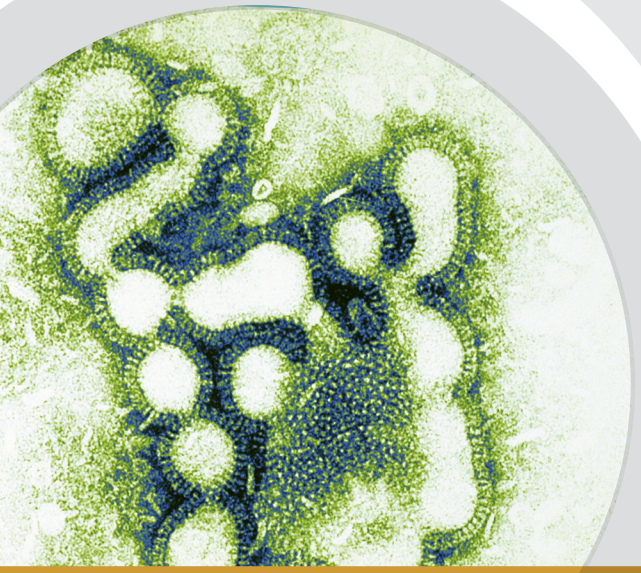

TECHNICAL REPORT

External quality assessment scheme for detection, isolation and characterisation of influenza viruses for the European Reference Laboratory Network for Human Influenza

2015

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External quality assessment scheme for detection, isolation and characterisation of influenza viruses for the European Reference Laboratory Network for Human Influenza

2015

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

Catherine Thompson and Joanna Ellis, Public Health England, London, UK and the contractor' s management team contributed to the design and planning of the exercise. The panel was produced by the Respiratory Virus Unit, Public Health England, London, UK.

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

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Abbreviations

EISN	European Influenza Surveillance Network
EQA	External quality assessment
ERLI-Net	European Reference Laboratory Network for Human Influenza
GISRS	Global Influenza Surveillance and Response System
HI	Haemagglutination inhibition
IF	Immunofluorescence
INF13	Influenza detection, isolation and characterisation EQA panel of ERLI-Net 2013
INF15	Influenza detection, isolation and characterisation EQA panel of ERLI-Net 2015
NAT	Nucleic acid amplification technologies
PCR	Polymerase chain reaction
PHE	Public Health England
QCMD	Quality Control for Molecular Diagnostics, Glasgow, UK
RIVM	National Institute for Public Health and the Environment
WHO	World Health Organization
WHO-CC	World Health Organization Collaborating Centre for Reference and Research on Influenza

Executive summary

In June and July 2015, an influenza virus rapid detection, isolation and characterisation external quality assessment (EQA) exercise was held for European influenza reference laboratories. This was the fourth rapid detection, isolation and characterisation EQA panel distributed by the European Reference Laboratory Network for Human Influenza (ERLI-Net; previously called CNRL).

The objectives of the exercise were to provide participants with an independent mechanism to check performance and to provide information for the entire network on the capacity and capability for rapid detection by PCR, influenza virus isolation and strain characterisation within a defined reporting timeframe. All 38 ERLI-Net member laboratories were invited to participate and accepted the opportunity, representing 30 European countries.

As with previous panels, the performance of laboratories in the rapid detection component of the exercise was encouraging. Thirty-four of the 38 laboratories achieved top marks, and an additional laboratory achieved top marks in spite of a lineage determination error. Errors reported by the remaining three laboratories included one false positive, a B/Vic reported instead of A/H3, and two influenza A samples that were not subtyped. The proportion of laboratories receiving a maximum score increased between 2008 and 2015: 71% (2008), 76% (2010), 79% (2013) and 92% (2015). This highlights the gradual but sustained development of modern molecular skills across the network.

A(H3N2) virus subclade 3C.2a and 3C.3a have been rapidly expanding around the world since early 2014. Subclade 3C.2a has proved to be particularly challenging to characterise antigenically due to poor haemagglutination (HA) of red blood cells preventing reliable haemagglutination inhibition (HI) assays. If laboratories also use HA to detect growth in samples being cultured, then successfully isolated viruses may miss detection. For these reasons, the 2015 EISN EQA panel specifically tested laboratories ability to first isolate and then characterise three A(H3N2) viruses, including a subgroup 3C.2a containing sample. There was no evidence that the participating laboratories found the A(H3N2) samples more difficult to isolate than other specimens (Figure 7). As expected, results from the antigenic characterisation reflected the difficulties that have been described with A(H3N2) subtypes.

The subgroup 3C.2a isolate proved challenging with only nine of the 26 laboratories reporting the correct strain characterisation (Figure 9A). Seventeen of 24 laboratories reported the correct strain characterisation of the subgroup 3C.3a virus, and 16 of 25 laboratories correctly characterised the older A(H3N2) isolate. Genetic characterisation was less of a problem, with 57 of 60 laboratories reporting the correct strain for the A(H3N2) containing samples.

Overall, virus characterisation results, based on the combined results from genetic and antigenic characterisation, have improved over the last three EQA panels. The network's capacity for virus characterisation has increased, with 32 laboratories correctly characterising a relatively conserved A(H1N1)pdm09 strain compared to between 25 and 28 laboratories in earlier panels (Figure 10). Also, the quality of reported data improved, with the number of laboratories correctly characterising the sample strains increasing from 22 in 2010 to 27 in 2013 and finally 29 in 2015. In comparison to 2010, the networks' capability for antigenic characterisation remained at a highly consistent level (Figure 9B) whereas the networks' capability for genetic characterisation has improved over the same period (Figure 8B).

Although genetic characterisation results were very accurate irrespective of the virus type, results for the older isolates suggest that some laboratories were less certain on how to report non-current seasonal viruses in the predefined TESSy reporting categories.

Introduction

The European Centre for Disease Prevention and Control (ECDC) is a European Union 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.' [1](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 viruses cause a highly contagious acute respiratory disease that can spread rapidly, 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 is unlikely to exist to animal zoonotic infections 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 cell culture or embryonated eggs and carry out serological tests to identify the antigenic characteristics and strain identity 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 EQA testing by a consortium of three European institutes, covering the period 2012–2016. Within this contract is the provision of biennial EQA of national influenza reference laboratories to ensure the reliability and comparability of results, and to identify needs for improvement in laboratory diagnostic capability. The 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 isolation, (sub)typing, antigenic and molecular characterisation for influenza virus.

This report contains the results of the influenza virus EQA, designed and prepared by the contractor and funded by ECDC.

The main purposes of external quality assessment schemes include:

- Assessment of the general performance standards
- Assessment of the effects of analytical procedures (method, principles and techniques)
- Evaluation of individual laboratory performance
- Identification and justification of problem areas
- Providing continuing education (testing against samples of known status) and enabling comparisons with other laboratories
- Identification of training needs.

Objectives

The major objective of the 2015 EISN influenza virus culture EQA panel was to assess individual European influenza reference laboratories' performance in the following areas:

- Rapid detection by PCR or other tests (within a defined reporting timeframe), including typing and subtyping
- Virus isolation (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 contractor's management team. The panel was prepared and tested by the Respiratory Virus Unit at PHE, London, UK. Further pre-testing was performed by the WHO-CC at the Francis Crick Institute, Mill Hill, London, UK, and the France South National Influenza Centre, Lyon, France. The panel contents were distributed in June 2015 to participants, frozen on dry ice, by specialist courier. Participants submitted results to a web-based database.

Participation

It was mandatory for all 38 ERLI-Net member laboratories to participate in at least the rapid detection component of this panel. All ERLI-Net influenza laboratory contact points were notified in advance of the EQA exercise. A list of participants in the influenza virus rapid detection and culture EQA can be found in Annex 2. Four laboratories were not able to participate in June 2015 during the ERLI-Net distribution but were able to join the WHO Regional Office for Europe distribution in early 2016 instead. This report contains the results from all 38 laboratories.

Panel description

The EQA panel consisted of eight simulated clinical samples containing influenza viruses from subtypes that are either current or have recently circulated in humans, including influenza A(H1N1)pdm09, influenza A(H3N2), and both genetic lineages of influenza B viruses. One negative sample, containing no virus, completed the panel. Viruses were grown in eggs and diluted to a suitable concentration for testing 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 PHE 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 (DG Global Forwarding) on 15 June 2015 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 isolation and characterisation results was within 28 days of receipt of the panel.

Testing

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

Data reporting

Rapid detection involves the detection, typing and subtyping of influenza A viruses. Laboratories were only required to detect and type influenza B viruses, although if lineage determination was performed, the data were included in the results tables. For virus isolation and strain characterisation, participants were asked to report the virus type/subtype (or 'negative') and the strain as determined by antigenic and/or genetic means.

QCMD operated a web-based reporting tool to collect data on the used methods and the results. Possible type/subtype/lineage and characterisation categories were predefined to reflect the viruses in the panel; the same predefined categories were also used as TESSy reporting categories for the 2015–2016 season.

Data analysis

The rapid detection scoring system awarded three points for the correct determination of a negative sample, one point for the 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 for correct typing. The maximum achievable score for rapid detection was 30 points.

