ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

SURVEILLANCE & MONITORING



European SARS-CoV-2 and influenza virus Bioinformatics External Quality Assessment (ESIB-EQA), 2025

ECDC SURVEILLANCE & MONITORING

European SARS-CoV-2 and influenza virus Bioinformatics External Quality Assessment (ESIB-EQA), 2025



This report was commissioned by the European Centre for Disease Prevention and Control (ECDC) under the framework contract ECDC/2022/001 and specific contract No.11 (request for services RS/2023/DPR/26559), coordinated by Olov Svartström and Eeva Broberg, and produced by AURORAE Consortium.

Authors
Samar Berreira, Jérôme Bourret, Kévin Da Silva, Maïwen Demeulle, and Frédéric Lemoine (National Reference Center for Respiratory viruses, Institut Pasteur, Paris, France);

Florian Zwagemaker, DGino Raaijmakers, Iveta Kršková, and Ivo Van Walle (National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands).

Suggested citation: European Centre for Disease Prevention and Control. European SARS-CoV-2 and influenza virus Bioinformatics External Quality Assessment (ESIB-EQA), 2025. Stockholm: ECDC; 2026.

Stockholm, November 2026

ISBN 978-92-9498-943-7
doi: 10.2900/4674278
Catalogue number TQ-01-26-088-EN-N

© European Centre for Disease Prevention and Control, 2026

Reproduction is authorised, provided the source is acknowledged.

Contents

Abbreviations....iv
Executive summary....1
1 Introduction....2
2 Study design and methods....4
Participants and process....4
Throughput time....4
Quality control....4
Consensus sequence generation....4
Clustering and classification....6
SARS-CoV-2 mutation detection....7
Influenza mutation detection and prediction of susceptibility to selected antivirals....8
3 Results....9
Participation....9
Throughput time....9
Quality control....10
Consensus sequence generation....11
SARS1 and SARS2 components....11
INFL1 and INFL2 components....12
Clustering and classification....13
SARS-CoV-2 mutation detection....13
Influenza virus susceptibility to antivirals prediction....13
4 Discussion....14
Throughput time....14
Quality control....14
Consensus sequence generation....14
SARS1 and SARS2 components....14
INFL1 and INFL2 components....14
Clustering and classification....15
SARS-CoV-2 mutation detection....15
Influenza virus susceptibility to antivirals prediction....15
Feedback from laboratories....15
5 Conclusions....16
6 Recommendations....17
References....19
Annex 1. Participating laboratories....21
Annex 2. Composition of the datasets....22

Tables

Table 1. Independent EQA components to which the laboratories could choose to participate....3
Table 2. Types of differences between the SARS-CoV-2 comparator sequence and the reported consensus sequences that were individually assessed....5
Table 3. Types of differences between the influenza virus comparator sequence and the consensus sequences that were individually assessed....6
Table 4. SARS-CoV-2 amino acid substitutions in the S-gene (S) that should be detected for the SARS4 component, by lineage....7
Table 5. Participation and completion of individual components by all laboratories that participated in at least one component....9
Table 6. Number of samples for which the quality assessment was identical (OK/OK or BAD/BAD) between comparator sequence/laboratory provided value, and number of samples where the assessment was different (OK/BAD or BAD/OK). (The latter two should ideally have zero samples.)....11
Table 7. Number and type of critical sequence differences versus the comparator sequence for SARS1 and SARS2, excluding BAD quality sequences and including educational samples....11
Table 8. Likely causes of different cluster assignments for the SARS3 component....13
Table 9. Recommendations based on this, third, ESIB-EQA (2025)....17

Figures

Figure 1. Phylogenetic tree of SARS3 samples, excluding BAD quality samples....7
Figure 2. Number of participating laboratories in the EU/EEA, the Western Balkan and Türkiye....9
Figure 3. Total and adjusted throughput time for the different components....10
Figure 4. Average number of sequence differences per sample for all OK quality, non-educational samples as compared to the comparator sequence....12

Abbreviations

CDS	Coding Sequence
COVID-19	Coronavirus disease 2019
DRM	Drug Resistance Mutation
EQA	External Quality Assessment
GR	Wuhan-1 global reference
GS	Comparator Sequence
HRI	Highly Reduced Inhibition
IAV	Influenza A virus
INDEL	Insertion/Deletion
INFL1-4	Influenza components 1 to 4
IQR	Inter-Quartile Range
NI	Normal Inhibition
NA	Influenza neuraminidase protein
PA	One of three influenza virus polymerase proteins
PB1	One of three influenza virus polymerase proteins
PB2	One of three influenza virus polymerase proteins
OCPs	Operational Contact Points
ORF	Open Reading Frame
RI	Reduced inhibition
RIVM	National Institute for Public Health and the Environment, the Netherlands
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SARS1-4	SARS-CoV-2 components 1 to 4
SNP	Single nucleotide polymorphism
S-gene	SARS-CoV-2 spike gene

Executive summary

SARS-CoV-2 and influenza viruses are significant public health threats with varying impacts on global health. SARS-CoV-2, the virus behind the COVID-19 pandemic, exhibits high transmissibility where variants such as Beta, Delta, and Omicron emerged and caused epidemiological waves. Seasonal influenza continues to cause significant illness and death worldwide, emphasising the importance of ongoing research and surveillance to improve prevention and control. Both SARS-CoV-2 and influenza challenge public health efforts due to their continuous evolution, necessitating ongoing surveillance, vaccine development, and strategic response measures.

Surveillance and Laboratory Networks: Surveillance for SARS-CoV-2 and influenza involves data collection, analysis, and interpretation to guide public health actions. The European Centre for Disease Prevention and Control (ECDC) sets surveillance objectives including monitoring epidemiological, clinical and virological changes and characteristics as well as assessing burden of disease and interventions. External Quality Assessment (EQA) programs support these objectives by ensuring laboratory accuracy and reliability.

ECDC coordinates the laboratory networks of the European Respiratory Viruses Network (ERVI-Net) which covers virological surveillance, laboratory capacity, and data sharing across Europe. These networks play a pivotal role in case detection, confirmation, and characterisation, contributing to effective public health responses.

Bioinformatics EQA: The ESIB-EQA assesses the capability of laboratories in public health bioinformatics for SARS-CoV-2 and influenza virus. It aims to provide troubleshooting advice and improve quality through training activities. This third iteration, conducted in 2025, focused on consensus sequence generation and quality control for both viruses, revealing several areas for improvement in laboratory practices.

Key findings

- **Consensus sequence generation**:
  - SARS-CoV-2: The main problem was over-reliance on the global (Wuhan-Hu-1) reference. When the comparator has a true deletion, some laboratories have put the reference bases back. Wrong nucleotides also appeared where bases were taken from the reference instead of the reads or from errors in or near primer regions. The primer-region errors point to incomplete primer trimming.
  - Influenza: Consensus generation was again harder than for SARS-CoV-2. The drivers of errors were: references covering only the coding sequence, so segment ends were truncated and scored as deletions; unmasked primers; wrong reference selection, the swine H1N2 sample read as an H1N1/H3N2 co-infection; defective viral genomes, which need manual curation.
- **Quality assessment**: Most samples were classified correctly. Co-infections were the main reason for sequences incorrectly being labelled 'OK'.
- **Clustering and classification**: The leading causes of error were: not inspecting the multiple sequence alignment, so that polymorphic positions masked by Ns were missed; indels ignored; wrong mutation cut-off; and the use of only a subset of the segments (HA/NA only).
- **Mutation detection and antiviral susceptibility**: The SARS-CoV-2 component revealed no systematic bioinformatic problem, but ambiguity codes were rarely taken into account. For influenza antiviral prediction, resistance was usually correctly reported. Discrepancies arose from interpreting World Health Organization (WHO) reference intervals against the >3-fold threshold, and from the H1N2sw sample.

Participation and feedback

- Participation: 20 of 30 EU/EEA Member States took part, as did two of six Western Balkan countries and Türkiye. This is a good level for a third iteration.
- Returning laboratories: 56% of laboratories had joined both earlier rounds, 36% one of them, and 8% were new.
- Feedback: Only two laboratories returned the questionnaire. Both were satisfied with the organisation and usefulness, but it is impossible to draw broader conclusions from just two responses.

Recommendations

- **Recurring issues**: Nine of the 14 recommendations were already made in the 2023 and 2024 reports. They cover indel handling, primer removal, ambiguity codes, coverage thresholds, indels in clustering and software versions.
- **For laboratories**: Avoid indels, and filled-in sequence at the genome ends, that come from alignment to a reference. Validate primer trimming. Report non-N ambiguity codes and use them in quality control. For influenza, use complete, up-to-date reference sets that include non-coding regions, and curate defective genomes manually. Inspect the multiple sequence alignment before clustering.
- **For ECDC**: Clearer guidance is needed on clustering cut-offs and on how to read antiviral susceptibility intervals.
- **Training**: Laboratories that struggle should be offered targeted training with emphasis on the recurring issues, and training can help close the identified gaps.

1 Introduction

SARS-CoV-2 and influenza viruses are pathogens capable of major public health impact [1-4]. These viruses pose a significant threat to global health, but their impact varies in terms of transmission, severity, and response measures. SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has demonstrated high transmissibility, leading to widespread outbreaks and a significant burden on healthcare systems worldwide. As of yet, there is little evidence of a seasonal pattern for SARS-CoV-2; instead new variants replace circulating ones [5] and their public health risk needs to be evaluated. Influenza viruses are responsible for seasonal outbreaks that can strain healthcare systems and lead to economic losses and increased work and school absenteeism. New strains of influenza also emerge, such as the A(H3N2) subclade K with a potential antigenic mismatch with the vaccine components of the 2025–2026 season [6]. While the impact of influenza and SARS-CoV-2 on populations are mitigated by vaccine development efforts, the continuous evolution of both viruses poses challenges for effective prevention and control efforts.

Understanding the public health impact of both SARS-CoV-2 and influenza is crucial for informing policy decisions, resource allocation, and the development of effective strategies to mitigate their respective impacts on population health. In this area, surveillance plays a crucial role in monitoring the spread and impact of SARS-CoV-2 and influenza [7]. It involves the collection, analysis, and interpretation of data to inform public health actions.

The European Centre for Disease Prevention and Control (ECDC) recognises the importance of disease surveillance and has set three overarching objectives to address respiratory viruses [8]: 1) Monitor epidemiological characteristics in time, person and place of acute respiratory illness due to influenza viruses, SARS-CoV-2, RSV and other respiratory pathogens to detect changes that can inform national and EU-level prevention and control measures; 2) Monitor virological changes and characteristics of circulating and emerging influenza, SARS-CoV-2 and RSV viruses to detect changes that can inform national and EU-level prevention and control measures; 3) Assess the burden of disease and the effectiveness and impact of interventions against acute respiratory illness due to influenza viruses, SARS-CoV-2, RSV and other respiratory pathogens.

External Quality Assessment (EQA) programs are designed to assess and improve the performance of laboratories involved in disease surveillance. These programs have specific objectives that align with and contribute to ECDC's surveillance objectives. The primary objectives of EQAs include evaluating the accuracy and reliability of laboratory data analysis methods and identifying areas for improvement. By ensuring standardised and high-quality laboratory testing, EQA programs aim to optimise laboratory performance and ultimately enhance the overall surveillance efforts. As part of this strategy, ECDC concluded a framework contract through public procurement with target to provide laboratory support for surveillance, preparedness and response to COVID-19 and influenza, and to specifically strengthen the capacity for genomic epidemiology and public health bioinformatics through interdisciplinary trainings. All these activities are crucial for response during a pandemic or unexpected major public health events. The geographic scope of these activities covers European Union/European Economic Area (EU/EEA) countries, the Western Balkans (Albania, Kosovo*, Montenegro, Serbia, North Macedonia, Bosnia and Herzegovina) and Türkiye.

In order to effectively monitor and respond to the public health impact of SARS-CoV-2 and influenza, a robust laboratory network is crucial. ECDC coordinates ERVI-Net with the aim of carrying out virological surveillance and strengthen laboratory capacity as well as foster data sharing for example through external quality assessments (EQAs) [9,10]. These networks comprise laboratories across Europe, which play a pivotal role in supporting disease surveillance by contributing to the detection, confirmation, and characterisation of cases. Through their coordinated efforts, this laboratory networks strengthen the surveillance infrastructure and enable the generation of accurate and reliable data for effective public health response.

