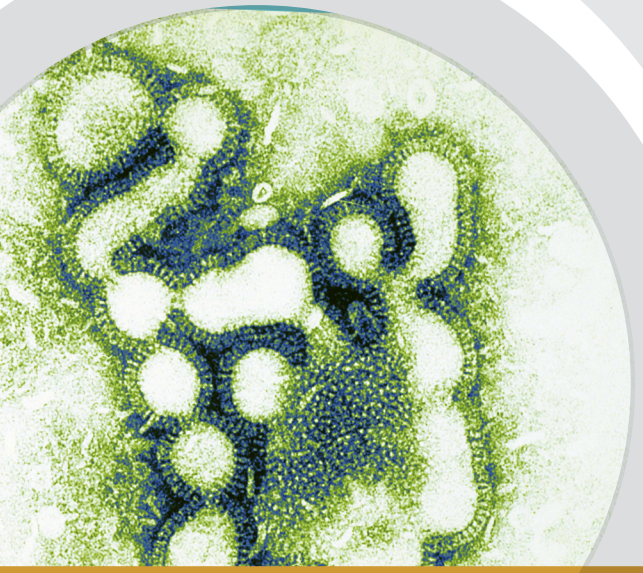

TECHNICAL REPORT

External quality assessment scheme for influenza antiviral susceptibility for the European Reference Laboratory Network for Human Influenza

2015

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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, and Adam Meijer, RIVM, the Netherlands, 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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Contents

Figures

Abbreviations

AANI	No amino acid substitution previously associated with highly reduced inhibition
AARI	Amino acid substitution previously associated with reduced inhibition
AAHRI	Amino acid substitution previously associated with highly reduced inhibition
AV	Antiviral
AV15	Influenza antiviral susceptibility external quality assessment panel of ERLI-Net
CNRL	Community Network of Reference Laboratories for Human Influenza in Europe
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
HA	Haemagglutinin
HRI	Highly reduced inhibition
IC50	50% inhibitory concentration
MUNANA	2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid sodium salt hydrate
NA	Neuraminidase
NAI	Neuraminidase inhibitors
NI	Normal inhibition
PCR	Polymerase chain reaction
PHE	Public Health England
QC	Quality control
QCMD	Quality Control for Molecular Diagnostics, Glasgow, UK
RI	Reduced inhibition
RIVM	National Institute for Public Health and the Environment, the Netherlands
SNP	Single nucleotide polymorphism
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 antiviral susceptibility external quality assessment (EQA) was held for European reference laboratories. This was the third antiviral EQA panel distributed by the European Reference Laboratory Network for Human Influenza (ERLI-Net; previously called CNRL) since the European Influenza Surveillance Network (EISN) was established in 2008.

The objectives of the exercise were to provide participants with an independent mechanism to check performance, to provide information for the entire network on capacity (i.e. the number of laboratories having the capability to conduct phenotypic and/or genotypic antiviral tests) and on capability (i.e. the ability of individual laboratories to conduct such tests), and to accurately report test results to TESSy, The European Surveillance System.

Overall results for the exercise were broadly encouraging. Twenty-four laboratories from 19 European countries participated in the exercise while 14 laboratories opted out as they did not perform assays capable of determining antiviral susceptibility. This represents increased capacity (24 laboratories) across the network, compared to the first EQA exercise in 2010 (20 laboratories).

Phenotypic antiviral susceptibility results were encouraging. Eighteen of the 24 laboratories (75%) returned phenotypic results, with only nine incorrect results out of a total of 261 (3.4%). One laboratory, using a NA-STAR-based assay failed to detect the mixed A(H1N1)pdm09 NA-H275Y sample, a problem that has been noted before. Three laboratories incorrectly reported 'normal inhibition' for zanamivir on the influenza type B NA-I221L [1] sample, all using kit-based assays (1 NA-STAR, 2 NA-FLUOR). With regard to the wild type specimens, five reports from three laboratories incorrectly interpreted viruses as 'reduced' or 'highly reduced' inhibition: one such error was due to a fold change just over the reduced inhibition threshold, three errors were caused by using the NA-FLUOR kit, and one error was merely an incorrect interpretation of an otherwise correct IC_{50} value.

All 24 laboratories participating in the EQA used some form of genetic characterisation. Depending on the laboratory and the sample involved, techniques varied from pyrosequencing, single nucleotide polymorphism PCRs to Sanger sequencing of the neuraminidase (NA) gene. Depending on the method employed, interpretations of antiviral susceptibility varied notably.

Strict marking criteria were applied to the interpretation of genotypic results in order to avoid a number of problems that had previously been identified in relation to the routine reporting of antiviral susceptibility data to TESSy. Overall, laboratories were able to identify nucleotide polymorphisms. A few issues were noted, e.g. results from samples for which only partial genetic information was available were over-interpreted, especially when polymorphisms associated with reduced susceptibility were missing (e.g. partial sequencing or SNP PCR). In some of these cases, laboratories incorrectly reported 'normal inhibited' instead of 'no interpretation possible'. Several laboratories used the wrong assays for the samples, e.g. by using SNP PCR on viruses with the N2 NA; SNP PCR is designed primarily for viruses with the N1 NA. These issues were addressed during the training webinars.

The 2015 EQA showed a general improvement in the technical ability of network laboratories, but also identified topics for future training and monitoring. Continued issues with incorrect interpretations of partial genetic information were addressed in two webinar training sessions. Some improvement has been seen in this area, although further training and support is required.

Introduction

Influenza viruses cause a highly contagious acute respiratory disease that can spread rapidly and cause high levels of morbidity and mortality. With the advent and increased clinical use of antiviral drugs against influenza the prevention and treatment of influenza has improved. The neuraminidase inhibitors (NAI) oseltamivir and zanamivir were developed through structure-based drug design to mimic the neuraminidase's natural substrate, sialic acid. The close similarity to the natural substrate was predicted to limit the emergence of resistance. WHO has standardised the terminology used to describe the susceptibility of influenza viruses in terms of the inhibitory effect of NAI on the NA enzyme activity as measured by the IC_{50} of a NAI: normal inhibition (NI), reduced inhibition (RI) and highly reduced inhibition (HRI). WHO also uses these three categorised for all amino acid substitutions [2,3]. ERLI-Net later adopted these categories.

Emergence of antiviral resistance is closely monitored through virological surveillance. Very few cases of resistance to NAIs were found during clinical trials and post-licensure surveillance. Amino acid substitutions in the NA gene associated with highly reduced inhibition or reduced inhibition to NAI were seen in a few cases of clinical resistance. In 2007, naturally occurring oseltamivir HRI due to a histidine-to-tyrosine amino acid substitutions at position 275 in the N1 NA (H275Y) was observed in former seasonal A(H1N1) viruses, and the oseltamivir HRI virus rapidly spread across Europe and worldwide [4,5]. NAI resistance in other influenza A subtypes and influenza B has not been widely observed. During the 2009 H1N1 pandemic, oseltamivir was commonly used for treatment and post-exposure prophylaxis. A relatively small number of A(H1N1)pdm09 viruses with HRI to oseltamivir, due to the H275Y substitution, have been observed since the emergence of this virus. On a few occurrences such viruses were detected in larger outbreaks that did not lead to global spread [6,7]. The situation is closely monitored by ERLI-Net and the WHO Global Influenza Surveillance and Response System (GISRS) [7,8,9,10].

The H275Y substitution in the A(H1N1) subtype is the most common mutation, and the only single polymorphism unequivocally considered to confer clinical resistance to oseltamivir in the absence of compensatory or secondary amino acid substitutions. Other (H)RI pathways have been described for A(H1N1) viruses so the absence of H275Y does not preclude (H)RI via alternative amino acid substitutions. While the H275Y substitution is relatively easily targeted by laboratory assays, the situation for the A(H3N2) subtype and influenza B virus is different because several substitutions were identified which generate RI [11]. For these viruses, a broader testing strategy is required. The gold standard phenotyping test requires isolated and propagated viruses and is therefore not as widely performed [12]. Phenotyping tests (IC_{50} assay) can detect changes in NAI susceptibility (RI/HRI) due to previously known – but also unknown – amino acid substitutions in influenza A and B viruses.

This report presents the results of the influenza virus antiviral susceptibility EQA. EQA panels were distributed in June 2015 by Public Health England. Quality Control for Molecular Diagnostics (QCMD) collated the results and performed the initial analyses.

This is the third EQA for influenza antiviral susceptibility conducted for EISN ERLI-Net and laboratories associated with the the former EISN Community Network of Reference Laboratories for Human Influenza in Europe. The first EQA was conducted in 2010 [13], the second one in 2013 [15,16]. As with all other aspects of influenza surveillance, it is essential that the accuracy of influenza antiviral susceptibility testing methods is assessed through effective quality control. EQAs are an integral part of quality control and provide a means of externally evaluating both individual laboratory performance and performance of the network as a whole.

Objectives

The specific objectives of the 2015 EISN influenza antiviral susceptibility testing EQA panel were to measure individual laboratory performance in four areas.

