TECHNICAL REPORT

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2013

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This report of the European Centre for Disease Prevention and Control (ECDC) was coordinated by Cornelia Adlhoch and Eeva Broberg (ECDC) and produced by Ian Harrison with assistance from Angie Lackenby and Catherine Thompson, Public Health England, London, on behalf of the European Reference Laboratory Network for Human Influenza (ERLI-Net).

Angie Lackenby and Joanna Ellis, Public Health England, London, UK and the EISN Coordination group contributed to the design and planning of the exercise. The panel was produced by the Respiratory Virus Unit, Public Health England, London.

Data services were provided by Quality Control for Molecular Diagnostics (QCMD), Glasgow. Analysis of the data was conducted by QCMD and Ian Harrison.

A(H7N9) sample was kindly provided by Dr Schweiger, Robert Koch Institute, Berlin

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Abbreviations

BPL	β-propiolactone
ECDC	European Centre for Disease Prevention and Control
EISN	European Influenza Surveillance Network
EQA	External quality assessment
ERLI-Net	European Reference Laboratory Network for Human Influenza
EU	European Union
GISRS	Global Influenza Surveillance and Response System
HI	Haemagglutination inhibition
IF	Immunofluorescence
INF13	Influenza detection, culture and isolation external quality assessment panel of ERLI-Net
NAT	Nucleic acid amplification technologies
NIMR	National Institute for Medical Research
PCR	Polymerase chain reaction
PHE	Public Health England
QC	Quality control
QCMD	Quality Control for Molecular Diagnostics
RIVM	Dutch National Institute for Public Health and the Environment
RVU	Respiratory virus unit
WHO	World Health Organization
WHO-CC	World Health Organization Collaborating Centre for Reference and Research on Influenza

Executive summary

In September/October 2013, the European Reference Laboratory Network for Human Influenza (ERLI-Net; previously called CNRL) conducted an external quality assessment (EQA) for the rapid detection of influenza virus, its isolation, and culture. This was the third rapid detection and culture EQA panel for European influenza reference laboratories distributed by ERLI-Net since the European Influenza Surveillance Network (EISN) was established in 2008. The objectives of the exercise were to a) measure the performance of participating laboratories through an independent mechanism and b) provide whole network information on the capacity and capability for rapid detection by PCR, influenza virus culture, and strain characterisation within a defined reporting timeframe.

All ERLI-Net member laboratories were invited to participate. Thirty-six laboratories from 29 European countries participated in the exercise. Each participant received a panel of ten coded samples (including a negative sample) of influenza A and B viruses that are circulating, or have recently circulated, in humans. All participating laboratories returned results for rapid detection of influenza viruses using PCR or other methods. Results for influenza virus culture were returned by 30 of the 36 participating laboratories, with 26 reporting strain characterisation results.

The proportion of laboratories achieving the maximum rapid detection proficiency score rose to 80% in 2013, compared with 76% in 2010 and 69% in 2008. There was particular improvement in the number of laboratories that were able to provide lineage determination for influenza B samples. Four false negative but no false positive samples were reported, which signifies an improvement from 2010 when three false negatives and two false positives were recorded, but still indicates possible sensitivity issues in some assays. Altogether, the panel was a comprehensive test of the laboratories' ability to detect recently circulating influenza viruses.

Avian influenza A(H7N9) in humans was first reported in China in March 2013. Network laboratories rapidly worked to design and implement assays that would enable them to detect and type this emerging virus. The 2013 EQA provided the first opportunity to assess A(H7N9) detection capabilities across the European network, and it proved that the emergency response mechanism worked well. Thirty-three of the 36 laboratories were able to detect, type and subtype the A/Anhui/1/2013 virus while the remaining three laboratories detected a non-subtypeable influenza A virus.

The laboratories which returned virus culture results performed well, with detailed strain characterisation being reported in 80% of cases. Five laboratories failed to recover virus from a total of 11 samples, highlighting the necessity for optimal cell culture systems and procedures, informed by guidance from the network on current best practise. In total, the number of laboratories participating remained constant with thirty participating laboratories.

Previous improvements in strain characterisation were consolidated in 2013, with modest improvements to the average score compared with 2010: 27/30, as compared to 26.4/30 earlier. The majority of participating laboratories used haemagglutination inhibition (HI) assay to determine strain identity rather than sequencing, emphasising the need for high-quality virus culture to provide virus isolates for HI. Genetic characterisation (sequencing) was performed by 13 laboratories and resulted in very accurate characterisation (11 laboratories achieved full scores). Three training courses in sequencing techniques have been provided since 2010, with ten of the 13 laboratories (77%) attending. This may account for increased EQA participation and the high quality of results obtained with this technique.

Overall, the 2013 influenza virus rapid detection and culture EQA demonstrated a modest improvement in performance in culture and strain characterisation. Rapid detection also continued to improve, especially with influenza B virus lineage determination. One of the most encouraging aspects of the 2013 EQA panel was the fact that it emphasised the strength of the network and its constituent laboratories. This was demonstrated by the rapid development of testing capabilities for influenza A(H7N9) virus, which was reconfirmed in this quality assurance exercise.

Introduction

The European Centre for Disease Prevention and Control (ECDC) is a European Union (EU) agency with a mandate to operate the dedicated surveillance networks and to identify, assess, and communicate current and emerging threats to human health from communicable diseases. Within its mission, ECDC shall 'foster the development of sufficient capacity within the community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes.' (Article 5.3, EC 851/2004).

The European Influenza Surveillance Network (EISN), which includes the European Reference Laboratory Network for Human Influenza (ERLI-Net), is a dedicated network for the epidemiological and virological surveillance of influenza.

Influenza virus is a highly contagious acute respiratory disease that can spread rapidly and widely, causing high levels of morbidity and mortality. Influenza viruses evolve rapidly from season to season, through point mutations leading to genetic and antigenic drift. Early detection and characterisation of circulating viral strains is of great importance for timely risk assessment, treatment recommendations, and vaccine formulation. The segmented nature of the influenza genome also makes genomic reassortment an important mechanism for generating genetic diversity (antigenic shift). This process is particularly important in influenza A virus because of its role in the generation of new pandemic strains of the virus [2,3]. Animal viral reservoirs also pose a particular risk, as prior population immunity to animal zoonotic infections is unlikely to exist, or infections from novel viruses produced by reassortment with animal subtypes.

The introduction of nucleic acid amplification technologies (NAT) has led to the development of sensitive tests that can rapidly identify the type of virus (A, B), the subtype of influenza A viruses (H1, H3, H7, etc.), and the genetic lineage of influenza B viruses (Victoria and Yamagata). As a result, these tests are assuming great practical and clinical relevance. However, the ability to accurately determine the antigenic profile of an influenza virus still requires the ability to isolate virus in culture and carry out serological tests to identify the antigenic characteristics of the virus.

It is essential that the reliability and robustness of technologies for influenza detection and typing are assessed through effective quality control. An integral part of quality control is external quality assessment (EQA), which provides a means of independently and objectively evaluating laboratory performance.

In 2012, a framework contract was put in place for the coordination of the ERLI-Net activities by a consortium representing three European institutes, covering the period 2012–2015. Within this contract is the provision of biennial EQAs of national influenza reference laboratories to ensure the reliability and comparability of results, and to identify needs for improvement in laboratory diagnostic capacity. The ERLI-Net EQA is designed to assess the performance of laboratories in all EU/EEA countries and includes panels of reference viruses for rapid detection and virus culture, (sub)typing, antigenic characterisation, and molecular typing for influenza virus.

The major objective of the 2013 EISN influenza virus culture EQA panel is to examine the ability of European influenza reference laboratories to accurately detect and rapidly report influenza virus-positive samples to be able to culture and characterise the antigenic profile of circulating human influenza virus strains, and to identify genetic characteristics of detected influenza viruses.

This report contains the results of the influenza virus EQA, designed and prepared by the ERLI-Net coordination team and funded by ECDC.

The main purposes of external quality assessment schemes include:

- assessment of the general standard of performance ('state of the art');
- assessment of the effects of analytical procedures (method principles and techniques);
- evaluation of individual laboratory performance;
- identification and justification of problem areas;
- provision of continuing education on testing against samples of known status;
- capacity to make comparisons with other laboratories; and the
- identification of needs for training activities.

