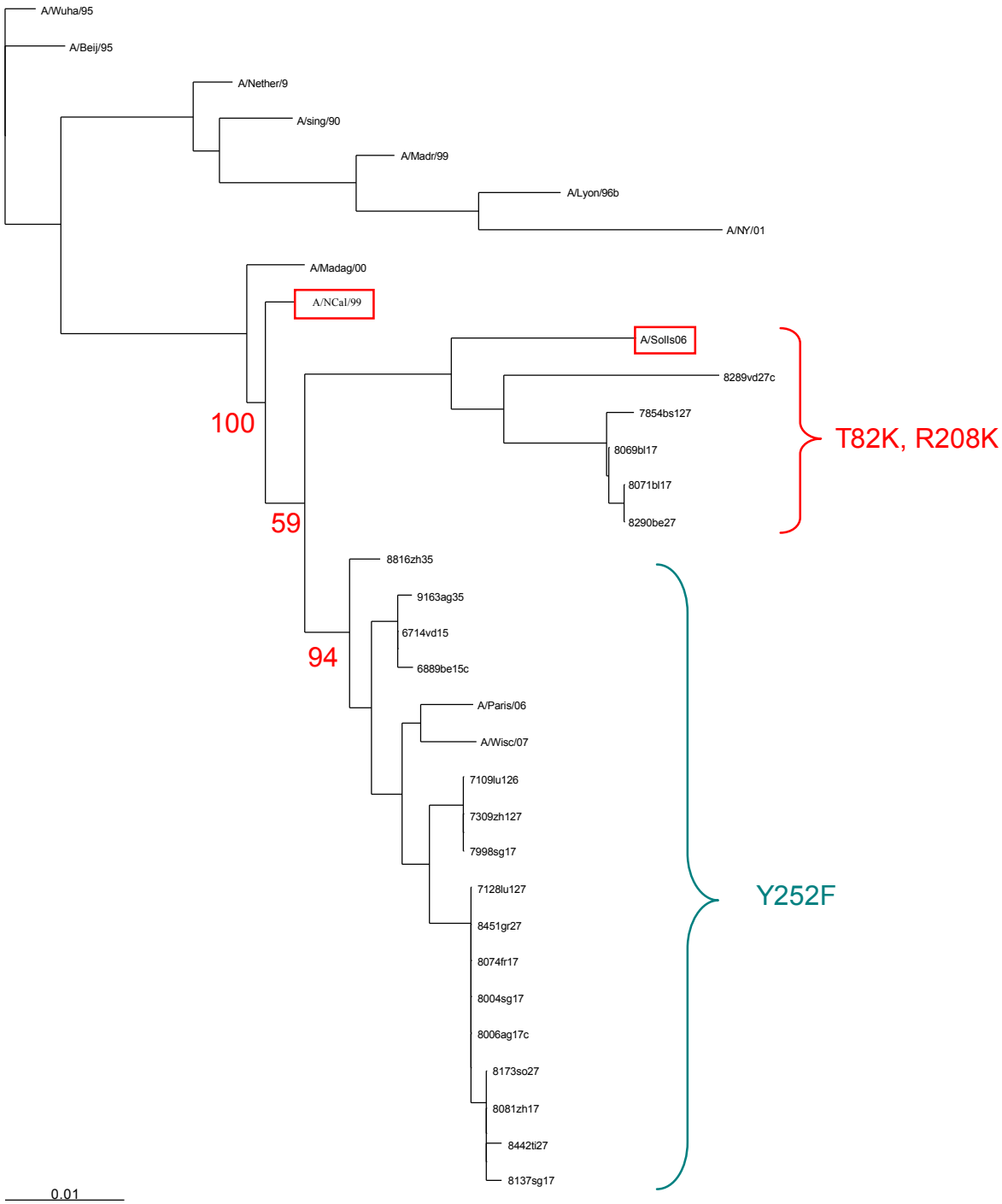



Figure 7 : Phylogenetic comparison of nucleotide sequences encoding hemagglutinins of influenza A (H1N1) viruses; reference strains are framed in red.
Viruses name is composed of the four registration numbers attributed by the laboratory, followed by the two letters of the canton, then the month and the number of the year (vaccine strains are framed in red). Red numbers represent the bootstrap values, validating the statistical significant of the phylogenetic tree (values >80% indicate a significant difference).

