Development of a Global External Quality Assessment Platform for Respiratory Syncytial Virus (RSV) Whole Genome Sequencing

Bi, S[1,2]., Cogle, K[2]., Mohareb, F[2]., Sadigh, M[1]. & Rughooputh, S[1*].
[1]UK NEQAS for Microbiology, [2]Cranfield University
[*]organiser@ukneqasmicro.org.uk

Introduction

Respiratory Syncytial Virus (RSV) is a leading viral pathogen responsible for severe respiratory infections in infants, older adults and immunocompromised individuals[1]. Effective global surveillance is crucial to monitor viral evolution, inform vaccine development, and support public health responses[2]. To address this need, the World Health Organization (WHO), in collaboration with UK NEQAS for Microbiology (UK NEQAS), established a global RSV surveillance programme aimed at enhancing laboratory detection capabilities[3] through a global RSV external quality assessment (EQA) scheme. In 2024, a whole genome sequencing (WGS) component was included to this EQA scheme involving participation from 21 laboratories globally (Figure 1). The WGS pipeline and data analysis was developed in collaboration with Cranfield University. This initiative integrated international performance evaluation with expert bioinformatics input to design a robust sequencing pipeline and assess participant data.

Figure 1: Map of participating laboratories globally

Methods

Figure 2 illustrates the stepwise methodology used in the WHO RSV WGS EQA Distribution 5791, highlighting the different steps from specimens distribution, specimen processing, genomic sequencing, data analysis by participating laboratories, followed by benchmarking by UK NEQAS.

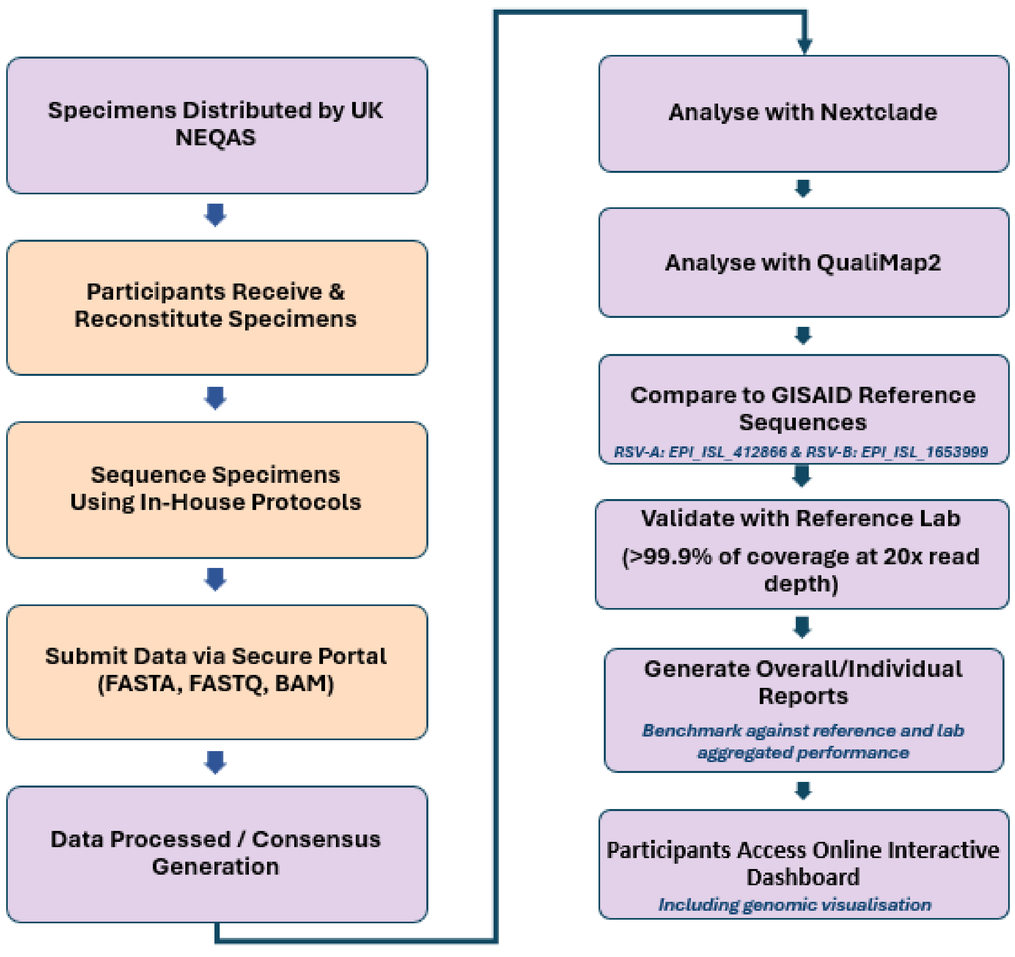

Figure 2: Workflow for the WHO RSV External Quality Assessment (EQA)

Results

RSV Subtyping

Strong concordance in subtyping across all three specimens: 94% for specimens 2524 and 2526, whilst for specimen 2525 concordance was 100%.

Results

Lineage Assignment

Strong concordance against the reference sequences across most participants results.