The main objectives of this bioinformatics EQA are (i) to assess the capability of participating laboratories for public health bioinformatics for SARS-CoV-2 and influenza; and (ii) to provide troubleshooting advice where needed and possible. Larger needs for improving quality are expected to be fulfilled through other training activities.

This bioinformatics EQA consisted of several components designed to each independently test one particular type of analysis for SARS-CoV-2 and influenza virus, as described in Table 1. The influenza virus components were managed by Institut Pasteur and the SARS-CoV-2 components by the Dutch National Institute for Public Health and the Environment (RIVM). This is the third iteration of this bioinformatics EQA, the previous ones being executed in 2023 and 2024 [11,12]. While the 2024 influenza part of the EQA was set up using scenarios specifically for viruses of zoonotic origin, the 2025 iteration was set up analogously to 2023 for both SARS-CoV-2 and seasonal influenza viruses.

* This designation is without prejudice to positions on status, and is in line with UNSCR 1244/1999 and the ICJ Opinion on the Kosovo declaration of independence.

Table 1. Independent EQA components to which the laboratories could choose to participate

Name	Organism	Samples	Description
SARS1	SARS-CoV-2	12	Consensus sequence generation from complete amplicons based on Nanopore reads.
SARS2	SARS-CoV-2	12	Consensus sequence generation from fragmented amplicons based on Illumina reads.
SARS3	SARS-CoV-2	20	Clustering and classification of full-length genomes.
SARS4	SARS-CoV-2	10	Detection of amino-acid substitutions of particular interest.
INFL1	Influenza virus	12	Consensus sequence generation from complete amplicons based on Nanopore reads.
INFL2	Influenza virus	12	Consensus sequence generation from fragmented amplicons based on Illumina reads.
INFL3	Influenza virus	20	Clustering and classification of full-length genomes.
INFL4	Influenza virus	11	Prediction of reduced susceptibility to antivirals.

2 Study design and methods

Participants and process

The invitation for the EQA was sent to ECDC's Operational Contact Points for COVID-19, for influenza and for bioinformatics, with ECDC's National Focal Points for Viral Respiratory Diseases and National Microbiology Focal Points (NMFPs) in copy. These contact points cover the 27 European Union Member States as well as the three European Economic Area countries. In addition, the Western Balkan countries and Türkiye were contacted by ECDC's Country Cooperation Office.

For both SARS-CoV-2 and influenza virus, each component had its own dataset consisting of several samples of which data could be downloaded by participants, processed, and the results provided back in an Excel template. Sequence data could also be provided in Fasta format. In addition to the assessment of the particular type of analysis for which the component was designed, two general aspects were assessed as well for each component: quality control of the input data for the analysis, and the throughput time of the analysis. The methodology used for all these aspects is described in the sections below.

For all SARS-CoV-2 and influenza virus components, a comparator sequence was prepared for each sample. The discrepancies were aggregated and compared to the methodology provided by the laboratory, to try to identify concrete methodological issues such as inappropriate parameter settings. No criteria were applied to classify the laboratory as having passed or failed each EQA component. Instead, the focus was on providing useful information for improvement, by highlighting potential issues and potential causes.

Upon conclusion of the allotted timeframe for result submission, individual reports were provided to laboratories, featuring an assessment of their results and anonymised comparisons with peer laboratory data. Laboratories were subsequently given the opportunity to discuss the report with the organisers, and finally feedback could be given through a questionnaire that allowed participants the option of remaining anonymous.

Throughput time

The total throughput time needed to process the component was measured for educational purposes only, from the moment the password was provided to decrypt the dataset for a particular component until the moment the results for that component were submitted. An adjusted version of the throughput time was calculated to better reflect actual throughput time by subtracting non-working hours from the total throughput time. The latter included weekend days as a whole and outside 8–18h on working days. In addition, when passwords were initially provided and/or results were submitted for two or more components at the same time, the corresponding throughput time was generally excluded from statistics on adjusted throughput time, since it no longer represents the throughput time for a single component.

Quality control

Quality control is an essential element of each step in a bioinformatic analysis. Analysing low-quality sequencing datasets often leads to misleading results such as missing mutations or unjustified clusters. In addition, the results from processed sequence reads can also be of bad quality because of the workflow used, and would then affect any downstream analyses. Each dataset therefore contained some clearly bad quality samples, and participants needed to indicate for each sample whether the quality was either OK or BAD.

For components 1 and 2 of SARS and INFL, the overall quality control needed to be carried out on the output genome sequences. For INFL1/2 components, all segments needed to be of OK quality for the entire genome to be OK. For SARS3 and INFL3, the overall quality control needed to be carried out on the input genome sequences. For SARS4 and INFL4, the specific quality control needed to be carried out on, respectively, the S-gene sequence and the HA, NA and PA input gene sequences.

Consensus sequence generation

The purpose of the SARS1/2 and INFL1/2 components (Table 1) are to assess the generation of full-length SARS-CoV-2 and influenza virus genomes from amplicons sequenced by respectively the Nanopore platform (SARS1 and INFL1 components) and the Illumina platform (SARS2 and INFL2 components), by comparing them with a comparator sequence for each sample in the dataset. The methodology for generating the comparator sequences and the composition of the respective datasets is described per component in the sections below.

SARS1 and SARS2 components

Two consensus sequences were generated (a strict and a less strict), corresponding to a high and a low coverage threshold. This was done using the ViroConstrictor pipeline v1.5.2 [13] with a modified version of TrueConsense specific for this EQA. The Nanopore reads were created with R10 flowcells and base calling was done with the High-Accuracy preset of Guppy [14], the high and low coverage thresholds were set to respectively 50x and 20x,

and for Illumina reads to 20x and 10x. Ambiguity symbols representing two nucleotides were put when coverage was sufficiently high and the difference between the most frequent and the second most frequent nucleotide was at most 10% or 20% respectively for the strict and the less strict consensus genome.

Low coverage regions were assigned Ns and subsequently manually curated by an expert used to perform curation in a routine context for SARS-CoV-2 national surveillance. A comparator sequence was created from that which allows, at each sequence position, both the strict and the less strict version. As such, an acceptable range of variation in the consensus sequence was created to reflect the fact that there are no exact criteria for defining correctness of a sequence in routine application. In addition, the sequence regions upstream and downstream the first/last primer were set to allow both N and gaps, and positions where an ambiguity symbol (not N) was allowed were also set to allow N.

The genome sequences returned by the participants were compared to the comparator sequence, and differences found were classified into eight types of differences listed in Table 2. Among these eight types, insertions, deletions and wrong nucleotides were considered critical differences since they are errors rather than reducing the information possible to extract. The SARS1, SARS2 datasets consisted of 12 samples, that were selected as described in Table A2.1 and Table A2.2.

Table 2. Types of differences between the SARS-CoV-2 comparator sequence and the reported consensus sequences that were individually assessed

Type of difference	Description
Insertion	An insertion was introduced versus the comparator sequence. The entire insertion is counted as a single difference.
Deletion	A deletion was introduced versus the comparator sequence. The entire deletion is counted as a single difference.
Wrong nucleotide	A different nucleotide or ambiguity symbol (other than N) than the one allowed was provided, or, in case an ambiguity symbol (not N) was allowed, the provided nucleotide is not represented by it.
Nucleotide instead of ambiguity	An actual nucleotide was provided where only an ambiguity symbol (other than N) was allowed.
Ambiguity instead of nucleotide	An ambiguity symbol (other than N) was provided where only an actual nucleotide was allowed.
Nucleotide stretch instead of stretch of Ns	A stretch of actual nucleotides or ambiguity (other than N) symbols was provided for a particular region (including a region of length 1), where only Ns were allowed. The entire stretch is counted as a single difference.
Stretch of Ns instead of nucleotide stretch	A stretch of Ns was provided for a particular region (including a region of length 1), where only actual nucleotides or ambiguity (other than N) symbols were allowed. The entire stretch is counted as a single difference.
Nucleotide stretch outside amplicon range	A stretch of actual nucleotides or ambiguity (other than N) symbols was provided before the first amplicon or after the last amplicon, instead of Ns or gaps. The entire stretch is counted as a single difference.

The types of differences are sorted in decreasing order of overall impact on the outcome. Insertions, deletions and wrong nucleotides are considered critical differences.

INFL1 and INFL2 components

The purpose of these two components was to assess the quality of the generation of full-length influenza genomes from RNA sequencing or from PCR amplicons sequenced by the Oxford Nanopore platform (INFL1 component) and the Illumina platform (INFL2 component). For each sample in the two respective components (described in Annex 2 Supplementary Table 15 and 16), a consensus genome was generated with the internal routine workflow for Illumina, and with three dedicated pipelines for Nanopore: wf-flu from EPI2ME labs (https://github.com/epi2me-labs/wf-flu), the Iterative Refinement Meta-Assembler (IRMA) package from CDC (https://wonder.cdc.gov/amd/flu/irma), and Viroconstrictor from RIVM labs (https://github.com/RIVM-bioinformatics/ViroConstrictor), with a coverage threshold of 10x for each [15]. Ambiguity symbols representing two nucleotides were used when the coverage was high (>10x), and the cumulative proportion of the two most frequent nucleotides fell between 60-40%.

Subsequently, the genomes were manually curated by an expert curator from the organising institutes, to correct errors such as false insertions/deletions or inappropriate ambiguous symbols, based on mapping results and consensus sequences from all three pipelines. The comparator sequence was then established from this curated dataset. In addition, sequence regions before and after the first/last primer were set to allow both Ns and gaps, and positions where an ambiguity symbol (not N) was allowed were also set to allow N.

Participant provided genomes were compared to the reference datasets and differences found were classified into categories described in Table 3. The samples for these two components are described in Table A2.5 and Table A2.6.

Table 3. Types of differences between the influenza virus comparator sequence and the consensus sequences that were individually assessed

Type of difference	Description
Wrong nucleotide	A different nucleotide (not N or any other ambiguous symbol) was provided, or if an ambiguity symbol (not N) was allowed, the provided nucleotide is not represented by it.
Extra ambiguity	An ambiguity symbol (not N) was provided where only an actual nucleotide was allowed.
Missing ambiguity	An actual nucleotide was provided where only an ambiguity symbol (not N) was allowed.
Missing N	A stretch of actual nucleotides or ambiguity (not N) symbols was provided for a region where only Ns were allowed. The entire stretch counts as a single difference.
Extra N	A stretch of Ns was provided for a region where only actual nucleotides or ambiguity (not N) symbols were allowed. The entire stretch counts as a single difference.
Extra deletion	A deletion was introduced versus the comparator sequence. The entire deletion counts as a single difference.
Extra insertion	An insertion was introduced versus the comparator sequence. The entire insertion counts as a single difference.
Missing segment	A segment was not reconstructed/analysed but was present in the comparator sequence.
Extra segment	A segment was reconstructed/analysed but was not present in the comparator sequence.

Clustering and classification

SARS components

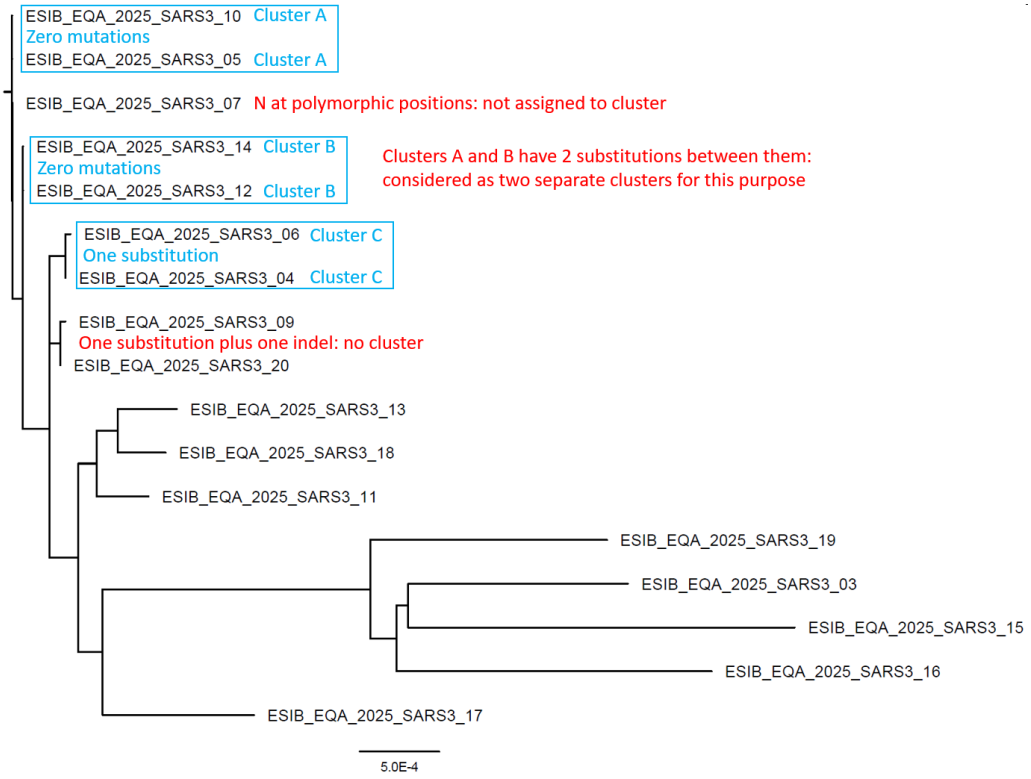
The purpose of the SARS3 component on clustering and classification was to assess the ability to identify clusters of samples that are phylogenetically so similar that they are likely part of a very short transmission chain – less than two weeks – and as such could be used as a basis for initiating contact tracing. In addition, the ability to perform classification at sub-species level was assessed. In the absence of official recommendation for such investigation, comparator sequence clusters were defined using a cut-off of a single mutation (substitution or indel). Participant provided cluster codes were mapped in all possible combinations to the comparator sequence cluster codes, removing first singleton clusters. The combination that scored best, i.e. had the least number of cluster assignment mismatches, was used for further assessment.