Fifteen influenza A (H1N1) viruses were able to be characterized by sequencing. This method allowed to confirm that the strains were genetically close to the influenza

A/New-Caledonia/20/99 strain (bootstrap value, 59; Figure 7) and indicate that the 2006-2007 flu vaccine was adapted to the strains circulating during this season. However, strains detected in five patients from the cantons of Bâle-city, Bâle-country, Berne and Vaud presented two mutations (T82K et R208K) in the HA which had been also observed in the recently-appeared strain, influenza A/Solomon Islands/3/2006 (Figure 7). This strain, although very close to the influenza A/New-Caledonia/20/99 strain, has been chosen to be included in the 2007-2008 vaccine to replace the latter. The other influenza strains detected and sequenced presented a mutation (Y252F) distinct from the influenza A/New-Caledonia/20/99 strain.

#### *5.2.3. Influenza B*

Only one influenza B strain was detected in a Sentinella patient sample. Unfortunately, the weak titer of this strain in the sample did not allow a more precise characterization.

### 5.3 Characteristics of patients with influenza infection

#### 5.3.1. Frequency of viruses detected in a particular age group

Influenza strains modify their genome and, consequently, their antigenicity. For these reasons, each season sees the appearance of new variants. An important aspect is the surveillance of the age categories of patients infected by these variants at the beginning of the season to determine if one category is more frequently affected than others. We are not all equal when it comes to flu infection and young children and the elderly are the main groups at risk. To evaluate this aspect, the proportions of virus detected in the samples received have been stratified into differing patient age groups (Figure 8).

The proportion of flu viruses present in the samples ranged between 25-35% in most age categories. No significant difference was observed between most groups which

indicates that the flu affected the population in a similar manner, independent of patient age.

Persons >60 years old, as well as children aged from 2-4 years old, represented the lowest percentage, between 15-21%, thus indicating that the impact of the flu was the least important for this population. This result is reassuring given that the elderly comprise one of the main groups at risk. The higher vaccination rate in this latter population is perhaps at the origin of the weaker impact of the influenza. No significant difference was observed between the different types of flu virus among the various age groups.

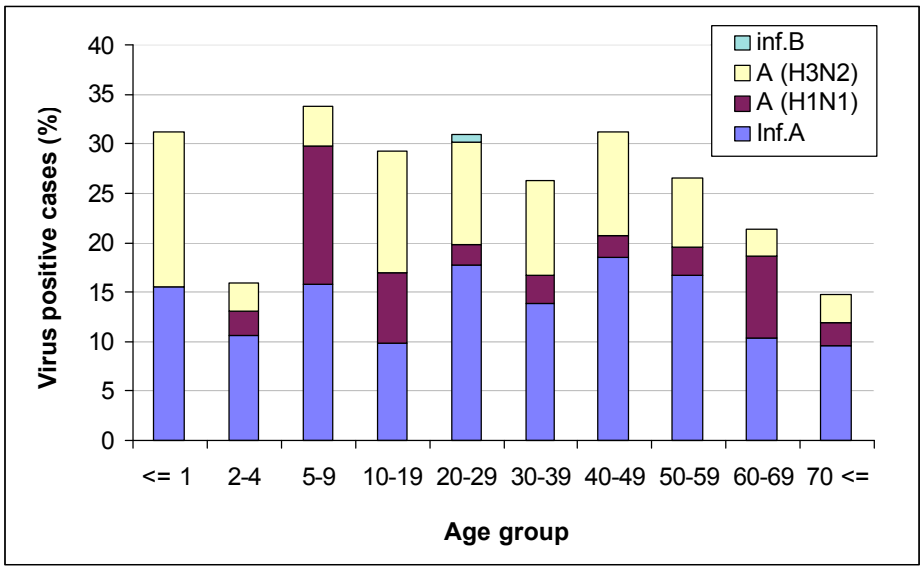



Figure 8: Percentage of viruses detected on number of samples according to age groups. A (H1N1): influenza A (H1N1) virus; A (H3N2): influenza A (H3N2) virus; inf.A: influenza A virus not subtyped; inf.B: influenza B virus

