Feb 17, 2025

Quality Metrics Report

**Introduction**

Thank you for participating in the distribution RSV 2024 Winter of UK NEQAS Microbiology pilot External Quality Assessment (EQA).  
  
Samples for this EQA are distributed by UK NEQAS Microbiology and those with detectable virus are either sequenced inhouse or forwarded to the appropriate laboratory for sequencing according to routine practice.  
  
A survey of sequencing technology is completed as part of the sequencing result upload process. FASTA, BAM and/or FASTQ files are requested to evaluate RSV sequencing quality. The sequence data submitted is also processed by the EQA to generate a lineage using Nextclade.  
  
Your individual sequencing quality and lineage assignment report (docx format) are available on the XXXXXX website. If you have any problems accessing your reports then please contact XXXXXXXXXXXXXXXXXX (email address).   
  
The purpose of this EQA is to assess:  
 ➢ The accuracy of RSV sequencing.  
 ➢ Provide a measurement of the quality of viral sequencing.  
  
The summary of the EQA participation, marking criteria applied, and the scoring is provided as Appendix 1.

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# Evaluation Report: 9999

## Table 1: RSV subtyping and lineage assignment

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Indicator** | **Specimen ID** | **Your result** | **Intended Result** | **Reference Lab result** | **Your score** | **Participant with intended results** |
| RSV Subtyping | 2525 | RSV-A | RSV-A | RSV-A | Pass | 3/5 (60%) |
| 2526 | RSV-B | RSV-B | RSV-B | Pass | 4/4 (100%) |
| 2524 | RSV-B | RSV-B | RSV-B | Pass | 4/4 (100%) |
| Lineage | 2525 | A.D.3 | A.D.3 | A.D.3 | Pass | 3/5 (60%) |
| 2526 | B.D.E.1 | B.D.E.1 | B.D.E.1 | Pass | 3/4 (75%) |
| 2524 | B.D.4.1.1 | B.D.4.1.1 | B.D.4.1.1 | Pass | 4/4 (100%) |
| Legacy lineage | 2525 | GA2.3.5 | GA2.3.5 | GA2.3.5 | Pass | 3/5 (60%) |
| 2526 | GB5.0.5a | GB5.0.5a | GB5.0.5a | Pass | 4/4 (100%) |
| 2524 | GB5.0.5a | GB5.0.5a | GB5.0.5a | Pass | 4/4 (100%) |

## Table 2: Sequencing Quality

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Indicator** | **Specimen ID** | **Your result** | **Recommended Value\*** | **Reference Lab result** | **Your score** | **Participant summary** | |
| Mean (IQR) | Participants meeting threshold |
| Genome Coverage (%) | 2525 | 97.5 | 90% or higher | 97.5 | Pass | 94 (92-99) | 3/4 (75%) |
| 2526 | 99.8 | 90% or higher | 99.8 | Pass | 59 (35-84) | 1/4 (25%) |
| 2524 | 95.7 | 90% or higher | 95.7 | Pass | 69 (64-84) | 1/4 (25%) |
| Ns in Sequence (%) | 2525 | 0.0 | 2% or lower | 0.0 | Pass | 5 (1-6) | 3/4 (75%) |
| 2526 | 0.0 | 2% or lower | 0.0 | Pass | 41 (16-65) | 1/4 (25%) |
| 2524 | 1.7 | 2% or lower | 1.7 | Pass | 31 (16-36) | 1/4 (25%) |
| Similarity (%) | 2525 | 96.1 | 98% or higher | 96.1 | Fail | 88 (79-97) | 1/4 (25%) |
| 2526 | 98.9 | 98% or higher | 98.9 | Pass | 58 (34-84) | 1/4 (25%) |
| 2524 | 95.0 | 98% or higher | 95.0 | Fail | 68 (63-84) | 1/4 (25%) |
| Read Coverage (mean) | 2525 | 23378.0 | 50 or higher | 23378.0 | Pass | 12817 (7536-18097) | 2/4 (50%) |
| 2526 | 11912.0 | 50 or higher | 11912.0 | Pass | 2973 (1508-4438) | 1/4 (25%) |
| 2524 | 355.0 | 50 or higher | 355.0 | Pass | 1536 (827-2245) | 2/4 (50%) |

\* Recommended Value

* Obtained sufficient genome coverage (90% or higher).
* Obtained sufficient Ns in Sequence (2% or lower).
* Obtained sufficient Similarity (98% or higher).
* Obtained sufficient Read Coverage (Mean depth of 50 or higher).