Objectives

The primary aim of this external quality assessment exercise was to assess individual laboratory performance in the following areas:

- Rapid detection by PCR or other tests (within a defined reporting timeframe), including typing and subtyping
- Virus culture (within a defined reporting timeframe)
- Virus typing after virus isolation (using HI or PCR)
- Influenza A virus subtyping and influenza B virus lineage determination after virus isolation (using HI or PCR)
- Virus strain identification (by HI and/or by sequencing)

Study design

Organisation

The EQA panel was designed by staff from PHE together with members of the ERLI-Net coordination team. The panel was prepared and tested by the Respiratory Virus Unit (RVU) at PHE, London. Further pre-testing was performed by the WHO-CC at the National Institute for Medical Research (NIMR) at Mill Hill, London, and the France South National Influenza Centre, Lyon. The panel contents were distributed in September 2013, frozen on dry ice by specialist courier. Participating laboratories submitted results electronically into a web-based database.

Participation

It was mandatory for all ERLI-Net member laboratories, including all EU countries, Norway and Iceland, to participate in at least the rapid detection component of this panel. All influenza laboratory contact points in the ERLI-Net were notified in advance of the EQA exercise. A list of participating laboratories in the influenza virus rapid detection and culture EQA can be found in Annex 5.

Panel description

The EQA panel consisted of ten simulated clinical samples containing influenza viruses from subtypes that are circulating, or have recently circulated, in humans, including influenza A(H1N1)pdm09, influenza A(H3N2) and both genetic lineages of influenza B viruses. In addition, an inactivated A(H7N9) virus was included in the 2013 panel, kindly provided by Dr B. Schweiger, Robert Koch Institute, Berlin. The purpose of this sample was to evaluate the laboratory workflow for dealing with influenza-A-positive samples that are not subtyped by routine assays. One negative sample, containing no virus, completed the panel. Viruses were grown in eggs and diluted to a suitable concentration for testing determined by viral plaque assay and haemagglutination assay. Viruses were aliquoted and stored frozen at -80 °C until dispatched. One panel was thawed and pre-tested at the PHE laboratory using in-house methods. Panels were sent frozen on dry ice to two independent laboratories for pre-testing. The final panels were shipped frozen on dry ice by specialist courier on the 24th September 2013 and were received by participating laboratories within two days. The deadline for rapid detection results was within seven days of receipt of the panel, the deadline for culture results was within 28 days of receipt of the panel.

Participant testing

Laboratories were expected to demonstrate their ability to detect, type and subtype positive samples (rapid detection) and to isolate influenza viruses in culture and provide characterisation using either reference antisera (isolation and characterisation) or sequencing. Participating laboratories were asked to test the panel using the standard laboratory protocols normally used by their laboratory for rapid detection, virus culture and antigenic characterisation including PCR, HI and sequencing.

Data reporting

For rapid detection, laboratories were required to detect, type and subtype influenza A viruses. For influenza B viruses, laboratories were only required to detect and type, although if lineage determination was performed, the data was included in the results tables. For virus culture and strain characterisation, participating laboratories were asked to report the virus type and subtype (or negative) and the strain as determined by antigenic and/or genetic means. The Quality Control for Molecular Diagnostics organisation (QCMD) operates a web-based reporting tool that was used to collect data on the results and methods used.

Data analysis

The rapid detection scoring system awarded three points for correct determination of a negative; one point for correct detection of influenza A virus; one point for correct typing; and one point for correct subtyping. For influenza B virus samples, one point was given for correct detection and two points were given for correct typing. The maximum achievable score for rapid detection was 30 points. For virus culture and strain characterisation, the scoring system gave one point for isolation of influenza virus A or B virus; one point for correct subtyping of influenza virus A or lineage-identification of influenza virus B; one point for correct strain identification; and three points for correct determination of a negative. The maximum achievable score for virus culture and strain characterisation was 27 points. As the same panel had been used for the rapid detection component of the EQA, laboratories had already determined the type/subtype or lineage of samples. Therefore, if no proof of further characterisation was provided, they were not given a mark for identifying the type or subtype.

Results

Panel composition

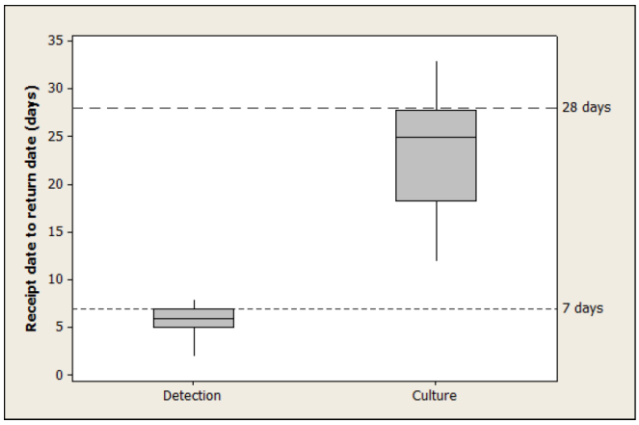
The influenza type, subtype, strain characterisation and plaque forming units/ml for each sample in the influenza detection, culture and isolation EQA panel (INF13) are shown in the expected results table (Table 1). Samples were diluted in a matrix consisting of Hep2C cells in virus transport medium (VTM) at a concentration of $1x10^5$ cells/ml. Sample EISN_INF13-10 was inactivated with β-propiolactone (BPL).

Table 1. INF13 panel composition

Sample code	Matrix*	Virus	Subtype	Plaque titre†
ISN_INF13-01	Hep2C cells in VTM	A/Texas/50/2012	A(H3N2)	5.09E+02
EISN_INF13-02	Hep2C cells in VTM	A/California/7/2009	A(H1N1)pdm09	3.79E+02
EISN_INF13-03	Hep2C cells in VTM	B/Brisbane/60/08	B-Vic	2.10E+02
EISN_INF13-04	Hep2C cells in VTM	B/Massachusetts/02/2012	B-Yam	8.10E+03
EISN_INF13-05	Hep2C cells in VTM	A/California/7/2009	A(H1N1)pdm09	3.79E+03
EISN_INF13-06	Hep2C cells in VTM	Influenza virus negative		
EISN_INF13-07	Hep2C cells in VTM	B/Massachusetts/02/2012	B-Yam	8.10E+02
EISN_INF13-08	Hep2C cells in VTM	A/Texas/50/2012	A(H3N2)	5.09E+03
EISN_INF13-09	Hep2C cells in VTM	B/Brisbane/60/08	B-Vic	2.10E+03
EISN_INF13-10	Hep2C cells in VTM	A/Anhui/1/2013	A(H7N9)	n/a

** Hep2C cells in virus transport medium (VTM) at a concentration of $1x10^5$ cells/ml.*
† Plaque forming units/ml. Sample EISN_INF13-10 was inactivated with β-propiolactone (BPL).

Figure 1. Time taken to return results



The number of days required by participating laboratories to return their rapid detection and culture results are presented. The date participating laboratories reported receipt of the panel samples was considered as the start date (courier date not considered). These data are presented as a box and whiskers plot, where the whiskers represent the range of values returned, and the box represents the first quartile, median and the third quartile.

Table 2. Number and percentage of results reported for INF13 rapid detection

Sample code	Sample contents	Plaque titre	Rapid detection results (n=36)								
			Type and subtype			Type			Incorrect result		
			Result	n	(%)	Result	n	(%)	Result	n	(%)
EISN_INF13-01	A/Texas/50/2012	5.09E+02	A, H3	33	91.7	A	1	2.8	Negative	2	5.6
EISN_INF13-02	A/California/7/2009	3.79E+02	A, H1/pdm09	34	94.4	A	1	2.8	Negative	1	2.8
EISN_INF13-03	B/Brisbane/60/08	2.10E+02	B, Victoria	17	47.2	B	19	52.8			
EISN_INF13-04	B/Massachusetts/02/2012	8.10E+03	B, Yamagata	16	44.4	B	20	55.6			
EISN_INF13-05	A/California/7/2009	3.79E+03	A, H1/pdm09	34	94.4	A	1	2.8	Negative	1	2.8
EISN_INF13-06	Influenza virus negative		Negative	36	100.0						
EISN_INF13-07	B/Massachusetts/02/2012	8.10E+02	B, Yamagata	16	44.4	B	20	55.6			
EISN_INF13-08	A/Texas/50/2012	5.09E+03	A, H3	35	97.2	A	1	2.8			

Sample code	Sample contents	Plaque titre	Rapid detection results (n=36)								
			Type and subtype			Type			Incorrect result		
			Result	n	(%)	Result	n	(%)	Result	n	(%)
EISN_INF13-09	B/Brisbane/60/08	2.10E+03	B, Victoria	17	47.2	B	19	52.8			
EISN_INF13-10	A/Anhui/1/2013	n/a	A, H7	33	91.7	A	3	8.3			

All results reported on each panel sample are shown, in addition to the number and percentage of participating laboratories reporting each result.