### 5.3.2. Symptoms of influenza-infected patients

The proportion of the five main symptoms observed in flu patients is shown in Figure 9. The most frequent symptoms this season were those most often observed during a classic flu season, i.e., fever and cough. Of note, headaches and a very rapid onset of the illness was also frequently observed this year.

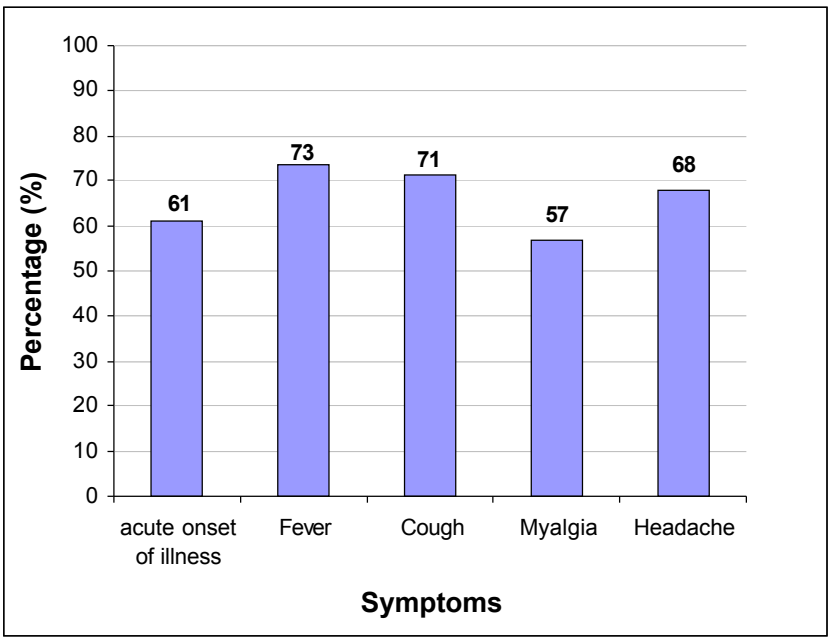



Figure 9: Frequency of symptoms recorded in influenza-infected patients

## 5.4. Comparison of the 13 previous influenza epidemics

The past 13 seasons were compared with regard to the duration of the epidemic phase, the maximum value of MC-ILI, and the week when the maximum value of medical consultations was observed (Table 2). Taken together, the maximum flu activity was observed generally during week 6. For the season 2006-2007, the maximum activity occurred during week 6 when a flu epidemic is often observed and was therefore not of a particularly late onset. The mean intensity of MC-ILI for the past 13 years is 55%. In comparison, the maximum activity reported during week 6 of the 2006-2007 season, 41%, is well below previous years. Finally, the duration of the epidemic was also very short and observed during only five weeks, the shortest reported for the past 13 years.

Table 2: Overview of the intensity of the last 13 seasons.