For the ability to perform classification at sub-species level, i.e. lineage, the assignment of Pango lineages was assessed. Comparator sequence Pango lineages were assigned with Pangolin version 4.3.1 (https://github.com/cov-lineages/pangolin; default settings) [16] and Pangolin data version 1.32 and 1.33. Provided values were compared with comparator sequence values on the basis of identical matches.

The SARS3 dataset consisted of 20 samples that were selected as described in Table A2.3. The annotated phylogenetic tree for the SARS3 component, limited to OK quality sequences, is shown in Figure 1. This tree was made using IQ_TREE COVID-19 release v2.2.0 [17].

Figure 1. Phylogenetic tree of SARS3 samples, excluding BAD quality samples

Samples or subtrees are annotated in blue with the cluster to which they belong, if any. Specific cases that may be included in a cluster but should not be, are indicated in red along with the explanation.



INFL components

Analogous to SARS-CoV-2, this component evaluates the ability to identify clusters of phylogenetically similar samples likely to be part of short transmission chains and suitable for initiating contact tracing. Additionally, it assesses the capability to classify samples at the sub-species level, including clade and subclade identification.

Comparator sequence clusters were defined using a threshold of maximum three mutations (substitutions or indels) across all influenza segments. This threshold was established based on the concatenated segment length (approximately 13,500 nucleotides) and an estimated annual substitution rate per site of around 2.21 x 10^{-3} [18]), resulting in approximately 2.25 mutations per month. Cluster codes provided by participants were mapped to these comparator sequence clusters. Comparator sequence clades, subclades, and any proposed subclades (indicated within brackets) were assigned using version 3.12.0 of nextclade.

Description of the samples, clade, and expected clustering results are given in Table A2.7, Figure A2.1, Figure A2.2 and Figure A2.3.

SARS-CoV-2 mutation detection

The purpose of this component, SARS4, was to assess the enumeration of amino acid substitutions present in a sample that have known phenotypic associations. The list of to be detected mutations for different Pango lineages was provided as part of the dataset for the component and was based on ECDC's list of variants of concern (including de-escalated ones) [19]. Additional mutations that were present in the sample were allowed to be reported as well, while any non-existing mutations or missed mutations compared to the list, were considered a difference.

The to be detected S-gene mutations for different lineages are given in Table 4. The SARS4 dataset consisted of 10 samples that were selected as described in Table A2.4.

Table 4. SARS-CoV-2 amino acid substitutions in the S-gene (S) that should be detected for the SARS4 component, by lineage

Lineage	Amino acid substitutions
BA.2.86	S:I332V, S:D339H, S:R403K, S:V445H, S:G446S, S:N450D, S:L452W, S:N481K, S:E484K, S:F486P
KP.3	S:Q493E, S:F456L
XEC	S:T22N, S:F59S, S:F456L, S:Q493E, S:V1104L

Influenza mutation detection and prediction of susceptibility to selected antivirals

This component evaluates the identification of Drug Resistance Mutations (DRMs) within the samples. These mutations are known to affect susceptibility to specific antiviral drugs: Oseltamivir, Zanamivir, Peramivir, Laninamivir (in the NA segment), and Baloxavir (in the PA segment). The list of target mutations for various subtypes was included in the dataset for this component and is derived from the WHO list of drug resistance mutations (https: //www.who.int/teams/global-influenza-programme/laboratory-network/quality-assurance/antiviral- susceptibility-influenza).

As it follows the WHO nomenclature for defining DRMs based on the level of susceptibility reduction, this component uses the following categorisation:

- NA segment: DRMs are classified as NI (normal inhibition), RI (reduced inhibition), HRI (highly- reduced inhibition), as well as intermediate values NI/RI and RI/HRI.
- PA segment: DRMs are categorised either as reduced inhibition (>3 fold inhibition) or not (≤3 fold inhibition). When the WHO list specifies a range of values (especially for the PA segment), we assume that the highest value indicates the DRM status. However, we still consider as valid the lowest value.

Participants are expected to use these classifications when identifying DRMs in the samples provided.

The list of expected results for susceptibility to antivirals is given in Table A2.8.

3 Results

Participation

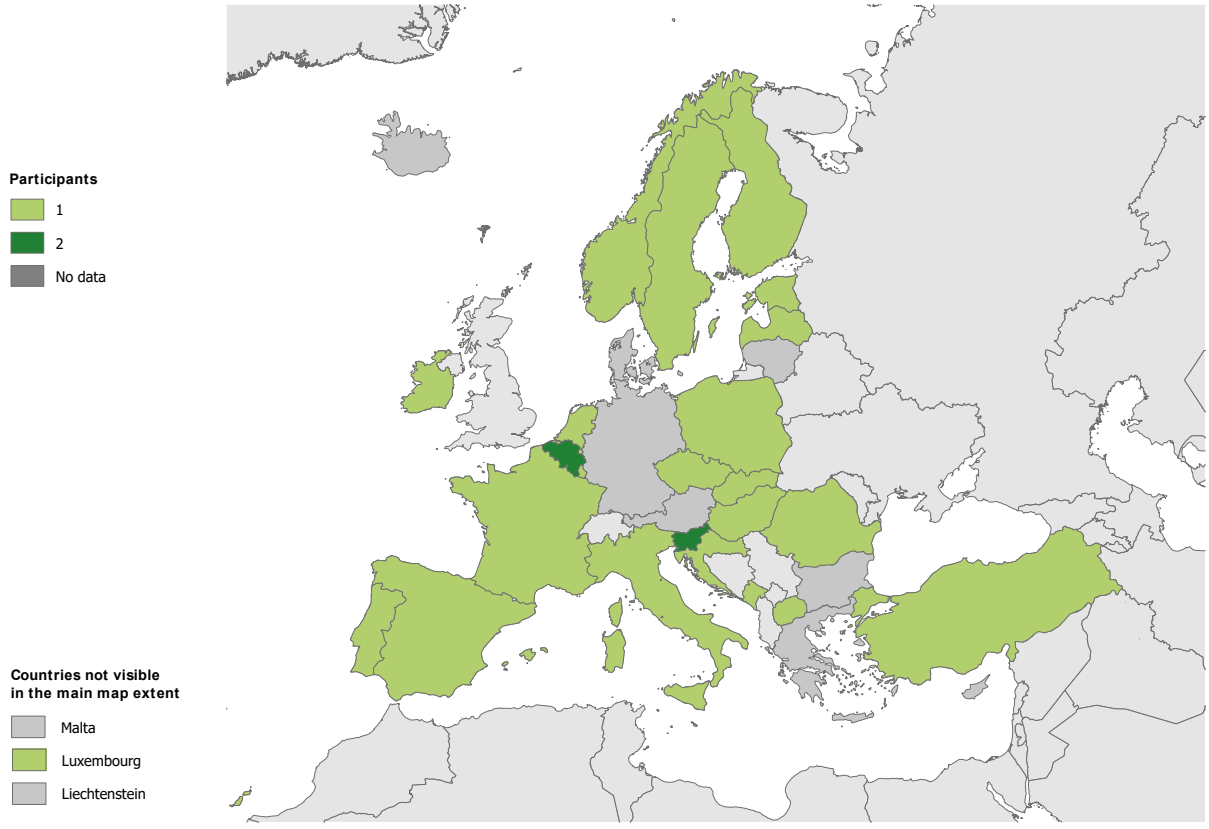
The EQA had participation from 20/30 EU/EEA Member States, from two Western Balkan countries and from Türkiye (Figure 2, Table A1.1). Two countries had two participating laboratories, all others one. Three laboratories that are not in the participation list withdrew from the EQA altogether because of unexpected resource or technical constraints. A total of 16 laboratories participated in both SARS1 and SARS2 (i.e. performing the same task from different sequencing platforms), whereas nine participated in only one of these two components (Table 5). For INFL1 and INFL2, these numbers were, respectively, 14 and nine laboratories. A total of 17 and 20 laboratories also participated in, respectively, the 2023 and 2024 EQAs.

Table 5. Participation and completion of individual components by all laboratories that participated in at least one component

The number of laboratories that participated in both iterations of the EQA so far and completed the corresponding component is also included.

Status	SARS1	SARS2	SARS3	SARS4	INFL1	INFL2	INFL3	INFL4
Completed	21	20	23	22	19	18	21	17
Participated but not completed	0	0	0	0	0	0	0	0
Not participating	4	5	2	3	6	7	4	8

Figure 2. Number of participating laboratories in the EU/EEA, the Western Balkan and Türkiye



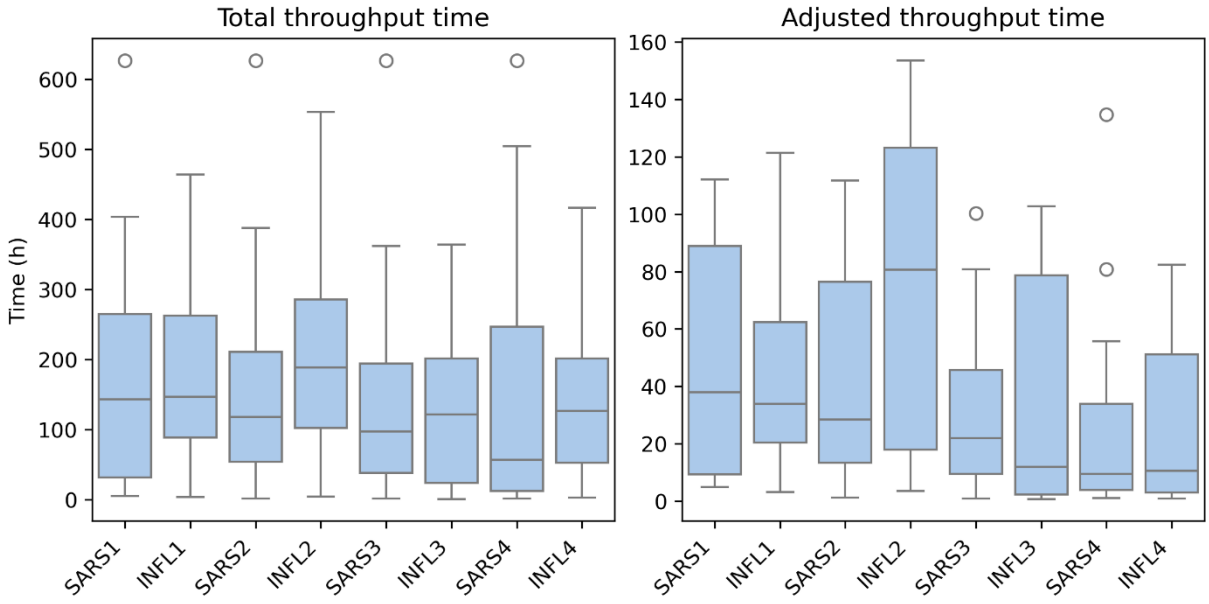
Map produced on: 13 Jun 2025. Administrative boundaries: © EuroGeographics © UN–FAO © Turkstat.The boundaries and names shown on this map do not imply official endorsement or acceptance by the European Union.