The scoring system for virus isolation and strain characterisation awarded one point for the isolation of influenza A or B virus, one point for correctly subtyping of influenza virus A (or lineage identification of influenza B virus), one point for correct strain identification, and three points for correct determination of a negative sample.

The maximum achievable score for virus isolation and strain characterisation was 27 points.

As the same panel was also used in the rapid detection component of the EQA, laboratories had already determined the type/subtype or lineage of the samples. Therefore, if no proof of further characterisation was provided, laboratories were not given additional points for identifying the type or subtype.

Initial report

QCMD and Ian Harrison worked jointly to perform an initial analysis on the submitted data. The report of the initial analysis along with the expected results were published by QCMD. Copies of these documents can be found on the ECDC extranet.

Results

Panel composition and participation

The influenza type, subtype, strain characterisation and plaque forming units/ml for each sample in the EQA panel 2015 (INF15) are shown in the expected results table below (Table 1). Samples were diluted in a matrix consisting of Hep2C cells in virus transport medium at a concentration of $1x10^5$ cells/ml.

Table 1. INF15 panel composition

Panel number	Virus	Subtype	Clade/ group	Plaque titre*	Expected antigenicity	Expected genetic group
EISN_INF15-1	B/England/531/2014	B-Yam	3	89	B/Phuket/3073/2013-like	B/Phuket/3073/2013 (Yamagata lineage clade 3)
EISN_INF15-2	A/Switzerland/9715293/2013	A(H3N2)	3C.3a	6306	A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)
EISN_INF15-3	Influenza virus negative	n/a	n/a	n/a	n/a	n/a
EISN_INF15-4	A/England/599/2014	A(H3N2)	3C.2a	541	A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)
EISN_INF15-5	A/England/215/2011	A(H3N2)	3C	1156	A(H3N2) not attributed to category	A(H3N2) not attributed to clade/group
EISN_INF15-6	A/ENG/579/2014	A(H1N1)pdm09	6B	953	A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)
EISN_INF15-7	A/England/226/2010	A(H1N1)pdm09	6	643	A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)
EISN_INF15-8	B/England/197/2014	B-Vic	1A	265	B/Brisbane/60/2008-like	B/Brisbane/60/2008 (Victoria lineage clade 1A)

** Plaque forming units/ml*
Note: Hep2C cells in virus transport medium at a concentration of $1x10^5$ cells/ml

All 38 ERLI-Net laboratories who submitted data were included in the analysis, irrespective of whether the data were submitted during the ERLI-Net or WHO Regional Office for Europe panel distributions.

Results for rapid detection of influenza virus

All 38 ERLI-Net laboratories participated in the rapid detection component of the exercise (Figure 1). The results of this component are presented in Figure 2. Thirty-eight participants (100%) reported results for rapid detection, including typing/subtyping results for influenza A virus and typing results for influenza B virus; 24 laboratories reported lineage determination results for influenza B virus. Thirty-four of the 38 (89.5%) participants who returned results for rapid detection achieved the maximum score of 24 points. The remaining four participants reported one false positive, one typing error, and three subtyping errors.

Figure 3 compares the 2015 rapid detection results with those from 2010 and 2013. The number of participating laboratories in this period was initially 31 (2008) and increased to 33 (2010) and then to 38 (2013 and 2015). The proportion of laboratories reporting no errors increased from 71% in 2008 to 76% in 2010 and to 79% in 2013, before reaching 92% in 2015. This documents the European networks' progress in performing accurate rapid detection testing.

Figure 1. Laboratories participating in the EISN-2015 EQA panel

Participant ID	Rapid detection	Isolation	Characterisation
75			
95			
112			
117			
200			
207			
1159			

Participant ID	Rapid detection	Isolation	Characterisation
1174			
1262			
1299			
1323			
1402			
1432			
1456			
1515			
1534			
1643			
1649			
2001			
2125			
2126			
2253			
2258			
2270			
2271			
2272			
2274			
2276			
2277			
2278			
2306			
2819			
2820			
3442			
4208			
4209			
4213			
4764			

Participating laboratories are identified by a unique anonymised participant ID code.
Green shading indicates participation in INF15 panel.
Grey shading indicates non-participation in certain components of the panel.

Figure 2. Rapid detection results for INF15

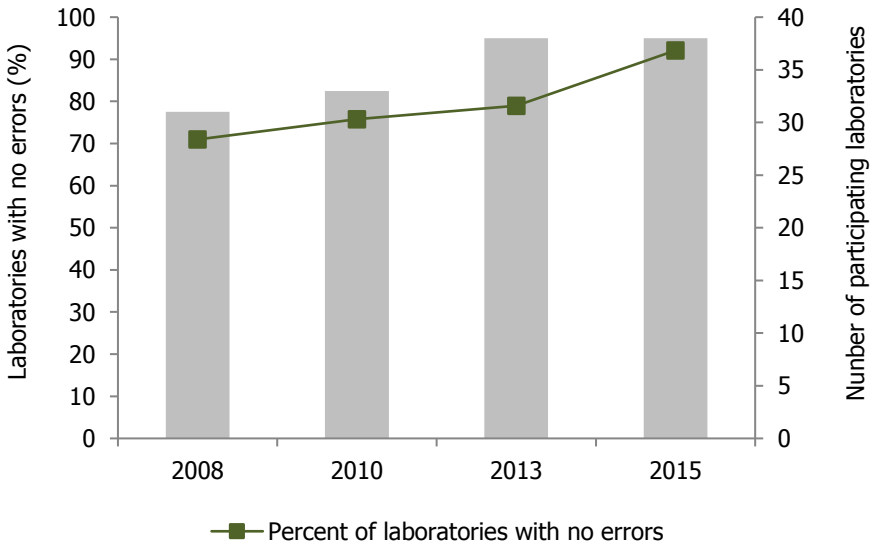
Participant ID	Overall score	INF15-01	INF15-02	INF15-03	INF15-04	INF15-05	INF15-06	INF15-07	INF15-08
		B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
75	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
95	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
112	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
117	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
200	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
207	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
1159	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
1174	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
1262	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
1299	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
1323	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
1402	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
1432	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
1456	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
1515	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
1534	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
1643	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
1649	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2001	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2125	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B

Participant ID	Overall score	INF15-01	INF15-02	INF15-03	INF15-04	INF15-05	INF15-06	INF15-07	INF15-08
		B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2126	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
2253	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2258	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2270	23	B/Yam	A/H3	Negative	A/H3	A/H3	A	A/H1/pdm09	B/Vic
2271	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2272	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2274	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
2276	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2277	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
2278	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
2306	20	B	A/H3	A	A/H3	A/H3	A	A/H1/pdm09	B
2819	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
2820	22	B/Yam	B/Vic	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
3442	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
4208	24	B	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B
4209	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
4213	24	B/Yam	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic
4764	24	B/Vic	A/H3	Negative	A/H3	A/H3	A/H1/pdm09	A/H1/pdm09	B/Vic

Typing error
Subtyping (lineage) error
False positive
False negative
No data returned

Individual laboratory data from INF15 rapid detection panel. Laboratories are identified by a unique anonymised participant ID code. Three points were available per sample; one for the correct detection of a positive sample, one for correct typing, and one for correctly subtyping influenza A samples.
Laboratories received two points for correctly typing influenza B samples.

Figure 3. Results of rapid detection, 2008–2015



Results for influenza virus isolation

Laboratories were required to isolate virus from influenza-positive samples in cell culture or embryonated eggs and then characterise the virus by means of antigenic or genetic assays or by a combination of both. Before characterisation, the virus had to be isolated and propagated from the sample.