Duration: number of weeks where the MC-ILI values were above or equal to the threshold (15 ‰); Maximum value of MC-ILI: maximum value observed during the whole season.

| Season | Duration (n = weeks) | Maximum value of MC-ILI (%) | Peak (week of the year) |
|---|---|---|---|
| 1994/95 | 10 | 55 | 11 |
| 1995/96 | 12 | 82 | 52 |
| 1996/97 | 13 | 57 | 1 |
| 1997/98 | 11 | 62 | 9 |
| 1998/99 | 11 | 77 | 7 |
| 1999/00 | 8 | 75 | 1 |
| 2000/01 | 5 | 33 | 6 |
| 2001/02 | 8 | 42 | 6 |
| 2002/03 | 8 | 43 | 9 |
| 2003/04 | 7 | 69 | 1 |
| 2004/05 | 8 | 60 | 6 |
| 2005/06 | 5 | 21 | 12 |
| 2006/07 | 5 | 41 | 6 |
| **Mean** | **9** | **55** | **6** |

## 5.5. Influenza in Europe

This year, the flu epidemics occurring in the various European countries were similar. The large majority of influenza viruses comprised influenza A (H3N2) strains, i.e., 82% of strains characterized antigenically and/or genetically (Figure 10). Similarly, the majority of strains were antigenically close to the vaccine strain, influenza A/Wisconsin/67/2005. Only 3% of influenza A (H3N2) strains were close to the strain included in the 2005-2006 vaccine, influenza A/California/7/2004. Twelve per cent of influenza strains were antigenically close to the vaccine strain influenza A/New-Caledonia/20/99 (H1N1). Finally, 6% of influenza viruses were of type B. The two lineages of type B strains, antigenically distinct, were detected: 90% were close to the vaccine strain influenza B/Malaysia/2506/2004, and only 10% were close to the influenza B/Jiangsu/10/2003 strain.

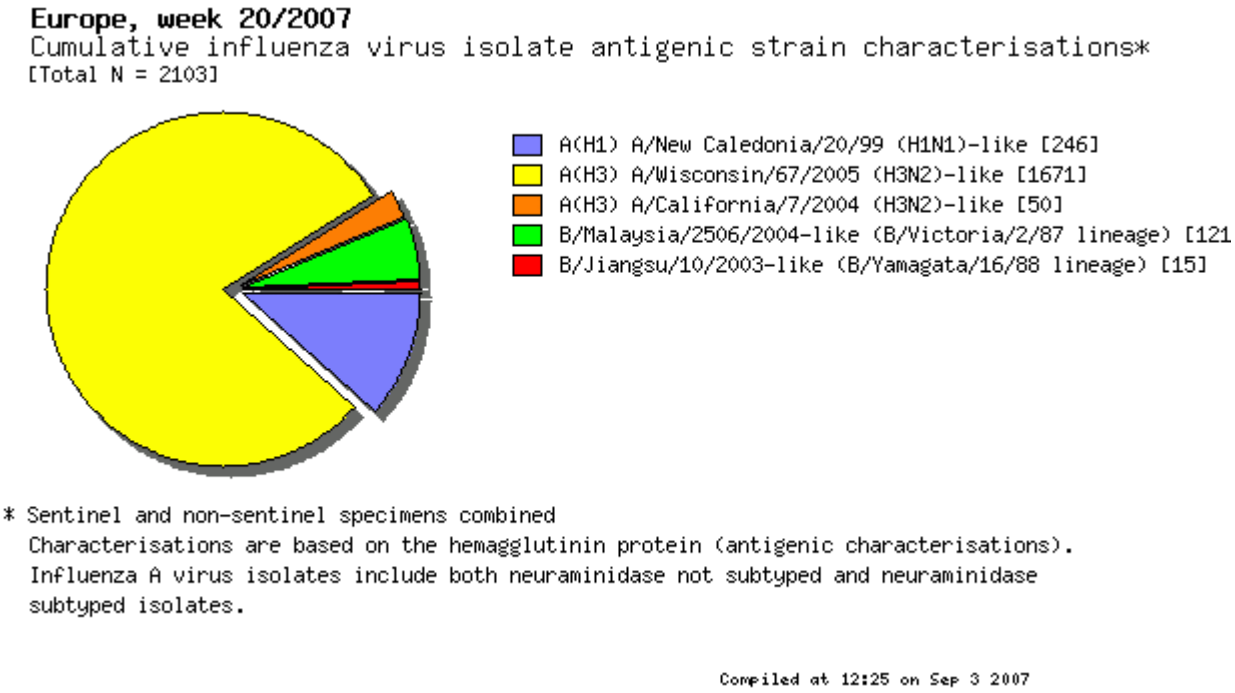



Figure 10: Number of specimens positive for influenza A and B viruses, season 2006-2007.
Source: European Influenza Surveillance Scheme