Throughput time

The total and adjusted throughput times for all components are shown in Figure 3. For the consensus sequence generation components, the first quartile of the adjusted throughput time for these four components, indicative of the more performant laboratories, ranged from 9.2 to 20.3 hours or 1-3 working days.

Figure 3. Total and adjusted throughput time for the different components

Distributions are represented as box plots with whiskers at max. 1.5x the inter-quartile range.



Quality control

The large majority of the samples were classified correctly according to their quality. Three laboratories had 100% correct classification for SARS1, one for SARS2, three for SARS3 and one for SARS4. Combining SARS1 and SARS2 results, 1/25 (4%), 5/25 (20%) and 7/25 (28%) of laboratories incorrectly classified respectively one, two or three sequences, while the remaining 9/25 (36%) incorrectly classified between four and seven sequences. The number of samples for which the quality assessment was identical or different between comparator sequence and provided value is given in Table 6. For the BAD/OK combination, i.e. where the sample was classified as BAD quality in the comparator sequence but not by the laboratory, respectively 13/18 (SARS1) and 12/18 (SARS2) occurred for the one sample in each component where a co-infection with distinct variants was observed (Table A2.1, Table A2.2), and which was most likely either not detected by the laboratory or not considered as BAD quality. Another 5/18 (SARS1) and 6/18 (SARS2) in the BAD/OK combination were most likely due to having too low a threshold on the maximum number of missing nucleotides (N's).

For the OK/BAD combination, i.e. where the sample was classified as OK quality in the comparator sequence but not by the laboratory, 8/12 (SARS1) and 9/24 (SARS2) were likely attributable to the consensus generation method introducing too many N's. This was followed by the incorrect introduction of a frameshift that was subsequently considered as BAD quality in 2/12 (SARS1) and 6/24 (SARS2) of the cases. Another 6/24 (SARS2) occurred for samples where most likely the presence of a substantial number of 'private' mutations, i.e. mutations that do not occur in a recent reference common ancestor, led to a classification as BAD quality. The likely cause for the remainder of the discrepancies was either a too high threshold on the maximum number of N's, or unclear. For SARS3, there were 11 laboratories that classified a sequence with clearly too many ambiguities as OK quality, and another two laboratories that considered a sequence with clearly too many N's as OK quality. There were nine laboratories that classified all six OK quality sequences with many 'private' mutations as BAD quality. For SARS4, a BAD quality S-gene sequence was classified as OK quality in 13 cases, likely because the overall sequence quality was considered OK even if the S-gene was BAD.

For INFL1 and INFL2 the differences in terms of quality assessment were generally due to distinct criteria on minimal segment coverage or read quality, or wrong reference selection. For INFL1 (resp. 2), 3 (resp. 2) samples were considered of BAD quality, due to low coverage or defective segments. Overall, a large majority of the laboratories correctly classified the samples according to their quality. Among all samples analysed by the participating laboratories, 157/228 (INFL1) and 166/216 (INFL2) samples were classified as OK quality (same as the comparator sequence), 45/228 (INFL1) and 18/216 (INFL2) were classified as low-quality sequences (same as the comparator sequence). However, 12/228 (INFL1) and 18/216 (INFL2) samples were classified as low quality while they were OK quality in the comparator sequence, 14/228 (INFL1) and 14/216 (INFL2) samples were classified as OK quality while they were low-quality sequences in the comparator sequence. In total, 26/228 (INFL1) and 32/216 (INFL2) samples failed to match the expected quality annotation of the comparator sequence. For INFL3, all sequences were of quality OK, however, two laboratories classified the sample INFL3.07 as low-quality sequence. For INFL4, all sequences were OK quality, but 11 were classified as low-quality sequence. Among those, seven corresponded to the sample INFL4.01, laboratories confused the H1N2sw sample for a low-quality sequence of seasonal flu.

Table 6. Number of samples for which the quality assessment was identical (OK/OK or BAD/BAD) between comparator sequence/laboratory provided value, and number of samples where the assessment was different (OK/BAD or BAD/OK). (The latter two should ideally have zero samples.)

Component	Correct classification		Incorrect classification	
	OK / OK	BAD / BAD	OK / BAD	BAD / OK
SARS1	188	24	22	18
SARS2	156	42	24	18
SARS3	331	55	60	14
SARS4	151	31	25	13
INFL1	157	45	14	12
INFL2	166	18	14	18
INFL3	418	0	2	0
INFL4	176	0	11	0

Consensus sequence generation

SARS1 and SARS2 components

The average number of sequence differences per good-quality sample, stratified by the type of difference, is shown in Figure 4. See Table 2 for a description of the types of differences that were distinguished between. Among these, the critical differences of insertions, deletions and wrong nucleotides are summarised by type in Table 7, and subdivided by potential cause. It should be noted that these differences, including insertions and deletions (indels), are with respect to the comparator sequence (GS) rather than the Wuhan-1 global reference (GR). An insertion in a provided sequence compared to the comparator sequence can therefore be at the same locus as where the GR has an insertion compared to the comparator sequence, or at a different locus. The same applies to deletions compared to the comparator sequence.

Table 7. Number and type of critical sequence differences versus the comparator sequence for SARS1 and SARS2, excluding BAD quality sequences and including educational samples

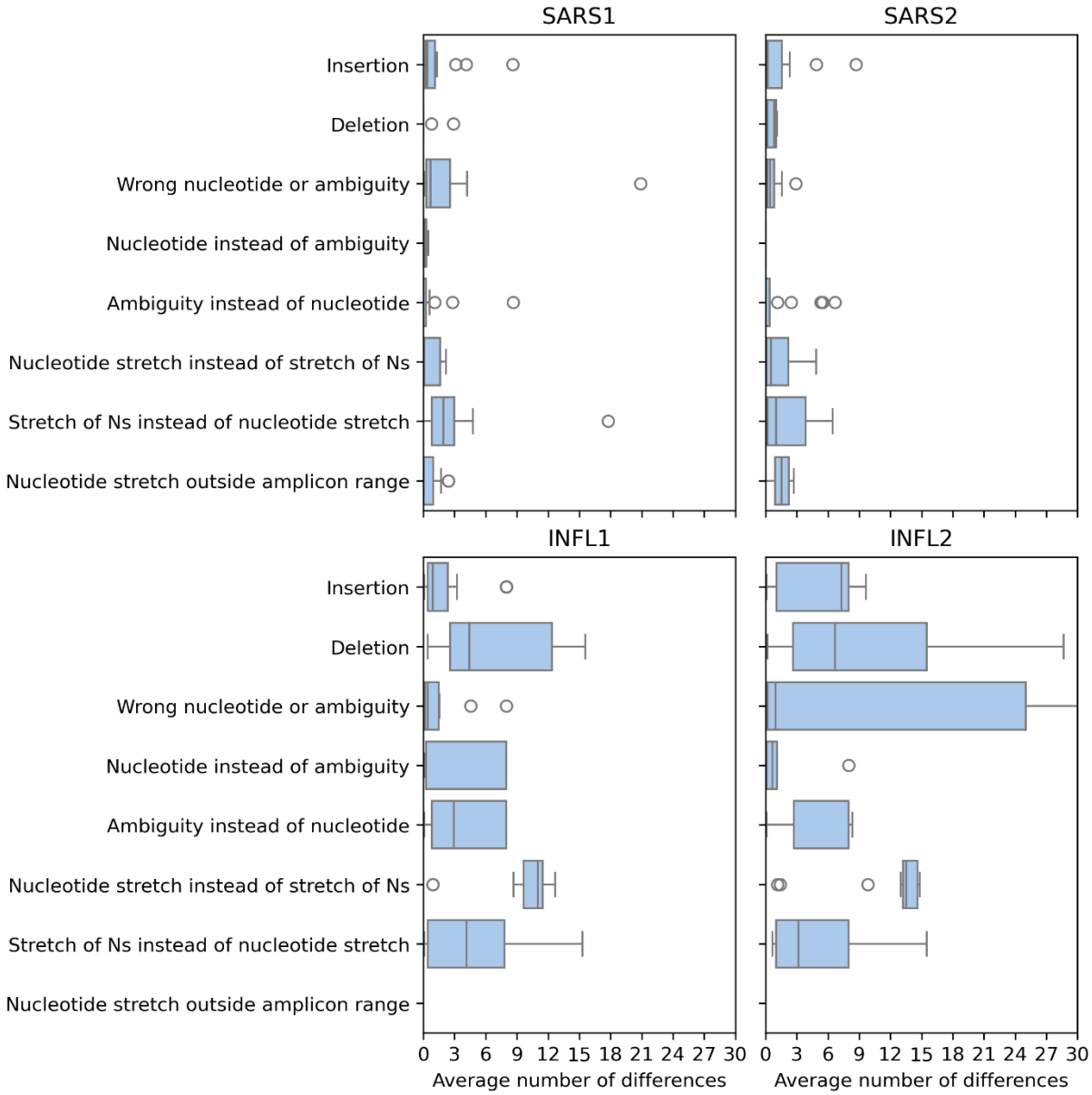
The main types are those described in Table 2. Subtypes are given when relevant, or when the cause of the difference is evident. Values for each subtype are expressed as the number and proportion (%) of the differences of that subtype within that type. The totals per type are shown in the header row for that type.

Type and subtype of sequence difference versus comparator sequence	SARS1 (%)	SARS2 (%)
Insertion	n=218	n=202
Same locus as GR insertion vs GS	130 (60)	183 (91)
Extra nucleotide in homopolymeric stretch	25 (11)	0 (0)
Insertion in primer region	20 (9)	0 (0)
Aligner unable to introduce very large deletions versus GR	18 (8)	16 (8)
Unclear cause	25 (11)	3 (1)
Deletion[1]	n=34	n=105
Same locus as GR deletion vs GS	8 (24)	89 (85)
Missing nucleotide in homopolymeric stretch	0 (0)	8 (8)
Deletion in primer region	0 (0)	2 (2)
Unclear cause	26 (76)	6 (6)
Wrong nucleotide[2]	n=928	n=114
Same nucleotide as GR rather than GS	40 (4)	73 (64)
In or near primer regions	260 (28)	27 (24)
Unclear cause	628 (68)	14 (12)

GS: comparator sequence; GR: global reference (Wuhan-Hu-1).
[1] For SARS1, two laboratories account for all the cases.
[2] For SARS1, two laboratories account for all the unclear cause cases.

Figure 4. Average number of sequence differences per sample for all OK quality, non-educational samples as compared to the comparator sequence

Results are stratified by the type of difference. Distributions are represented as box plots with whiskers at max. 1.5x the inter-quartile range.



INFL1 and INFL2 components

Out of 20 laboratories that participated in INFL1, 18 completed the component and for INFL2, 18 out of 18 completed the component.

The main sources of differences between consensus sequences and comparator sequences were the following:

1. Lab consensuses were truncated, i.e. starting at the initial start codon (ATG) instead of the beginning of segment (due to usage of references consisting only of the coding sequence part). In that case, nucleotides before ATG could not be called and were counted as deletion compared to comparator sequence.
2. In other cases, primer matching sequences were not masked (in 5' and 3'), and thus counted as nucleotides instead of Ns when compared to comparator sequences.
3. In the manual curation phase, ambiguity in nucleotide calling was not resolved the same way in comparator sequences and in the consensus. This case ended up with wrong nucleotides, ambiguity instead of nucleotide, or nucleotide instead of ambiguity.
4. Diversity of pipelines used for influenza components may explain the number of insertion-deletions and wrong nucleotides called.
5. Selection of the wrong reference caused some issues for the reconstruction of good consensus sequences.

Clustering and classification

For the SARS3 component, and across 23 laboratories that completed the component (out of 25 participants), a total of 377/425 (88.7%) OK quality sequences were correctly assigned either to the correct cluster or as not belonging to any cluster. The 48 different assignments were inspected, and the likely cause determined (Table 8). Fourteen (61%) of the laboratories likely did not inspect the multiple alignment to determine that one sample could not be assigned to a cluster due to the polymorphic positions being masked. Four laboratories used a cluster cut-off of two instead of one, and another four likely did not take into account indels when performing the clustering, i.e. only considered substitutions, leading to around half of the wrong assignments

With respect to the assignment of the Pango lineage, two (8%) laboratories likely used an outdated version of the software, as a result, an ancestral, i.e. less high resolution, lineage was assigned. The remainder of the Pango lineage assignments was identical and correct.