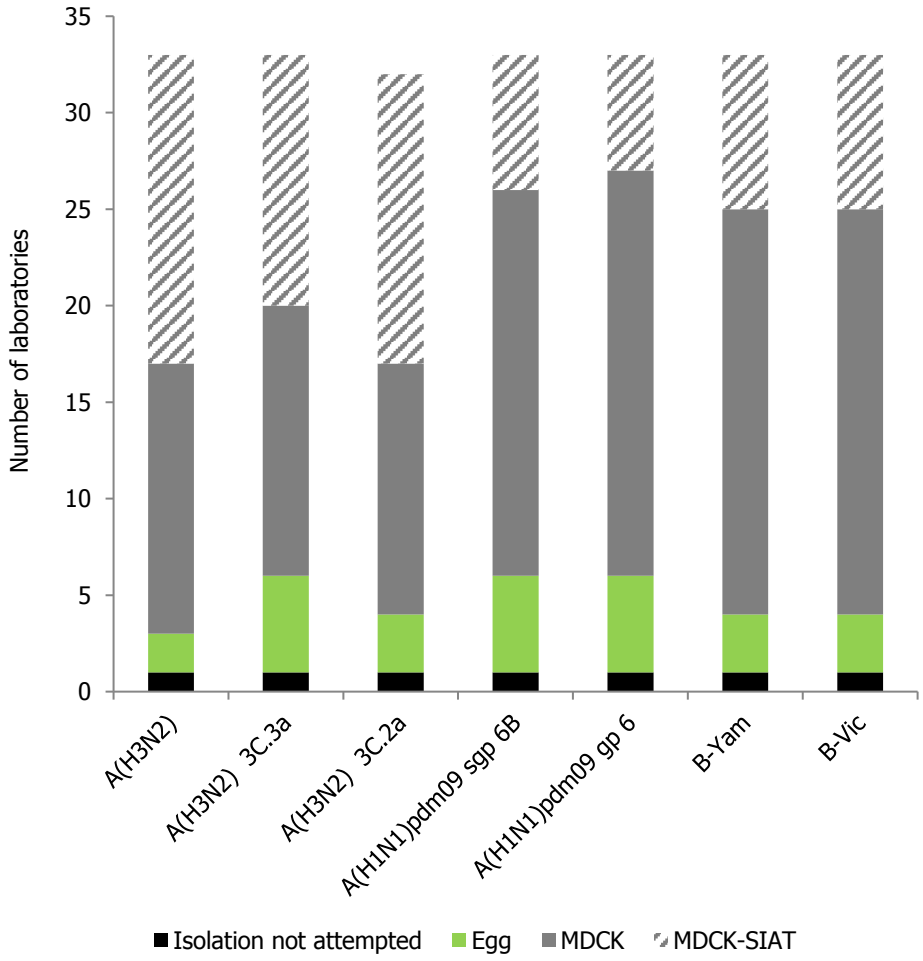
The recently emerged group 3C H3N2 viruses have proved particularly challenging to isolate and characterise. This is due to a number of factors including i) the variable agglutination of red blood cells from guinea pigs, turkeys and humans, particularly for H3N2 subgroup 3C.2a; ii) increased levels of agglutination caused by binding of NA; iii) poor recognition of samples by reference antiserum raised against egg-propagated viruses; and iv) variable levels

of cytopathic effect in culture. In response to these challenges, three H3N2 samples were included in the INF15 panel to test the networks' ability to isolate and characterise these viruses.

The rate of successful isolation of H3N2 3C viruses can be affected by the reduced affinity of HA for sialic acid, its receptor, which results in reduced replication in tissue culture cells. The use of tissue culture cell lines with increased receptor expression (MDCK-SIAT) has been suggested to improve the isolation rate of viruses with reduced receptor affinity. Figure 4 depicts the cell line used by each laboratory for successful isolation of samples from the INF15 panel.

One laboratory (participant ID 1174) did not attempt isolation but characterised the samples directly from the simulated material. Up to five laboratories used eggs for virus isolation while the remaining laboratories (~30) used members of the broad range of MDCK or MDCK-SIAT cell lines [4]. Of the laboratories that used MDCK-based cell lines, approximately half used the SIAT line, which has an over-expression of receptors for the isolation of H3N2 samples. Approximately a quarter of the laboratories used the SIAT line for the non-H3 samples.

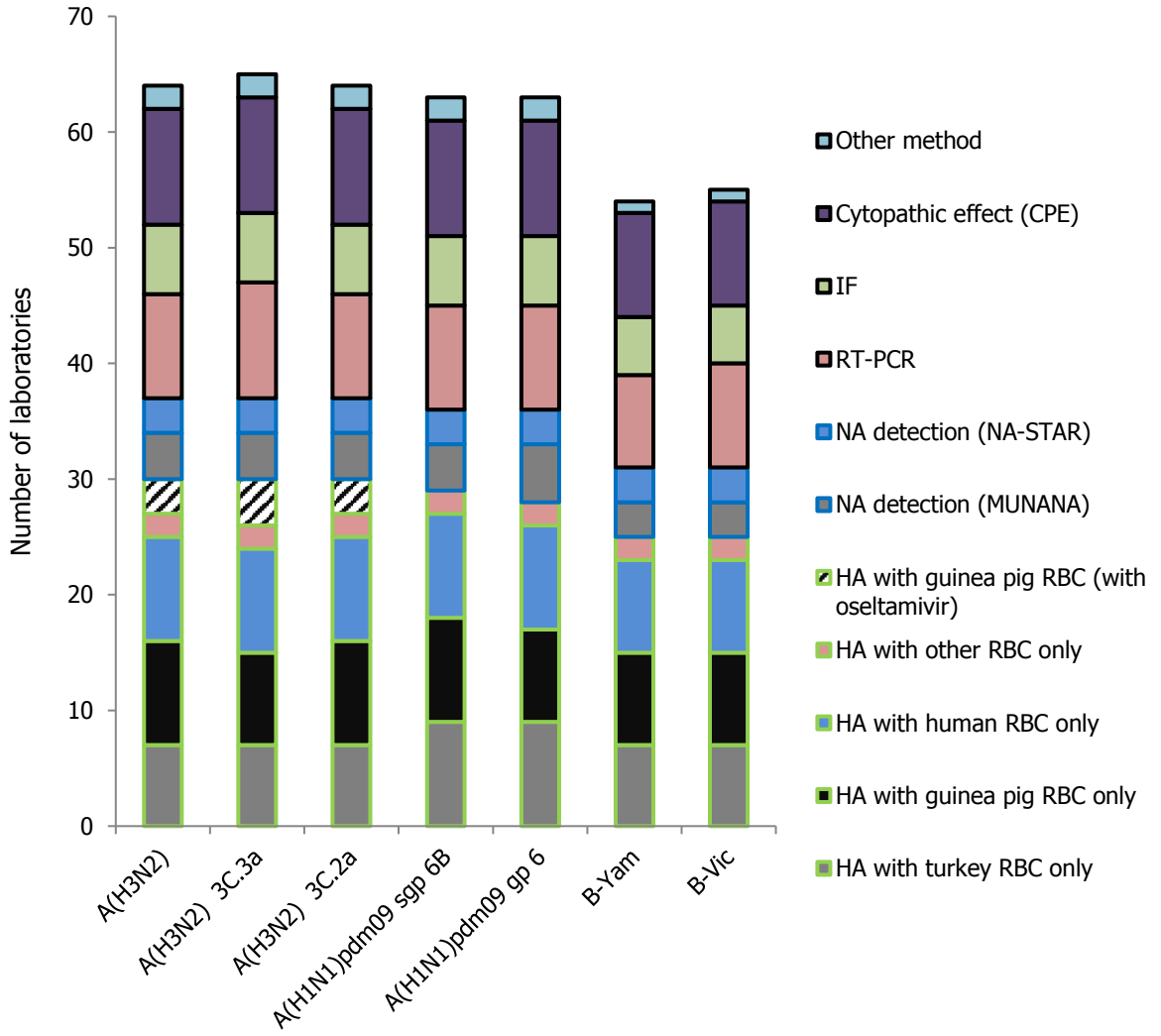
Figure 4. Materials used for virus isolation of IFN15 samples



The combination of variable cytopathic effect in culture and the variable (or complete lack of) agglutination of red blood cells can make H3N2 3C viruses difficult to recover from culture due to lack of a signal indicating successful propagation of the sample. To assess if this is a problem, the ERLI-Net laboratories were asked to report which methods they used to successfully detect and isolate viruses (Figure 5). The majority of laboratories did not rely on a single method but used a combination of approaches to determine whether virus had been isolated. Approximately seven laboratories used a neuraminidase activity assay, 28 laboratories used some form of HA assay, but only three or four included oseltamivir for the HA assay on H3 viruses.

Figure 6 shows the number of laboratories returning virus isolation results. The total number of laboratories participating in virus isolation increased from 30 (2010) to 32 (2013, 2015) while the number of laboratories with no isolation errors increased from 21 in 2010 and 24 in 2013 to 26 in 2015.

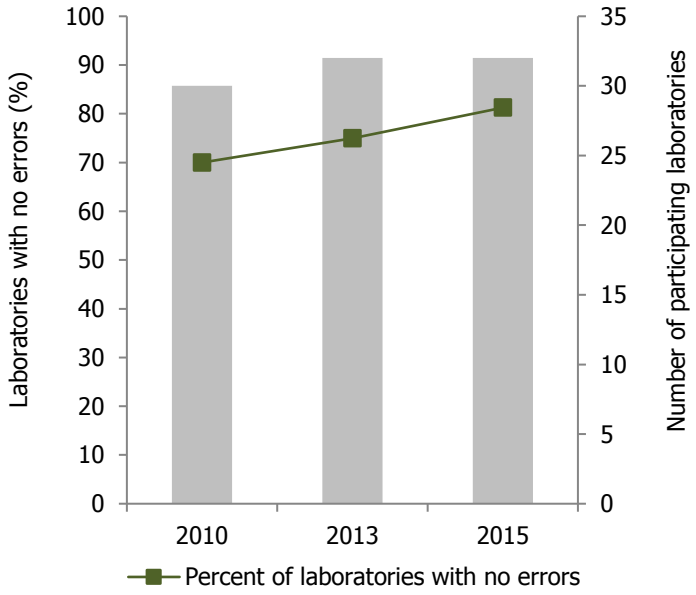
Figure 5. Methods used to detect successful virus propagation



Methods used by laboratories to detect successful virus isolation and propagation, by virus type. Laboratories often used multiple methods per sample.
Colour of column outline represents groups of techniques.