# 6. INFLUENZA VACCINE COMPOSITION FOR THE 2007-08 SEASON FOR THE NORTHERN HEMISPHERE

The annual meeting for the composition of the influenza vaccine took place on 12-14 February 2007 at WHO headquarters in Geneva. The following recommendations were given for the composition of the influenza vaccine for the 2007-08 season (WHO, 2007):

- A/Solomon Islands/3/2006(H1N1)-like virus
- an A/Wisconsin/67/2005 (H3N2)-like virus[a]
- a B/Malaysia/2506/2004-like virus[b]

[a] *A/Wisconsin/67/2005 (H3N2) and A/Hiroshima/52/2005 can be used*

[b] *B/Malaysia/2506/2004 virus and B/Ohio/1/2005 can be used*

# 7. DISCUSSION

The 2006-2007 influenza season was of moderate intensity in Switzerland and similar to other European countries. Influenza A viruses mainly dominated this year. The two classic influenza A viruses circulated in parallel, namely, a large majority of influenza A (H3N2) viruses and some influenza A (H1N1) viruses. These two virus populations were close to the 2006-2007 vaccine strains. Thus, no severe impact was observed for those persons at risk such as the population >60 years old and very young children. Indeed, the number of viruses detected in these age groups was not particularly high and were even inferior to rates observed for other age categories. Finally, the mortality rate for those >60 years old showed no significant difference for the weeks consecutive to the peak of the flu epidemic (results not shown).

Influenza strains circulating this year grew badly on culture medium. Only 42 of these strains rendered a titer sufficient for study by HA inhibition. Study by HA inhibition was made difficult by the use of an antiserum against the viruses grown on egg which gave low HA inhibition titers with strains circulating this year. This observation was confirmed by the WHO Collaborating Centre (MRC) in London (Hay et al., 2007).

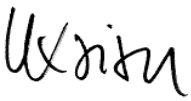
Analysis by sequencing allowed to compare genetically the HA sequences of more than 70 additional strains and to compare these with the sequences of the vaccine strains. These two analyses allowed to show that the influenza A (H3N2) strains which represented the majority circulating this season were genetically very close to the vaccine strain influenza A/Wisconsin/67/05 as demonstrated by the HA sequence. Moreover, two populations were able to be distinguished on the basis of modified amino acids on the C-terminal of the HA1. The first population (40% of the strains sequenced) presented an HA1 sequence according to the A/Wisconsin/67/2005 strain. The second population (60%) showed some amino acids modified with regard to the A/Wisconsin/67/2005 and A/California/7/2004 strains. As described in section 5.2.1., these amino acids are present in the exposed part of the HA and probably intervene in the virus antigenicity (Figure 10). These same modified amino acids have also been observed in the HA1 sequence of the reference strain A/Népal/921/06 detected recently. Recent studies conducted at the Center for Disease Control and Prevention (CDC) at Atlanta, USA (Klimov A., 2007), have

confirmed the presence of two populations of influenza A (H3N2) viruses circulating in 2007 in the Americas (Missouri, Texas, Washington, Brazil, Mexico, etc.), in Asia (Korea, India, Japan, etc.), and in Europe (Norway, France) : one close to the A/Wisconsin/67/2005 strain, and the other sharing the same modified amino acids (N6I, R142G, L157S, K173E, T128A).