- To test the participants' ability to detect nucleotide mutations in the NA gene; these mutations result in amino acid substitutions known to confer RI/HRI by NAI in a panel of influenza A and B viruses by genotypic and/or phenotypic antiviral susceptibility methods.
- To provide participating laboratories with an independent mechanism to assess the performance of their influenza antiviral susceptibility testing methodology.
- To gain insights into the performance of different techniques used for influenza antiviral susceptibility testing in European laboratories, thus helping ERLI-Net and ECDC to determine training priorities and produce guidelines on the reporting of results and on how to harmonise the interpretation of antiviral data.
- To evaluate test results and assess the quality of data uploaded to TESSy.

EQA testing also promotes the following overall objectives:

- 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

Study design

Organisation

The EQA panel was designed by staff from Public Health England, together with members of the contractor's management team and several ERLI-Net network members. The panel was prepared and tested by the Respiratory Virus Unit at Public Health England, London. Further pre-testing was performed by the WHO-CC at the Francis Crick Institute at Mill Hill, London, and the France South National Influenza Centre, Lyon, France. Data provided by the participating laboratories were collected online and managed by Quality Control for Molecular Diagnostics (QCMD). The panel contents were distributed frozen on dry ice and by specialist courier.

Participation

All influenza laboratory contact points in the ERLI-Net were notified in advance of the EQA exercise. Laboratories were given the option to opt out of the EQA if they were not equipped for antiviral susceptibility testing. Annex 4A lists all EQA participants.

Panel description

The panel consisted of two simulated samples containing viruses with amino acid substitutions conferring NAI (H)RI alongside eight samples used for the Influenza_2015 (IFN15) panel of which the included viruses did not contain amino acid substitutions conferring NAI H(RI). Two simulated clinical samples were provided, containing inactivated influenza A(H1N1)pdm09 and influenza B viruses. These inactivated samples contained a viral load that allowed direct measurement of IC_{50} without a virus isolation and propagation step.

Virus type and subtype were provided for each sample to guide the selection of appropriate tests and analysis. Viruses were inactivated with Triton X-100 retaining functional NA enzyme activity [14], aliquoted and stored frozen at -80° C until dispatched. Laboratories were required to also characterise the eight samples and viruses that had been isolated and tested as part of the INF15 panel. One panel was thawed and pre-tested at Public Health England using in-house methods. Panels were sent frozen on dry ice to two independent laboratories for pre-testing. The final panel contents were shipped frozen on dry ice by specialist courier (DG Global Forwarding) on 15 June 2015, and all were received within two days. The deadline for reporting results was within 28 days of receipt of the panel.

Testing

Laboratories were expected to only use those genotypic and/or phenotypic methods for antiviral susceptibility testing which were readily available in their facility. Laboratories were not penalised for reporting 'not tested' if they had no suitable testing methods available for one of more samples in the panel. Laboratories were assessed on their final interpretation of the obtained results ('normal', 'reduced', 'highly reduced inhibition' or 'no interpretation possible', based on genotypic and/or phenotypic data).

Data reporting

For genotypic characterisation, participants were asked to report the predicted amino acid substitutions based on nucleotide sequence detected in the sample and their interpretation of the impact on NAI susceptibility (amino acid substitutions associated with (highly) reduced inhibition (AARI, AAHRI), or no amino acid substitution previously associated with highly reduced inhibition (AANI)). For phenotypic characterisation, participants were asked to report the IC_{50} value measured and submit their interpretation of the data ('reduced', 'highly reduced' or 'normal' inhibition (RI, HRI, NI)). Participants were asked to report if an interpretation was not possible with the used methodology, or if a negative result was obtained. Data on the genotypic and phenotypic methods used in the participating laboratories were collected online and managed by QCMD.

Data analysis

WHO phenotypic classification definitions are shown in Annex 1 [2]. The phenotypic scoring system awards a maximum of one point for each analysed sample, irrespective of whether the participating laboratory tested for both oseltamivir and zanamivir, or oseltamivir alone. One point was deducted for each incorrect result. No distinction was made between samples that were classified as HRI and samples that were classified as RI. The maximum achievable score for phenotypic characterisation was 9 points. The genotypic scoring system awarded

one point for a 'correctly interpreted' result if the results were obtained through an appropriate test and an equally appropriate interpretation. If present, amino acid substitutions associated with reduced inhibition had to be correctly identified and reported. The maximum score for genotypic characterisation was 27 points.

Initial report

QCMD and Ian Harrison worked jointly to perform an initial analysis of 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 expected results

The influenza type, subtype, strain characterisation and antiviral susceptibility profile with associated amino acid substitutions in the NA protein for each sample in the EQA panel are shown in the table below. (Figure 1). Amino acid numbering corresponding to the relevant NA subtype is used throughout. The influenza subtype was identified in the description of each inactivated panel sample so that participants could target subtype-specific tests appropriately. The virus type and subtype for samples tested as part of the Rapid Detection, Isolation and Characterisation panel were determined by the receiving laboratories as part of the routine laboratory testing workflow.

Figure 1. Panel composition

Sample code	Subtype	Designation	Amino acid substitutions associated with (H)RI	Oseltamivir interpretation	Zanamivir interpretation
EISN_INF15-01	B-Yam	B/England/531/2014	none	No inhibition	No inhibition
EISN_INF15-02	A(H3N2)	A/Switzerland/9715293/2013	none	No inhibition	No inhibition
EISN_INF15-03	n/a	n/a	n/a	n/a	n/a
EISN_INF15-04	A(H3N2)	A/England/599/2014	none	No inhibition	No inhibition
EISN_INF15-05	A(H3N2)	A/England/215/2011	none	No inhibition	No inhibition
EISN_INF15-06	A(H1N1)pdm09	A/ENG/579/2014	none	No inhibition	No inhibition
EISN_INF15-07	A(H1N1)pdm09	A/England/226/2010	none	No inhibition	No inhibition
EISN_INF15-08	B-Vic	B/England/197/2014	none	No inhibition	No inhibition
AV15-01	A(H1N1)pdm09	H275Y mix	275H/Y	(Highly) reduced inhibition	No inhibition
AV15-02	B-Vic	B/Lyon/CHU/15.216/2011	I221L	Highly reduced inhibition	Reduced inhibition

AV15-01 and AV15-02 participants were provided with information on type/subtype of samples. AV15-01 reduced susceptibility to oseltamivir as a result of viruses with mixed NI and HRI NAI phenotypes.

An 'expected results letter' with strain designations was sent to all laboratories after testing was completed. The strain designations together with the complete gene sequences (downloadable from GISAID) allowed the laboratories to check the specificity of primers and probes used for testing.

EISN-AV15 participation

The number of laboratories participating in the 2015 antiviral susceptibility EQA (EISN-AV15) is shown in Figure 2, alongside the participation data for the previous two EQA panels in 2010 and 2013. After an increase in participation in 2013, the number of laboratories reporting antiviral susceptibility results has remained relatively stable at approximately 24. The number of laboratories using phenotypic characterisation has increased from 12 to 18 while the number of laboratories using genotypic characterisation has increased from 20 to 24 since 2010 (Figure 2).

The number of laboratories using phenotypic, genotypic or a combination of antiviral susceptibility testing methods is shown in Figure 3. Six laboratories used only genotypic analysis while 18 laboratories used a combination of genotypic and phenotypic analysis. No laboratory used phenotypic analysis if genetic analysis was not possible.

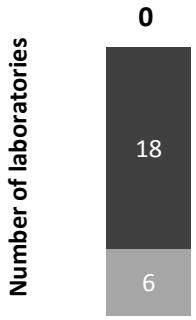
Figure 2. Participating laboratories: antiviral susceptibility testing, EISN-AV15 EQA panel

	2010		2013		2015	
	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic
Austria	■	■	■	■	■	■
Belgium			■		■	■
Bulgaria						
Croatia						
Cyprus						
Czech Republic				■	■	■
Denmark		■	■	■	■	■

	2010		2013		2015	
	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic
Estonia						
Finland						
France – Lyon						
France – Paris						
Germany						
Greece – Athens						
Greece–Thessaloniki						
Hungary						
Iceland						
Ireland						
Italy						
Latvia						
Lithuania						
Luxembourg						
Malta						
Netherlands – Bilthoven						
Netherlands – Rotterdam						
Norway						
Poland						
Portugal						
Romania						
Slovak Republic						
Slovenia						
Spain – Barcelona						
Spain – Madrid						
Spain – Valladolid						
Sweden						
UK – Belfast						
UK – Cardif						
UK – Glasgow						
UK – London						
	12	**20**	**17**	**24**	**18**	**24**
	20		**25**		**24**	