Individual laboratories results for INF15 isolation and culturing (2010–2015) are shown in Figure 7. This figure shows that multiple isolation errors are often associated with individual laboratories rather than being evenly distributed across all participants. This fact is reinforced by calculating the average number of isolation failures associated with each laboratory reporting some form of error; the mean across the last three EQA panels is 1.78 isolation failures. Figure 7 also highlights that particular virus types/subtypes, for example A(H3N2), do not appear to be more difficult to isolate than others.

Figure 6. Number of laboratories returning virus isolation results; percentage of laboratories reporting no isolation errors, ERLI-Net, 2010–2015



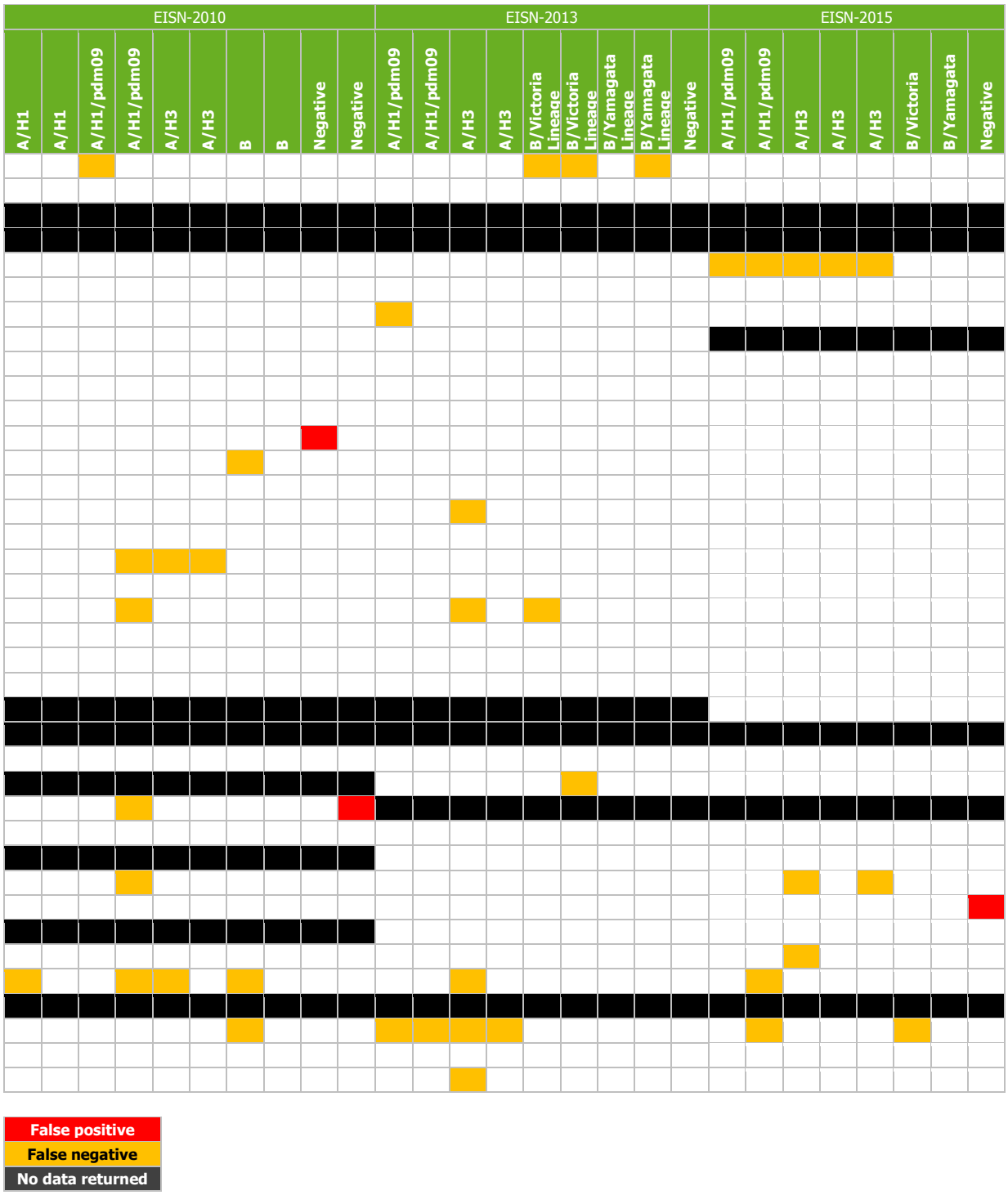
Results for influenza virus characterisation – genetic

After isolation of the INF15 panel viruses, laboratories could use either antigenic, genetic or both characterisation techniques to analyse the samples. Laboratories were not penalised for using only one characterisation method. If both methods were employed and returned discrepant results, participants were scored on the technique which gave the correct answer.

The number of laboratories reporting genetic characterisation results is presented in Figure 8A. Overall, genetic characterisation was very accurate. Eight incomplete genetic characterisations (correct type, subtype or lineage in the absence of detailed strain information) were recorded; two of which were for the A(H1N1)pdm09 gp 6 sample, three for the A(H3N2) sample and three for the B-Victoria sample. All errors were associated with older, non-seasonal (at the time of the exercise) viruses, which seems to indicate that the errors were associated with uncertainties on how to report non-current seasonal isolates through the predefined TESSy reporting categories rather than the actual analysis of the nucleotide sequence.

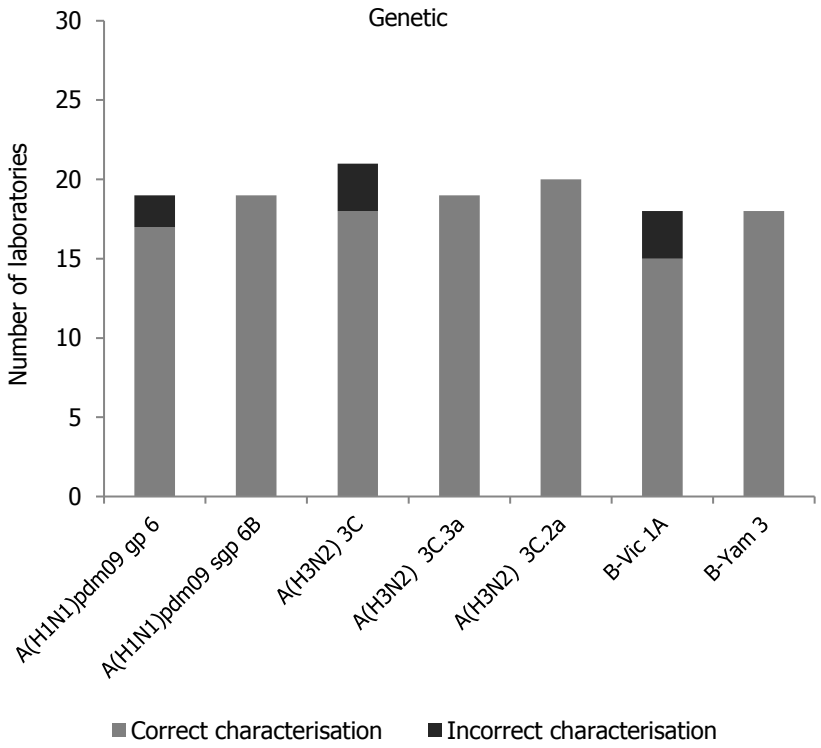
To assess whether the networks' capability and quality for genetic characterisation of samples had improved since 2013, the genetic characterisation results for a relatively conserved A(H1N1)pdm09 sample were compared between the two EQA exercises. Figure 8B shows the characterisation of the H1N1pdm09 sample in the 2013 (A/California/7/2009) and 2015 (A/England/226/2010) EQA panels. The number of laboratories which used genetic characterisation for this type of sample increased from 12 to 19 in 2015, which is a 58% increase. No errors were reported in 2013 but two of the 19 laboratories reported incomplete characterisations in 2015.

Figure 7. Individual laboratories virus isolation results, EQA panels 2010–2015



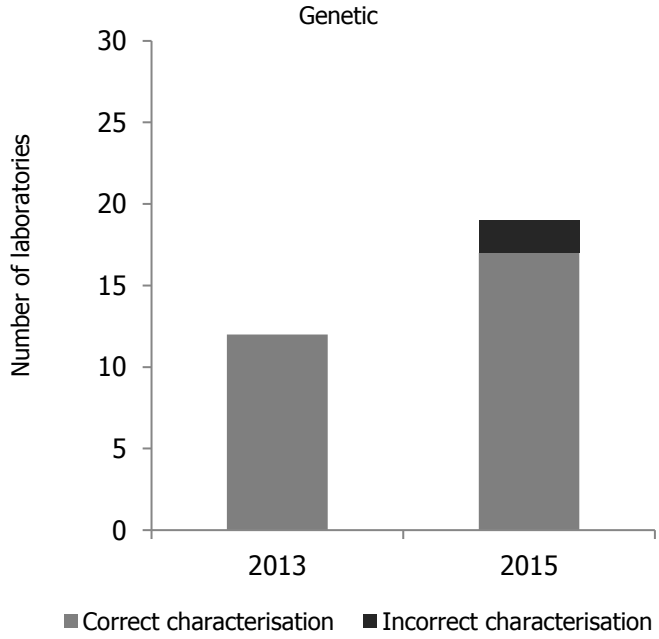
Comparison of individual laboratory isolation results across ERLI-Net region between 2010 and 2015. Laboratories are identified by a unique anonymised participant ID code.

