TECHNICAL REPORT

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Contents

Summary

Following the emergence of swine influenza A(H3N2) variant (v) viruses with sporadic human infections in North America, the European Centre for Disease Prevention and Control (ECDC) and the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL) have considered whether European influenza laboratories would be able to detect this novel virus. Although an ECDC risk assessment [1] found no indication that A(H3N2)v viruses are yet prevalent in pigs in Europe, ECDC and CNRL distributed an online questionnaire to all influenza reference laboratories in European Union (EU)/European Economic Area (EEA) countries aiming to explore their capability to detect A(H3N2)v viruses by reverse transcription-polymerase chain reaction (RT-PCR) in their day-to-day diagnostics, and to subtype these viruses as swine-origin variants. Twenty-eight (78%) of 36 laboratories from 23 (79%) of 29[1] EU/EEA Member States responded. Twenty-seven (96%) of 28 responding laboratories considered the M gene primers and probes, based on *in silico* analysis, appropriate for detection of A(H3N2)v virus. A majority (57%) of the responding laboratories considered their H3 subtyping RT-PCR inappropriate for identifying variant viruses. Detecting A(H3N2)v virus at type level seems less problematic than actually receiving the specimens at the national reference laboratory and selecting them for subtyping. Not all influenza A(H3N2) viruses will be subtyped, since the dominant virus strain now is A(H3N2). This survey indicates that with current capabilities, the variant viruses would be detected in Europe although some of them would not be subtyped and identified as variant viruses. ECDC's future influenza virology network activities will take into account emergence of animal influenza viruses in human samples and rapid information exchange between laboratories in such situations for actions required to update their methods of detection for such new viruses.

Background

A small number of human infections with swine-origin triple-reassortant (SOtr) influenza A(H3N2) virus have been identified in the United States since 2009 [2]. Of these cases, eleven were reported during 2011 and all of them were infected with SOIVtrH3N2 viruses containing the matrix (M) gene from the pandemic 2009 influenza A(H1N1) virus, now known as A(H1N1)pdm09. In this document, these influenza variant A(H3N2) viruses are called A(H3N2)v viruses as agreed by the World Health Organization (WHO), Food and Agriculture Organization (FAO) and World Organisation for Animal Health (OIE).

As of 9 December 2011, the United States Centers for Disease Control and Prevention (CDC) has reported influenza A(H3N2)v virus infection in humans in five states: Indiana (two cases), Iowa (three), Maine (two), Pennsylvania (three) and West Virginia (one). The most recent case from West Virginia was reported in a child in December 2011 [2–4]. These cases are not representative of the extent of infection in pigs and humans in the USA where, as in Europe, surveillance of animal disease is incomplete and not all human influenza A virus cases are subtyped meaning full identification of human cases will not take place [2].

Although preliminary evidence from the investigation in Iowa showed no evidence of ongoing transmission among humans [2], the United States is moving towards the development of an A(H3N2)v-based vaccine as a precautionary measure [4]. It should be noted that surveillance of influenza in animals and humans in Europe has not detected any triple reassortant viruses in general or this virus in particular. However, surveillance of these influenza viruses in animals in Europe is weak as they are not notifiable, being of little economic relevance. Equally, surveillance of pig-to-human infections is weaker than in the US, although European swine viruses are known to infect humans on occasion [1]. Should these viruses adapt to humans in North America, they would likely spread quickly to Europe as A(H1N1)pdm09 did. Hence, there is a need to assess the diagnostic capacity in Europe for these viruses.

Diagnostic RT-PCR targeting the M gene of generic influenza A virus will detect swine-origin triple-reassortant A(H3N2)v virus as human influenza A. However, the subtype-specific RT-PCR for either H3 or N2 of human influenza A virus will probably have a decreased sensitivity or will result in no detection of A(H3N2)v virus. Probes directed against other genes, e.g. the nucleoprotein (NP) gene as was used during the early phase of the 2009 pandemic with the A(H1N1)pdm09 virus, will enable preliminary differentiation between human seasonal and zoonotic H3N2 viruses. Therefore, swine-origin-specific subtype RT-PCR, antigenic characterisation, and partial or full genome sequencing are the most appropriate techniques to distinguish between human and these new zoonotic influenza viruses.