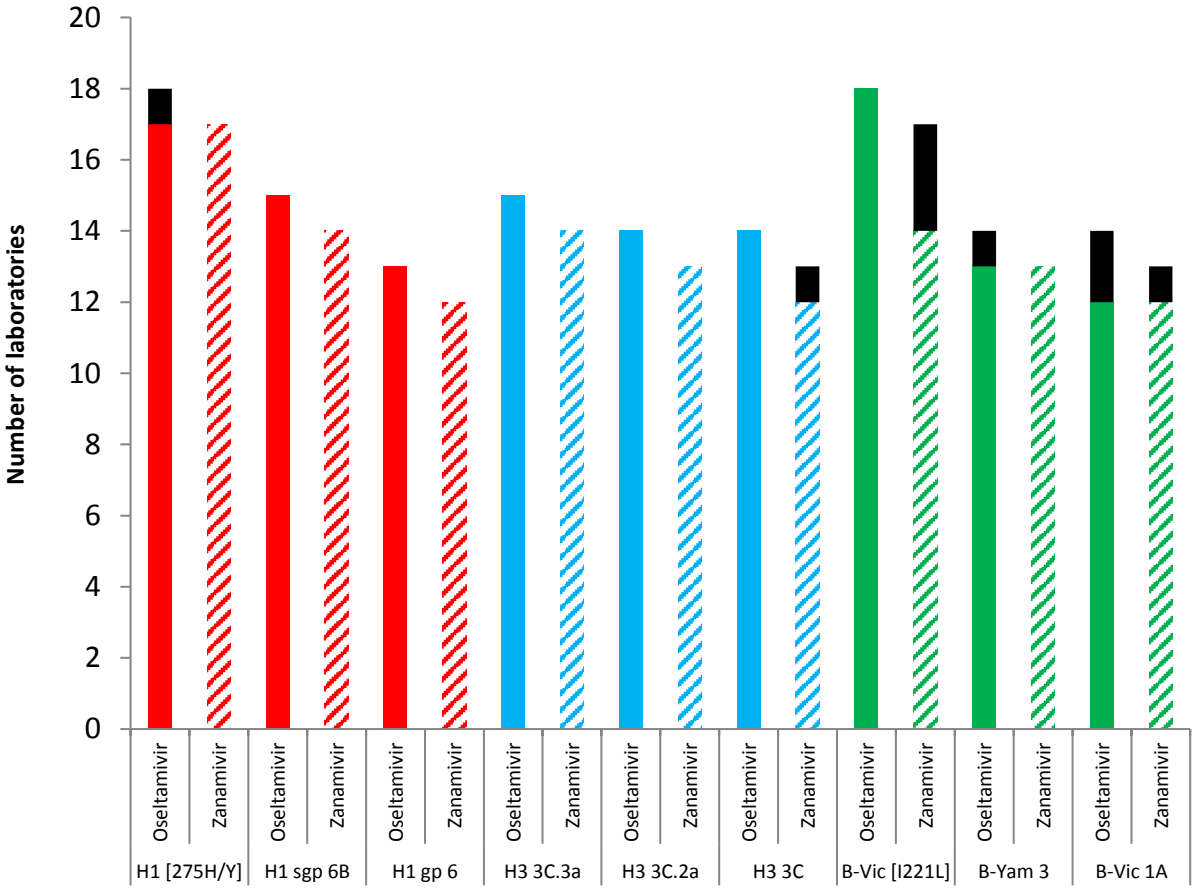
Participating laboratories were identified by a unique anonymised participant ID code (Annex 4). Green shading indicates participation in AV15. Grey shading indicates non-participation in the panel (i.e. no results returned or no panel requested). Participation over the last three EQA panels (2010, 2013, 2015) is shown.

Figure 3. Methods used for antiviral susceptibility testing



Number of laboratories reporting the use of phenotypic, genotypic or a combination of both techniques for the analysis of the EISN-AV15 panel
Black: phenotypic; dark grey: genotypic and phenotypic; light grey: genotypic

Figure 4A. Analysis of phenotypic antiviral susceptibility per sample in the AV15-EQA panel



Overview of AV15 phenotypic results, with the number of laboratories reporting the correct result depicted in colour and incorrect results shown with black fill. The number of laboratories reporting results for each sample is shown. Samples are divided and colour coded into virus type/subtype. Oseltamivir results are in bold fill and zanamivir results are in hatched fill.

Results of phenotypic testing

Eighteen of the 24 (75%) ERLI-Net laboratories that participated in the AV15 panel used phenotypic testing to determine the susceptibility of the EQA panel against oseltamivir. Seventeen of the participants also determined the susceptibility of the EQA panel to zanamivir. Analysis of the phenotypic results presented by individual laboratories (Figure 4A) shows that phenotypic testing was generally accurate. Figure 4B shows the results presented by individual laboratories; in total, nine errors were reported; one was associated with a laboratory using the NA-Star

assay which failed to detect the mixed NI/HRI population of A(H1N1) due to the NAI mutation H275Y. Three laboratories incorrectly reported 'NI' for zanamivir on the influenza B NA-I221L sample. The remaining five errors were with wild-type samples, one with an A(H3N2) sample and four with influenza B specimens.

Two fundamental assay platforms can be used for phenotypic testing: chemiluminescence-substrate-based assays (e.g. NA-Star and NA XTD Kit) and fluorescent-substrate-based assays (e.g. MUNANA in-house method and NA Fluor Kit). Figure 5 represents the number of laboratories using each of the assay formats; 14 use fluorescent-substrate-based assays while four laboratories use chemiluminescence assays.

Figure 4B. Individual laboratories results for phenotypic antiviral susceptibility testing

Participant ID	A(H1N1)pdm09		A(H1N1)pdm09 sgp 6B		A(H1N1)pdm09 gp 6		A(H3N2) 3C.3a		A(H3N2) 3C.2a		A(H3N2)		B-Vic [I221L]		B-Yam 3		B-Vic 1A		Score	Out of	% score
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir			
75	RI	NI											HRI	RI					2	2	100
1174	RI	NI											HRI	RI					2	2	100
1299	RI	NI	NI	NI			NI	NI	NI	NI	NI	NI	RI	RI	NI	NI	NI	NI	8	8	100
1323	RI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI	9	9	100
1456	RI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI	9	9	100
1534	RI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	RI	RI	NI	NI	NI	NI	9	9	100
1643	RI	NI	NI	NI	NI	NI	NI	NI					HRI	RI					5	5	100
2001	RI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	RI	HRI	RI	NI	NI	NI	NI	8	9	89
2126	HRI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI	9	9	100
2271	HRI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI	9	9	100
3442	RI	NI	NI	NI			NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI	8	8	100
4213	RI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI	9	9	100

Above: Phenotypic antiviral susceptibility testing, MUNANA assay

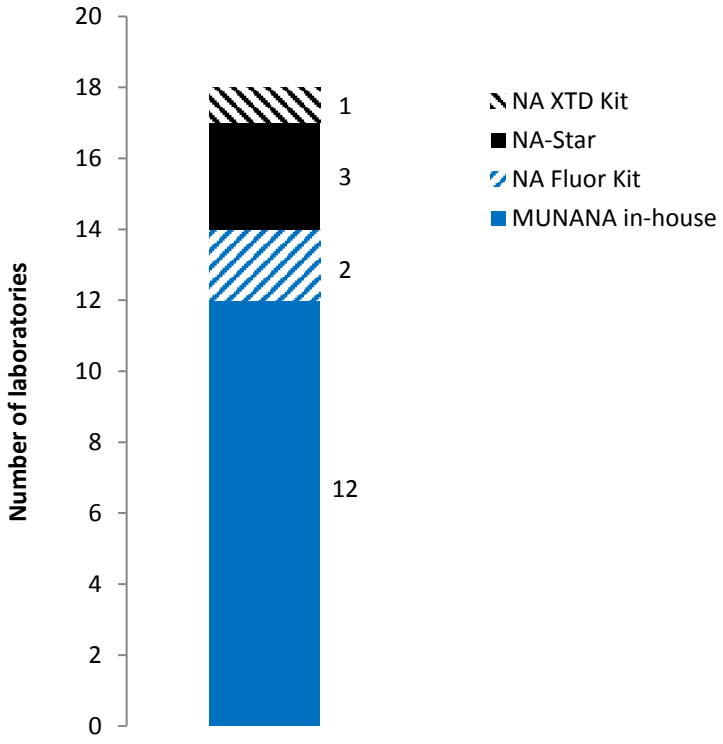
Participant ID	A(H1N1)pdm09		A(H1N1)pdm09 sgp 6B		A(H1N1)pdm09 gp 6		A(H3N2) 3C.3a		A(H3N2) 3C.2a		A(H3N2)		B-Vic [I2221L]		B-Yam 3		B-Vic 1A		Score	Out of	% score
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir			
95	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	NI	NI	NI	RI	NI			
200	RI	NI											HRI	NI							
1159	RI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI			
1402	RI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	NI	RI	NI	HRI	RI			
2125	RI		NI		NI		NI		NI		NI		HRI		NI		NI				
2276	HRI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	HRI	RI	NI	NI	NI	NI			

Above: Phenotypic antiviral susceptibility testing, other assays

HRI: highly reduced inhibition, RI: reduced inhibition, NI: normal inhibition

Participating laboratories are identified by a unique anonymised participant ID code. Oseltamivir and zanamivir interpretations are presented per panel sample for each laboratory that returned results. Shaded cells indicate incorrect or incompletely correct results. Blacked out cells indicate that no result was submitted. The scoring system used was as follows: a maximum of one point was awarded for each sample, irrespective of whether the participating laboratory tested for both oseltamivir and zanamivir, or oseltamivir alone. One point was deducted for each incorrect answer.