Figure 8A. Genetic characterisation of INF15 panel



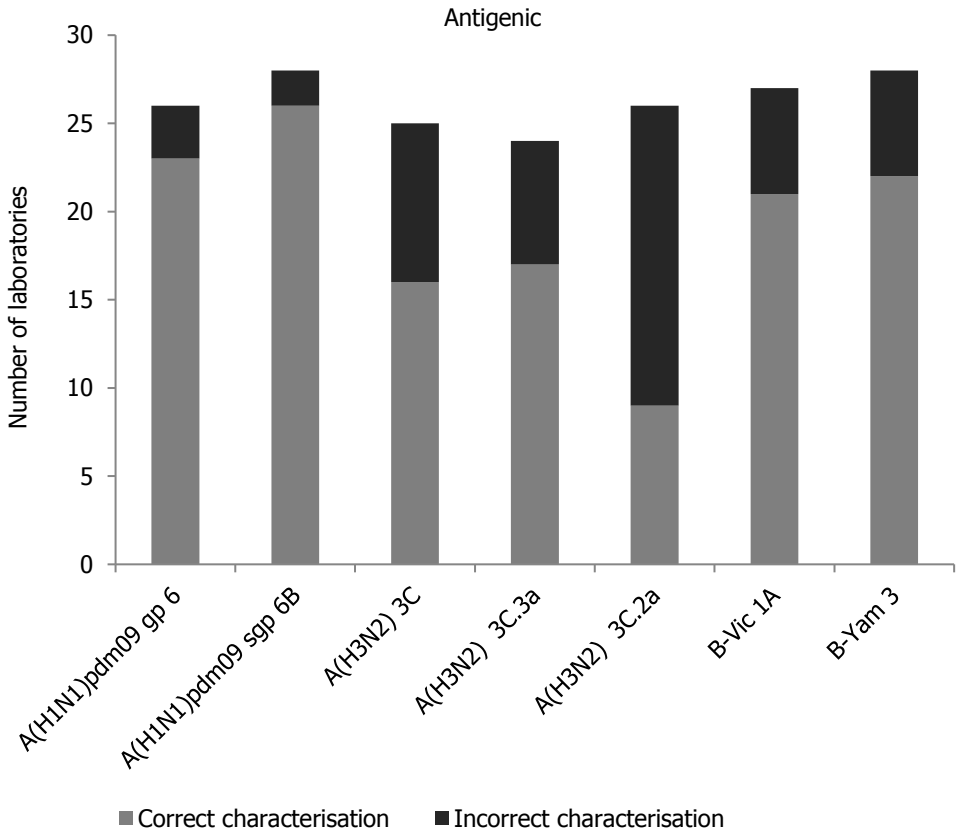
The number of laboratories reporting genetic characterisation results for INF15 samples depicted per virus type in each sample.

Figure 8B. Genetic characterisation of an A(H1N1)pdm09 sample in 2013 and 2015



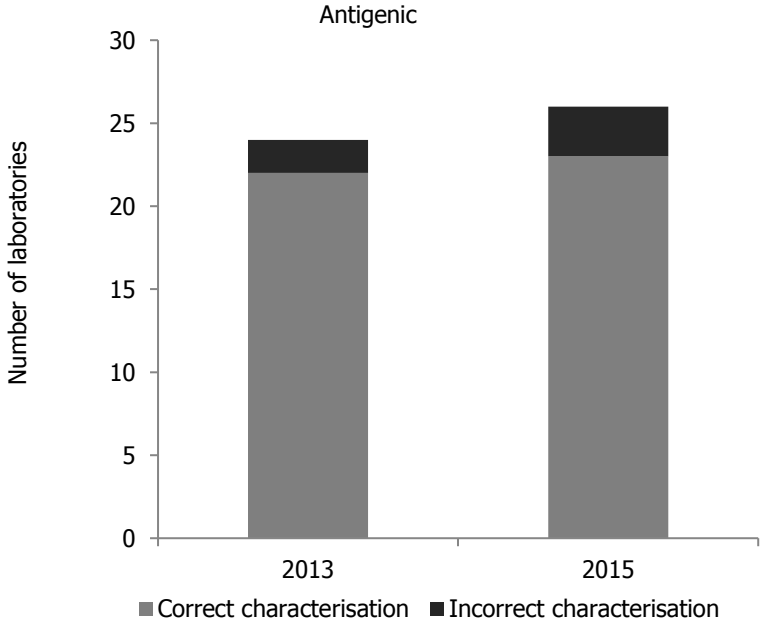
Comparison of the number of laboratories reporting genetic characterisation results for the H1N1pdm09 sample in the 2013 and 2015 EQA panels.
2013 sample: A/California/7/2009.
2015 sample: A/England/226/2010

Figure 9A. Antigenic characterisation of INF15 panel



The number of laboratories reporting antigenic characterisation results for INF15 samples depicted per virus type in each sample.

Figure 9B. Comparison of the number of laboratories reporting antigenic characterisation results for the A(H1N1)pdm09 sample in the 2013 and 2015 EQA panels



2013 sample: A/California/7/2009.
2015 sample: A/England/226/2010

Results for influenza virus characterisation – antigenic

The number of laboratories reporting antigenic characterisation results is presented by virus sample (type/subtype) and correct characterisation (Figure 9A). Between 24 and 28 laboratories used antigenic means to characterise samples from the IFN15 panel. For the A(H1N1) samples only 2 or 3 incomplete characterisations were reported. This increased to six incomplete characterisations for the influenza B samples. As mentioned previously, the A(H3N2) viruses, particularly from subgroup 3C.2a, have proved difficult to characterise due to reduced reactivity with reference antiserum raised against egg-propagated viruses. Of the 26 laboratories that used antigenic characterisation on the 3C.2a sample, only nine laboratories reported the correct full characterisation. This improved for the A(H3N2) 3C.3a sample: 17 of the 24 laboratories reported a complete and correct characterisation. Sixteen of the 25 laboratories correctly characterised the A(H3N2) sample, again suggesting that some laboratories were less certain about how to report non-current seasonal samples through the predefined TESSy reporting categories.

To assess whether the technical ability of the network has improved, the authors of this study compared the characterisations of two relatively stable A(H1N1)pdm09 samples: A/California/7/2009 in 2013 and A/England/226/2010 in 2015 (Figure 9B).

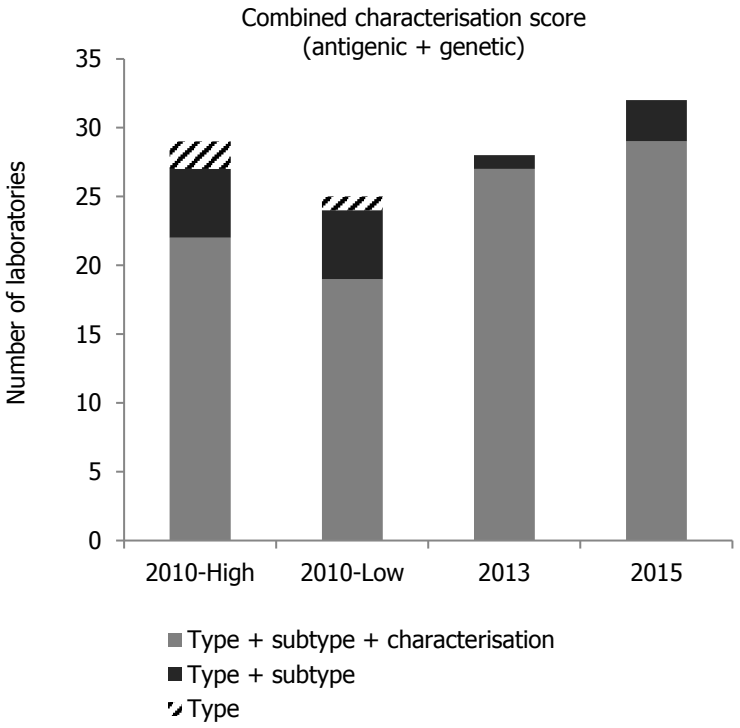
The number of laboratories using antigenic characterisation increased from 24 in 2013 to 26 in 2015 while the number of incomplete characterisations increased from two to three over the same period. This is a modest improvement compared with 2013, but not as pronounced as the improvement in genetic characterisation. Since antigenic characterisations have been performed to a high technical standard, further improvements are difficult. By contrast, genetic characterisation, being a relatively recent addition to most laboratory workflows, showed a much greater potential for improvement.

The combined antigenic and genetic raw data are presented in Annex 1.