Table 8. Likely causes of different cluster assignments for the SARS3 component

Likely cause	Different assignments	Laboratories
Cluster cut-off of 0 instead of 1	2 (4%)	1 (4%)
Cluster cut-off of 2 instead of 1	8 (17%)	4 (17%)
Too large cluster cut-off	2 (4%)	1 (4%)
Not taking into account indels	6 (13%)	4 (17%)
Incorrect interpretation of tree	28 (58%)	14 (61%)
Unknown or other	2 (%4)	1 (4%)

For the INFL1/2 component, and across 21 laboratories that completed the component, a total of 238/420 (56.7%) sequences were correctly assigned either to the correct cluster or as not belonging to any cluster. One laboratory completed only the clade assignment part but not the clustering. Recurring misassignments involved mainly the sample INFL3.15 being grouped with cluster A, sample INFL3.13 being grouped with cluster B, and sample INFL3.17 being grouped with cluster C (Figures A2.1, A2.2 and A2.3). Discrepancies between comparator sequence and laboratories can be explained by (i) a different definition of the cut-off between the comparator sequence and what laboratories consider as a cluster; (ii) different ways of counting the number of mutations; (iii) poor quality consensus construction that did not allow a complete comparison of sequences; and (iv) the comparison being based only on the clades or subclades instead of analysing the sequences and counting the number of mutations.

SARS-CoV-2 mutation detection

There were 22 out of 25 participants that completed the SARS4 component. Twenty labs likely did not interpret an ambiguity symbol in one sample and in codons coding for either the wild type or one of the mutations on the list.

Influenza virus susceptibility to antivirals prediction

For INFL4 component, resistance mutations to antivirals were expected (Annex 2 Supplementary Table 18) among major variants.

For oseltamivir, resistance was correctly reported for 141/187 (75.4%) samples. For zanamivir, resistance was correctly reported for 155/187 (82.9%) samples. For Peramivir, resistance was correctly reported for 148/187 (79.1%) samples. For Laninamivir, resistance was correctly reported for 165/187 (88.2%) samples. For Baloxavir, resistance was correctly reported for 158/187 (84.5%) samples. Discrepancies were probably due to a different interpretation of the interval given by the WHO guidelines [20-22] compared to the threshold given in the component (> 3-fold).

Many discrepancies involved the sample INFL4.01, a H1N2sw sample either considered as a low-quality sample or laboratories did not know how to handle such samples for antiviral prediction.

4 Discussion

Throughput time

Throughput time was measured for both the SARS and INFL components in this third iteration of the EQA. For consensus sequence generation, around 1-3 working days were needed by the first quartile of the laboratories, to complete one of these components, which is similar to the first two iterations. For the SARS3/4 and INFL3/4 components, it is less clear what may have driven the throughput time.

Quality control

For SARS-CoV-2 consensus sequences, the majority of sequences incorrectly classified as OK quality were likely due to not properly taking into account the possibility of samples corresponding to co-infections with distinct variants. This was also an issue in the first and second iterations. Such co-infections should lead to ambiguity symbols in the consensus sequence, and should subsequently be taken into account for the assessment of the quality. At the same time, co-infections are rare events and this dataset was enriched in them, so the overall impact of this issue is likely not very high. Among the sequences incorrectly classified as BAD quality, the main cause that could be identified was the introduction of too many N's or a frameshift by the participant's consensus generation method and that was subsequently considered as BAD quality. The second main cause was considering the presence of a substantial amount of 'private' mutations, i.e. mutations that do not occur in a recent reference common ancestor, as an indicator for BAD quality. In the absence of ambiguities, which would indicate a co-infection or otherwise mixed sample, such samples however need not necessarily be considered to be of BAD overall quality since the 'private' mutations may simply be a result of a true evolutionary event.

For influenza sequences (INFL1 and INFL2) the differences in terms of quality assessment were generally due to distinct criteria on minimal segment coverage or read quality, or not accounting for all segments.

Consensus sequence generation

A total of eight types of differences were distinguished as part of the assessment (Table 2/Table 3). The clearly wrong – and therefore critical – types of differences are insertions, deletions or a wrong nucleotide compared to the comparator sequence. The other types have less of an impact as they are not necessarily wrong or merely reduce the available information, and are therefore considered non-critical differences: nucleotide instead of ambiguity, ambiguity instead of nucleotide, nucleotide stretch instead of stretch of Ns, stretch of Ns instead of nucleotide stretch, nucleotide stretch outside amplicon range.

SARS1 and SARS2 components

Insertions with respect to the comparator sequence were in the majority of the cases likely caused by over-reliance on the global reference sequence in reference-sequence based pipelines, irrespective of the sequencing platform, i.e. Nanopore for SARS1 or Illumina for SARS2. Similarly, true large deletions from the global reference in the comparator sequence are often not handled. On the other hand, insertions or deletions in a homopolymeric stretch, a type of error specific to Nanopore reads, were not observed very frequently. Overall, deletions were also observed much less frequently than insertions and were entirely due to two laboratories. The large majority of wrong nucleotides occurred either in or near primer regions, or had the global reference nucleotide assigned rather than that of the actual reads. Both these types of errors occurred with around the same frequency and irrespective of the sequencing platform.

The problem of 'filling in' the global reference is one of the main issues that should clearly be improved about consensus sequence generation for SARS-CoV-2. In addition, the absence of or incomplete trimming of primers from reads is the likely root cause for most of the wrong nucleotides in primer regions. As a result, when the primer has a difference compared to the actual sequence, the reference (primer) nucleotide is called, or an ambiguous nucleotide is called. This is a second main issue that should be improved about consensus generation for SARS-CoV-2.

INFL1 and INFL2 components

One of the main issues found in these components relate to missing nucleotides at the beginning and at the end of segments which, in most cases, corresponded to flanking and non-coding stretches in 5' or 3' regions; some laboratories produced segments beginning at the first start codon and ending at the last stop codon of the related genes. This is mainly due to the usage of software or pipelines which focus on the coding part of segments, instead of the whole segment. In addition, some laboratories did not mask the primers at the beginning and at the end of the segments. Both particularities explain the numbers of deletions and nucleotides instead of Ns.

Another main issue appeared with some influenza virus sequences that were not analysed thoroughly by some laboratories. As an example, and for some laboratories, the H1N2 sample was not reconstructed and confused for a co-infection of H1N1 and H3N2, or BYAM were identified as BVIC. This issue reveals that 1) some currently used

software do not propose a diverse enough reference dataset, and ii) comprehensive influenza reference sequence dataset that laboratories could readily use would be desirable.

One last point is related to defective genomes, that are particularly difficult to analyse and require time-consuming manual curation when detected. These samples are one of the main sources of differences between comparator sequences and lab consensus.

Clustering and classification

For SARS-CoV-2 clustering, most of the discrepancies arose from not inspecting the multiple alignment that is the basis for generating the phylogenetic tree. This allows to detect indels, in case these are not considered by the tree generation method, and which some laboratories indeed did not detect. In addition, N's in polymorphic regions may also be detected, which led to the majority of discrepancies in this dataset. This is an issue that would need to be improved since especially indels are at least equally important to take into account, in particular when deciding on public health actions. On the other hand, only one laboratory had a clearly too high cluster cut-off. For the SARS-CoV-2 Pango lineage classification, and in contrast to the previous iterations of the EQA, only two laboratories used an outdated version of the software or lineage definition data.

For influenza viruses, most of the discrepancies came from the way to detect clusters: It is important to use all the segments, otherwise mutations on other segments than HA/NA can be hidden. Also, it is important to build phylogenetic trees to visualise sample clusters, and then refine them using information provided by insertions/deletions. Finally, the cutoff in terms of number of allowed mutations is important. In the future these issues could be addressed by more specific guidelines on how to perform the exercise, with a clearer definition of the cut-off and analysis strategy.

SARS-CoV-2 mutation detection

Similar to the first iteration of the EQA, this component did not reveal any particular issues that would need to be addressed.

Influenza virus susceptibility to antivirals prediction

This component did not reveal any major issues. The main challenge was how to report resistance for H1N2sw which are not specifically detailed in the WHO guidelines.

Feedback from laboratories

A total of two laboratories provided feedback through the questionnaire. Both deemed the time period to complete all the components as sufficiently long. With respect to how satisfied laboratories were with the practical organisation of the EQA in general, regardless of the content of the different components, one answered Very satisfied, and one somewhat satisfied. The same was true for the usefulness of the individual EQA report. One laboratory commented that the instructions for influenza virus should be stand-alone and not refer to SARS-CoV-2, and also that the SARS3 and INFL3 components took them more time than they had anticipated. Finally, one laboratory commented that the SARS1 dataset was much more challenging than SARS2 due to the issues introduced, although both datasets were in fact selected to have more or less the same biological issues.

5 Conclusions

The EQA had participation from 20 of 30 EU/EEA Member States and two of six Western Balkan countries and from Türkiye. Overall, this is a good level of participation in the EU/EEA for the third iteration of this EQA, and also an improvement in the number of third countries participating compared to the previous iteration. There was a slightly higher participation rate for SARS-CoV-2 than for influenza virus: 56% of the laboratories took part in both of the previous iterations, while 36% participated in at least one of them. Only 8% of the laboratories participated for the first time.

For the consensus sequence generation, and similar to the two first iterations, about 60% of the laboratories signed up for both Nanopore and Illumina reads based components, and the other 40% only for one, indicating that both technologies are often available in these laboratories. No substantial difference in throughput time for both technologies could be observed for the second quartile of laboratories, indicating a similar workload for the consensus generation process. Several recurring issues in the generation of the consensus sequence were observed and comparatively more issues were observed for influenza virus than for SARS-CoV-2. This may mainly be due to (i) usage of references with only CDS sequences, (ii) usage of the wrong reference sequence (per segment), (iii) non masking of primer sequences, and (iv) difficulty to analyse (either automatically or manually) data corresponding to defective genomes. Altogether, consensus sequence generation should be improved for a number of laboratories and in general for influenza virus. The most important of these improvements are described further in the Recommendations section.

For clustering, and similar to the two first iterations, the main issue that was observed was not taking into account indels when determining phylogenetic relatedness. Apart from that, for SARS-CoV-2, a few laboratories used a cluster cut-off of 2 instead of 1, which was used for the assessment. Contrary to the first iteration, no laboratories used a cut-off of 0. However, in the absence of guidance on which cut-off or cut-off range to use for which purpose, such differences cannot be considered as critical issues. For classification, in some cases the data definitions used were outdated for SARS-CoV-2, which should be avoided. For influenza virus, main classification and clustering issues were due to wrongly reconstructed consensus sequences.

The SARS-CoV-2 mutation detection component did not reveal significant issues. Susceptibility to influenza antivirals was generally correct, with some difficulties related to interpretation of the reference interval for reduced inhibition.

6 Recommendations

With the first iteration of this EQA, a baseline has been established for the expected performance on consensus sequence generation, clustering, classification, mutation detection and antiviral resistance prediction. Subsequent iterations can also assess the evolution of the quality of these processes, and apply more stringent quality criteria where necessary.

All the recommendations derived from the previous sections have been compiled in Table 9. In order to improve the performance, laboratories that have quality issues and that are at present not well-placed to address them, should be given the opportunity to participate in relevant training. This training can cover consensus sequence generation e.g. understanding of the differences between and limitations of existing tools, appropriate parameterisation of tools, local installation of tools, quality control and manual curation. For clustering, the usage and limitations of tools, as well as guidance on appropriate cluster cut-offs may also be helpful. Over time, results from this EQA can then also be used to monitor the impact of training and support.