Figure 5. Assay platforms used for phenotypic testing



Number of laboratories using each of the assay platforms for phenotypic antiviral susceptibility testing. Blue fill denotes fluorescence-based assays (MUNANA and NA Fluor); black fill denotes chemiluminescence (NA-Star and NA-XTD).

Of a total of 88 samples tested by 12 laboratories with the MUNANA assay, only one error was recorded (1.1% of all tests); five errors were recorded out of a total of 11 samples tested by the two laboratories which used NA Fluor (45.5%); three errors were recorded out of a total of 27 samples tested by the three laboratories which used NA-STAR (11.1%); one laboratory tested a total of nine samples with the NA XTD kit and reported no errors.

As previously stated, one of the three laboratories that used NA-Star encountered errors with a sample that contained a mixed population of NI/HRI viruses. The reduced sensitivity of the NA-Star assay for mixed-phenotype samples had previously been noted.

Genotypic testing for NA gene substitutions

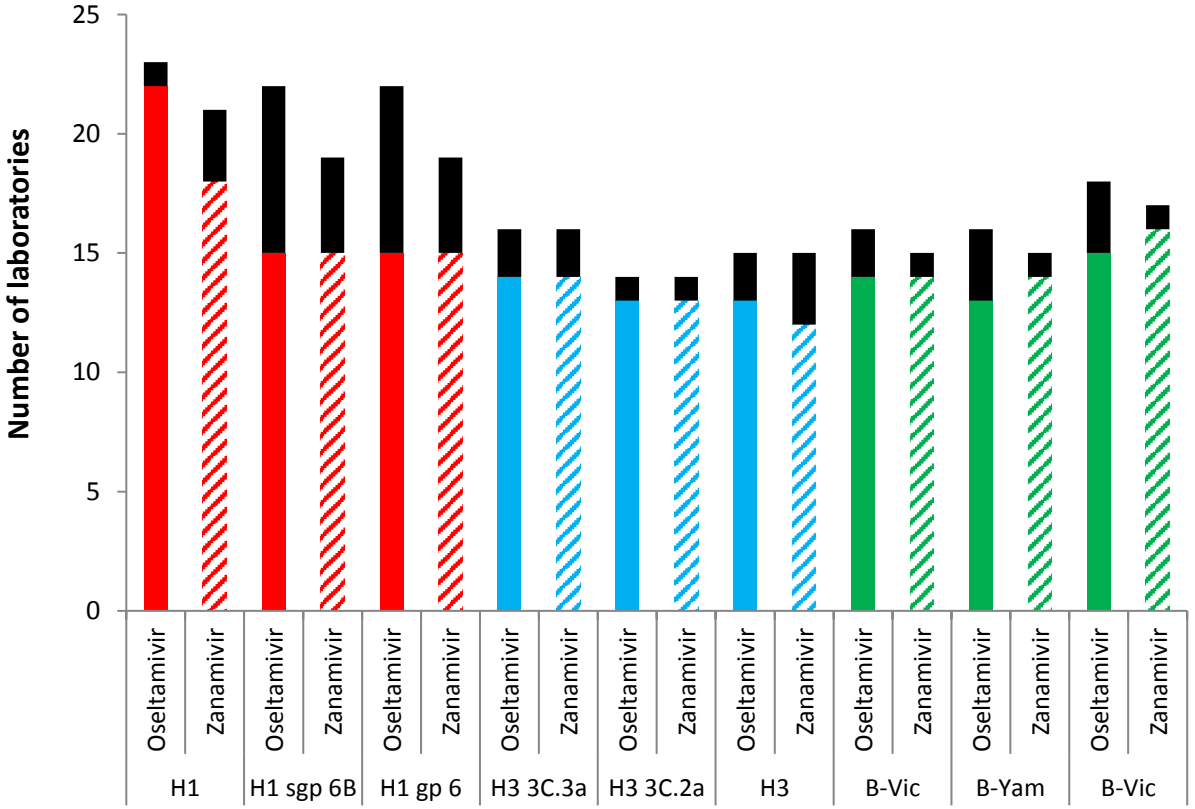
Twenty-four laboratories (100%) participated in at least one aspect of the genotypic testing. A correct genotypic characterisation was defined as results obtained through an appropriate test with appropriate interpretation. Where present, amino acid substitutions associated with reduced inhibition must be correctly identified and reported, which requires an appropriate assay and interpretation.

Participants were not given a score if a result was over-interpreted. For example, the detection of H275 in an A(H1N1)pdm09 sample by single nucleotide polymorphism (SNP) PCR can only be interpreted as 'no interpretation' for oseltamivir susceptibility because we do not know what other amino acid substitutions might be present. Laboratories that only use pyrosequencing or SNP PCR are therefore more restricted in the interpretation of results. Participants were also asked to identify the amino acid substitutions that they considered important for antiviral susceptibility.

Figure 6 provides an overview of the laboratories which reported genetic results for each sample in the EISN-AV15 panel; the figure also indicates whether the antiviral susceptibility interpretation for oseltamivir and zanamivir was correct. Annex 3 shows the raw data by individual laboratory and the technique used to analyse the sample and any amino acid substitutions associated with (highly) reduced inhibition (AARI, AAHRI). If the sample contained no amino acid substitution previously associated with highly reduced (AANI), this is also mentioned.

Approximately 15 laboratories reported antiviral susceptibility data for the influenza A(H3) and influenza B samples. Over 20 laboratories reported data for the influenza A(H1) samples, but the number of errors increased with the number of laboratories which over-interpreted SNP and pyrosequencing results for A(H1N1) samples where no amino acid substitutions associated with (highly) reduced inhibition had been detected.

Figure 6. Genotypic characterisation of antiviral susceptibility per sample in the EISN-AV15 EQA

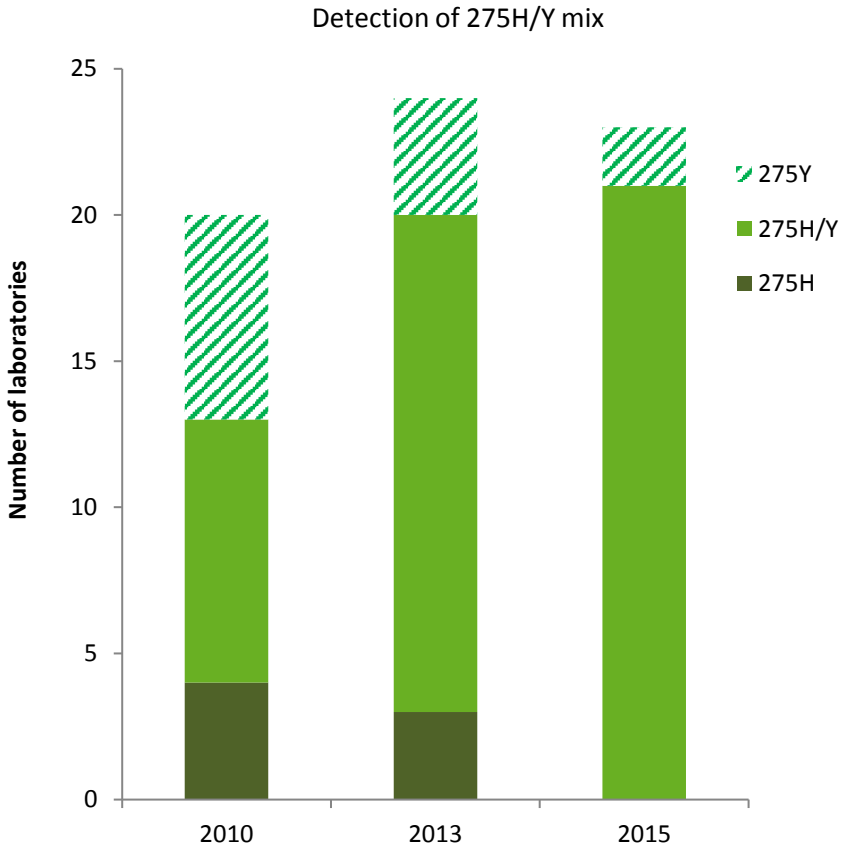


Overview of AV15 genotypic results, with the number of laboratories reporting the correct result depicted in colour and incorrect results shown with black fill. The number of laboratories reporting results for each sample is shown. Samples are divided and colour coded into virus type/subtype. Oseltamivir results are in bold fill and zanamivir results are in hatched fill.

A correct genotypic characterisation is defined as results obtained through an appropriate test with appropriate interpretation. Where present, amino acid substitutions associated with reduced inhibition must be correctly identified.

To determine whether the technical ability of the network has improved, we compared the ability of laboratories to detect NAI-associated nucleotide mutation in a sample containing mixed NI/HRI viruses. The last three EQA panels all contained a similar sample, i.e. a mixed population of A(H1N1)pdm09 virus containing the NAI-associated nucleotide mutation H275Y. The number of laboratories returning results increased from 20 in 2010 to 24 in 2013 and then decreased to 23 in 2015. Figure 7 shows the number of laboratories which detected 275H/Y within a mixed virus population. The percentage of laboratories which detected 275H/Y within a mixed virus population increased from 45% in 2010 to 71% in 2013 and then to 90% in 2015. The proportion of laboratories who were able to detect H275 genotype associated with AANI for this mixed sample dropped from 20% in 2010 to 13% in 2013 and then to 0% by 2015. Together these results suggest that that the quality of genetic analysis has improved over the last three EQA panels.