Reporting time and participation

Figure 1 summarises the time taken from receipt of the panel to the reporting of rapid detection and culture results. The time period between courier delivery and panel receipt was not considered. For rapid detection, 34 of 36 participating laboratories (94.4%) reported receipt of a panel, and results and were included in the analysis shown in Figure 1. Two participating laboratories did not report receipt of a panel and could not be considered for Figure 1, but did return results. For culture, 28 of 36 participating laboratories (77.8%) reported a panel receipt, and results and were included in the analysis shown in Figure 1. Of the participating laboratories not included in Figure 1 (n=8; 22.2%), two reported results which were included in the analysis for the remainder of the report. One did not report panel receipt but returned results while the second participant uploaded results within the timeframe but did not submit them until after the deadline. No mechanism was provided for laboratories to participate only in the rapid detection but not the culture component of the EQA. Five of the remaining laboratories reported a panel receipt but did not return results, and one participant did not report a panel receipt or return results.

The median time taken for participating laboratories to return detection results (from the reported date of receipt) was 6 days (range 2–8 days), and the mean time taken was 5.5 days. The median time taken for participating laboratories to return culture results (from the reported date of receipt) was 25 days, and the mean time taken was 23.1 days (range 12–33 days). Participating laboratories were expected to return rapid detection results within 7 days of receipt and culture results within 28 days of receipt. These deadlines are indicated by dotted lines on the plot.

All laboratories which submitted data were included in the remaining analysis, irrespective of the data submission deadline. The number of laboratories participating in the EISN-INF13 rapid detection panel increased from 34 (2010) to 36 (2013). The number of laboratories completing the culture and characterisation panel remained at 30 (Figure 2).

Results for rapid detection of influenza virus

The number and percentage of results reported for rapid detection of influenza virus by participating laboratories is presented in Table 2. Thirty-six participating laboratories (100%) reported results for rapid detection. All 36 reported typing and subtyping results for influenza A virus and typing results for influenza B virus; 16 and 17 laboratories reported lineage determination results for influenza B virus lineage B(Yamagata) and B(Victoria), respectively. Twenty-nine of the 36 (80.6%) participating laboratories returning results for rapid detection achieved the maximum score (30 points). Participating laboratories that did not achieve a full score received scores from 24 to 29 points (Annex 1). Four false negative, and no false positive, results were reported.

An inactivated A(H7N9) sample was included with the INF13 panel, marking the first time that an EQA has tested the ability of European influenza reference laboratories to correctly detect the novel H7N9 subtype. Previous panels included H7N7 samples after this virus caused an epidemic in the Netherlands in 2003. Thirty-three of the 36 participating laboratories detected, typed and subtyped the sample. The remaining three laboratories detected it as a non-subtypable influenza A which would have warranted further analysis. Nine of the laboratories also subtyped the neuraminidase although there was no mechanism to report this data.

Five laboratories used antigen detection techniques in addition to PCR-based methods (Table 3), which resulted in false negative results from three laboratories. A commercial immunofluorescence kit failed to identify the influenza A(H7N9) sample, whereas in another laboratory an A(H3N2) sample was not detected by an in-house assay. The third laboratory used a commercial rapid test kit which recorded negative results for all samples except the A(H7N9). There are two possible explanations for this result: the Anhui A(H7N9) sample preparation was at a higher titre compared with the other samples in the panel. Alternatively, sample inactivation with BPL may have affected the sensitivity of the assay by exposing more antigens. The laboratory in question no longer uses this rapid test assay for routine samples.

Figure 2. Laboratories participating in rapid detection and virus isolation and characterisation in 2010 and 2013

2013 participant ID	2010		2013	
	Rapid detection	Virus isolation and culture	Rapid detection	Virus isolation and culture
75				
95				
112				
117				
200				
207				
1159				
1174				
1262				
1299				
1323				
1402				
1433				
1456				
1515				
1534				
1643				
1649				
2001				
2125				
2126				
2253				
2258				
2270				
2271				
2274				
2275				
2276				
2277				
2278				
2306				
2820				
3442				
4208				
4209				
4213				
	Total number			
	34	30	36	30

Participating laboratories are identified by a unique anonymised participant ID code. Green shading indicates participation in INF13 panel. Grey shading indicates non-participation in the panel. Dark grey shading indicates non-participation in EQA exercise. Participation in the EISN 2010 and 2013 EQA is shown.

Table 3. Rapid detection results obtained by laboratories using antigen detection techniques

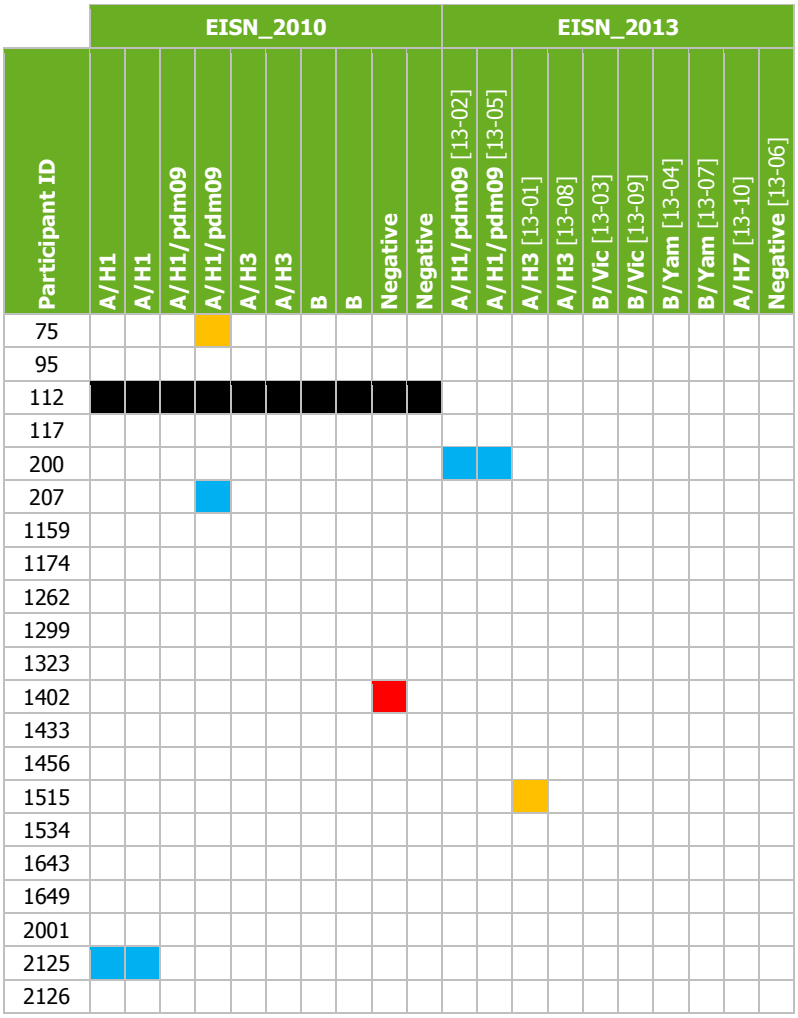
Participant ID	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
95	A	A	B	B	A	-ve	B	A	B	A
2253	A	A	B	B	A	-ve	B	A	B	-ve
1456	A	A	B	B	A	-ve	B	A	B	A
2270	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	A
2275	-ve	A	B	B	A	-ve	B	A	B	A

Results obtained by laboratories using direct antigen detection techniques are shown. A and B indicated influenza A/B-positive result. -ve indicates a negative result; red shading indicates false negative results.

Figure 3 depicts – by laboratory – the rapid detection results compared with those from 2010. Other than the three laboratories that failed to subtype the A(H7N9) sample, two laboratories had difficulties with subtyping A(H1N1)pdm09 and A(H3N2), resulting in a total of seven subtyping errors. Finally, four laboratories reported false negative results, which is a matter of concern because samples were provided at a titre for which there should have been sufficient sensitivity. Despite these problems, the 2013 results represent an improvement over 2010 when two false positives, three false negatives and eleven (sub)typing errors were recorded.