[1] Liechtenstein does not participate in the Community Network of Reference Laboratories for Human influenza in Europe (CNRL)

The European influenza reference laboratories (National Influenza Centres or NICs) were informed of the detection challenges by ECDC and WHO Regional Office for Europe leading to a number of laboratories reviewing and updating their detection protocols to be able to make this distinction.

Should any unusual influenza virus be identified in a human in any EU or EEA country and be reported to ECDC, then it would be included in the Weekly Influenza Surveillance Overview [5]. Such a situation would also meet the criteria for the Member State to consider communicating this under the obligation of the International Health Regulations through WHO,[6] and through the Early Warning and Response System.[2]

In 2011, in addition to this questionnaire, ECDC and CNRL undertook an in silico (a computer-based analysis) exercise with the national reference laboratories to understand the existing strategies for detection of novel viruses across national laboratories in the EU/EEA countries.

Results

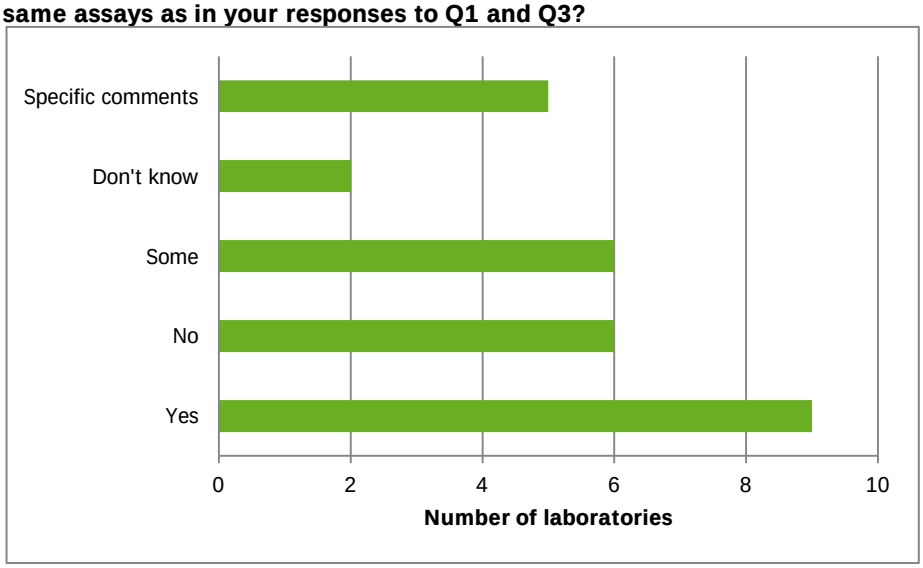
The objective of the questionnaire was to explore the RT-PCR capability of influenza reference laboratories in EU/EEA countries to detect A(H3N2)v viruses in their day-to-day diagnostics and to subtype them as swine-origin variant viruses. On 12 November 2011, the online questionnaire was distributed to all these laboratories and the results were compiled.

Twenty-eight (78%) of 36 laboratories, from 23 (79%) of 29 EU/EEA Member States, responded to the questionnaire (see Annex 2). Five countries did not respond while one country reported that they are not performing RT-PCR testing for influenza. All respondents reported the influenza virus M gene being the target for their real-time or conventional RT-PCR detection of influenza A viruses. One laboratory uses NP as conventional RT-PCR target. Based on *in silico* analysis, 27 (96%) of 28 responding laboratories considered the M gene primers and probes appropriate for detection of A(H3N2)v virus.

Answers to questions 3–9 (see Annex 1) were more diverse. Twenty-two (79%) laboratories never use subtype-specific RT-PCR targeting the H gene (e.g. H3) for direct detection of influenza A virus, but use an RT-PCR for generic detection of influenza A virus beforehand. The remaining six laboratories use subtype-specific RT-PCR targeting the H gene in specific situations, e.g. high sample numbers with highly predominant subtype, or on all sentinel samples for surveillance testing purposes and for confirmation of non-sentinel samples.

Nine (32%) of the reference laboratories know that other laboratories in their country use the same assays as the reference laboratory in detecting generic influenza A and in subtyping (Figure 1). In six countries, the other laboratories do not use the same assays as the reference laboratory. However, in some countries, only limited regional testing is carried out or all positive samples are sent to the reference laboratory for subtyping.