Overall characterisation performance

In order to determine whether the network's overall characterisation capability had improved since 2010, the genetic and antigenic characterisations of a relatively conserved sample were compared. Each EQA panel since 2010 has contained an A(H1N1)pdm09 sample, which has remained genetically and antigenically relatively stable over the time period in question. Both the 2010 and 2013 panels contained the A/California/7/2009 sample while the 2015 panel contained A/England/226/2010. All samples where provided to recipients at between 100 and 1000 pfu/ml, and the majority of laboratories successfully isolated the sample from the simulated clinical sample. Data for both the high and low titre samples from 2010 are shown. Figure 10 shows that the number of laboratories reporting a complete and correct characterisation increased from 22 in 2010 (high) to 27 in 2013 and 29 in 2015. The number of incomplete characterisations dropped from seven in 2010 to one in 2013 and three in 2015. These figures not only show that the number of laboratories which genetically characterise influenza viruses is increasing, but that data quality is also improving.

Figure 10. Comparison of the combined characterisation score (genetic and antigenic) for the A(H1N1)pdm09 sample, 2010–2015 EQA panels



Comparison of combined genetic and antigenic virus characterisation data for a relatively conserved A(H1N1)pdm09 samples across three EQA panels.
2010: A/California/7/2009 @ 1 000 plaque forming units/ml, 2010-High; 100 pfu/ml, 2010-Low.
2013: A/California/7/2009 @ 379 pfu/ml
2015: A/England/226/2010 @ 643 pfu/ml

Number of laboratories reporting either full characterisation (type, subtype, characterisation) compared to incomplete characterisation (type, subtype) or just virus type.

Training

The contractor recognises the ability to isolate influenza virus in cell culture or embryonated eggs as a core capability. Wet laboratory training courses in November 2012 and April 2013 were supplemented by individual twinning applications. Webinar training courses with a focus on virus characterisation were delivered in December 2014 and January 2016. For laboratories performing genetic characterisation, sequencing and sequence analysis, techniques were presented in October 2013 and January 2016 (Annex 3).

Discussion and conclusions

All thirty-eight ERLI-Net laboratories participated in the 2015 EQA exercise. The results showed a high level of competency and further improvements since the previous panel in 2013.

Each participant received a panel of eight coded samples, including a negative sample. At the time of distribution, the included influenza A and B viruses were either circulating or had recently been circulating in humans. All participants returned results for rapid detection of influenza viruses. Thirty-two participants returned results for influenza virus isolation, and 33 (87%) laboratories reported strain characterisation results.

Analysis of the rapid detection results from all thirty-eight ERLI-Net laboratories showed that 35 of the 38 laboratories (92%) achieved full marks. Of the five errors, only two were serious: one false positive and one example of an A(H3) sample reported as a B/Vic (Figure 2). The other three errors were mistaken lineage determinations or incomplete subtyping. This is an improvement over the 2013 panel where four false negatives and seven subtyping errors were reported. Assessing the last four EQA panels (Figure 3) showed the improved quality of detection data, combines with an improved testing capacity. The quality of reported data also improved, with 92% of the laboratories achieving top marks in 2015, a significant increase over the three previous panels

where top marks were given to 71%, 76% and 79% of the participants. Over the same period, the number of laboratories reporting results increased from 31 laboratories to 38.

Laboratories were not required to determine the lineage of influenza B viruses during rapid detection; however, 24 of the 38 participants (63%) supplied lineage information. This is a marked increase from 2013 when 16 of 36 (45%) laboratories reported viral lineage. This information is particularly relevant in conjunction with the increasing uptake of quadrivalent influenza vaccines.

Overall, across the period of the framework contract (2012–2016) the progressive increase in the number of laboratories performing rapid detection was matched by an increase in data quality.

Of the 38 laboratories that returned rapid detection results, 32 also returned data on virus isolation in culture from the simulated specimens. Twenty-six of the laboratories isolated virus from all of the positive samples (81%), showing a steady improvement over the previous two EQA panels (2010: 70%, 2013: 75%; see Figure 6).

The INF15 panel contained three A(H3) samples which were added in response to the described difficulties with isolation and characterisation of viruses from this subtype, particularly subgroup 3C.2a. Approximately seven laboratories used MDCK-SIAT cells to isolate all samples from the INF15 panel, with an additional seven laboratories selectively using MDCK-SIAT specifically for isolation of the A(H3) samples (Figure 4). Of the 11 false negative isolations, six errors were associated with the three A(H3) samples (two errors/H3 sample). This was a similar error rate as detected in the two A(H1) samples, where four A(H1) false negatives were reported (two errors/H1 sample). This suggests that the A(H3) samples present the same level of difficulty as other samples (Figure 7).

An additional challenge is to detect growth of A(H3) samples in culture due to poor HA titres, especially for subgroup 3C.2a samples. Laboratories were asked to describe assays used to detect successful culture of samples (Figure 5). The majority of laboratories reported the use of multiple methods.

Five laboratories reported false-negative isolation results, accounting for a total of 11 samples. Only three of these 11 samples (27%), from two laboratories, were not characterised in response to the isolation error. The remaining eight samples, from three laboratories, were genetically characterised directly from the simulated material. Although genetic characterisation is possible from the primary material, it is not possible to recover sufficient material for phenotypic antiviral susceptibility testing. Some of these samples were therefore only genetically characterised for the Antiviral_2015 (AV15) EQA panel.

Thirty-three laboratories (86%) returned characterisation results for the INF15 panel, representing a consistent level of participation: in 2013 (INF13), 30 of 38 participating laboratories (83%) returned results, and in 2010, 30 of 34 laboratories (88%) reported characterisation results.

Genetic characterisation was used by approximately 19 laboratories (Figure 8), with highly consistent results. A total of eight incomplete or inaccurate characterisations were reported, all with virus strains that had not been recently classified as seasonal at the time of testing. This suggests that some of these errors could be attributed to uncertainties on how to report non-seasonal results to TESSy.

A comparison of the genetic characterisation of a relatively stable A(H1N1)pdm09 strain between the 2013 and 2015 EQA panels (Figure 8B) showed the increased use of genetic characterisation techniques in routine laboratory workflows: the number of laboratories submitting results increased from 12 to 19. The number of laboratories reporting incomplete characterisation, however, increased to two in 2015 compared with zero in 2013. Two laboratories reported using next generation sequencing techniques for sample analysis rather than the traditional Sanger sequencing methodologies, providing insight into future developments to expect across the network.

Antigenic characterisation was performed by approximately 26 laboratories (Figure 9), a similar participation rate as in the IFN13 panel. An average of two to three characterisation errors were reported for the A(H1) samples. This increased to an average of six errors with the influenza B samples. The A(H3) samples were selected to be challenging; with an historical A(H3) sample and samples from subgroup 3C.3a and 3C.2a included. Nine, seven and seventeen characterisation errors, respectively, were reported for these samples, confirming the previously described difficulties, particularly with the 3C.2a subgroup.

Improvements in the networks' competency for performing antigenic characterisations was monitored by comparing the results for similar A(H1N1)pdm09 samples that were included in both the 2013 and 2015 EQA panels (Figure 9B). Laboratory capability and quality of results appear to be stable or slightly improving, with an increase in the number of reporting laboratories from 24 to 26. Over the same period, the number of incomplete characterisations also increased from two to three.

A comparison of the combined genetic and antigenic characterisation results for the relatively stable A(H1N1)pdm09 strain (Figure 10) over the period of the framework contract (2012−2016) showed improvements in both network capacity and quality of strain characterisation results. The number of complete virus characterisation results increased from 22 in 2010 to 27 in 2013 and to 29 in 2015.

Training on virus isolation, antigenic and genetic characterisation was provided throughout the period of the framework contract. The contractor's management team supported training delivered via wet laboratory courses, webinars and twinning activities. It is notoriously difficult to directly link training to improvements in EQA results, but there were noticeable and consistent improvements in both network capacity and quality with regard to rapid detection and virus characterisation. The largest improvements have been seen in molecular testing techniques such as sequencing, which has also been a main focus of by ERLI-Net training.

The inclusion of three challenging influenza A(H3N2) isolates was intended as a test of the network's ability to isolate and characterise difficult seasonal viruses. As expected, antigenic characterisation of these viruses proved difficult; however, all 20 laboratories that genetically characterised the 3C.2a and 3C.3a reported correct results.

Recommendations

Rapid detection

- One participant (ID 2306) reported a false positive in the rapid detection and the isolation components of the panel. This indicates an issue with sample contamination or switching, and therefore a sample handling error rather than a testing protocol issue. A review of standard operating procedures (SOPs) for specimen reception and sample handling should resolve this issue.
- Another participant (ID 2820) reported a B/Vic result instead of A/H3 in the rapid detection panel and failed to isolate the virus from this sample. This may also suggest an issue with sample handling as the alternative explanation requires two independent errors. A review of SOPs for specimen reception and sample handling should resolve this issue.
- Of the three remaining rapid detection subtyping/lineage determination errors, two were corrected during the detailed strain characterisation component of the panel.
- Training on assay validation and methods for primer/probe assessment with seasonal viruses will be delivered by webinar later this year.