A comparison of the overall rapid detection scores between 2008 and 2013 shows that the number of laboratories participating in the EQA increased from 31 in 2008 to 36 in 2013. Over the same period, the average score increased from 28.5/30 to 29.5/30 (Figure 4).

Figure 3. Rapid detection results for 2010 and 2013 presented by individual laboratory



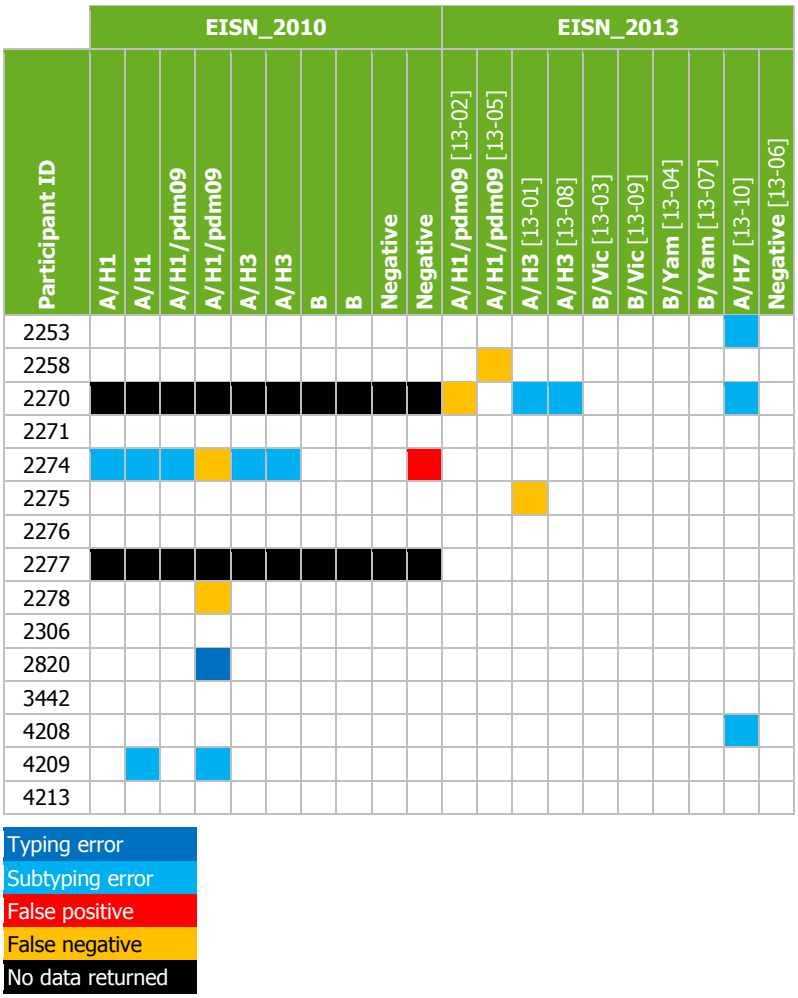
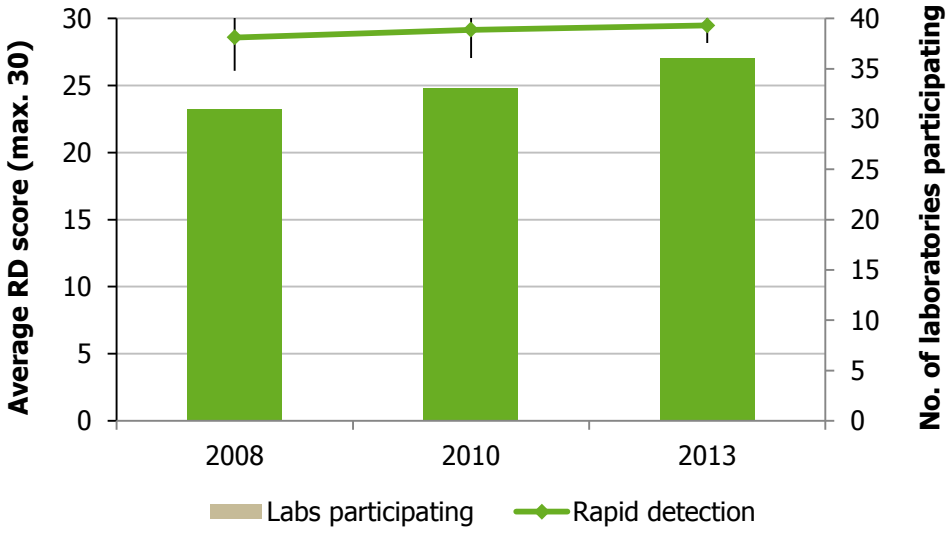


Figure 4. Average rapid detection score since 2008



Comparison of the number of participating laboratories (shown on the second Y-axis) and the average rapid detection score returned in the 2008, 2010 and 2013 EQA panels (first Y-axis). RD=rapid detection. Error bars represent the standard deviation.

Results for culture of influenza virus

Laboratories were required to culture the positive samples and characterise the virus by means of antigenic or genetic assays, or a combination of both. Laboratories were not penalised for performing only one method of characterisation. If both methods were employed and returned discrepant results, participating laboratories were scored on the technique which gave the correct answer. The number and percentage of results reported for culture is presented in Table 4.

Successfully isolating and culturing virus from the sample is a prerequisite before characterisation can be performed. The total number of laboratories with and without culturing errors is shown in comparison to the 2010 results (Figure 5). The total number of false positives and false negatives are also indicated. The total number of laboratories participating in virus isolation remained at 30, the number of laboratories with no culture errors increased from 21 to 23. The number of false negatives declined from 15 to 13 and false positives dropped from 2 to 0. Nine of the 13 false negative results are associated with just three laboratories. See Annex 2 for details.

Twenty-six participating laboratories used antigenic methods, and 10 laboratories reported correct results. The average score for antigenic characterisation was 92% (Figure 6). Twelve laboratories performed genetic characterisation, with 10 reporting correct results; the average score for genetic characterisation was 97%. Raw data and the combined antigenic and genetic data (by laboratory) are presented in Annexes 3 and 4a–c.

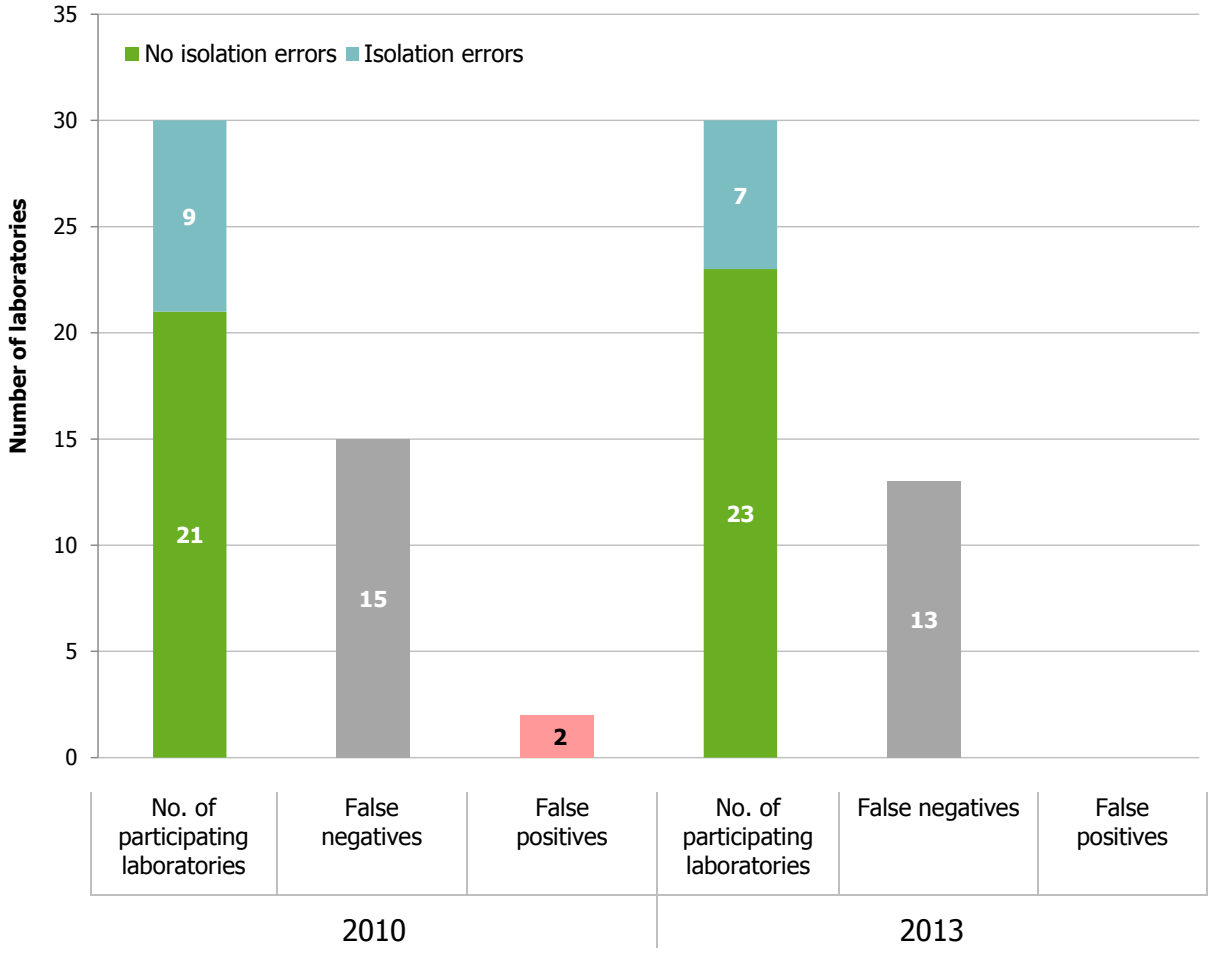
For the last three EQAs (2008, 2010 and 2013), the average combined antigenic and genetic scores improved from 25.8 to 27, out of a total of 30. The number of participating laboratories has remained constant between 2008 and 2013.