# Sample 2524

## Lineage assignment

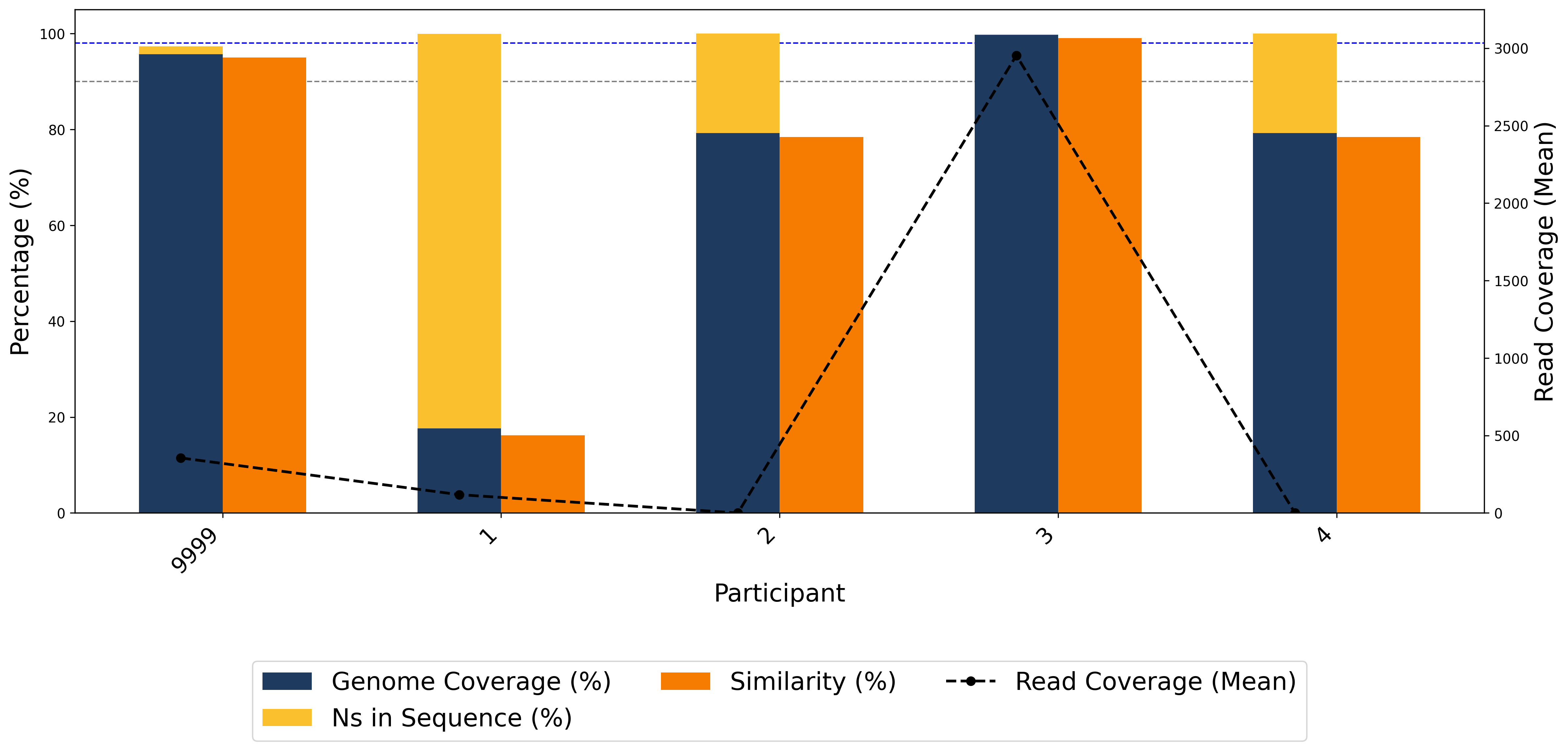
* All participants matched lineage assignments with reference lab.