Virus isolation

- One laboratory reported five errors and two more had two errors each. A review of all relevant SOPs is recommended as these errors are usually due to reagent, cell or sensitivity issues.
- Continued support and training for virus isolation is important to maintain capability for this technique within Europe. This is particularly relevant for countries with diminishing capacity, especially with the increased use of direct sequence analysis from clinical specimens.

Genetic characterisation

- Genetic testing resulted in very accurate strain characterisations. Errors were only reported in non-seasonal samples, indicating that these errors may be associated with uncertainties on how to report non-seasonal isolates through the predefined TESSy categories. This topic should be addressed in a future webinar training session.

Antigenic characterisation

- Issues with characterisation of H3 have been well described; however, the overall capability for antigenic characterisation is still good. Continued updates on virus evolution and characterisation issues should be delivered during webinars.

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Annex 1a. Characterisation results presented by individual laboratory – A(H1N1)pdm09

Participant ID	A(H1N1)pdm09 gp 6 [EISN_INF15-07]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
75	Virus isolated: A/H1/pdm09	Genetic - NGS		A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
95	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
200	No virus islolated	Genetic - Sanger		A(H1N1)pdm09 not attributed to clade/group	2
207	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)pdm09 not attributed to category		2
1159	Virus isolated: A/H1/pdm09	Antigenic - HI with oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1174	Isolation not attempted	Genetic - Sanger		A(H1N1)pdm09 not attributed to clade/group	2
1262	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1299	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1323	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1402	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1432	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1456	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1515	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
1534	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1643	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
1649	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2001	Virus isolated: A/H1/pdm09	Genetic - Sanger		A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
2125	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
2126	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2253	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2258	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2271	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
2272	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2276	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2277	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3

Participant ID	A(H1N1)pdm09 gp 6 [EISN_INF15-07]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
2278	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)pdm09 not attributed to category	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
2306	Virus isolated: A/H1/pdm09	Genetic - Sanger		A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
2819	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2820	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
3442	No virus islolated	Genetic - Sanger		A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
4209	No virus islolated				0
4213	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - NGS	A(H1N1)pdm09 not attributed to category	A(H1N1)pdm09 A/St Petersburg/27/2011 (group 6)	3
4764	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3

Participant ID	A(H1N1)pdm09 sgp 6B [EISN_INF15-06]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
75	Virus isolated: A/H1/pdm09	Genetic - NGS		A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
95	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
200	No virus islolated	Genetic - Sanger		A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
207	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)pdm09 not attributed to category		2
1159	Virus isolated: A/H1/pdm09	Antigenic - HI with oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1174	Isolation not attempted	Genetic - Sanger		A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1262	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1299	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1323	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1402	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1432	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)pdm09 not attributed to category	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1456	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1515	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
1534	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1643	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
1649	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2001	Virus isolated: A/H1/pdm09	Genetic - Sanger		A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3

Participant ID	A(H1N1)pdm09 sgp 6B [EISN_INF15-06]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
2125	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
2126	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2253	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2258	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2271	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
2272	Virus isolated: A/H1/pdm09	Antigenic - HI with oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2276	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2277	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2278	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
2306	Virus isolated: A/H1/pdm09	Genetic - Sanger		A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
2819	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
2820	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
3442	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - Sanger	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3
4209	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir	A(H1N1)/pdm09 A/California/7/2009-like		3
4213	Virus isolated: A/H1/pdm09	Antigenic - HI without oseltamivir, genetic - NGS	A(H1N1)/pdm09 A/California/7/2009-like	A(H1N1)pdm09 A/South Africa/3626/2013 (subgroup 6B)	3

Annex 1b. Characterisation results presented by individual laboratory – A(H3N2)

Participant ID	A(H3N2) 3C.2a [EISN_INF15-04]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
75	Virus isolated: A/H3	Genetic - NGS		A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
95	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Victoria/361/2011-like		2
200	No virus islolated	Genetic - Sanger		A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
207	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		2
1159	Virus isolated: A/H3	Antigenic - HI with oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1174	Isolation not attempted	Genetic - Sanger		A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1262	Virus isolated: A/H3	Antigenic - HI with oseltamivir,Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1299	Virus isolated: A/H3	Antigenic - HI with oseltamivir, genetic - Sanger	A(H3N2) not attributed to category	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1323	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1402	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Texas/50/2012-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1432	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1456	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1515	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Victoria/361/2011-like		2
1534	Virus isolated: A/H3	Antigenic - HI with oseltamivir,Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1643	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Texas/50/2012-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
1649	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Texas/50/2012-like		2
2001	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
2125	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Texas/50/2012-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
2126	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Texas/50/2012-like		2
2253	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Texas/50/2012-like		2
2258	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		2
2271	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
2272	Virus isolated: A/H3	Antigenic - HI with oseltamivir	-		2

Participant ID	A(H3N2) 3C.2a [EISN_INF15-04]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
2276	Virus isolated: A/H3	Antigenic - HI with oseltamivir,Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
2277	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3
2278	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
2306	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
2819	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		2
2820	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3
3442	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Victoria/361/2011-like	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
4209	Virus isolated: A/H3	Antigenic - HI without oseltamivir	-		2
4213	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - NGS	A(H3N2) not attributed to category	A(H3N2) A/Hong Kong/5738/2014 (subgroup3C.2a)	3
4764	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		2

Participant ID	A(H3N2) [EISN_INF15-05]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
75	Virus isolated: A/H3	Genetic - NGS		A(H3N2) not attributed to clade/group	3
95	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Victoria/361/2011-like		3
200	No virus islolated	Genetic - Sanger		A(H3N2) not attributed to clade/group	3
207	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		3
1159	Virus isolated: A/H3	Antigenic - HI with oseltamivir, genetic - Sanger	A(H3N2) A/Perth/16/2009-like	A(H3N2) not attributed to clade/group	3
1174	Isolation not attempted	Genetic - Sanger		A(H3N2) not attributed to clade/group	3
1262	Virus isolated: A/H3	Antigenic - HI with oseltamivir, antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Texas/50/2012-like	A(H3N2) not attributed to clade/group	3
1299	Virus isolated: A/H3	Antigenic - HI with oseltamivir, genetic - Sanger	A(H3N2) not attributed to category	A(H3N2) not attributed to clade/group	3
1323	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Victoria/361/2011-like	A(H3N2) not attributed to clade/group	3
1402	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Texas/50/2012-like	A(H3N2) A/Texas/50/2012 (subgroup 3C.1)	2
1432	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) not attributed to clade/group	3
1456	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Perth/16/2009-like	A(H3N2) not attributed to clade/group	3
1515	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Perth/16/2009-like		3
1534	Virus isolated: A/H3	Antigenic - HI with oseltamivir, antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) not attributed to clade/group	3
1643	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) not attributed to clade/group	3
1649	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Texas/50/2012-like		2
2001	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) not attributed to clade/group	3

Participant ID	A(H3N2) [EISN_INF15-05]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
2125	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Texas/50/2012-like	A(H3N2) not attributed to clade/group	3
2126	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) not attributed to category	A(H3N2) not attributed to clade/group	3
2253	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Victoria/361/2011-like		3
2258	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		3
2271	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Victoria/361/2011-like	A(H3N2) not attributed to clade/group	3
2272	Virus isolated: A/H3	Antigenic - HI with oseltamivir	-		2
2276	Virus isolated: A/H3	Antigenic - HI with oseltamivir, antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Perth/16/2009-like	-	3
2277	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		2
2278	No virus islolated	Genetic - Sanger		A(H3N2) not attributed to clade/group	3
2306	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Stockholm/1/2013 (subgroup 3C.2)	2
2819	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		3
2820	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Texas/50/2012-like		2
3442	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Victoria/361/2011-like	A(H3N2) not attributed to clade/group	3
4209	Virus isolated: A/H3	Antigenic - HI without oseltamivir	-		2
4213	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - NGS	A(H3N2) not attributed to category	A(H3N2) not attributed to clade/group	3
4764	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		3

Annex 1c. Characterisation results presented by individual laboratory – A(H3N2)

Participant ID	A(H3N2) 3C.3a [EISN_INF15-02]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
75	Virus isolated: A/H3	Genetic - NGS		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
95	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Victoria/361/2011-like		2
200	No virus islolated	Genetic - Sanger		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
207	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		2
1159	Virus isolated: A/H3	Antigenic - HI with oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1174	Isolation not attempted	Genetic - Sanger		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1262	Virus isolated: A/H3	Antigenic - HI with oseltamivir, antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1299	Virus isolated: A/H3	Antigenic - HI with oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1323	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1402	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1432	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1456	Virus isolated: A/H3	Antigenic - HI with oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1515	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3
1534	Virus isolated: A/H3	Antigenic - HI with oseltamivir, antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1643	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
1649	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Texas/50/2012-like		2
2001	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
2125	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
2126	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3
2253	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3
2258	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		2
2271	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
2272	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Texas/50/2012-like		2