Table 4. Number and percentage of results reported for INF13 influenza virus culture and characterisation

Sample code	Sample contents	Correct strain results (n=30)		Antigenic characterisation					Genetic characterisation				
				Total results	Correct		Incorrect		Total results	Correct		Incorrect	
		n	%		n	%	n	%		n	%	n	%
EISN_INF13-01	A/Texas/50/2012	20	66.7	25	17	68.0	8	32.0	12	9	75.0	3	25.0
EISN_INF13-02	A/California/7/2009	27	90.0	25	22	88.0	3	12.0	12	12	100.0	0	0.0
EISN_INF13-03	B/Brisbane/60/08	24	80.0	25	21	84.0	4	16.0	11	9	81.8	2	18.2
EISN_INF13-04	B/Massachusetts/02/2012	22	73.3	25	15	60.0	10	40.0	12	11	91.7	1	8.3
EISN_INF13-05	A/California/7/2009	28	93.3	26	23	88.5	3	11.5	13	13	100.0	0	0.0
EISN_INF13-06	Influenza virus negative	30	100.0										
EISN_INF13-07	B/Massachusetts/02/2012	19	63.3	25	13	52.0	12	48.0	11	10	90.9	1	9.1
EISN_INF13-08	A/Texas/50/2012	22	73.3	26	17	65.4	9	34.6	13	10	76.9	3	23.1
EISN_INF13-09	B/Brisbane/60/08	26	86.7	26	23	88.5	3	11.5	11	10	90.9	1	9.1

***Correct strain results**: Number and percentage of participating laboratories reporting the correct viral strain, or who correctly reported the negative sample. **Total results**: Participating laboratories were asked to specify their characterisation method as 'antigenic', 'genetic, both or neither for each panel sample. This total indicates the number of results for each characterisation method. **Correct**: Number and percentage of results correctly identifying the viral strain. **Incorrect**: Number and percentage of results incorrectly identifying the viral strain.*

Figure 5. Number and type of virus culture error, 2010 and 2013

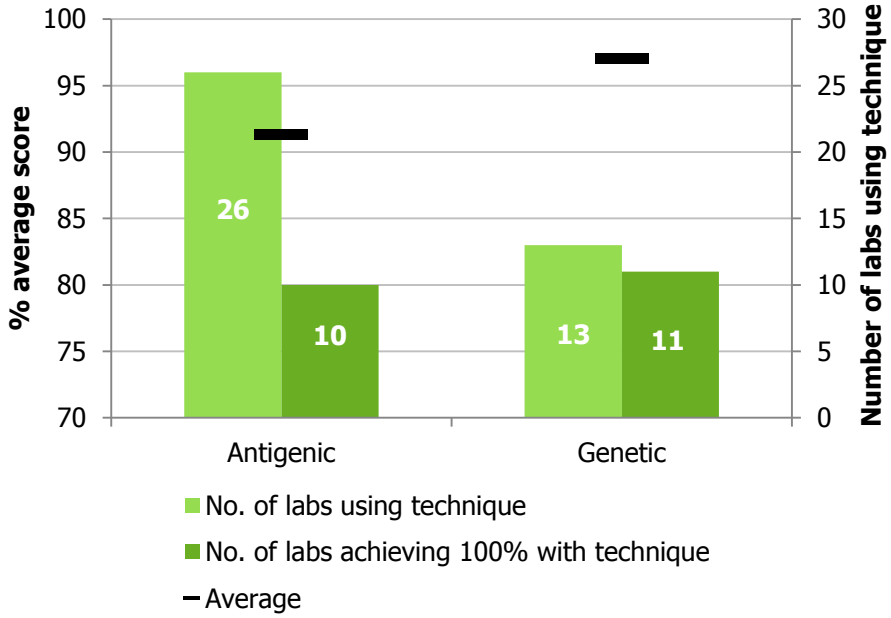


The number of laboratories with/without isolation errors is shown for the 2010 and 2013 EQA panels. Errors were grouped into two categories: false negatives and false positives.

Summary of overall performance

The number and percentage of participating laboratories that reported correct rapid detection and culture results for each of the panel samples is summarised in Table 5. Thirty participating laboratories returned datasets for both rapid detection and culture out of the total of 36 (83.3%). Strain data were not included in this analysis, therefore the expected result for culture was taken as the influenza virus type/subtype. Of the 30 laboratories participating in both components of the INF13 EQA, between 25 and 30 (83 and 100% respectively) successfully detected each panel sample, with an average of 92% across all samples being reported correctly.

Figure 6. Average score achieved with antigenic or genetic characterisation technique for the INF13 panel



Virus characterisation data for the INF13 panel is divided into either genetic or antigenic characterisation. The number of laboratories using each techniques and the number of laboratories with no mistakes are shown. The average score achieved with the technique is also depicted.

Conclusion and discussion

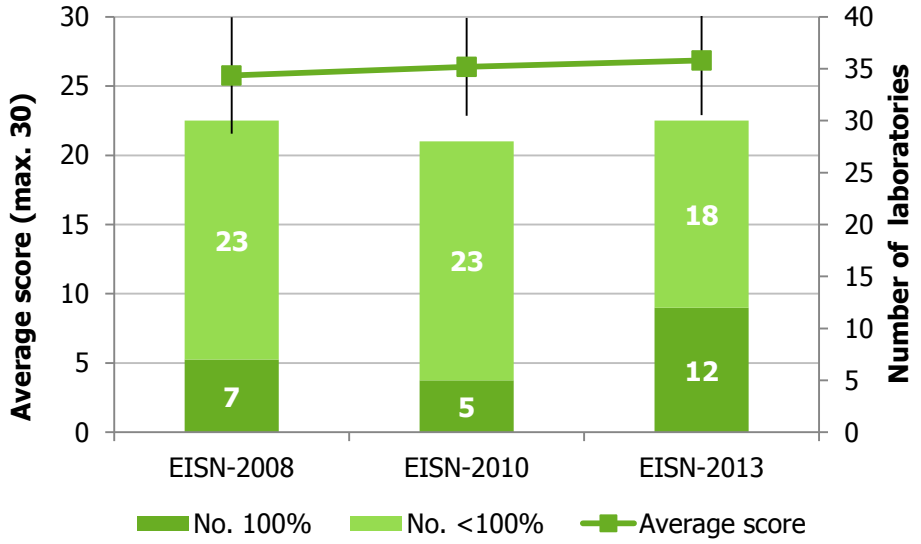
The 2013 EQA panel showed a high level of competence across the European network for detection, rapid reporting and characterisation of influenza viruses with 97% of rapid detection samples being correctly identified, 80.7% of culture and characterisation samples being isolated and the strain being correctly characterised.