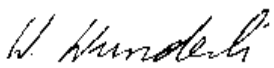
The influenza A (H1N1) strains detected during this season were analyzed primarily by sequencing (RT-PCR). All were showed to be genetically close to the vaccine strain influenza A/New Caledonia/20/99. However, two groups of strains could be differentiated by the presence of modified amino acids which comprised of T99K and R225K on the one hand and of Y269F on the other. The former strain group is very close to the recently-detected influenza A/Solomon Islands/3/2006 strain and this strain has been included in the 2007-2008 vaccine.

Geneva, 12 September 2007

Yves Thomas



Laurent Kaiser



Werner Wunderli

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## Annex 1: Detection of respiratory viruses during the 2006/07 season

MC-ILI: proportion of medical consultations for influenza-like illness; influ A: influenza A virus; unsubt.: influenza A which could not be subtyped. Undet.: influenza A (H3N2) which could not be characterized by inhibition of the Hemagglutination reaction; A/Calif: influenza A/California/7/04; A/Wis: A/Wisconsin/67/2005; A/NC: A/New-Caledonia/20/99; Influ. B: influenza B virus; B/Mal: B/Malaysia/2506/2004; B/Jiang: B/Jiangsu/10/2003

| Sentinelle Surveillance - Season 2006-07 | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weeks | Dates | | MC-ILI (‰) | Samples received | Influ. A Unsubt. | A (H3N2) | | | | A (H1N1) | | | Influenza B | | | | Total virus (n) |
| | | | | | | Undet. | A/Calif | A/Wis | Total | Undet. | A/NC | Total | Undet. | B/Mal | B/Jiang | Total | |
| 38 | 16-sept-06 | 22-sept-06 | 0.6 | 4 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 39 | 23-sept-06 | 29-sept-06 | 1 | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 40 | 30-sept-06 | 06-oct-06 | 1.3 | 8 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 41 | 07-oct-06 | 13-oct-06 | 1.4 | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 42 | 14-oct-06 | 20-oct-06 | 0.7 | 4 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 43 | 21-oct-06 | 27-oct-06 | 0.7 | 4 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 44 | 28-oct-06 | 03-nov-06 | 1.6 | 15 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 45 | 04-nov-06 | 10-nov-06 | 1.9 | 21 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 46 | 11-nov-06 | 17-nov-06 | 2.1 | 8 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 47 | 18-nov-06 | 24-nov-06 | 2.4 | 22 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 48 | 25-nov-06 | 01-dec-06 | 2 | 16 | **1** | - | - | - | - | - | - | - | - | - | - | - | **1** |