Participant ID	A(H3N2) 3C.3a [EISN_INF15-02]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
2276	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
2277	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3
2278	No virus islolated	Genetic - Sanger		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
2306	Virus isolated: A/H3	Genetic - Sanger		A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
2819	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) not attributed to category		2
2820	No virus islolated				0
3442	Virus isolated: A/H3	Antigenic - HI without oseltamivir, genetic - Sanger	A(H3N2) A/Switzerland/9715293/2013-like	A(H3N2) A/Switzerland/9715293/2013 (subgroup 3C.3a)	3
4209	Virus isolated: A/H3	Antigenic - HI without oseltamivir	-		2
4213	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3
4764	Virus isolated: A/H3	Antigenic - HI without oseltamivir	A(H3N2) A/Switzerland/9715293/2013-like		3

Annex 1d. Characterisation results presented by individual laboratory – influenza B

Participant ID	B-Vic [EISN_INF15-08]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
75	Virus isolated: B/Victoria Lineage	Genetic - NGS		B/Brisbane/60/2008 (Victoria lineage clade 1A	3
95	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Victoria lineage not attributed to category		2
200	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
207	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Victoria lineage not attributed to category		2
1159	Virus isolated: B/Victoria Lineage	Antigenic - HI with oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Victoria lineage - not attributed to clade/group	3
1174	Isolation not attempted	Genetic - Sanger		B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1262	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1299	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Victoria lineage not attributed to category	B/Victoria lineage - not attributed to clade/group	2
1323	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1402	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1432	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1456	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1515	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
1534	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1643	Virus isolated: B/Victoria Lineage	Genetic - Sanger		B/Brisbane/60/2008 (Victoria lineage clade 1A	3
1649	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
2001	Virus isolated: B/Victoria Lineage	Genetic - Sanger		B/Brisbane/60/2008 (Victoria lineage clade 1A	3
2125	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Victoria lineage not attributed to category		2
2126	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
2253	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
2258	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
2271	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
2272	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
2276	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	-	3

Participant ID	B-Vic [EISN_INF15-08]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
2277	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Phuket/3073/2013-like (Yamagata lineage)		1
2278	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
2306	Virus isolated: B/Victoria Lineage	Genetic - Sanger		-	2
2819	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Victoria lineage not attributed to category		2
2820	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
3442	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Brisbane/60/2008-like (Victoria lineage)	B/Brisbane/60/2008 (Victoria lineage clade 1A	3
4209	No virus islolated				0
4213	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3
4764	Virus isolated: B/Victoria Lineage	Antigenic - HI without oseltamivir	B/Brisbane/60/2008-like (Victoria lineage)		3

Participant ID	B-Yam [EISN_INF15-01]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
75	Virus isolated: B/Yamagata Lineage	Genetic - NGS		B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
95	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Massachusetts/02/2012-like (Yamagata lineage)		3
200	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
207	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Yamagata lineage not attributed to category		2
1159	Virus isolated: B/Yamagata Lineage	Antigenic - HI with oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1174	Isolation not attempted	Genetic - Sanger		B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1262	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Massachusetts/02/2012-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1299	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1323	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1402	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1432	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Massachusetts/02/2012-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1456	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1515	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Florida/4/2006-like (Yamagata lineage)		2
1534	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Florida/4/2006-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1643	Virus isolated: B/Yamagata Lineage	Genetic - Sanger		B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
1649	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Massachusetts/02/2012-like (Yamagata lineage)		3
2001	Virus isolated: B/Yamagata Lineage	Genetic - Sanger		B/Phuket/3073/2013 (Yamagata lineage clade 3)	3

Participant ID	B-Yam [EISN_INF15-01]				
	Subtype	Method	Characterisation antigenic	Characterisation genetic	Score
2125	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Phuket/3073/2013-like (Yamagata lineage)		3
2126	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Phuket/3073/2013-like (Yamagata lineage)		3
2253	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Phuket/3073/2013-like (Yamagata lineage)		3
2258	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Yamagata lineage not attributed to category		2
2271	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
2272	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Massachusetts/02/2012-like (Yamagata lineage)		3
2276	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Wisconsin/1/2010-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
2277	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Phuket/3073/2013-like (Yamagata lineage)		3
2278	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
2306	Virus isolated: B/Yamagata Lineage	Genetic - Sanger		B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
2819	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Yamagata lineage not attributed to category		2
2820	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Phuket/3073/2013-like (Yamagata lineage)		3
3442	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir, genetic - Sanger	B/Phuket/3073/2013-like (Yamagata lineage)	B/Phuket/3073/2013 (Yamagata lineage clade 3)	3
4209	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	-		2
4213	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Massachusetts/02/2012-like (Yamagata lineage)		3
4764	Virus isolated: B/Yamagata Lineage	Antigenic - HI without oseltamivir	B/Phuket/3073/2013-like (Yamagata lineage)		3

Annex 1e. Characterisation results presented by individual laboratory – influenza negative sample

participantID	Negative [EISN_INF15-03]					Overall culture score
	Subtype	Method	CharacterisationAntigenic	Characterisation Genetic	Score	
75	No virus islolated				3	24
95	No virus islolated				3	21
200	No virus islolated				3	23
207	No virus islolated	Antigenic	Negative	-	3	24
1159	No virus islolated				3	18
1174	Isolation not attempted				3	24
1262	Isolation not attempted				3	23
1299	Isolation not attempted				3	24
1323	No virus islolated				3	23
1402	No virus islolated	Antigenic - HI without oseltamivir, genetic - Sanger	Negative	Negative	3	24
1432	No virus islolated				3	23
1456	No virus islolated				3	24
1515	No virus islolated				3	24
1534	No virus islolated				3	22
1643	No virus islolated				3	24
1649	No virus islolated	Antigenic - HI without oseltamivir	Negative		3	24
2001	No virus islolated				3	21
2125	No virus islolated				3	24
2126	No virus islolated				3	23
2253	No virus islolated				3	23
2258	No virus islolated	Antigenic	Negative		3	24
2271	No virus islolated	Antigenic - HI without oseltamivir, genetic - Sanger	Negative	Negative	3	23
2272	No virus islolated	Antigenic - HI with oseltamivir	Negative		3	21
2276	No virus islolated				3	24
2277	No virus islolated				3	21
2278	Isolation not attempted				3	24
2306	Virus isolated: type not Determined	Genetic - Sanger		Not determined	0	21
2819	No virus islolated	Antigenic	Negative	Negative	3	14
2820	No virus islolated				3	24
3442	No virus islolated				3	19
4209	No virus islolated				3	20
4213	No virus islolated				3	20
4764	No virus islolated	Antigenic	Negative	Not determined	3	23

Annex 2. Laboratories participating in the EISN-INF15

Country	City	Organisation
Austria	Vienna	AKH Wien – Medical Uni of Vienna
Belgium	Brussels	Institute of Public Health
Bulgaria	Sofia	Center of Infectious & Parasitic Disease
Croatia	Zagreb	National WHO Influenza Centar, Croatian national institute of public health
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
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
Greece	Thessaloniki	National Influenza Centre for N Greece, Aristotle University of Thessaloniki
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	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 3. List of training provided

Country	Influenza surveillance	Sequencing and bioinformatics tools	AntiViral	Sequencing and bioinformatics tools	Virus culture	Virus culture	Sequencing and bioinformatics Tools	Virus characterisation	AntiViral	NGS Bioinformatics	EQA + season start
	Jun 2010	Nov 2010	Jul 2011	Nov 2011	Nov 2012	Apr 2013	Oct 2013	Dec 2014	Jan 2015	Jan 2016	Jan 2016
Sweden							x		**		
Netherlands-Rotterdam							x				
UK – Cardiff											*
UK – Glasgow										*	**
Denmark			xx		x			**	**	**	**
Iceland					x			**			
Austria	x		x	x		x		**			**
Belgium		x	x			x		**			**
Finland	x		x	x		x	x	**	**		**
France – Paris											
Germany										**	
Hungary	x		x		x			**			**
Ireland	x		x		x				**	**	**
Italy	x			x				**	**		**
Luxembourg		x				x	x	**		**	
Norway						x		**	**	**	**
Spain – Madrid		x	x		x			**		*	
Spain – Valladolid				x			x	**			**
Netherlands – Bilthoven				x						*	
Czech Republic	x	x									
UK – London											**
Latvia	x	x					x	**			
Estonia	x	x			x			**			**
Cyprus											
Greece – Athens		x	x	x	x		x			*	**
Greece – Thessaloniki	x			x						**	
Malta	x			x	x					*	**
Romania	x	x	x			x			**	*	**
Slovak Republic						x					
Slovenia	x	x	x		x		x	**			**
Spain – Barcelona			x	x	x				**		**
Croatia					x						
Bulgaria						x					
Portugal	x		x	x	x		x	**	**	**	**
UK – Belfast		x									
Lithuania	x		x		x				**	**	**
France – Lyon				x			x				
Poland		x					x	**			**

Training courses offered to ERLI-Net (CNRL) laboratories. Attendance of a wet training course is marked with an X.
*Webinar training courses are marked with ** for countries who dialled into the live presentation.*
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Countries with access to the ECDC extranet can view the presentations online.

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