The rapid detection results were expected to be reported within seven days. Analysis of data from 34 of the 36 laboratories showed the median time to report data was in fact 6 days and at least 94% of the participating laboratories returned results within the time-limit. Two additional laboratories participated in this panel compared to the 2010 EQA. Laboratories were not required to determine the lineage of influenza B viruses during rapid detection, however at least sixteen of the 36 participating laboratories (45%) did produce this information. Of the rapid detection errors, the four false-negative results warrant the most attention. Analysis of the extraction process, PCR protocol and reagents will be required to assess and rectify these issues. Overall, a progressive increase in the number of laboratories performing rapid detection has been matched by an increase in average score achieved.

The first human case of influenza A(H7N9) was reported in China on the 31st March 2013 and there have subsequently been over 270 cases until February 2014. ERLI-Net laboratories have worked to rapidly develop and implement assays to detect this virus [Broberg et al., 2014]. The EISN-INF13 EQA provided the first opportunity to test the networks' detection capability and proved that H7 assays had successfully been implemented. Thirty-three of the 36 participating laboratories identified the sample as influenza A(H7) and nine of these laboratories also typed the neuraminidase gene. The three remaining laboratories determined the sample to be a non-subtypeable influenza A, which would routinely warrant further investigations. This supports the results of a questionnaire that was circulated in May 2013 that showed that twenty-nine laboratories in 27 countries had implemented assays for H7 subtyping [Broberg et al., 2014].

Five laboratories utilised direct antigen detection assays to analyse the rapid detection panel. False negatives were reported by three laboratories, including in one instance with the A(H7N9) sample a commercial IF assay. This will warrant further investigations as the manufacturer and users of the assay may need to be informed of this weakness.

Figure 7. Average score for virus culture and characterisation temporally since 2008



Comparison of the number of participating laboratories and the average culture and characterisation score returned in the 2008, 2010 and 2013 EQA panels. Average scores were calculated for samples in which specific techniques was attempted. Error bars represent the standard deviation.

The reporting deadline for the culture and characterisation results was 28 days; twenty-four of the 28 participating laboratories (85%) achieved this; the overall median was 25 days for all analysed samples. Of the 36 laboratories that returned rapid detection results, 30 also returned culture and characterisation results. In 2010, 88% (30/34) of the laboratories participated in the culture and characterisation component of the panel whereas in 2013 the proportion dropped to 83% (30/36). (The ERLI-Net coordination team considers the ability to culture influenza virus a core capability and used parts of the ERLI-Net 2013 twinning budget to support a laboratory in need of funds to implement virus isolation.)

The average score for the culture and characterisation component of the EQA has shown a steady increase over the last three panels (Figure 7). The ability of laboratories to successfully isolate and culture viruses has also improved compared with 2010. Nine of the thirteen false negatives in the 2013 panel were reported by three laboratories, suggesting that targeted training activities may help to improve this component. Two false negatives were rapid detection errors rather than related to problems with virus culture.

The genetic characterisation of isolated viruses was very accurate, with an average score of 97%; 10 of the 13 laboratories using this technique received full marks. A general increase in the use of genetic techniques has been observed over the last few years, and the three training courses on sequencing and bioinformatics tools that ERLI-Net offered were well attended. These courses, which took place in November 2010, November 2011 and October 2013, may help explain the increased use of this technique and the quality of the results produced by direct sequencing as ten of the 13 (77%) laboratories reporting genetic characterisation results had attended an ERLI-Net sequencing training course.

The antigenic characterisation of isolated viruses was also of high quality, with an average score of 92%. Ten of the 25 laboratories using this technique achieved full scores while the majority of the remaining laboratories had minor problems distinguishing the various strains. Wet laboratory training courses in these techniques were provided in November 2012 and April 2013, and 17 (65%) of the laboratories later using antigenic characterisation in the EQA attended. The five (of nine) laboratories that did not participate in any training courses (1299, 4213, 2126, 95, and 1323) would probably not have benefitted from these courses as they are already well-versed in antigenic characterisation. The majority of errors was caused by incorrect interpretation and inaccurate reporting of successfully isolated and characterised viruses; additional training in this area might improve the overall score.

Information on the participation of network laboratories in ERLI-Net training courses over the last three years can be found in Annex 6; the results of a survey on virus culture training and its implementation are shown in Annex 7.

In order to conduct antigenic characterisations of viruses, laboratories participating in the EQA need appropriate reference antisera. When the EQA panel was distributed in September 2013, some laboratories did not receive the WHO kit with up-to-date reference antiserum and therefore could not participate in this antigenic characterisation component of the EQA. ERLI-Net will have to find a solution for this problem before the next EQA distribution.

Table 5. Number and percentage of participating laboratories reporting correct results for rapid detection and virus culture

Sample code	Sample contents	Summary of results by number of participants (30/36 returned)			
		Rapid detection	Culture	Correct on both	
		Expected result	Expected result	n	%
EISN_INF13-01	A/Texas/50/2012	A, H3	A, H3	25	83.3
EISN_INF13-02	A/California/7/2009	A, H1/pdm09	A, H1/pdm09	27	90.0
EISN_INF13-03	B/Brisbane/60/08	B	B, Victoria	25	83.3
EISN_INF13-04	B/Massachusetts/02/2012	B	B, Yamagata	30	100.0
EISN_INF13-05	A/California/7/2009	A, H1/pdm09	A, H1/pdm09	28	93.3
EISN_INF13-06	Influenza virus negative	Negative	Negative	30	100.0
EISN_INF13-07	B/Massachusetts/02/2012	B	B, Yamagata	28	93.3
EISN_INF13-08	A/Texas/50/2012	A, H3	A, H3	28	93.3
EISN_INF13-09	B/Brisbane/60/08	B	B, Victoria	28	93.3

The number of participating laboratories that returned datasets for both rapid detection and culture was n=29/36 (80.6%). Strain data were not included in this analysis. ***Rapid detection****: Expected rapid detection result for each panel sample.* ***Culture****: Expected culture result (type and either influenza A subtype or influenza B lineage) for each panel sample.* ***Correct on both****: Number and percentage of laboratories reporting the correct results for both rapid detection and culture.*

Recommendations

Rapid detection

- Four participating laboratories reported false negative results in the rapid detection component of the panel; laboratory directors will be contacted to discuss questions relating to the sensitivity of assays. If required, additional PCR detection samples might have to be obtained to re-evaluate any protocol modifications. Quality assurance companies will be able to provide previous panels directly to laboratories, or laboratories may be able to use national/international panels to judge assay improvements.
- Two laboratories had subtyping issues. The coordination team will offer technical assistance to these laboratories.

Virus culturing

- Three laboratories accounted for nine of the 13 false negatives. Issues should be discussed with the laboratory directors, and possible training might be considered. Two false negatives were the consequence of rapid detection errors.
- It is essential to maintain capability for virus culturing in Europe; continued support and training is needed to counteract the gradually diminishing capacity in many countries.

Genetic characterisation

- Increased participation would be beneficial for the network.
- In general, the EQA showed a good genetic characterisation performance of the ERLI-Net laboratories; no immediate need for training was indicated.

Antigenic characterisation

- Some issues with data interpretation and reporting remain. A training course on theoretical concepts might help address these issues.

References

[1] Broberg E, Pereyaslov D, Struelens M, Palm D, Meijer A, Ellis J, et al. Laboratory preparedness in EU/EEA countries for detection of novel avian influenza A(H7N9) virus, May 2013. Euro Surveill. 2014 Jan 30;19(4). pii: 20682.

[2] Westgeest KB, Russell CA, Lin X, Spronken MI, Bestebroer TM, Bahl J, et al. Genomewide analysis of reassortment and evolution of human influenza A(H3N2) viruses circulating between 1968 and 2011. J Virol. 2014 Mar;88(5):2844-57.

[3] Wu A, Su C, Wang D, Peng Y, Liu M, Hua S, et al. Sequential reassortments underlie diverse influenza H7N9 genotypes in China. Cell Host Microbe. 2013 Oct 16;14(4):446-52.