Figure 7. Comparison of the analysis and interpretation for relatively conserved A(H1N1)pdm09 samples containing mixed populations of NAI NI and RI viruses across three EQA panels: number and proportion of correctly interpreted genotypic results from similar A(H1N1)pdm09 275H/Y samples tested in 2010, 2013 and 2015



Number of laboratories reporting either 275Y or 275H/Y (AARI/AAHRI) or 275H (AANI).
Sample compositions:
2010: A/England/1434/2009 and A/California/7/2009
2013: A/England/428 and A/England/356 mix
2015: A(H1N1)pdm09 mixed 275H/Y

Training

Three wet-laboratory training courses covering the techniques of sequencing and bioinformatics tools were held in November 2010, November 2011 and October 2013. The ability to sequence the NA gene is fundamental to the accurate interpretation of NAI susceptibility; especially in non-A subtypes where SNP and pyrosequencing assays are constrained by more limited interpretations. A wet-laboratory course and a webinar on antiviral susceptibility monitoring were held in July 2011 and January 2015, respectively, and an additional webinar was held in January 2016. Both webinars discussed the results from the 2013 and 2015 EISN-AV EQA panels and included extensive discussions on issues such as over-interpretation of molecular testing results. A full list of all training activities can be found in Annex 5.

Discussion and conclusion

In the 2015 EQA, participating laboratories showed a reasonable level of competence with regard to antiviral susceptibility testing. Twenty-four laboratories participated (out of a potential 38), which represents 63% of the network laboratories invited to join the EQA exercise – an increase compared to 2010.

The panel contained a range of samples encompassing both wild-type strains and samples with amino acid substitutions affecting NAI susceptibility most likely to be encountered during surveillance. The panel also represented a diverse selection of type/subtype, including influenza A(H1N1)pdm09, A(H3N2), B(Victoria) and B(Yamagata). It also included a range of possible phenotypes represented by samples with NI, RI and HRI to both oseltamivir and zanamivir. As participants had the option to use phenotypic and/or genotypic testing, samples for addressing quality for all possible techniques for antiviral susceptibility testing were available in the AV15 EQA panel.

Eighteen laboratories used phenotypic testing on the EQA panel for oseltamivir susceptibility, and seventeen for zanamivir susceptibility; as phenotypic testing can identify novel resistance mechanisms it is encouraging that 75% of the network laboratories returning antiviral susceptibility results can use this testing method.

Neuraminidase enzyme inhibition assays have a second benefit for laboratories because the neuraminidase enzyme activity measuring component of the assay can be used to detect positive virus isolation from cultures, especially with influenza A(H3) specimens that might fail in the traditionally used haemagglutination inhibition assay.

The fact that 18 laboratories used phenotypic testing in 2015 represents an increase from the number of laboratories using phenotypic testing in 2010 (12) and 2013 (17). A number of laboratories only tested the inactivated samples, which were distributed in sufficient quantity for direct analysis, but did not test samples that required prior virus isolation and propagation. In some cases this was due to failed virus isolation whereas in other examples this appeared to be due to specific sample processing workflows in the laboratory.

Overall, phenotypic testing was very accurate (Figure 4), with only a single error recorded for the A(N1N1)pdm09 sample containing a mixed population of NI and HRI viruses. This error was reported by a laboratory using a chemiluminescent-based assay (NA-Star), which may explain the mistake. Fourteen of the 18 laboratories (78%) that performed phenotypic testing used fluorescence-based assays, which are more sensitive at detecting mixed populations of NI and RI viruses within a sample (Figure 5). Seven of the nine phenotypic errors were related to interpretation of influenza B samples, with three errors associated with incorrect interpretation of zanamivir susceptibility for influenza B virus carrying NA I221L mutation. Of the remaining five errors in 'wild-type' samples, one was attributed to an A(H3) sample while the remaining four were in influenza B samples. A combination of incorrect interpretation and IC_{50} fold-changes close to the NI/RI threshold (Annex 1) appears to have caused the errors.

Genotypic analysis was generally of a satisfactory standard. Unlike phenotypic testing, where the number of laboratories returning results remained stable regardless of the virus type, results for genotypic testing fluctuated depending on the virus type. Fifteen to 16 laboratories returned genotypic results for samples that contained the influenza A(H3) and B viruses, slightly more than for phenotypic testing. Even more laboratories (21) returned results for the samples containing influenza A virus, which probably reflects the ease of performing some of the SNP-based assays available for influenza A virus.

Concerns have been raised that the quality of data uploaded to TESSy could be compromised by the over-interpretation of genotypic data. For this reason, strict marking rules were employed to point out earlier over-interpretations.

It is encouraging that only 11% of influenza B and 12% of influenza A(H3N2) virus genotypic results were incorrect. This number increased to 20% for the samples that contained influenza A(H1N1) virus, highlighting the problem of over-interpretation of SNP and partial sequencing assay results.

Two training webinars addressed over-interpretation of genotypic results, particularly when only partial genetic information is available. Although the number of errors in the reported genotypic results is still too high, interpretations seem to have improved compared to 2013. Other training activities delivered through the framework contract addressed sequencing and genetic data analysis (Annex 5), techniques that are essential for successful antiviral analysis by whole or partial gene sequencing. We believe that these training activities contributed towards a general improvement in the network's capability and capacity to perform antiviral testing; however, directly linking training to improvements is always difficult.

As the aim of the first antiviral susceptibility EQA in 2010 differed from later EQAs, it is difficult to compare global genotypic results. In 2010, the focus was on evaluating whether participating laboratories could apply genotypic methods to detect amino acid substitutions affecting NAI susceptibility. In 2013 and 2015, the focus shifted to evaluating the interpretation of the genotypic results; particularly whether participating laboratories had a sound knowledge of the methodological limitations, especially of those methods targeting SNPs as opposed to

full segment length sequencing. Common to both the 2010, 2013 and 2015 exercises was the inclusion of a sample containing a mixture of oseltamivir HRI (H275Y) and NI viruses. A comparison of reporting on this sample type across the three panels provided an indication of how the technical ability of the network has developed (Figure 8). The number of laboratories returning genotypic results for this sample has increased since the first EQA panel. The proportion of laboratories that detected the 275H/Y mixed amino acid substitution increased from 45% (2010) to 71% (2013) and reached 91% in 2015. This is a significant improvement in capability because samples with a (highly) reduced inhibited virus quasispecies are a common scenario for laboratories which receive samples from patients as part of a surveillance programme.

The 2015 EISN-AV EQA panel has provided evidence of the continued improvements in the technical capacity to perform both phenotypic and genotypic antiviral testing across the ERLI-Net network. The quality assessment has confirmed the gradual improvements in the quality of genotypic data reported to the TESSy system.

There is, however, further work required, particularly with regard to the limitations of specific assays and over-interpretation of results. Training courses to address this issue were already held, but need to be repeated within laboratories to ensure a unified and robust level of genotypic interpretation.

Recommendations

Phenotypic characterisation

- Eighteen laboratories utilised phenotypic characterisation; an increase in this number would arguably be beneficial for ERLI-Net. Either method-specific training courses or twinning activities may help increase the number of laboratories using this technique.
- A number of laboratories incorrectly interpreted zanamivir IC_{50} data for samples containing influenza B virus, suggesting that a refresher webinar might be beneficial for data interpretation.

Genotypic characterisation

Two webinar theoretical training courses on genotypic data interpretation and TESSy reporting were held. Improvements have been seen, however the message needs further reinforcement:

- The use of appropriate assays; SNP-based assays may be suitable for screening of A(H1N1)pdm09 because the majority of HRI viruses have H275Y) or A(H3N2) because the majority of HRI viruses have R292K or E119V. SNP is less appropriate for influenza B viruses due to the number of different amino acid substitutions which have been described to impact NAI susceptibility, all of which occur infrequently.
- Interpretation of genotypic data without over-interpretation; if SNP-based data or partial sequencing data are used, interpretations for wild-type viruses are not possible.
- Data reporting; if reporting a HRI/RI virus, the associated amino acid substitutions must also be reported as soon as possible, especially if the sequencing is performed by a third party.

Training courses should be delivered as a webinar training sessions and supplemented by written material on the extranet.