***Table 1. Lineage assignments for sample 2524, including RSV subtype.***

|  |  |  |  |
| --- | --- | --- | --- |
| **Participant** | **RSV Subtype** | **Lineage** | **Legacy lineage** |
| *9999* | RSV-B | B.D.4.1.1 | GB5.0.5a |
| *1* | RSV-B | B.D.4.1.1 | GB5.0.5a |
| *2* | RSV-B | B.D.4.1.1 | GB5.0.5a |
| *3* | RSV-B | B.D.4.1.1 | GB5.0.5a |
| *4* | RSV-B | B.D.4.1.1 | GB5.0.5a |

## Sequencing quality

* Participants 1, 2, 4 failed to satisfy the threshold for genome coverage (90%).
* Participants 1, 2, 4 failed to satisfy the threshold for Ns in Sequence (greater than 2%).
* Participants 9999, 1, 2, 4 failed to satisfy the threshold for Similarity (98%).
* Participants 2, 4 failed to satisfy the threshold for Read Coverage (Mean depth of 50).



***Figure 1. Quality metrics for sample 2524 for participants that submitted appropriate data files***

***Table 2. Quality metrics data for sample 2524***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Participant** | **Genome Coverage (%)** | **Ns in Sequence (%)** | **Similarity (%)** | **Read Coverage (Mean)** |
| *9999* | 95.7 | 1.7 | 95.0 | 355.0 |
| *1* | 17.6 | 82.4 | 16.2 | 117.9 |
| *2* | 79.3 | 20.7 | 78.4 |  |
| *3* | 99.7 | 0.1 | 99.1 | 2953.7 |
| *4* | 79.3 | 20.7 | 78.4 |  |