Annex 1. Rapid detection results, by laboratory code

Participant ID	Overall score	EISN_INF13-01	EISN_INF13-02	EISN_INF13-03	EISN_INF13-04	EISN_INF13-05	EISN_INF13-06	EISN_INF13-07	EISN_INF13-08	EISN_INF13-09	EISN_INF13-10
		A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
75	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
95	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
112	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
117	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
200	28	A/H3	A	B/Vic	B/Yam	A	Negative	B/Yam	A/H3	B/Vic	A/H7
207	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
1159	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
1174	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
1262	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
1299	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
1323	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
1402	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
1433	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
1456	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
1515	27	Negative	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
1534	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
1643	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
1649	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
2001	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
2125	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
2126	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
2253	29	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A
2258	27	A/H3	A/H1/pdm09	B	B	Negative	Negative	B	A/H3	B	A/H7
2270	24	A	Negative	B	B	A/H1/pdm09	Negative	B	A	B	A
2271	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
2274	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
2275	27	Negative	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
2276	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
2277	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
2278	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
2306	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
2820	30	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A/H7
3442	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7
4208	29	A/H3	A/H1/pdm09	B	B	A/H1/pdm09	Negative	B	A/H3	B	A
4209	30	A/H3	A/H1/pdm09	B/Vic	B	A/H1/pdm09	Negative	B	A/H3	B/Vic	A/H7
4213	30	A/H3	A/H1/pdm09	B/Vic	B/Yam	A/H1/pdm09	Negative	B/Yam	A/H3	B/Vic	A/H7

Participating laboratories are identified by a unique anonymised participant ID code. Shaded cells indicate incorrect or partially correct results.

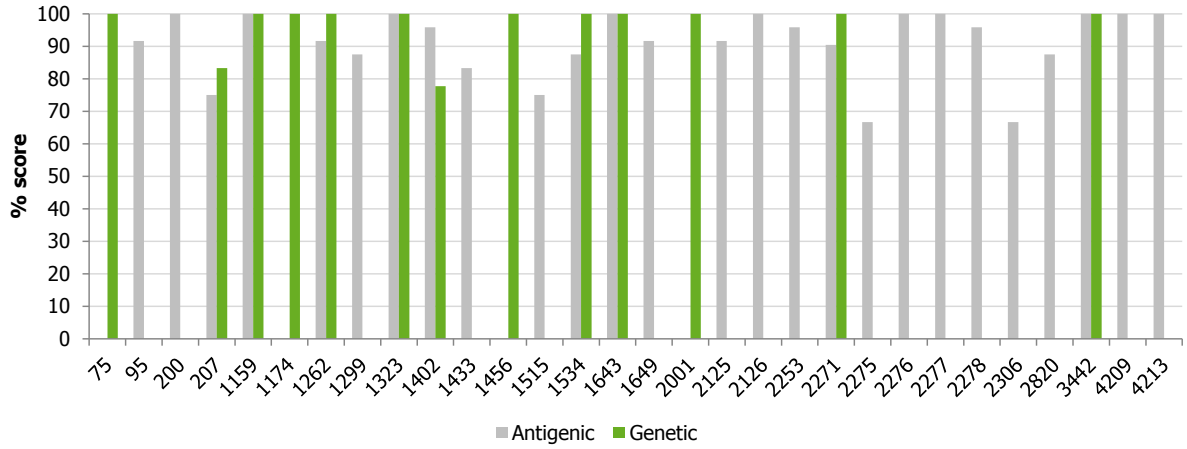
Annex 2. Comparison of influenza virus isolation and culture success, reported in 2010 and 2013, by laboratory code

	EISN-2010											EISN-2013									
Participant ID	A/H1	A/H1	A/H1/pdm09	A/H1/pdm09	A/H3	A/H3	B	B	Negative	Negative	Virus isolation errors	A/H1/pdm09	A/H1/pdm09	A/H3	A/H3	B/Victoria lineage	B/Victoria lineage	B/Yamagata lineage	B/Yamagata lineage	Negative	Virus isolation errors
75											1										3
95																					
112																					
117																					
200																					
207																					
1159																					1
1174																					
1262																					
1299																					
1323																					
1402											1										
1433											1										
1456																					
1515																					1
1534																					
1643											3										
1649																					
2001											1										2
2125																					
2126																					
2253																					
2258																					
2270																					
2271																					
2274											2										
2275																					1
2276																					
2277																					
2278											1										
2306																					
2820																					
3442											4										1
4208																					
4209											1										4
4213																					

False positive
False negative
No data returned

Summary of the virus isolation and culture results from 2010 and 2013. Individual laboratories are identified by a unique anonymised participant ID code.

Annex 3. Relative score for antigenic and genetic characterisation, INF13 panel, by laboratory code



Annex 4a. Characterisation results, by laboratory code

Participant ID	EISN_INF13-06					Overall culture score
	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score	
75	Negative	Antigenic	Negative		3	18
95	Negative	-			3	25
200	Negative	-			3	27
207	Negative	-			3	21
1159	Negative	-			3	24
1174	Negative	Antigenic	Negative		3	27
1262	Negative	Antigenic	Negative		3	27
1299	Negative	Antigenic	Negative		3	24
1323	Negative	Antigenic	Negative		3	27
1402	Negative	-			3	26
1433	Negative	Antigenic	Negative		3	23
1456	Negative	Antigenic	Negative		3	27
1515	Negative	Antigenic	Negative		3	21
1534	Negative	Antigenic	Negative		3	27
1643	Negative	Antigenic	Negative		3	27
1649	Negative	Antigenic	Negative		3	25
2001	Negative	Antigenic	Negative		3	18
2125	Negative	Both	Negative	Negative	3	25
2126	Negative	-			3	27
2253	Negative	-			3	26
2271	Negative	Both	Negative	Negative	3	27
2275	Negative	Both	Negative	Negative	3	17
2276	Negative	Antigenic	Negative		3	27
2277	Negative	Both	Negative	Negative	3	27
2278	Negative	Both	Negative	Negative	3	26
2306	Negative	Both	Negative	Negative	3	19
2820	Negative	-			3	24
3442	Negative	Genetic		Negative	3	24
4209	Negative	-			3	15
4213	Negative	-			3	27

Annex 4b. Characterisation results, by laboratory code

Participant ID	EISN_INF13-01					EISN_INF13-08					EISN_INF13-02					EISN_INF13-05				
	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score	Subtype	Method	Antigenic characterisation	Characterisation genetic	Score	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score
75	A/H3	Genetic		A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Genetic		A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Genetic		A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3	A/H1/pdm09	Genetic		A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3
95	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	Not attributed to category		2	A/H1/pdm09	Antigenic	Not attributed to category		2
200	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
207	A/H3	Both	A/H3/ not attributed to category	A/H3 not attributed to clade/group	2	A/H3	Both	A/H3/ not attributed to category	A/H3 not attributed to clade/group	2	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3
1159	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	Not determined	-			0	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3
1174	Not determined	Genetic		A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	Not determined	Genetic		A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	Not determined	Genetic		A/H1/pdm09 not attributed to clade/group	3	Not determined	Genetic		A/H1/pdm09 not attributed to clade/group	3
1262	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3
1299	A/H3	Antigenic	A/H3/ A/Perth/16/2009 H3N2-like		2	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
1323	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3
1402	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	-	3	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	-	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3
1433	A/H3	Antigenic	A/H3/ not attributed to category		2	A/H3	Antigenic	A/H3/ not attributed to category		2	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
1456	A/H3	Genetic		A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Genetic		A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Genetic		A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3	A/H1/pdm09	Genetic		A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3
1515	Negative	-			0	A/H3	Antigenic	A/H3/ A/Perth/16/2009 H3N2-like		2	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
1534	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Both	A/H3/ A/Perth/16/2009 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Both	Not attributed to category	A/H1/pdm09 not attributed to clade/group	3	A/H1/pdm09	Both	Not attributed to category	A/H1/pdm09 not attributed to clade/group	3
1643	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3
1649	A/H3	Antigenic	A/H3/ not attributed to category		2	A/H3	Antigenic	A/H3/ not attributed to category		2	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2001	Negative	-			0	A/H3	Genetic		Negative	0	A/H1/pdm09	Genetic		A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3	A/H1/pdm09	Genetic		A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3
2125	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2126	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2253	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2271	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H3	Both	A/H3/ A/Victoria/361/2011 H3N2-like	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 – clade representative A/California/7/2009 / A/Christchurch/16/2010 group	3
2275	Negative	Antigenic	Negative		0	A/H3	Antigenic	A/H3/ not attributed to category		2	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2276	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2277	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2278	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2306	A/H3	Antigenic	A/H3/ not attributed to category		2	A/H3	Antigenic	A/H3/ not attributed to category		2	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
2820	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3
3442	Negative	Both	Negative	Negative	0	Negative	Both	Negative	A/H3 – clade representative A/Victoria/208/2009 / A/Victoria/361/2011 group 3C	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3	A/H1/pdm09	Both	A/California/7/2009 H1N1-like	A/H1/pdm09 not attributed to clade/group	3
4209	Negative	Antigenic	Negative		0	Negative	Antigenic	Negative		0	Negative	Antigenic	Negative		0	Negative	Antigenic	Negative		0
4213	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H3	Antigenic	A/H3/ A/Victoria/361/2011 H3N2-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3	A/H1/pdm09	Antigenic	A/California/7/2009 H1N1-like		3

** Note on laboratory #1534: The two A(H1)pdm09 viruses conform closely to the parental A/California/07/2009 genetic group and cannot be placed in any of the reporting groups offered in the form. The two A(H3) viruses closely match the HA sequence of the new vaccine virus A/Texas/50/2012; however, one of them displayed a slightly better reactivity to Perth/16/09 in antigenic analysis and has been recorded as such; it is also reactive to the Vic/361-like reference virus.*

Grey shading indicates an incorrect or incomplete result.