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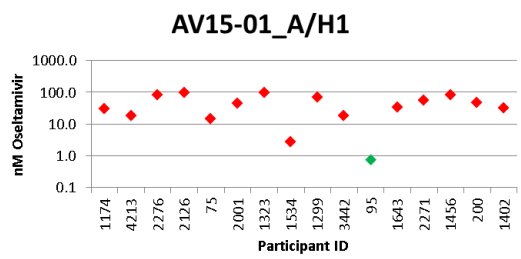
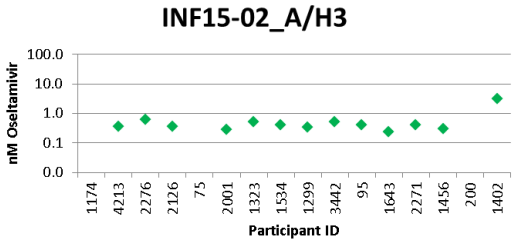
Annex 1. Definition of phenotypic classification

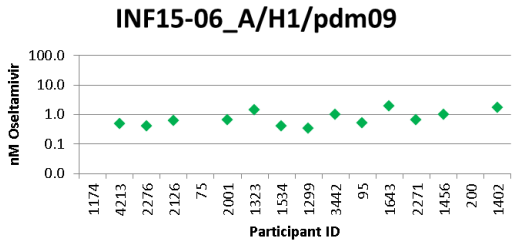
Category	Fold IC_{50} change*	
	Type A viruses	Type B viruses
Normal inhibition (NI)	<10	<5
Reduced inhibition (RI)	10–100	5–50
Highly reduced inhibition (HRI)	>100	>50

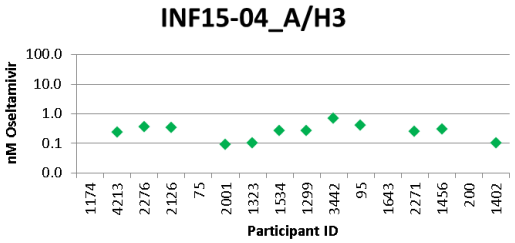
** Fold change against the median/mean IC_{50} for the (sub)type, previous or current season, after removal of obvious outliers, or against a known wild type virus of the same subtype.*

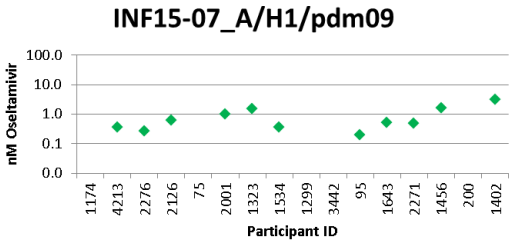
Annex 2A. Oseltamivir phenotypic IC_{50} results presented by laboratories

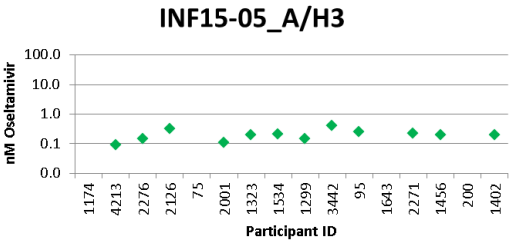


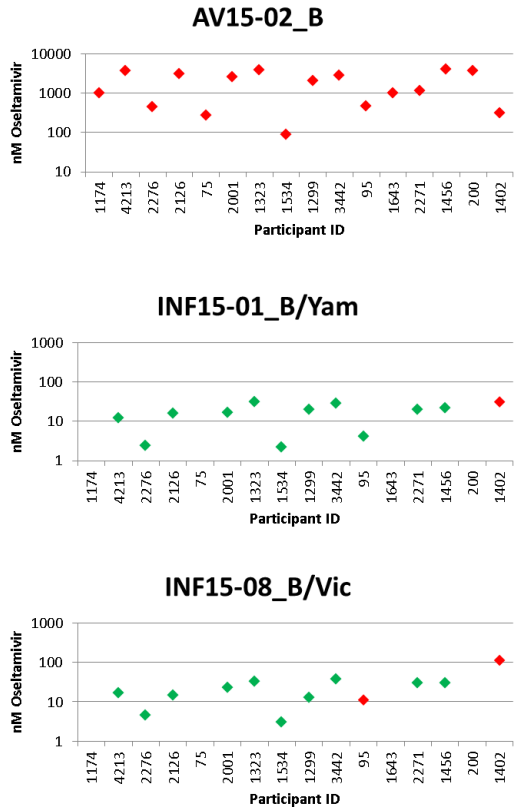






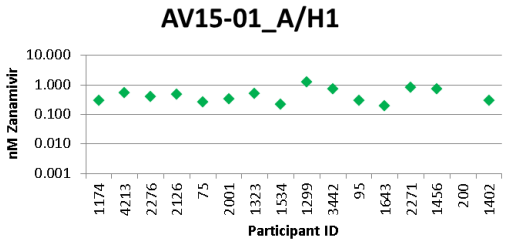


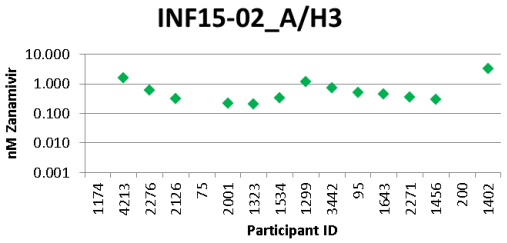


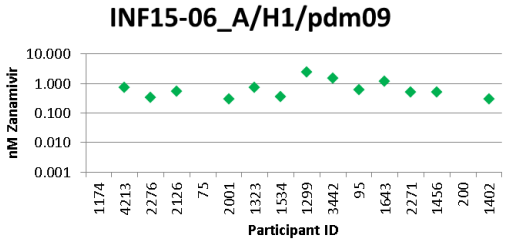


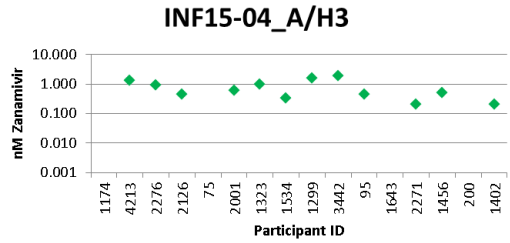
Individual laboratories' IC50 (nM) for each sample of the AV15 panel are shown. Interpretation based on the IC50 in comparison to controls is indicated by the colour of the data point (red; RI/HRI or green; NI).

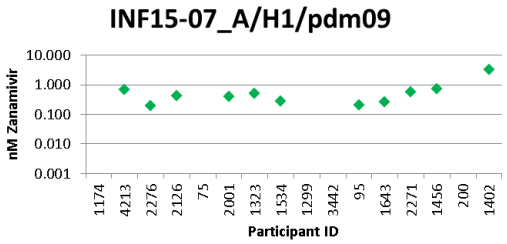
Annex 2B. Zanamivir phenotypic IC_{50} results presented by laboratories

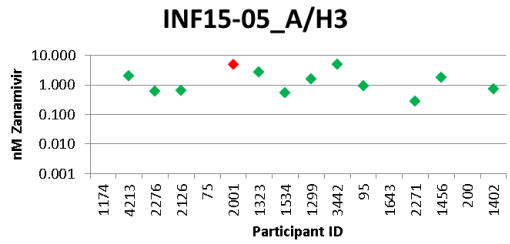


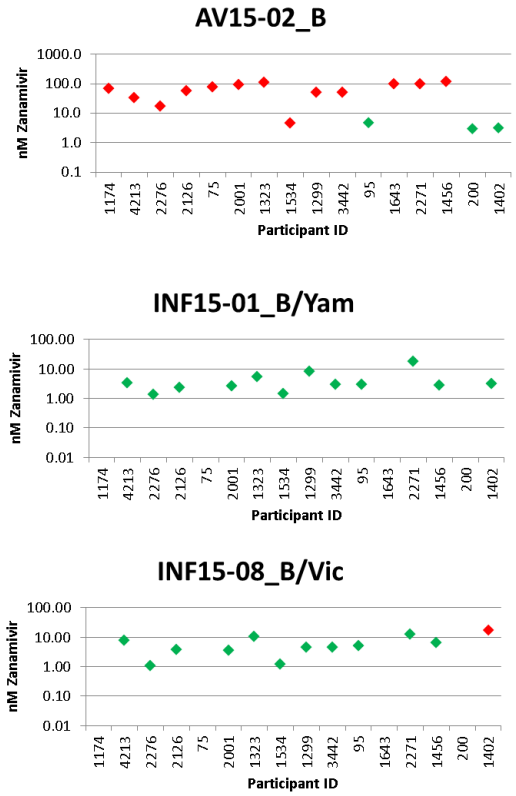












Individual laboratories' IC50 (nM) for each sample of the AV15 panel are shown. Interpretation based on the IC_{50} in comparison to controls is indicated by the colour of the data point (red; RI/HRI or green; NI).