Table 9. Recommendations based on this, third, ESIB-EQA (2025)

Id	Target audience	Process	Description
1[a,b]	Individual laboratories	Consensus sequence generation	The introduction of indels in the consensus sequence that are not present in the raw data but are instead due to alignment to a reference sequence, should be avoided. This can be done through e.g. manual curation, less stringent mapping of reads with respect to gaps, and/or an additional steps of aligning reads to the generated (intermediate) consensus sequence.
2[a,b]	Individual laboratories	Consensus sequence generation	The introduction of wrong nucleotides or wrong non-N ambiguities due to absence of or incomplete removal of primers should be avoided. The primer removal step should be well-validated.
3[a,b]	Individual laboratories	Consensus sequence generation	Clearly ambiguous positions should be assigned a non-N ambiguity symbol, so that subsequent analyses, including quality controls, can take this information into account. When e.g. the two most frequent nucleotides at a certain position have less than 10% difference in frequency, the corresponding ambiguity symbol should be put instead of that of the most occurring nucleotide.
4[a,b]	Individual laboratories	Consensus sequence generation	The minimum coverage threshold that is normally applied should be neither too high nor too low, to avoid stretches of Ns where actual nucleotides can be put and vice versa. For Nanopore reads, a threshold in the range between e.g. 20-50x could be applied whereas for Illumina reads, a threshold in the range between e.g. 10-20x could be applied. It should be kept in mind these figures also depend on the protocol applied and that as technology evolves, increased accuracy may lower these thresholds.
5[a,b]	Individual laboratories	Consensus sequence generation	The completion of the genome in 5' of the first amplicon and in 3' of the last amplicon with the corresponding part of a reference sequence need to be avoided, since this wrongly assumes that no mutations may be present in these regions. Instead, an equivalent number of Ns can be put or no nucleotides at all.
6[b]	Individual laboratories	Consensus sequences generation influenza	Up-to-date and complete reference influenza genomes should be used. Each reference segment may be chosen among a large database of possible sequences, and should include sequence outside CDS, as well as properly named segments.
9[b]	Individual laboratories	Consensus sequences generation influenza	Samples indicative of defective viral genomes, which may lead to many wrong nucleotides reporting, should be detected and manual curation performed on them.
10[a,b]	Individual laboratories	Sequence quality control	The presence of non-N ambiguity symbols in the sequence should be part of quality control since this can be indicative of co-infection with distinct variants or contamination with material from other samples. Such sequences should not be considered to be of OK quality, and only used in specific analyses such as on co-infections.
11[b]	Individual laboratories	Sequence quality control SARS-CoV-2	The presence of a substantial number of 'private' mutations, i.e. mutations that do not occur in a recent

Id	Target audience	Process	Description
			reference common ancestor, as an indicator for bad quality, is not necessarily wanted. In the absence of ambiguities, which would indicate a co-infection or otherwise mixed sample, such samples need not necessarily be considered to be of BAD overall quality since the 'private' mutations may simply be correct. If no ambiguities are assigned, i.e. contrary to Recommendation 3, this distinction cannot be made in the first place
12[a,b]	Individual laboratories	Clustering	When determining the phylogenetic distance between sequences, indels should also be taken into account rather than only substitutions.
13[a,b]	Individual laboratories	Classification	Sufficiently recent versions should be used for classification algorithms such as Pangolin for SARS-CoV-2. This also applies to any (reference) dataset that they use. If these algorithms are run as part of a broader pipeline, the versions should be checked as well.
14[a,b]	ECDC	Clustering	Guidance in terms of which cut-offs should be used for clustering and for which purpose could be helpful.
15	ECDC	Susceptibility to antivirals influenza	Some susceptibilities are given as intervals, which leads to some heterogeneity in interpretations. Clearer guidelines are needed.
16	Individual laboratories	Reconstruction of rare reassortants	Some laboratories had difficulties to reconstruct the H1N2 swine sample. Pipelines with diverse enough references are recommended.

a) Recommendation also made in report for the first iteration of this EQA.
b) Recommendation also made in report for the second iteration of this EQA.

References

1. Grubaugh ND, Hodcroft EB, Fauver JR, Phelan AL, Cevik M. Public health actions to control new SARS-CoV-2 variants. Cell. 2021 Mar 4;184(5):1127-32. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33581746
2. Abdool Karim SS, de Oliveira T. New SARS-CoV-2 Variants - Clinical, Public Health, and Vaccine Implications. N Engl J Med. 2021 May 13;384(19):1866-8. Available at: https://www.ncbi.nlm.nih.gov/pubmed/33761203
3. World Health Organization (WHO). Factsheet - Influenza (Seasonal) 2025. Geneva: World Health Organization; 2023. Available at: https://www.who.int/en/news-room/fact-sheets/detail/influenza-(seasonal)
4. European Centre for Disease Prevention and Control (ECDC). Public health impact of SARS-CoV-2 variants of concern: scoping review protocol. Stockholm: ECDC; 2021. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/Public-health-impact-of-SARS-CoV-2-variants-of-concern-scoping-review-protocol.pdf
5. WHO TAG-VE Risk Evaluation for SARS-CoV-2 Variant Under Monitoring: BA.3.2. Geneva: World Health Organization; 2025. Available at: https://cdn.who.int/media/docs/default-source/documents/epp/tracking-sars-cov-2/05122025_ba.3.2_ire.pdf?sfvrsn=a29c3612_4
6. European Centre for Disease Prevention and Control. Threat Assessment Brief – Assessing the risk of influenza for the EU/EEA in the context of increasing circulation of A(H3N2) subclade K – 20 November 2025. ECDC: Stockholm; 2025.
7. World Health Organization (WHO). Interim guidance - Maintaining surveillance of influenza and monitoring SARS-CoV-2 – adapting Global Influenza surveillance and Response System (GISRS) and sentinel systems during the COVID-19 pandemic. Geneva: World Health Organization; 2020 Available at: https://iris.who.int/bitstream/handle/10665/336689/WHO-2019-nCoV-Adapting_GISRS-2020.1-eng.pdf?sequence=1
8. European Centre for Disease Prevention and Control (ECDC). Integrated respiratory virus surveillance guidance for the EU/EEA: A framework for surveillance to inform public health action. Available at: https://www.ecdc.europa.eu/en/publications-data/integrated-respiratory-virus-surveillance-guidance-eueea-framework-surveillance
9. European Centre for Disease Prevention and Control (ECDC). Website - European Reference Laboratory Network for Human Influenza (ERLI-Net). Stockholm: ECDC; 2017. Available at: https://www.ecdc.europa.eu/en/about-us/networks/disease-and-laboratory-networks/erlinet-about
10. European Centre for Disease Prevention and Control (ECDC). Website - European COVID-19 reference laboratory network (ECOVID-LabNet). Stockholm: ECDC; 2022. Available at: https://www.ecdc.europa.eu/en/about-ecdc/partners-and-networks/disease-and-laboratory-networks/european-covid-19-reference
11. European Centre for Disease Prevention and Control. European SARS-CoV-2 and influenza Bioinformatics External Quality Assessment (ESIB-EQA), 2023. Stockholm: ECDC; 2024. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/SARS-CoV-2-influenza-bioinformatics-external-quality-assessment-2023.pdf
12. European Centre for Disease Prevention and Control. External quality assessment of European SARS-CoV-2 and zoonotic influenza Bioinformatics (ESIB-EQA), 2024. Stockholm: ECDC; 2025. Available at: https://www.ecdc.europa.eu/en/publications-data/external-quality-assessment-european-sars-cov-2-and-zoonotic-influenza
13. Zwagemaker F HK, Schmitz D, Kroneman A, The RIVM-IDS Bioinformatics team. ViroConstrictor: Zenodo; 2024. Available at: https://github.com/RIVM-bioinformatics/ViroConstrictor/tree/v1.4.6
14. Oxford Nanopore Technologies. Guppy basecalling software. Available at: https://nanoporetech.com/document/Guppy-protocol
15. Shepard SS, Meno S, Bahl J, Wilson MM, Barnes J, Neuhaus E. Viral deep sequencing needs an adaptive approach: IRMA, the iterative refinement meta-assembler. BMC Genomics. 2016 Sep 5;17(1):708.
16. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nature Microbiology. 2020 2020/11/01;5(11):1403-7. Available at: https://doi.org/10.1038/s41564-020-0770-5
17. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution. 2020;37(5):1530-4. Available at: https://doi.org/10.1093/molbev/msaa015
18. Bhattacharjee, U., Chakrabarti, A. K., Kanungo, S., & Dutta, S. (2023). Evolutionary dynamics of influenza A/H1N1 virus circulating in India from 2011 to 2021. *Infection, Genetics and Evolution*, *110*, 10542419.
19. European Centre for Disease Prevention and Control (ECDC). Website - SARS-CoV-2 variants of concern as of 20 December 2024. Stockholm: ECDC; 2024. Available at: https://www.ecdc.europa.eu/en/covid-19/variants-concern
20. World Health Organization (WHO). Website - Laboratory methodologies for testing the antiviral susceptibility of influenza viruses. Geneva: World Health Organization; 2024. Available at:

https://www.who.int/teams/global-influenza-programme/laboratory-network/quality-assurance/antiviral-susceptibility-influenza

21. World Health Organization (WHO). Laboratory methodologies for testing the antiviral susceptibility of influenza viruses: Neuraminidase inhibitor (NAI); 2024. Available at: https://cdn.who.int/media/docs/default-source/influenza/laboratory---network/quality-assurance/human-nai-marker-table_for-publication_final_20240918.pdf?sfvrsn=c6d153ec_3
22. World Health Organization (WHO). Laboratory methodologies for testing the antiviral susceptibility of influenza viruses: Polymerase acidic (PA) inhibitor, Baloxavir; 2024. Available at: https://cdn.who.int/media/docs/default-source/influenza/laboratory---network/quality-assurance/antiviral-susceptibility-influenza/pa-marker-who-table_07-08-2024_updated_final-version.pdf?sfvrsn=5307d6fe_2
23. Klivleyeva N, Saktaganov N, Glebova T, Lukmanova G, Ongarbayeva N, Webby R. Influenza A Viruses in the Swine Population: Ecology and Geographical Distribution. Viruses. 2024 Nov 1;16(11):1728. doi: 10.3390/v16111728. PMID: 39599843; PMCID: PMC11598916.
24. Chastagner, A.; Hervé, S.; Quéguiner, S.; Hirchaud, E.; Lucas, P.; Gorin, S.; Béven, V.; Barbier, N.; Deblanc, C.; Blanchard, Y.; et al. Genetic and Antigenic Evolution of European Swine Influenza A Viruses of HA-1C (Avian-Like) and HA-1B (Human-Like) Lineages in France from 2000 to 2018. *Viruses* 2020, *12*, 1304. https://doi.org/10.3390/v12111304
25. Chepkwony, S., Parys, A., Vandoorn, E., Stadejek, W., Xie, J., King, J., ... & Van Reeth, K. (2021). Genetic and antigenic evolution of H1 swine influenza A viruses isolated in Belgium and the Netherlands from 2014 through 2019. *Scientific Reports*, *11*(1), 11276.

Annex 1. Participating laboratories

Table A1.1. Participating laboratories, sorted by country and name

Country	Name
Belgium[b]	National Influenza Center in Belgium, Sciensano
Belgium[a]	National reference centre for respiratory pathogens, University Hospitals UZ Leuven, Leuven
Croatia[a,b]	Croatian Institute of Public Health
Czech Republic[b]	National Institute of Public Health
Estonia[a,b]	Health Board
Finland[a,b]	The National Institute for Health and Welfare
France[a,b]	CNR des virus à transmission respiratoire (dont la grippe), Groupement Hospitalier Nord, Lyon
Hungary[b]	National Center for Public Health and Pharmacy
Ireland[a,b]	National Virus Reference Laboratory
Italy[b]	Istituto Superiore di Sanità
Latvia[a,b]	National Microbiology Reference Laboratory, Riga East University Hospital
Luxembourg[a,b]	Laboratoire Nationale de la santé
Montenegro	Institute of Public Health of Montenegro
Norway[a,b]	Norwegian Institute of Public Health
Poland[a,b]	National Institute of Public Health NIH - NRI
Portugal[a,b]	National Reference Laboratory For Influenza and Other Respiratory Viruses, Instituto Nacional de Saúde Doutor Ricardo Jorge
Republic of North Macedonia[a]	Institute of Public Health
Romania[a,b]	National Institute of Public Health-Romania / Institutul National de Sanatate Publica
Slovak Republic[b]	Public Health Authority of the Slovak Republic
Slovenia[a,b]	National Laboratory of Health, Environment and Food
Slovenia[a,b]	University of Ljubljana, Faculty of Medicine, Institute of Immunology and Microbiology
Spain[a,b]	Institute of Health Carlos III
Sweden[b]	Public Health Agency of Sweden
The Netherlands[a]	Department Viroscience, Unit Clinical Virology, Erasmus MC, Rotterdam
Türkiye	Republic of Türkiye, Ministry of Health, General Directorate of Public Health

[a] Also participated in the 2023 EQA.
[b] Also participated in the 2024 EQA.