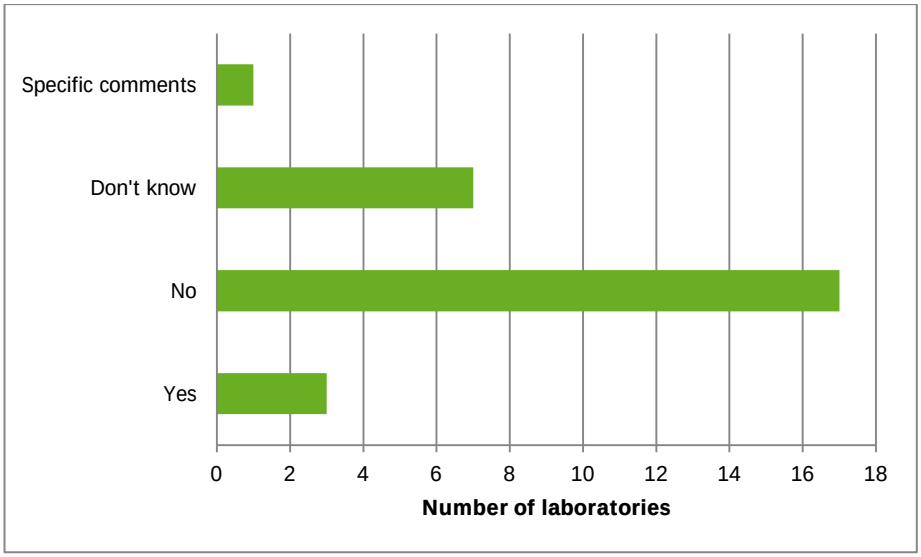
Figure 1. Results of question 4: Are other laboratories in your country to your knowledge using the same assays as in your responses to Q1 and Q3?



In the majority of countries, diagnostic laboratories are not using subtype-specific RT-PCR without using generic influenza A virus detection methods beforehand (Figure 2).

[2] European Union Early Warning and Response System (EWRS). Available at https://ewrs.ecdc.europa.eu

Figure 2. Results of question 5: Are other laboratories in your country to your knowledge using subtype specific RT-PCR targeting the H gene (e.g. H3) for direct detection of influenza A virus without using a RT-PCR for generic detection of influenza A virus?



Twelve (43%) laboratories consider their primers and probes for the H3 RT-PCR appropriate for detecting/subtyping SOIVtrH3N2-M virus whereas sixteen (57%) laboratories do not (Figure 3a). Primers and probes for the N2 RT-PCR are considered appropriate for detecting/subtyping the SOIVtrH3N2-M virus by nine (32%) laboratories (Figure 3b). One laboratory reported that they are not testing for N2 and one that the primers and probes may detect the virus, but with lower sensitivity.

Figure 3a Results of question 6: Do you consider your primers and probes for the H3 RT-PCR appropriate for detecting/subtyping the SOIVtrH3N2-M virus?