Annex 3A. Genotypic results presented by individual laboratories – influenza A(H1N1)pdm09 samples

Participant ID	EISN_AV15-01					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	117; 119; 136; 151; 155; 199; 223; 247; 275; 293; 295; 313; 334; 427	H275Y	AAHRI	AANI	2	3
95	SNP	275	275H/Y	AARI	No interpretation	3	3
117	SNP	H275Y	H275Y	AAHRI	AANI	1	3
200	SNP, partial gene sequencing	275H/Y and 1-339	275H/Y	AAHRI	AANI	3	3
1159	Full gene sequencing	1-305	275H/Y	AAHRI	AANI	3	3
1174	-		-	-	-		
1262	Full gene sequencing	465	275H/Y	AAHRI	AANI	3	3
1299	SNP		275H/Y	AARI	No interpretation	3	3
1323	Full gene sequencing	14-469	275H/Y	AAHRI	AANI	3	3
1402	Full gene sequencing	0-471 AA	119E, 275Y (mixed H/Y)	AARI	AANI	3	3
1432	Partial gene sequencing	262-320	275H/Y	AAHRI	AANI	3	3
1456	Full gene sequencing	1-457 aa	275H/Y	AAHRI	AANI	3	3
1534	Full gene sequencing, pyrosequencing	Complete NA reading frame, focused on known resistance sites 275	275Y	AAHRI	AANI	2	3
1643	Full gene sequencing	1-462	116V; 119E; 136Q; 155Y; 199D; 223I; 247S; 262K; 275H/Y; 295N	AAHRI	AANI	3	3
2001	SNP, partial gene sequencing	275 position only and range covering all known aa substitutions previously associated with reduced antiviral susceptibility: 19-456	275H/Y	AAHRI	AANI	3	3
2125	Full gene sequencing	1-469	275H/Y	AARI	AANI	3	3
2126	SNP	275 SNP test	H275Y	AAHRI	No interpretation	2	3
2253	SNP		274Y	AAHRI	Not tested	1	2
2271	SNP, full gene sequencing	1-470	275H/Y	AAHRI	AANI	3	3
2272	SNP	H275Y H1N1pdm09	275Y	AAHRI	Not tested	1	2
2276	SNP, full gene sequencing	275 and full gene	mix 275H/Y	AANI	AANI	2	3
2306	Partial gene sequencing	35-462 and 35-462	275 H/Y	AAHRI	AAHRI	2	3
3442	SNP, full gene sequencing	275H; 275Y 11-466	275H/Y	AAHRI	AANI	3	3
4213	SNP	275H	275H/Y	AARI	AANI	2	3

Participant ID	EISN_INF15-06					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	117;119;136;151;155;199;223;247;275;293;295;313;334;427	I117M	AANI	AANI	3	3
95	SNP	275		AANI	No interpretation	2	3
117	SNP	H275Y	None	AANI	AANI	1	3
200	SNP, full gene sequencing	275 1-470		AANI	AANI	3	3
1159	Full gene sequencing	1-305		AANI	AANI	3	3
1174	Full gene sequencing	1-469		AANI	AANI	3	3
1262	Full gene sequencing	465		AANI	AANI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	14-469	None	AANI	AANI	3	3
1402	Full gene sequencing	0-471 AA	119E, 275H	AANI	AANI	3	3
1432	Partial gene sequencing	262-320	None	AANI	AANI	1	3
1456	Full gene sequencing	1-457 aa	None	AANI	AANI	3	3
1534	Full gene sequencing, pyrosequencing	Complete NA reading frame, focused on known resistance sites275	275H	AANI	AANI	3	3
1643	Full gene sequencing	1-462	116V; 119E; 136Q; 155Y; 199D; 223I; 247S; 262K; 275H; 295N	AANI	AANI	3	3
2001	SNP, partial gene sequencing	275 position only and range covering all known aa substitutions previously associated with reduced antiviral susceptibility: 1-345	None	AANI	AANI	3	3
2125	Full gene sequencing	1-469		AANI	Not tested	2	3
2126	-		-	-	-		
2253	SNP		NO 274Y	AANI	Not tested	1	2
2271	SNP, full gene sequencing	1-470		AANI	AANI	3	3
2272	SNP	H275Y H1N1pdm09		AANI	Not tested	1	2
2276	SNP	275		AANI		1	2
2306	Partial gene sequencing	35-462	No	AANI	AANI	3	3
3442	SNP, full gene sequencing	275H; 275Y and 11-466	275H	AANI	AANI	3	3
4213	SNP	275H		AANI	AANI	1	3

Participant ID	EISN_INF15-07					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	117;119;136;151;155;199;223;247;275;293;295;313;334;427	None	AANI	AANI	3	3
95	SNP	275		AANI	No interpretation	2	3
117	SNP	H275Y	None	AANI	AANI	1	3
200	SNP, full gene sequencing	275 1-470		AANI	AANI	3	3
1159	Full gene sequencing	1-305		AANI	AANI	3	3
1174	Full gene sequencing	1-469		AANI	AANI	3	3
1262	Full gene sequencing	465		AANI	AANI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	14-469	None	AANI	AANI	3	3
1402	Full gene sequencing	0-471 AA	119E, 275H	AANI	AANI	3	3
1432	Partial gene sequencing	262-320	None	AANI	AANI	1	3
1456	Full gene sequencing	1-457 aa	None	AANI	AANI	3	3
1534	Full gene sequencing, pyrosequencing	Complete NA reading frame, focused on known resistance sites275	275H	AANI	AANI	3	3
1643	Full gene sequencing	1-462	116V; 119E; 136Q; 155Y; 199D; 223I; 247S; 262K; 275H; 295N	AANI	AANI	3	3
2001	SNP, full gene sequencing	275 position only and 1-468	None	AANI	AANI	3	3
2125	Full gene sequencing	1-469		AANI	Not tested	2	3
2126	-		-	-	-		
2253	SNP		No 274Y	AANI	Not tested	1	2
2271	SNP, full gene sequencing	1-470		AANI	AANI	3	3
2272	SNP	H275Y H1N1pdm09		AANI	Not tested	1	2
2276	SNP	275		AANI		1	2
2306	Partial gene sequencing	35-462	No	AANI	AANI	3	3
3442	SNP, full gene sequencing	275H; 275Y 12-466	275H	AANI	AANI	3	3
4213	SNP	275H		AANI	AANI	1	3

Annex 3B. Genotypic results presented by individual laboratories – influenza A(H3N2)pdm09 samples

Participant ID	EISN_INF15-02					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	119;136;148;151;222;224;245;246;247;248;276;292;294;371	None	AANI	AANI	3	3
95	SNP	119, 292		AANI	No interpretation	2	3
117	-		-	-	-		
200	Full gene sequencing	1-470		AANI	AANI	3	3
1159	Full gene sequencing	1-440		AANI	AANI	3	3
1174	-		-	-	-		
1262	Full gene sequencing	469		AANI	AANI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	15-460	None	AANI	AANI	3	3
1402	Full gene sequencing	0-471 AA	119E, 292R	AANI	AANI	3	3
1432	Partial gene sequencing	15-342	None	AANI	AANI	1	3
1456	Full gene sequencing	1-457 aa	None	AANI	AANI	3	3
1534	Full gene sequencing	Complete NA reading frame, focused on known resistance sites		AANI	AANI	3	3
1643	Full gene sequencing	1-463	119E; 136Q; 151D; 152R; 222I; 224R; 226Q; 276E; 292R; 294N; 371R	AANI	AANI	3	3
2001	SNP,Full gene sequencing	119 and 292 position 1-469	None	AANI	AANI	3	3
2125	Full gene sequencing	1-469		AANI	Not tested	2	3
2126	-		-	-	-		
2253	-		-	-	-		
2271	Full gene sequencing	1-470		AANI	AANI	3	3
2272	-		-	-	-		
2276	Full gene sequencing	Full gene		AANI	AANI	3	3
2306	Full gene sequencing	1-469	No	AANI	AANI	3	3
3442	Full gene sequencing	No PCR amplification		No interpretation	No interpretation		
4213	-		-	-	-		

Participant ID	EISN_INF15-04					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	119; 136; 148; 151; 222; 224; 245; 246; 247; 248; 276; 292; 294; 371	T148K; D151G/N	AANI	AANI	3	3
95	SNP	119, 292		AANI	No interpretation	2	3
117	-		-	-	-		
200	Full gene sequencing	1-469		AANI	AANI	3	3
1159	Full gene sequencing	1-440		AANI	AANI	3	3
1174	-		-	-	-		
1262	Full gene sequencing	469	148K	AANI	AANI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	15-460	None	AANI	AANI	3	3
1402	Full gene sequencing	0-471 AA	119E, 292R	AANI	AANI	3	3
1432	Partial gene sequencing	None; sample did not amplify		No interpretation	No interpretation		
1456	Full gene sequencing	1-457 aa	None	AANI	AANI	3	3
1534	Full gene sequencing	Complete NA reading frame, focused on known resistance sites		AANI	AANI	3	3
1643	Full gene sequencing	1-463	119E; 136Q; 151D; 152R; 222I; 224R; 226Q; 276E; 292R; 294N; 371R	AANI	AANI	3	3
2001	SNP, full gene sequencing	119 and 292 position and 1-469	None	AANI	AANI	3	3
2125	Full gene sequencing	1-469		AANI	Not tested	2	3
2126	-		-	-	-		
2253	-		-	-	-		
2271	Full gene sequencing	1-470		AANI	AANI	3	3
2272	-		-	-	-		
2276	-		-	-	-		
2306	Full gene sequencing	1-469	No	AANI	AANI	3	3
3442	Full gene sequencing	No PCR amplification		No interpretation	No interpretation		
4213	-		-	-	-		