| 49 | 02-dec-06 | 08-dec-06 | 1.9 | 17 | - | - | - | **3** | **3** | **1** | **1** | **2** | - | - | - | - | **5** |
| 50 | 09-dec-06 | 15-dec-06 | 2.3 | 25 | **1** | - | - | **1** | **1** | | **2** | **2** | - | - | - | - | **4** |
| 51 | 16-dec-06 | 22-dec-06 | 2.2 | 19 | - | - | - | **2** | **2** | **1** | **1** | **2** | - | - | - | - | **4** |
| 52 | 23-dec-06 | 29-dec-06 | 3.1 | 16 | - | - | - | **3** | **3** | - | - | - | - | - | - | - | **3** |
| 1 | 30-dec-06 | 05-jan-07 | 6 | 24 | **1** | **1** | - | **4** | **5** | - | - | - | - | - | - | - | **6** |
| 2 | 06-jan-07 | 12-jan-07 | 6 | 43 | **3** | **2** | - | **12** | **14** | **1** | **1** | **2** | - | - | - | - | **19** |
| 3 | 13-jan-07 | 19-jan-07 | 7.7 | 63 | **14** | **7** | - | **11** | **18** | **1** | - | **1** | - | - | - | - | **33** |
| 4 | 20-jan-07 | 26-jan-07 | 12 | 79 | **16** | **32** | - | - | **32** | **5** | **3** | **8** | **1** | - | - | **1** | **57** |
| 5 | 27-jan-07 | 02-feb-07 | 20.5 | 99 | **28** | **34** | - | - | **34** | **4** | **4** | **8** | - | - | - | - | **70** |
| 6 | 03-feb-07 | 09-feb-07 | 40.6 | 106 | **15** | **58** | - | **1** | **59** | **4** | **2** | **6** | - | - | - | - | **80** |
| 7 | 10-feb-07 | 16-feb-07 | 39.8 | 102 | **18** | **51** | - | **4** | **55** | **1** | - | **1** | - | - | - | - | **74** |
| 8 | 17-feb-07 | 23-feb-07 | 38.3 | 75 | **10** | **44** | - | - | **44** | **2** | - | **2** | - | - | - | - | **56** |
| 9 | 24-feb-07 | 02-mar-07 | 24.1 | 49 | **10** | **24** | - | - | **24** | **1** | - | **1** | - | - | - | - | **35** |
| 10 | 03-mar-07 | 09-mar-07 | 10.4 | 30 | **8** | **8** | - | **4** | **12** | **1** | - | **1** | - | - | - | - | **21** |
| 11 | 10-mar-07 | 16-mar-07 | 7.3 | 27 | **7** | **4** | - | **1** | **5** | - | - | - | - | - | - | - | **12** |
| 12 | 17-mar-07 | 23-mar-07 | 4.8 | 35 | **4** | **2** | - | **4** | **6** | **2** | - | **2** | - | - | - | - | **12** |
| 13 | 24-mar-07 | 30-mar-07 | 1.7 | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14 | 31-mar-07 | 06-apr-07 | 1.9 | 8 | **1** | **1** | - | - | **1** | - | - | - | - | - | - | - | **2** |
| 15 | 07-apr-07 | 13-apr-07 | 1.2 | 4 | - | - | - | - | - | - | **1** | **1** | - | - | - | - | **1** |
| 16 | 14-apr-07 | 20-apr-07 | 0.8 | 6 | - | - | - | - | - | - | | - | - | - | - | - | - |
| | | | | **948** | **137** | **268** | **0** | **50** | **318** | **24** | **15** | **39** | **1** | **0** | **0** | **1** | **495** |
| | | | | | **494** | | | | | | | | **1** | | | | |