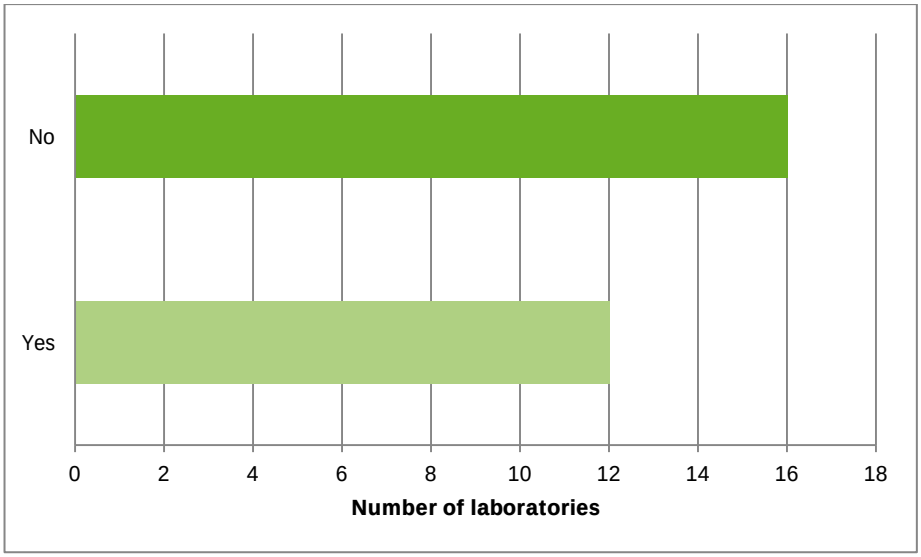
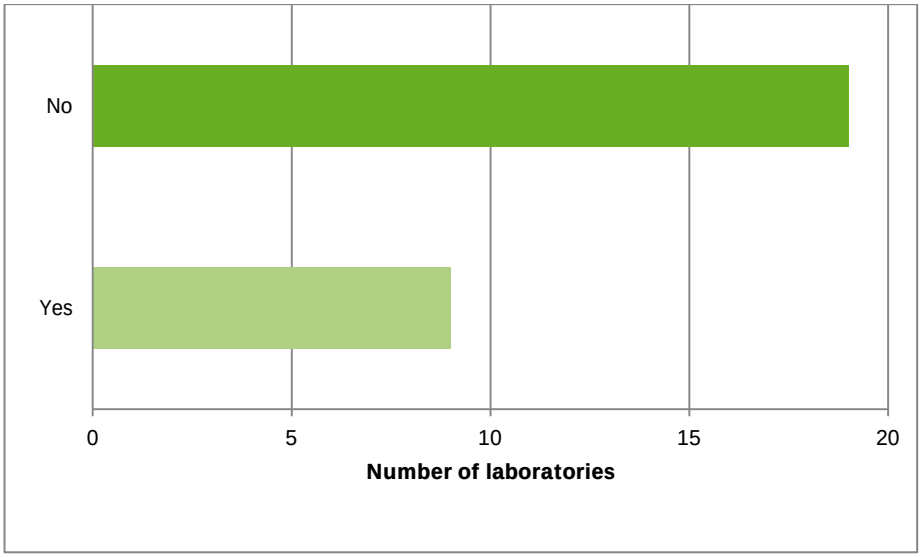
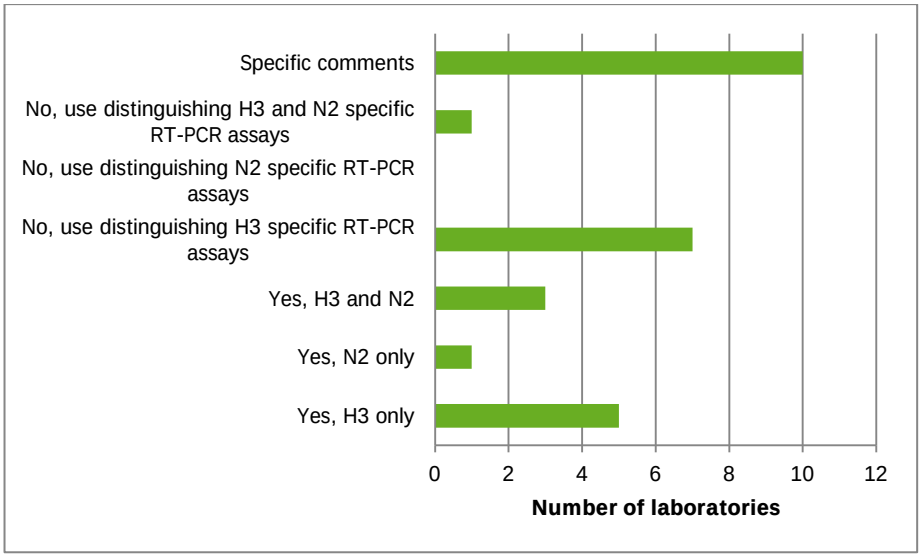


Figure 3b Results of questions 7: Do you consider your primers and probes for the N2 RT-PCR appropriate for detecting/subtyping the SOIVtrH3N2-M virus?