Annex 2. Composition of the datasets

Table A2.1. Composition of the SARS1 dataset

Sample	Description
1	An alternative primer sequence introduces an incorrect ambiguity position in a sample with real ambiguous positions.
2	Contains a specific motif of deletions and insertions in the spike gene, which may cause issues for certain analysis methodologies.
3	Contain reads originating from a mismatching primer site which binds in ORF1b, leading to a frameshift.
4	Contains a local frameshift in ORF1a.
5	Mixed sample with a high number of ambiguous positions.
6	A relatively new recombinant strain which contains a larger than usual deletion in the Nucleocapsid gene.
7	No specific challenges.
8	An alternative primer sequence introduces incorrect nucleotide calls.
9	Contains reads originating from two mismatching primer sites, leading to multiple insertion calls.
10	Contains a deletion in two parts within the Spike gene, incorrect resolution of the deletion leads to a frameshift in the Spike gene.
11	Contains a knockout of both ORF7a and ORF8 in two separate deletion events.
12	Contains various amplicon dropouts.

Table A2.2. Composition of the SARS2 dataset

Sample	Description
1	Contains a deletion in two parts within the Spike gene, incorrect resolution of the deletion leads to a frameshift in the Spike gene.
2	Contains a large deletion that spans over most of ORF8.
3	Contains a specific motif of deletions and insertions in Spike may cause issues for certain analysis methodologies.
4	No specific challenges.
5	Mixed sample with a high number of ambiguous positions.
6	No specific challenges.
7	No specific challenges.
8	Contains various amplicon dropouts.
9	No specific challenges.
10	Contains a large frameshift in ORF1a.
11	Contains various amplicon dropouts.
12	An alternative primer sequence introduces incorrect ambiguity calls.

Table A2.3. Composition of the SARS3 dataset

Samples	Pangolin lineage	Description
5, 10	MC.24	Cluster A, zero SNPs and indels difference between both samples. Cluster A has two SNPs difference from cluster B.
12, 14	MC.24	Cluster B, zero SNPs and indels difference between both samples. Cluster B has two SNPs difference from cluster A.
4, 6	KP.3.1.1	Cluster C, one SNP difference between both samples.
9, 20	KP.3.1.1	One SNP and one indel differences between both samples and therefore not considered a cluster.
7	MC.24	Has zero SNPs and indels difference with both cluster A and cluster B, but the two polymorphic positions that differentiate both clusters are missing in this sample. The sample can therefore not reliably be assigned to a cluster.
3	BA.2.3.2	Not in a cluster.
11	XEC	Not in a cluster.
13	XEK.1	Not in a cluster.
15	BF.11.2	Not in a cluster.
16	BN.1.5.2	Not in a cluster.
17	JN.1.7	Not in a cluster.
18	XEV	Not in a cluster.
19	XBB.2.3.3	Not in a cluster.
1, 2, 8		Clearly BAD overall quality.

Table A2.4. Composition of the SARS4 dataset

The S gene substitutions column only contains those substitutions that were included in the list of to be detected mutations.

Sample	S gene substitutions	Description
1	S:F486P, S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:V1104L, S:R403K, S:F456L, S:N481K, S:I332V	KP.3.2.4. Overall quality is OK. The S gene is completely resolved and thus the specific quality is OK as well.
2	S:F486P, S:E484K, S:F59S, S:R403K, S:N481K, S:Q493E, S:I332V, S:T22N	XEC. Bad overall quality due to more than 3000 nucleotides missing. The S gene is missing amino acid positions 115-184,408-471,684-772,785-878,1085-1172, obscuring F456L, V1104L (2/5 mutations to detect). Because more than one of the five mutations to detect is obscured, we considered this as BAD specific quality.
3	S:F486P, S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:F59S, S:V1104L, S:R403K, S:F456L, S:N481K, S:Q493E, S:I332V, S:T22N	KP.3.3. Overall quality is OK. The S gene is missing amino acid positions 408-423, but these are not in the list of mutations and thus considered OK specific quality.
4	S:F486P, S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:R403K, S:N481K, S:I332V	BA.2.86. Bad overall quality due to more than 3000 nucleotides missing. The S gene is missing amino acid positions 115-184,785-790,877-878,995-1070, but this obscures none of the mutations on the list and thus the specific quality is OK as well.
5	S:F486P, S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:V1104L, S:R403K, S:F456L, S:N481K, S:Q493E, S:I332V, S:T22N	XEC. Overall quality is OK. The S gene is completely resolved and thus the specific quality is OK as well.
6	S:F486P, S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:R403K, S:N481K, S:I332V	BA.2.86.1. Overall quality is OK. The S gene is completely resolved and thus the specific quality is OK as well.
7	S:F486P, S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:F59S, S:V1104L, S:R403K, S:F456L, S:N481K, S:Q493E, S:I332V, S:T22N	XEC. Overall quality is OK. The S gene is completely resolved and thus the specific quality is OK as well.
8	S:F486P, S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:F59S, S:R403K, S:F456L, S:N481K, S:Q493E, S:I332V, S:T22N	XEC.2. Overall quality is OK. The S gene is missing amino acid positions 1085-1172, obscuring V1104L (1/5 mutations to detect). Because only one of five mutations to detect is obscured, we still considered this as OK specific quality.
9	S:F59S, S:V1104L, S:R403K, S:I332V	KP.3.3. Overall quality is OK. The S gene is missing amino acid positions 408-521, obscuring F456L, Q493E (2/2 mutations to detect) and thus the specific quality is BAD.
10	S:V445H, S:E484K, S:G446S, S:N450D, S:L452W, S:R403K, S:N481K, S:I332V	BA.2.86.1. Overall quality is OK. The S gene is completely resolved and thus the specific quality is OK as well.

Table A2.1. Composition of the INFL1 dataset

Sample	Subtype	Quality	Description
INFL1.1	H3N2	OK	
INFL1.2	YAM	BAD	A low-quality B/Yamagata sample with low coverage on various segments; NP, PB1, and PB2 are not present in the comparator sequence. The challenge is to analyse this sample and identify it as B/Yamagata. It is recommended to exclude it from further analysis.
INFL1.3	H3N2	OK	
INFL1.4	H1N1	OK	A H1N1pdm with defective PA, PB1, and PB2 segments. PB1 has low coverage and is not in the comparator sequence.
INFL1.5	H3N2	OK	A H3N2 with a missing PB2 segment. Since most segments can still be analysed, this sample is accepted.
INFL1.6	H3N2	OK	
INFL1.7	H1N2 (Swine)	OK	A H1N2 (swine) sample with defective PA, PB1, and PB2 segments.
INFL1.8	B/VIC	BAD	A B/Victoria sample with several dropouts (NA, MP, NP, and NS segments). Despite these dropouts, it can still be typed, so this sample is accepted. It may be excluded from further analysis, especially given the low coverage of the NA segment.
INFL1.9	H3N2	OK	
INFL1.10	H1N1	OK	
INFL1.11	N1	BAD	A low-quality H1N1pdm with low coverage in various segments and some defective segments. It is recommended to exclude it from further analysis.
INFL1.12	H1N1	OK	

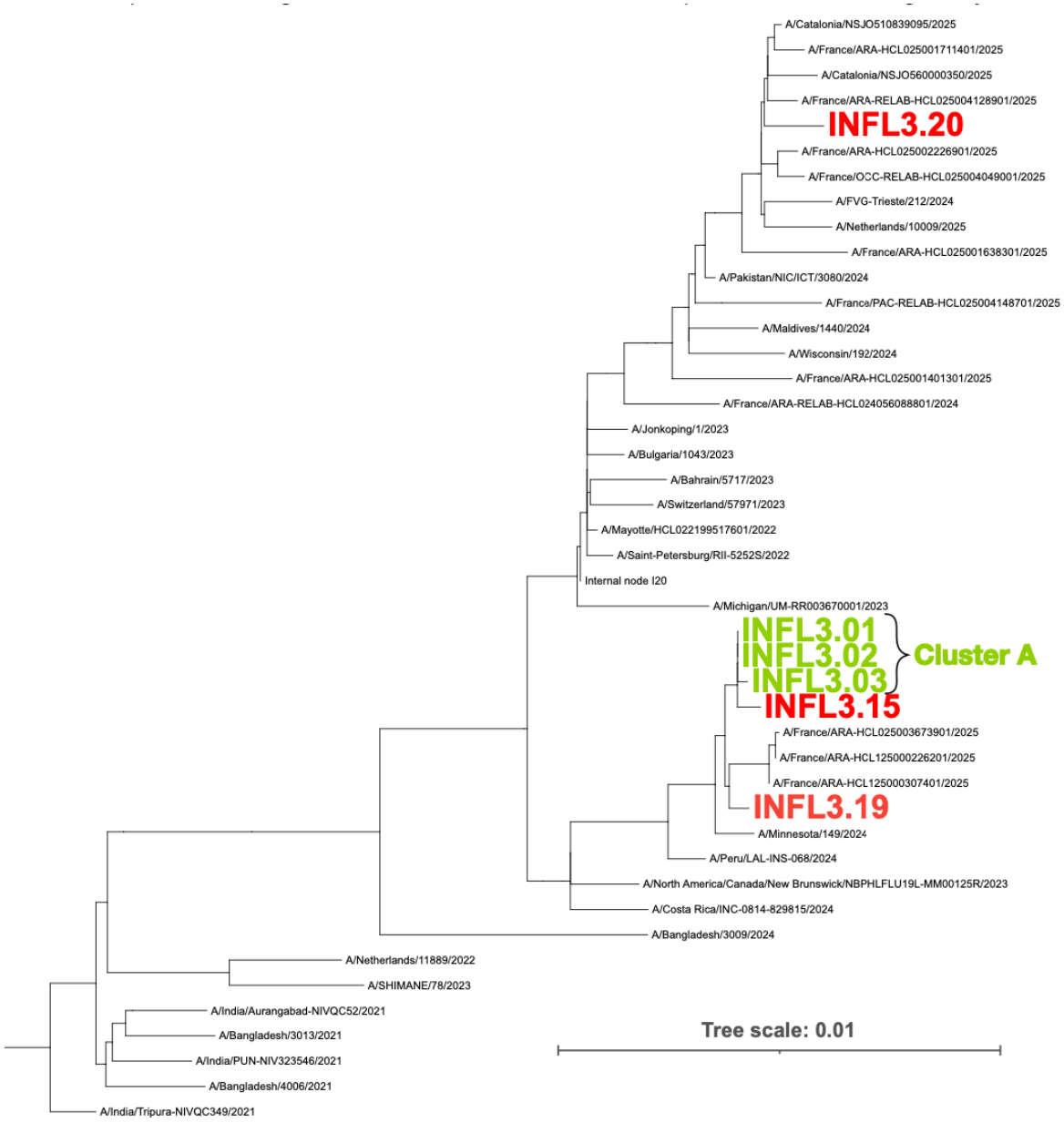
Table A2.6. Composition of the INFL2 dataset

Sample	Subtype	Quality	Description
INFL2.1	N1	BAD	A low-quality H1N1pdm sample with low coverage and defective PA and PB2 segments. The HA segment is absent from this sample. It is recommended that such samples be excluded from further analysis
INFL2.2	H1N1	OK	
INFL2.3	H1N1	OK	PA, PB1, and/or PB2 segments are defective, potentially complicating analysis. Some segments may have occasional low-coverage sections or mixtures at various positions.
INFL2.4	H1N1	OK	
INFL2.5	H1N1	OK	A H1N1pdm sample with a defective PB2 segment. A mixture position exists at the break- point between defective and full-length genome, challenging participants to identify that the predom- inant nucleotide is actually from the defective genome. The consensus should report the minor nu- cleotide.
INFL2.6	H1N2 (Swine)	OK	A H1N2 (swine) sample. The primary challenge is the ability to analyse the sample and correctly identify it as H1N2 (swine).
INFL2.7	H3N2	OK	
INFL2.8	H3N2	BAD	A low-quality H3N2 sample with low coverage across various segments. PB1 is not present in the comparator sequence. It is recommended to exclude such samples from further analysis.
INFL2.9	H3N2	OK	PA, PB1, and/or PB2 segments are defective, potentially complicating analysis. Some segments may have occasional low-coverage sections or mixtures at various positions.
INFL2.10	H3N2	OK	PA, PB1, and/or PB2 segments are defective, potentially complicating analysis. Some segments may have occasional low-coverage sections or mixtures at various positions.
INFL2.11	B/VIC	OK	
INFL2.12	B/YAM	OK	A B/Yamagata sample. The primary challenge is analysing the sample to correctly detect it as B/Yamagata.