## Annex 2: Titers of the Hemagglutination inhibition obtained with influenza A (H3N2) viruses grown on cell culture

| Seq N° | Typisation | A/Calif | A/NY | A/WISC | A/Wyo | A/Nep | London |
|---|---|---|---|---|---|---|---|
| | A/Calif | **512** | 128 | 64 | 256 | 64 | |
| | A/NY | 128 | **256** | 128 | 64 | 128 | |
| | A/Wisc | 128 | 128 | **512** | 16 | 256 | |
| | A/Wyo | 512 | 256 | 256 | **2048** | 128 | |
| | A/Nepal | 64 | 128 | 128 | 32 | **512** | |
| 05/6936 | A/California/7/04 | 128 | 128 | 128 | 32 | | |
| 11/7073 | A/California/7/04 affaibli | 64 | 32 | 64 | 32 | | # |
| 15/7192 | A/California/7/04 affaibli | 64 | 32 | 64/64 | 16 | 64 | # |
| 29/7397 | A/Wisconsin/67/05 | 32 | 64 | 64/128 | 128 | 128 | # |
| 04/7441 | | 32 | 32 | 32 | 16 | | # |
| 04/7443 | A/Wisconsin/67/05 | 512 | 128 | 2048 | 128 | | # |
| 04/7448 | | 32 | 32 | 32 | 16 | | # |
| 09/7530 | A/Wisconsin/67/05 | 128 | 64 | 256 | 64 | | # |
| 09/7534 | | 16 | 16 | 32/16 | 32 | 16 | |
| 10/7558 | A/New York/55/04 | 64 | 64 | 64 | 16 | | # |
| 22/7859 | A/Wisconsin/67/05 | 128 | 64 | 128 | 64 | | |
| 24/7929 | | 16 | 8 | 16 | 16 | | |
| 24/7930 | | 16 | <8 | 16/32 | 32 | 16 | |
| 07/8343 | A/Wisconsin/67/05 | 128 | 128 | 256 | 16 | | |
| 08/8393 | A/Nepal/921/06 | 32 | 32 | 64/64 | 16 | 256 | |
| 13/8525 | A/Wisconsin/67/05 | 64 | 32 | 256 | 16 | | |
| 13/8528 | A/Wisconsin/67/05 | 256 | 64 | 1024 | 64 | | |
| 14/8582 | A/Wisconsin/67/05 | 256 | 64 | 2048 | 128 | | |
| 15/8918 | A/Wisconsin/67/05 | 64 | 64 | 256 | 32 | | |
| 15/8631 | | 32 | 32 | 64/32 | 32 | 64 | |
| 09/9185 | A/Wisconsin/67/05 | 32 | 64 | 64/128 | 32 | 128 | |
| 09/9187 | A/Wisconsin/67/05 | 32 | 32 | 128 | 16 | | |
| 09/9188 | A/Wisconsin/67/05 | 32 | 32 | 256 | 16 | | |
| 13/9228 | A/Wisconsin/67/05 | 256 | 64 | 1024 | 32 | | |
| 16/9310 | A/Wisconsin/67/05 | 64 | 32 | 256 | 32 | | |
| 20/9360 | | 16 | 16 | 32 | 8 | | |
| 20/9361 | | 16 | 32 | 32 | <8 | | |
| 21/9403 | A/Wisconsin/67/05 | 1024 | 64 | 4096 | 64 | | |
| 22/9434 | A/Wisconsin/67/05 | 64 | 32 | 512 | 32 | | |
| 27/9523 | A/Wisconsin/67/05 | 128 | 64 | 1024 | 32 | | |
| 27/95240 | A/Wisconsin/67/05 | 64 | 32 | 128 | 32 | | |
| 05/9716 | | 32 | 32 | 32/32 | 16 | 16 | |
| 18/7779 | A/Nepal/921/06 | 128 | 32 | 256 | 32 | 512 | |
| 24/7924 | A/Nepal/921/06 | 32 | 32 | 64 | 16 | 128 | |
| 24/7942 | A/Nepal/921/06 | 128 | 64 | 256 | 16 | 512 | |
| 06/6980 | | 32 | 32 | 32 | 8 | 64 | |
| 08/8395 | A/New York/55/04 | 32 | 64 | 64 | 32 | 64 | |

## Annex 3: Titers of the Hemagglutination inhibition obtained with influenza A (H1N1) viruses grown on cell culture

| Seq N° | Typisation | A/New Cal | A/Nethe | A/Egyp | A/Fuku | A/HK | A/Solo.Is. | London* |
|---|---|---|---|---|---|---|---|---|
| | A/New Cal | **128** | 128 | 256 | 32 | 16 | 32 | |
| | A/Nethe | 256 | **2048** | 2048 | 64 | 32 | 16 | |
| | A/Egyp | 64 | 256 | **1024** | 64 | 32 | 64 | |
| | A/Fuku | 8 | 32 | 128 | **2048** | 1024 | 256 | |
| | A/HK | 8 | 32 | 64 | 256 | **1024** | 128 | |
| | A/Solomon Island | 32 | 32 | 32 | 64 | 64 | **128** | |
| 07/7022 | A/Netherlands/128/04 | 256 | 2048 | 1024 | 64 | 32 | | |
| 12/7109 | A/New Caledonia/20/99 | 128 | 512 | 512 | 64 | 32 | | |
| 13/7128 | A/New Caledonia/20/99 | 64 | 256 | 256 | 32 | 32 | | |
| 13/9815 | A/New Caledonia/20/99 | 64 | 512 | 512 | 32 | 16 | | |
| 09/8451 | A/Egypt/39/05 | 128 | 256 | 1024 | 64 | 32 | 64 | |
| | | | | | | | | |
| | | | | | | | | |

** London : sample sent to London*