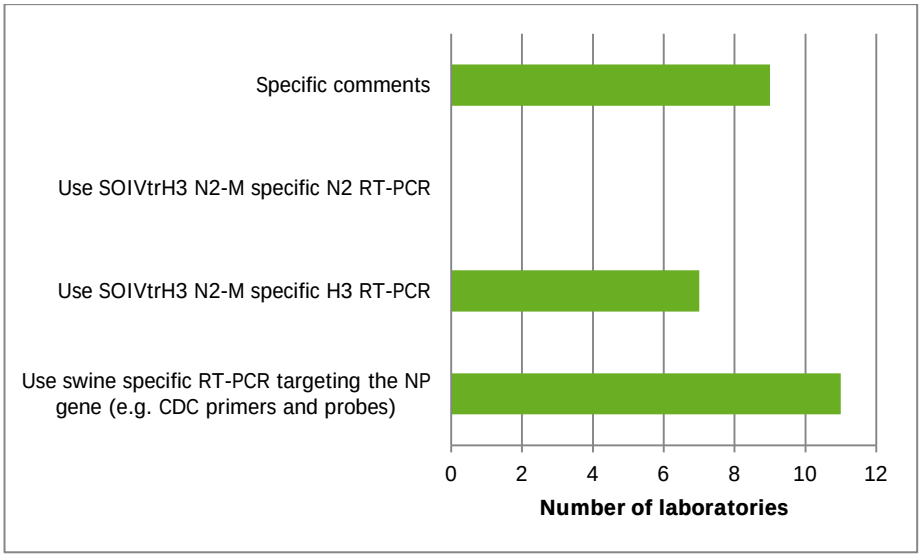
The last two questions asked for more details on whether the laboratories had adapted their H3 and/or N2 RT-PCR to react both with SOIVtrH3N2-M and seasonal H3N2 (sH3N2) virus, and 27 laboratories responded. Since detection of the A(H3N2)v cases in the US, five laboratories had updated their H3 assays, one had updated their N2 assay and three laboratories both their H3 and N2 assays (Figure 4). Seven laboratories use a distinguishing H3-specific RT-PCR assay and one uses H3 and N2-specific RT-PCRs (Figure 4).

Figure 4. Results of question 8: If Q6 and/or Q7 are answered with No, have you adapted your H3 and/or N2 RT-PCR to react both with the SOIVtrH3N2-M and seasonal H3N2 virus?



The majority of laboratories use swine-specific primers and probes targeting the NP gene (11 of 27 laboratories; 41%), or an A(H3N2)v-specific H3 RT-PCR (7 of 27 laboratories; 26%) to distinguish seasonal A(H3N2) viruses from variant viruses (Figure 5). Nine laboratories replied in free text, e.g. that they use sequencing for distinguishing purposes.

Figure 5. Results of question 9: If Q8 has been answered with Yes, how do you distinguish the SOIVtrH3N2-M virus from seasonal H3N2 virus?



Conclusions

With a reasonably high response rate, the results of this questionnaire show that CNRL laboratories have a very good capability to identify influenza A(H3N2)v viruses as A viruses. A fifth of the respondents use subtype-specific RT-PCR targeting the H gene in specific situations, e.g. during weeks with high sample numbers. They risk overlooking A(H3N2)v viruses which would remain negative in assays targeting the human H3 gene. The same applies to primary diagnostic laboratories that do not use the same assay as the reference laboratory and have not targeted their initial detection reaction to capture variant influenza virus strains. On the other hand, detection assays should not be fully harmonised within Europe, as this would increase the risk of missing a change in the influenza virus population and shortage of reagents.

A majority of the respondents considered their H3 subtyping RT-PCR inappropriate for detecting variant viruses. Only nine laboratories considered themselves able to subtype the variant viruses based on the N2 gene. Many laboratories have already updated their protocols and some possibly after responding to the questionnaire. The majority of respondents use the US CDC approach to distinguish between seasonal A(H3N2) and A(H3N2)v viruses. Their RT-PCR targets the swine-specific NP gene if the specimen is influenza A-positive (M gene), but remains negative in human H3 and N2 assays. Some laboratories have developed specific H3 and/or N2 RT-PCR assays to target variant viruses.

On the basis of this questionnaire, it is reasonable to state that the detection capability for influenza A(H3N2)v viruses at type level in Europe is high, but subtyping the viruses could prove challenging with the current assays. Many laboratories have updated their assays or are targeting the NP gene for subtyping when the specimen remains influenza A-positive but H3- and N2-negative with the human H3 and N2 virus assays. Detecting A(H3N2)v virus at subtype level seems less problematic than actually receiving the specimens at the national reference laboratory and selecting them for subtyping. Not all influenza A(H3N2) viruses will be subtyped, especially now that the dominant virus strain is A(H3N2) [5].

This survey indicates that with current capabilities, the variant viruses would be detected in Europe, yet some of them would not be subtyped and identified as variant viruses. ECDC's future influenza virology network activities will take into account emergence of animal influenza viruses in human samples and rapid information exchange between laboratories in such situations for actions required to update their methods of detection for such new viruses.

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Annexes

Annex 1 Questionnaire

- 1. What is the target gene of your real-time or conventional RT-PCR for generic detection of influenza A viruses?