- 94% correct for specimens 2524 and 2526.
- 100% correct for specimen 2525

Indicator	Specimen ID	Your result	Intended Result	Reference Lab result	Your score	Participant with intended results
RSV Subtyping	2524	RSV-B	RSV-B	RSV-B	Met	16/17 (94%)
	2525	RSV-A	RSV-A	RSV-A	Met	21/21 (100%)
	2526	RSV-B	RSV-B	RSV-B	Met	16/17 (94%)
Lineage	2524	B.D.4.1.1	B.D.4.1.1	B.D.4.1.1	Met	16/17 (94%)
	2525	A.D.3	A.D.3	A.D.3	Met	21/21 (100%)
	2526	B.D.E.1	B.D.E.1	B.D.E.1	Met	16/17 (94%)
Legacy lineage	2524	GB5.0.5a	GB5.0.5a	GB5.0.5a	Met	15/17 (88%)
	2525	GA2.3.5	GA2.3.5	GA2.3.5	Met	21/21 (100%)
	2526	GB5.0.5a	GB5.0.5a	GB5.0.5a	Met	16/17 (94%)

Table 1: Result summary for participating laboratories

Sequencing Quality Metrics*

- Genome coverage ≥90%: achieved by 41–90% of participants
- Low ambiguity (% Ns ≤2%): observed in 29–85% of sequences
- Sequence similarity ≥95%: reported in 41–85% of submissions
- Mean read depth ≥50: reached by 52–57% of labs

Indicator	Specimen ID	Your result	Recommended Value*	Reference Lab result	Your score	Participant summary	
						Mean (IQR)	Participants meeting threshold
Genome Coverage (%)	2524	95.7	90% or higher	95.7	Met	79 (77-97)	7/17 (41%)
	2525	97.5	90% or higher	97.5	Met	98 (98-100)	19/21 (90%)
	2526	99.8	90% or higher	99.8	Met	87 (84-100)	11/17 (64%)
Ns in Sequence (%)	2524	1.7	2% or lower	1.7	Met	16 (1-21)	5/17 (29%)
	2525	0.0	2% or lower	0.0	Met	2 (0-1)	18/21 (85%)
	2526	0.0	2% or lower	0.0	Met	13 (0-16)	8/17 (47%)
Similarity (%)	2524	95.0	95% or higher	95.0	Met	78 (77-96)	7/17 (41%)
	2525	96.1	95% or higher	96.1	Met	96 (97-98)	18/21 (85%)
	2526	98.9	95% or higher	98.9	Met	85 (82-99)	10/17 (58%)
Read Coverage (mean)	2524	355.0	50 or higher	355.0	Met	7408 (696-9138)	9/17 (52%)
	2525	23378.0	50 or higher	23378.0	Met	15761 (2947-27042)	12/21 (57%)
	2526	11912.0	50 or higher	11912.0	Met	11277 (1292-19301)	9/17 (52%)

Table 2: Sequence quality for participating laboratories

The interactive dashboard as illustrated in Figure 3, provided participants with IGV-style interface[4], enabling direct inspection of read alignments, genome coverage, and areas of ambiguity within their submitted RSV sequences.

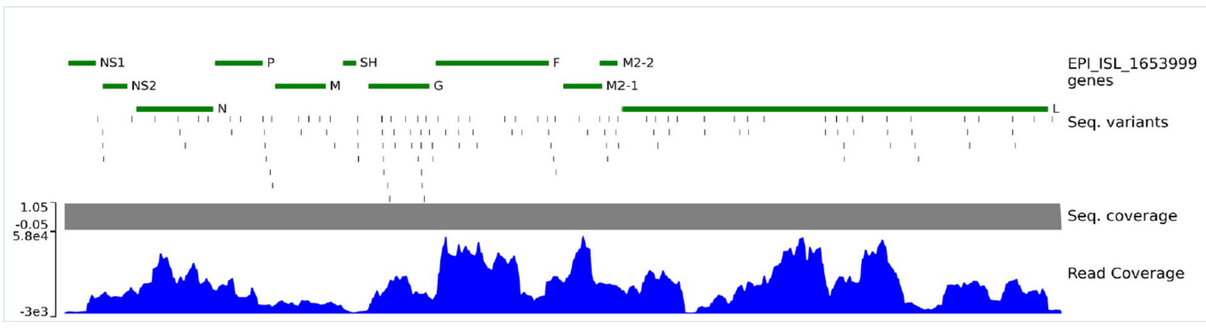

Figure 3: Interactive genomic visualisation (IGV) for the submitted sequences and reads

Discussion/Conclusion

- Data collection, storage analysis and quality control (QC), was carried out by UK NEQAS in collaborations with Cranfield University.
- The RSV sequencing EQA platform effectively assessed laboratory performance and data quality.
- While subtyping and lineage assignment accuracy were high, variability in genome coverage and % Ns highlight the need for continued standardisation and training.
- This initiative supports global efforts to enhance pathogen genomics (PG) capacity and ensure reliable molecular surveillance of RSV.
- The collaboration marks a significant advancement toward the standardisation of WGS EQA for RSV and other respiratory viruses.

Acknowledgements

Thank you to World Health Organization, Centers of Disease Control & Prevention, WHO RSV Collaborating Centres, Reference Labs for performing WGS, staff at UK NEQAS for Microbiology & UKHSA for assisting with this project.

References

1. Kaler, J., *et al.* (2023). Respiratory Syncytial Virus: A Comprehensive Review of Transmission, Pathophysiology, and Manifestation. Cureus, 15(3), e36342. https://doi.org/10.7759/cureus.36342
2. Broor, S., *et al.* (2020). Leveraging the Global Influenza Surveillance and Response System for global respiratory syncytial virus surveillance-opportunities and challenges. Influenza and other respiratory viruses, 14(6), 622–629. https://doi.org/10.1111/irv.12672
3. Williams, T., *et al* (2023). Results from the second WHO external quality assessment for the molecular detection of respiratory syncytial virus, 2019-2020. Influenza and other respiratory viruses, 17(1), e13073. https://doi.org/10.1111/irv.13073
4. Thorvaldsdóttir, *et al*. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Briefings in bioinformatics, 14(2), 178–192. https://doi.org/10.1093/bib/bbs017