Annex 4c. Characterisation results, by laboratory code

Participant ID	EISN_INF13-03					EISN_INF13-09					EISN_INF13-04					EISN_INF13-07				
	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score	Subtype	Method	Antigenic characterisation	Genetic characterisation	Score
75	Not determined	-			0	Negative	-			0	B/Yamagata Lineage	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3	Not determined	-			0
95	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Estonia/55669/2011-like		3	B/Yamagata Lineage	Antigenic	B/Estonia/55669/2011-like		3
200	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3
207	B/Victoria Lineage	Antigenic	B/Vic lineage not attributed to category		2	B/Victoria Lineage	Antigenic	B/Vic lineage not attributed to category		2	B/Yamagata Lineage	Antigenic	B/Yam lineage not attributed to category		2	B/Yamagata Lineage	Antigenic	B/Yam lineage not attributed to category		2
1159	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3
1174	Not determined	Genetic		B/Vic lineage – clade representative B/Brisbane/60/2008	3	Not determined	Genetic		B/Vic lineage – clade representative B/Brisbane/60/2008	3	Not determined	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3	Not determined	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3
1262	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Both	B/Florida/4/2006-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Both	B/Florida/4/2006-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3
1299	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Florida/4/2006-like		2	B/Yamagata Lineage	Antigenic	B/Florida/4/2006-like		2
1323	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3
1402	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage - not attributed to clade/group	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage - not attributed to clade/group	3	B/Yamagata Lineage	Both	B/Florida/4/2006-like	B/Yam lineage - not attributed to clade/group	2	B/Yamagata Lineage	Both	B/Wisconsin/1/2010-like	B/Yam lineage - not attributed to clade/group	3
1433	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Yam lineage not attributed to category		2	B/Yamagata Lineage	Antigenic	B/Yam lineage not attributed to category		2
1456	B/Victoria Lineage	Genetic		B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Genetic		B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3
1515	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Florida/4/2006-like		2	B/Yamagata Lineage	Antigenic	B/Bangladesh/3333/2007-like		2
1534	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3
1643	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3
1649	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3
2001	B/Victoria Lineage	Genetic		Negative	0	B/Victoria Lineage	Genetic		B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Genetic		B/Yam-lineage – clade representative B/Estonia/55669/2011	3
2125	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Florida/4/2006-like		2	B/Yamagata Lineage	Antigenic	B/Florida/4/2006-like		2
2126	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3
2253	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Florida/4/2006-like		2
2271	B/Victoria Lineage	Genetic		B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Both	B/Florida/4/2006-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Both	B/Florida/4/2006-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3
2275	B/Yamagata Lineage	Antigenic	B/Yam lineage not attributed to category		1	B/Victoria Lineage	Antigenic	B/Vic lineage not attributed to category		2	B/Yamagata Lineage	Antigenic	B/Yam lineage not attributed to category		2	B/Victoria Lineage	Antigenic	B/Vic lineage not attributed to category		1
2276	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3
2277	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3
2278	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Estonia/55669/2011-like		3	B/Yamagata Lineage	Antigenic	B/Florida/4/2006-like		2
2306	B/Yamagata Lineage	Antigenic	B - lineage not determined		1	B/Yamagata Lineage	Antigenic	B - lineage not determined		1	B/Yamagata Lineage	Antigenic	B - lineage not determined		2	B/Yamagata Lineage	Antigenic	B - lineage not determined		2
2820	Negative	Antigenic	B - lineage not determined		1	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Yam lineage not attributed to category		2
3442	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Victoria Lineage	Both	B/Brisbane/60/2008-like	B/Vic lineage – clade representative B/Brisbane/60/2008	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3	B/Yamagata Lineage	Both	B/Estonia/55669/2011-like	B/Yam-lineage – clade representative B/Estonia/55669/2011	3
4209	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3	B/Yamagata Lineage	Antigenic	B/Wisconsin/1/2010-like		3
4213	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Victoria Lineage	Antigenic	B/Brisbane/60/2008-like		3	B/Yamagata Lineage	Antigenic	B/Estonia/55669/2011-like		3	B/Yamagata Lineage	Antigenic	B/Estonia/55669/2011-like		3

Annex 5. Laboratories participating in the EISN-INF13

Country	City	Organisation
Austria	Vienna	AKH Wien – Medical University of Vienna
Belgium	Brussels	Institute of Public Health
Bulgaria	Sofia	Center of Infectious and Parasitic Disease
Cyprus	Nicosia	Nicosia General Hospital
Czech Republic	Prague	National Institute of Public Health
Denmark	Copenhagen	Statens Serum Institute
Estonia	Tallinn	Health Protection Inspectorate
Finland	Helsinki	National Institute for Health and Welfare (THL)
France	Paris	CNR de la Grippe – Institute Pasteur
France	Bron, Lyon	CNR Virus Influenza – HCL Lyon
Germany	Berlin	Robert Koch Institute
Greece	Athens	National Influenza Center for S Greece
Hungary	Budapest	Országos Epidemiológiai Központ
Iceland	Reykjavik	Landspitali-University Hospital
Ireland	Dublin	University College Dublin
Italy	Rome	Istituto Superiore di Sanita (NIH)
Latvia	Riga	National Microbiology Reference Laboratory, NIC of Latvia
Lithuania	Vilnius	National Public Health Surveillance Laboratory
Luxembourg	Luxembourg	Laboratoire National de Sante
Malta	Msida	Mater Dei Hospital
Netherlands	Bilthoven	RIVM
Netherlands	Rotterdam	Erasmus MC
Norway	Oslo	Norwegian Institute of Public Health
Poland	Warsaw	National Institute of Hygiene
Portugal	Lisbon	Instituto Nacional de Saúde Doutor Ricardo Jorge
Romania	Bucharest	Cantacuzino Institute
Slovak Republic	Bratislava	Public Health Authority of the Slovak Republic
Slovenia	Ljubljana	National Institute of Public Health
Spain	Majadahonda, Madrid	Instituto de Salud Carlos III
Spain	Valladolid	Hospital Clinico Universitario
Spain	Barcelona	Hospital Clinic i Provincial
Sweden	Solna	Folkhälsomyndigheten
United Kingdom	London	Public Health England
United Kingdom	Belfast	Belfast City Hospital
United Kingdom	Glasgow	Gartnavel General Hospital
United Kingdom	Cardiff	Public Health Wales

Annex 6. ERLI-Net training courses and participation, by laboratory code

	Influenza surveillance	Influenza antiviral susceptibility surveillance	Virus culture	Virus culture	Sequencing and bioinformatics tools	Sequencing and bioinformatics tools	Sequencing and bioinformatics tools
	June 2010	July 2011	November 2012	April 2013	November 2010	November 2011	October 2013
75							x
95							x
112							
117							
200		x	x				
207			x				
1159	x	x		x		x	
1174		x		x	x		
1262	x	x		x		x	x
1299							
1323							
1402	x	x	x				
1433	x	x	x				
1456	x					x	
1515				x	x		x
1534				x			
1643		x	x		x		
1649						x	x
2001						x	
2125	x				x		
2126							
2253	x				x		x
2258	x		x		x		
2270							
2271		x	x		x	x	x
2274	x		x			x	
2275					x		x
2276	x	x		x	x		
2277				x			
2278	x	x	x		x		x
2306		x	x			x	
2820				x			
3442	x	x	x			x	x
4208					x		
4209	x	x	x				
4213						x	x

X *indicates course attendance*