  ☐M
  ☐NP
  ☐NS
  ☐Other, namely (below):
  ☐Specify your own value:

- 2. Do you consider these primers and probes, based on *in silico* analysis, appropriate for detection of SOIVtrH3N2-M virus?

  ☐Yes
  ☐No

- 3. Are you using subtype-specific RT-PCR targeting the H gene (e.g. H3) for direct detection of influenza A virus WITHOUT using an RT-PCR for generic detection of influenza A virus?

  ☐Yes, always
  ☐Yes, in high sample load periods
  ☐Yes, in specific situations, namely (below):
  ☐No, never
  ☐Specify your own value:

- 4. Are other laboratories in your country to your knowledge using the same assays as in your responses to Q1 and Q3?

  ☐Yes
  ☐No
  ☐Some
  ☐Don't know
  ☐Comments below
  ☐Specify your own value:

- 5. Are other laboratories in your country to your knowledge using subtype-specific RT-PCR targeting the H gene (e.g. H3) for direct detection of influenza A virus WITHOUT using an RT-PCR for generic detection of influenza A virus?

  ☐Yes
  ☐No
  ☐Some
  ☐Don't know
  ☐Comments (below)
  ☐Specify your own value:

- 6. Do you consider your primers and probes for the H3 RT-PCR appropriate for detecting/subtyping the SOIVtrH3N2-M virus?

  ☐Yes
  ☐No

- 7. Do you consider your primers and probes for the N2 RT-PCR appropriate for detecting/subtyping the SOIVtrH3N2-M virus?

  ☐Yes
  ☐No

- 8. If Q6 and/or Q7 are answered with No, have you adapted your H3 and/or N2 RT-PCR to react both with the SOIVtrH3N2-M and seasonal H3N2 (sH3N2) virus?

  ☐Yes, H3 only
  ☐Yes, N2 only
  ☐Yes, H3 and N2
  ☐No, use distinguishing H3-specific RT-PCR assays
  ☐No, use distinguishing N2-specific RT-PCR assays
  ☐No, use distinguishing H3- and N2-specific RT-PCR assays
  ☐Comments (below)
  ☐Specify your own value:

- 9. If Q8 has been answered with Yes, how do you distinguish the SOIVtrH3N2-M virus from seasonal H3N2 (sH3N2) virus?

  ☐Use swine-specific RT-PCR targeting the NP gene (e.g. CDC primers and probes)
  ☐Use SOIVtrH3N2-M-specific H3 RT-PCR
  ☐Use SOIVtrH3N2-M-specific N2 RT-PCR
  ☐Comments (below)
  ☐Specify your own value:

Annex 2 Participating laboratories

Organisation name and city	Country
Medical University Vienna, Vienna	Austria
Scientific Institute of Public Health, Brussels	Belgium
National Center of Infectious and Parasitic Diseases, Sofia	Bulgaria
National Institute of Public Health, Prague	Czech Republic
Statens Serum Institut, Copenhagen	Denmark
Health Board, Tallinn	Estonia
National Institute for Health and Welfare, Helsinki	Finland
Centre Hospitalier Lyon Sud, Lyon	France
Pasteur Institute of Paris, Paris	France
Robert Koch Institute, Berlin	Germany
Hellenic Pasteur Institute, Athens	Greece
National Center for Epidemiology, Budapest	Hungary
University College Dublin, Dublin	Ireland
Istituto Superiore di Sanità, Rome	Italy
State Agency "Infectology Center of Latvia", Riga	Latvia
Center for Communicable Diseases and AIDS, Vilnius	Lithuania
National Institute for Public Health and the Environment (RIVM), Bilthoven	Netherlands
Norwegian Institute of Public Health, Oslo	Norway
National Institute of Health Dr. Ricardo Jorge, Lisbon	Portugal
National Institute of Research and Development for Microbiology and Immunology "Cantacuzino", Bucharest	Romania
Public Health Authority of Slovak Republic, Bratislava	Slovakia
Centro Nacional de Gripe, Barcelona	Spain
National Centre for Microbiology, Madrid	Spain
Swedish Institute for Communicable Disease Control, Solna	Sweden
Health Protection Agency, Colindale	United Kingdom
Royal Victoria Hospital, Belfast	United Kingdom
Health Protection Agency Scotland, Glasgow	United Kingdom
Specialist Virology Centre for Wales, Cardiff	United Kingdom