Table A2.7. Composition of the INFL3 dataset

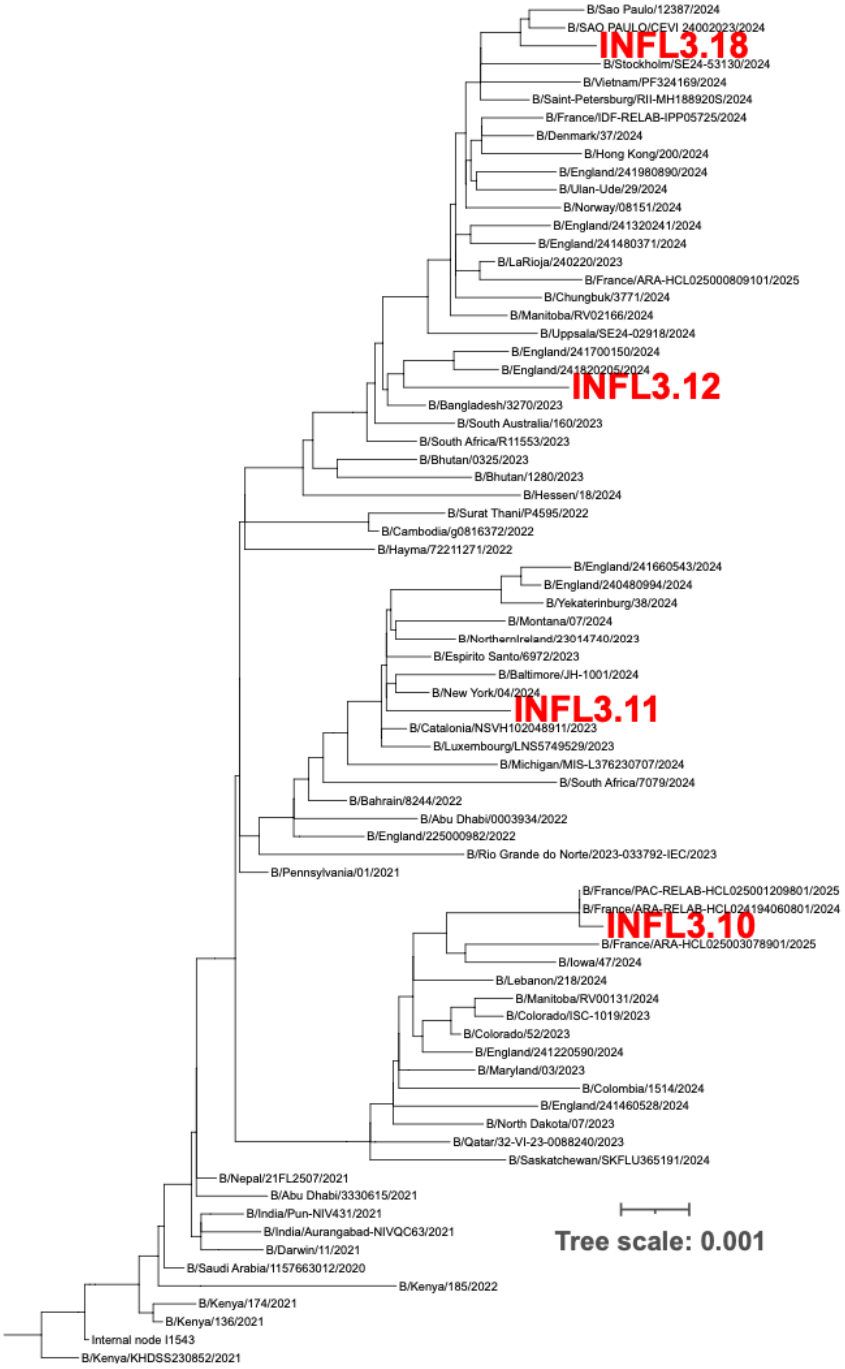
Sample	Quality	Clade	Subclade
INFL3.1	OK	6B.1A.5a.2a.1	D
INFL3.2	OK	6B.1A.5a.2a.1	D
INFL3.3	OK	6B.1A.5a.2a.1	D
INFL3.4	OK	3C.2a1b.2a.2a.3a.1	J.2
INFL3.5	OK	3C.2a 1b.2a.2a.3a.1	J.2
INFL3.6	OK	3C.2a 1b.2a.2a.3a.1	J.2
INFL3.7	OK	3C.2a1b.2a.2a.3a.1	J.2
INFL3.8	OK	3C.2a1b.2a.2a.3a.1	J.2
INFL3.9	OK	3C.2a1b.2a.2a.3a.1	J.2
INFL3.10	OK	V1A.3a.2	C.5.6
INFL3.11	OK	V1A.3a.2	C.5.1
INFL3.12	OK	V1A.3a.2	C. 5.7
INFL3.13	OK	3C.2a1b.2a.2a.3a.1	J.2
INFL3.14	OK	3C.2a1b.2a.2a.3a.1	J.2.2
INFL3.15	OK	6B.1A.5a.2a.1	D
INFL3.16	OK	3C.2a1b.2a.2a.3a.1	J.2.2
INFL3.17	OK	3C.2a1b.2a.2a.3a.1	J.2
INFL3.18	OK	V1A.3a.2	C. 5.7
INFL3.19	OK	6B.1A.5a.2a.1	D
INFL3.20	OK	6B.1A.5a.2a	C. 1.9.3

Figure A2.1. Phylogenetic tree of INFL3 H1N1pdm samples based on the concatenation of all eight segments



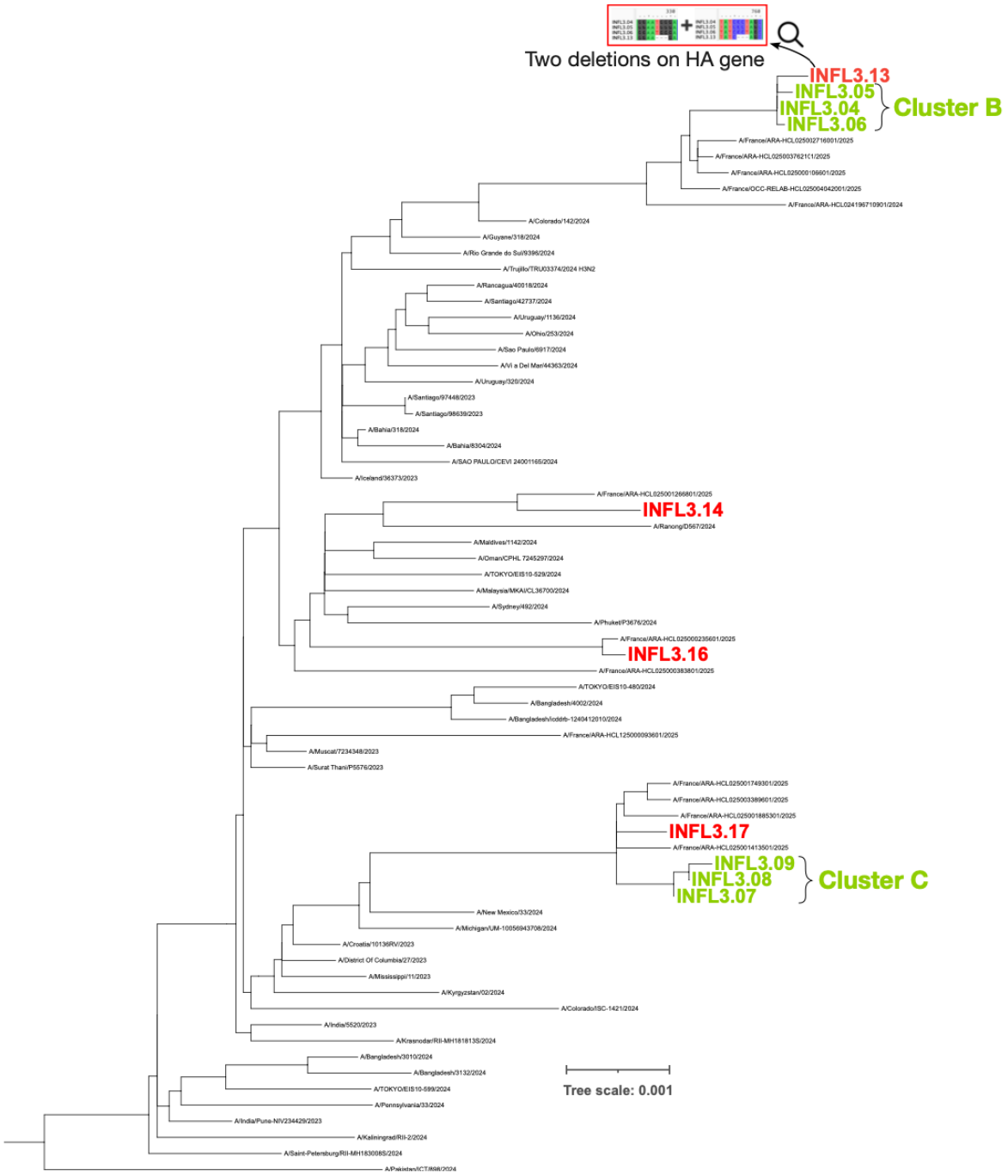
Samples (total of 6) have been analysed along a contextual dataset (total of 727) gathered on 28 February 2025. To lighten the visual representation of the phylogeny, only 37 contextual samples have been kept. Green annotates samples that belong to cluster A and red annotates the samples that do not belong to any cluster.

Figure A2.2. Phylogenetic tree of INFL3 B/Victoria samples based on the concatenation of all eight segments



Samples (total of 4) have been analysed along a contextual dataset (total of 961) gathered on 28 February 2025. To lighten the visual representation of the phylogeny, only 71 contextual samples have been kept. Red annotates the samples that do not belong to any cluster. There is no cluster among the B/Victoria samples.

Figure A2.3. Phylogenetic tree of INFL3 H3N2 samples based on the concatenation of all eight segments



Samples (total of 10) have been analysed along a contextual dataset (total of 914) gathered on 28 February 2025. To lighten the visual representation of the phylogeny, only 60 contextual samples have been kept. Green annotates samples that belong to clusters B and C, and red annotates the samples that do not belong to any cluster. In this figure, two deletions on HA gene of INFL3.13 are shown, demonstrating that this sample does not belong to the cluster B.

Table A2.8. Composition of the INFL4 dataset

Sample	Status	Osel.	Zana.	Pera.	Lani.	Balo.	NA DRM	PA DRM
INFL4.01*	OK	HRI	HRI	RI	RI	Reduced Susceptibility	N142S	E23K
INFL4.02	OK	HRI	NI	RI/HRI	NI	Normal Susceptibility	H275Y	-
INFL4.03	OK	RI	NI	NI	NI	Normal Susceptibility	I223V + S247N	-
INFL4.04	OK	NI	NI	NI	NI	Reduced Susceptibility	-	I38V
INFL4.05	OK	RI	NI	NI	NI	Normal Susceptibility	N329R	-
INFL4.06	OK	RI/HRI	NI	NI	NI	Normal Susceptibility	E119V	-
INFL4.07	OK	NI	NI	NI	NI	Reduced Susceptibility	-	E23K
INFL4.08	OK	NI/RI	NI/RI	RI	NI	Normal Susceptibility	D197N	-
INFL4.09	OK	NI	NI	NI	NI	Normal Susceptibility	I361V	-
INFL4.10	OK	NI/RI	NI/RI	NI/RI	NI	Normal Susceptibility	G247D + I361V	-
INFL4.11	OK	NI	NI	NI	NI	Normal Susceptibility	-	-

** Due to its porcine origin, it may be difficult to draw conclusions about the NA DRMs of this sample. Nevertheless, and because we feel that no risks should be taken, we are considering that this H1N2 has human-like N2 profile (and to a lesser extent an avian-like one). Furthermore, this sample is a H1N2 belonging to the HA-1C.2.4 clade (swine H1 global classification of BV-BRC), which is known to contain a N2 segment derived from A/swine/Gent/1/1984 [23,24]. A/swine/Gent/1/1984 itself seems to have a human-derived N2, reinforcing our belief that such a sample should be considered human when looking at DRMs [25]. DRMs in the NA segment of this sample were therefore identified based on the human A(H3N2) list.*

European Centre for Disease Prevention and Control (ECDC)

Gustav III:s Boulevard 40
16973 Solna, Sweden

Tel. +46 858 60 10 00
ECDC.info@ecdc.europa.eu

www.ecdc.europa.eu



Publications Office
of the European Union