Influenza Genomic Analysis EQA – Participant Guide

1. Introduction

A regional hospital has submitted 13 nasopharyngeal swab samples from patients admitted over a 3-day period with severe acute respiratory illness (SARI). Initial rapid antigen tests indicated Influenza A (H1N1)pdm09 infection. The patients are all from the same district and attended the same event. An additional sample from an individual not associated with the event was also sent.

Referral Lab (Your Role as Participant)

As the referral lab, you are tasked with sequencing and characterizing these samples to determine:

- Whether they are genetically related (i.e., part of the same outbreak/cluster)
- Which influenza subtype and clade they belong to
- Whether they harbor mutations of concern (e.g., increased virulence, antibody evasion or antiviral resistance)
- The potential public health implications

2. Objective

This EQA aims to assess the capacity to analyze Influenza virus NGS data, including genome assembly, phylogenetics, mutational profiling, and detection of antiviral resistance.

- Evaluate consistency and accuracy of influenza genome assembly from NGS data.
- Phylogenetic analysis and clade assignment.
- Assess mutation profiles of the NA, especially for antiviral resistance.

3. Materials Provided

- 5 paired-end FASTQ datasets containing human seasonal influenza (H1N1pdm09).
- Sample metadata (ID, collection year, country), below.
- Submission templates (results matrix, mutation report, consensus sequences).

Sample metadata

sample name	collection date	country	Age	Sex	province	Sequencing technology

barcode01	2022/06/07	South Africa	28	F	Gauteng	ONT
barcode02	2022/06/07	South Africa	16	M	Gauteng	ONT
barcode03	2022/06/07	South Africa	30	F	Gauteng	ONT
barcode04	2022/06/07	South Africa	18	F	Gauteng	ONT
barcode05	2022/06/08	South Africa	21	F	Gauteng	ONT
barcode06	2022/06/08	South Africa	35	M	Gauteng	ONT
barcode07	2022/06/08	South Africa	31	M	Gauteng	ONT
barcode08	2022/06/08	South Africa	23	F	Gauteng	ONT
barcode09	2022/06/08	South Africa	27	M	Gauteng	ONT
barcode10	2022/06/09	South Africa	30	F	Gauteng	ONT
barcode11	2022/06/09	South Africa	35	F	Gauteng	ONT
barcode18	2022/06/09	South Africa	37	M	Gauteng	ONT
barcode20	2022/06/09	South Africa	26	M	Gauteng	ONT

4. Analysis Tasks (e.g Tools that can be use)

A. Quality Control & Assembly:

- Assemble genomes (reference-based) (MIRA, InsaFlu, Epi2Me).
- Output: Consensus FASTA per sample.

B. Phylogenetic Analysis:

- Align HA (Haemagglutinin) (MAFFT, MUSCLE, Nextclade)
- Construct phylogenetic trees (IQ-TREE, RAxML, NextStrain).
- Assign lineage or clade (NextClade).
- Output: Newick tree + labeled image (Tree can be annotated as desired but must include subclades, Paste image to the excel spreadsheet, Identify the sample not involved in the outbreak).

C. Mutation Identification:

- Identify nucleotide and amino acid changes (Nextclade, FluServer (https://flusurver.bii.a-star.edu.sg/)

- For the HA, identify substitutions associated with antigenic drift: Increased virulence/ antibody evasion: To assist you with this assessment, find positions of interest below:

The H1N1 HA protein has five major antigenic sites and the receptor binding domain (RBD) on the globular head of HA1.

Site	Amino Acid positions	Notes
Sa	128–129, 156–160, 162–167	Surface-exposed; near receptor-binding site
Sb	187–198	Highly variable; close to RBS
Ca1	169–173, 206–208	Cross-reactive; at HA1-HA2 interface
Ca2	140–145, 220–225	Surface-exposed; near fusion peptide
Cb	74–78	Less variable; contributes to broader immunity
RBD	98–105, 130–138, 190–198, 220–229	Directly interact with sialic acid receptors on host cells

-For the NA (Neuraminidase), identify substitutions associated with antiviral resistance:
- Find known markers below:
  - H275Y in NA (Oseltamivir)
  - S31N in M2 (Adamantanes)
  - E199G in PA (Baloxavir)

References

- Caton AJ et al. (1982). Antigenic variation in influenza virus. Evidence for a high frequency of amino acid changes in HA antigenic sites. Cell, 31(2 Pt 1), 417–427.
- Wiley DC et al. (1981). Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. Nature.
- Xu R et al. (2010). Structural basis of preexisting immunity to the 2009 H1N1 pandemic influenza virus. Science, 328(5976), 357–360.
- Gamblin SJ, Skehel JJ. (2010). Influenza hemagglutinin and neuraminidase membrane glycoproteins. Journal of Biological Chemistry, 285(37), 28403–28409.
- Moscona A. (2005). Neuraminidase inhibitors for influenza. New England Journal of Medicine, 353(13), 1363–1373.
- Dong G. et al. (2015). Adamantane-resistant influenza A viruses in the world (1902–2013): frequency and distribution of M2 gene mutations. PLoS One, 10(3), e0119115.
- Omoto S. et al. (2018). Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. Scientific Reports, 8, 9633.

6. Timeline

Activity | Date

Dataset release | June 13, 2025
Submission deadline | July 15, 2025
Feedback & results | July 18, 2025 (We will have a zoom call to discuss analysis and results)

7. Evaluation Criteria

Category | Points (%) | Criteria

Assembly quality | 25% | Coverage reporting and quality assessment
Phylogenetic classification | 25% | Correct lineage/clade assignment, tree topology
Antigenic drift interpretation | 15% | Correct identification of substitutions on the HA gene and possible effect.
Resistance interpretation | 15% | Correct identification of resistance substitutions on the NA gene
Reporting & documentation | 10% | Clarity, completeness, methods stated