Participant ID	EISN_INF15-05					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	119; 136; 148; 151; 222; 224; 245; 246; 247; 248; 276; 292; 294; 371	T148I; D151G/N	AANI	AANI	3	3
95	SNP	119, 292		AANI	No interpretation	2	3
117	-		-	-	-		
200	Partial gene sequencing	1-343		AANI	AANI	1	3
1159	Full gene sequencing	1-440		AANI	AANI	3	3
1174	-		-	-	-		
1262	Full gene sequencing	469	148I	AANI	AANI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	15-460	None	AANI	AANI	3	3
1402	Full gene sequencing	0-471 AA	119E, 292R,	AANI	AANI	3	3
1432	Partial gene sequencing	None; sample did not amplify		No interpretation	No interpretation		
1456	Full gene sequencing	1-457 aa	148T/I	AANI	AARI	2	3
1534	Full gene sequencing	Complete NA reading frame, focused on known resistance sites		AANI	AANI	3	3
1643	Full gene sequencing	1-463	119E; 136Q; 151D; 152R; 222I; 224R; 226Q; 276E; 292R; 294N; 371R	AANI	AANI	3	3
2001	SNP,Full gene sequencing	119 and 292 position and 1-469	None	AANI	AANI	3	3
2125	Full gene sequencing	1-469		AANI	Not tested	2	3
2126	-		-	-	-		
2253	-		-	-	-		
2271	Full gene sequencing	1-470		AANI	AANI	3	3
2272	-		-	-	-		
2276	-		-	-	-		
2306	Full gene sequencing	1-469	No	AANI	AANI	3	3
3442	Full gene sequencing	8-469		AANI	AANI	3	3
4213	-		-	-	-		

Annex 3C. Genotypic results presented by individual laboratories – influenza B samples

Participant ID	EISN_AV15-02					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	105; 117; 138; 139; 140; 144; 145; 150; 197; 200; 221; 245; 273; 292; 294; 360; 374; 395; 407; 432	I221L	AAHRI	AARI	3	3
95	SNP	150, 197, 294					
117	-		-	-	-		
200	Full gene sequencing	1-467		AANI	AANI	0	3
1159	Full gene sequencing	1-430 and 1-430	I221L	AAHRI	AARI	3	3
1174	-		-	-	-		
1262	Full gene sequencing	466	221L	AAHRI	AARI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	1-498	221L	AAHRI	AARI	3	3
1402	Full gene sequencing	0-467 AA	150R, 197D, 221L	AAHRI	AARI	3	3
1432	Partial gene sequencing	17-336	221L	AAHRI	AARI	3	3
1456	Partial gene sequencing	88-244 aa	I221L	AAHRI	AARI	3	3
1534	Full gene sequencing	Complete NA reading frame, focused on known resistance sites	221L	AAHRI	AARI	3	3
1643	Full gene sequencing	1-493	117E; 150R; 197D; 221L; 292R; 294N; 374R; 407G	AAHRI	AARI	3	3
2001	Partial gene sequencing	Range covering all known aa substitutions previously associated with reduced antiviral susceptibility: 111-431	I221L	AAHRI	AARI	3	3
2125	Partial gene sequencing	42-191		AANI	Not tested	0	2
2126	SNP	152, 198 and 222 SNP tests	R152; D198; I222L	AAHRI	AARI	3	3
2253	-		-	-	-		
2271	Full gene sequencing	1- 466	I221L	AAHRI	AARI	3	3
2272	-		-	-	-		
2276	-		-	-	-		
2306	Full gene sequencing	1-466	221L	AAHRI	AAHRI	3	3
3442	Full gene sequencing	1-467	I221L	AAHRI	AARI	3	3
4213	-		-	-	-		

Participant ID	EISN_INF15-01					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	105; 117; 138; 139; 140; 144; 145; 150; 197; 200; 221; 245; 273; 292; 294; 360; 374; 395; 407; 432	None	AANI	AANI	3	3
95	SNP	150, 197, 294		AANI	No inter-pretation	2	3
117	-		-	-	-		
200	Full gene sequencing	1-467		AANI	AANI	3	3
1159	Full gene sequencing	1-430 and 1-430		AANI	AANI	3	3
1174	Partial gene sequencing	1-305		No interpretation	No interpretation		
1262	Full gene sequencing	466		AANI	AANI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	1-498	None	AANI	AANI	3	3
1402	Full gene sequencing	0-467 AA	150R, 197D, 221I	AANI	AANI	3	3
1432	Partial gene sequencing	17-197	None	AANI	AANI	1	3
1456	Full gene sequencing	1-467 aa	None	AANI	AANI	3	3
1534	Full gene sequencing	Complete NA reading frame, focused on known resistance sites		AANI	AANI	3	3
1643	Full gene sequencing	1-493	117E; 150R; 197D; 221I; 292R; 294N; 374R; 407G	AANI	AANI	3	3
2001	Partial gene sequencing	Range covering all known aa substitutions previously associated with reduced antiviral susceptibility: 111-431	None	AANI	AANI	3	3
2125	Partial gene sequencing	42-191		AANI	Not tested	0	2
2126	-		-	-	-		
2253	-		-	-	-		
2271	Full gene sequencing	1- 466		AANI	AANI	3	3
2272	-		-	-	-		
2276	-		-	-	-		
2306	Full gene sequencing	1-466	No	AANI	AANI	3	3
3442	Full gene sequencing	1-467		AANI	AANI	3	3
4213	-		-	-	-		

Participant ID	EISN_INF15-08					Score	
	Method	NucAA range	NA mutation	Oseltamivir	Zanamivir	points	out of
75	Full gene sequencing	105; 117; 138; 139; 140; 144; 145; 150; 197; 200; 221; 245; 273; 292; 294; 360; 374; 395; 407; 432	None	AANI	AANI	3	3
95	SNP	150, 197, 294		AANI	No interpretation	2	3
117	-		-	-	-		
200	Full gene sequencing	1-467		AANI	AANI	3	3
1159	Full gene sequencing	1-430		AANI	AANI	3	3
1174	Full gene sequencing	1-466		AANI	AANI	3	3
1262	Full gene sequencing	466		AANI	AANI	3	3
1299	-		-	-	-		
1323	Full gene sequencing	1-498	None	AANI	AANI	3	3
1402	Full gene sequencing	0-467 AA	150R, 197D, 221I	AANI	AANI	3	3
1432	Partial gene sequencing	17-333	None	AANI	AANI	1	3
1456	Full gene sequencing	1-449 aa	None	AANI	AANI	3	3
1534	Full gene sequencing	Complete NA reading frame, focused on known resistance sites		AANI	AANI	3	3
1643	Full gene sequencing	1-493	117E; 150R; 197D; 221I; 292R; 294N; 374R; 407G	AANI	AANI	3	3
2001	Partial Gene sequencing	Range covering all known aa substitutions previously associated with reduced antiviral susceptibility: 111-431	None	AANI	AANI	3	3
2125	Partial gene sequencing	42-191		AANI	Not tested	1	2
2126	-		-	-	-		
2253	-		-	-	-		
2271	Full gene sequencing	1-466		AANI	AANI	3	3
2272	-		-	-	-		
2276	Full gene sequencing	Full gene		AANI	AANI	3	3
2306	Full gene sequencing	1-466	No	AANI	AANI	3	3
3442	Full gene sequencing	17-467		AANI	AANI	3	3
4213	-		-	-	-		

Participating laboratories are identified by a unique anonymised participant ID code. Participants' genotypic assay types are categorised as 'Sequencing', 'partial gene sequencing', 'SNP/Pyro' or both SNP and sequencing 'Both'. Oseltamivir and zanamivir interpretations are presented per panel sample for each laboratory that returned results. Shaded cells indicate incorrect result. Scores per sample are indicated to the right of each drug interpretation column.

Annex 4. List of participating laboratories

Austria	Vienna	AKH Wien – Medical University of Vienna
Belgium	Brussels	Institute of Public Health
Czech Republic	Prague	National Institute of Public Health
Denmark	Copenhagen	Statens Serum Institute
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
Ireland	Dublin	University College Dublin
Italy	Rome	Istituto Superiore di Sanita (NIH)
Latvia	Riga	National Microbiology Reference Laboratory, NIC of Latvia
Netherlands	Bilthoven	RIVM
Netherlands	Rotterdam	Erasmus MC
Norway	Oslo	Norwegian Institute of Public Health
Portugal	Lisbon	Instituto Nacional de Saúde Doutor Ricardo Jorge
Romania	Bucharest	Cantacuzino Institute
Spain	Madrid	Instituto de Salud Carlos III
Spain	Barcelona	Hospital Clinic i Provincial de Barcelona
Sweden	Solna	Folkhälsomyndigheten
United Kingdom	London	Public Health England
United Kingdom	Glasgow	Gartnavel General Hospital

Annex 5. List of training activities

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.*
Countries that expressed an interest and received the video of the presentation are marked with an asterisk (*).

Countries with access to the ECDC extranet can view the recorded presentations online.

European Centre for Disease Prevention and Control (ECDC)

Postal address:
Granits väg 8, SE-171 65 Solna, Sweden

Visiting address:
Tomtebodavägen 11A, SE-171 65 Solna, Sweden

Tel. +46 858601000
Fax +46 858601001
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