# Sample 2525

## Lineage assignment

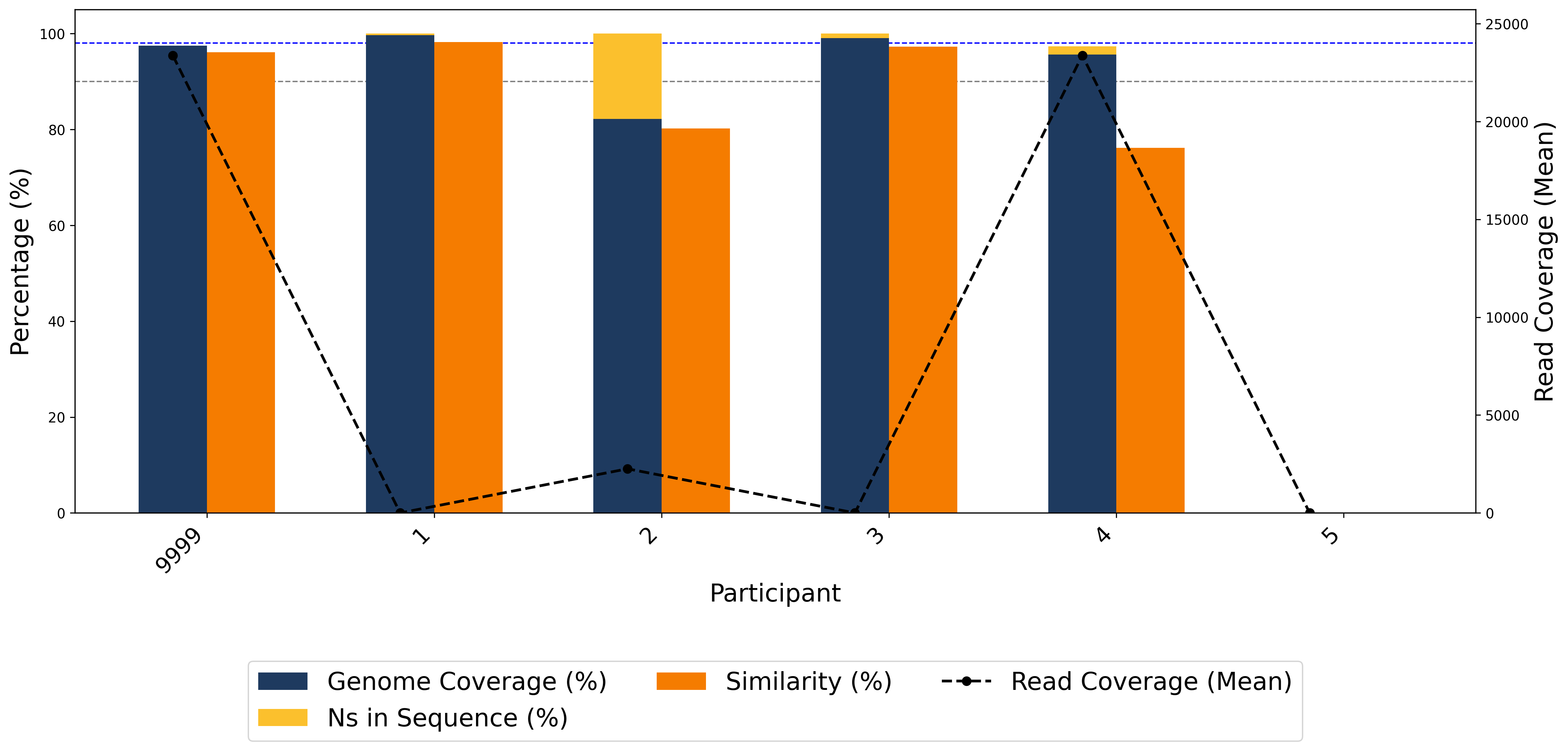
* Some participants' clade assignments differed from the reference lab.

***Table 3. Lineage assignments for sample 2525, including RSV subtype.***

|  |  |  |  |
| --- | --- | --- | --- |
| **Participant** | **RSV Subtype** | **Lineage** | **Legacy lineage** |
| *9999* | RSV-A | A.D.3 | GA2.3.5 |
| *1* | RSV-A | A.D.3 | GA2.3.5 |
| *2* | RSV-A | A.D.3 | GA2.3.5 |
| *3* | RSV-A | A.D.3 | GA2.3.5 |
| *4* | RSV-B | A.3.1.1 | GA2.3.3 |
| *5* | N/A | N/A | N/A |

## Sequencing quality

* Participants 2, 5 failed to satisfy the threshold for genome coverage (90%).
* Participants 2, 5 failed to satisfy the threshold for Ns in Sequence (greater than 2%).
* Participants 9999, 2, 3, 4, 5 failed to satisfy the threshold for Similarity (98%).
* Participants 1, 3, 5 failed to satisfy the threshold for Read Coverage (Mean depth of 50).



***Figure 2. Quality metrics for sample 2525 for participants that submitted appropriate data files***

***Table 4. Quality metrics data for sample 2525***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Participant** | **Genome Coverage (%)** | **Ns in Sequence (%)** | **Similarity (%)** | **Read Coverage (Mean)** |
| *9999* | 97.5 | 0.0 | 96.1 | 23378.1 |
| *1* | 99.7 | 0.3 | 98.2 |  |
| *2* | 82.2 | 17.8 | 80.2 | 2254.9 |
| *3* | 99.1 | 0.9 | 97.2 |  |
| *4* | 95.6 | 1.7 | 76.2 | 23378.2 |
| *5* |  |  |  |  |

# Sample 2526

## Lineage assignment

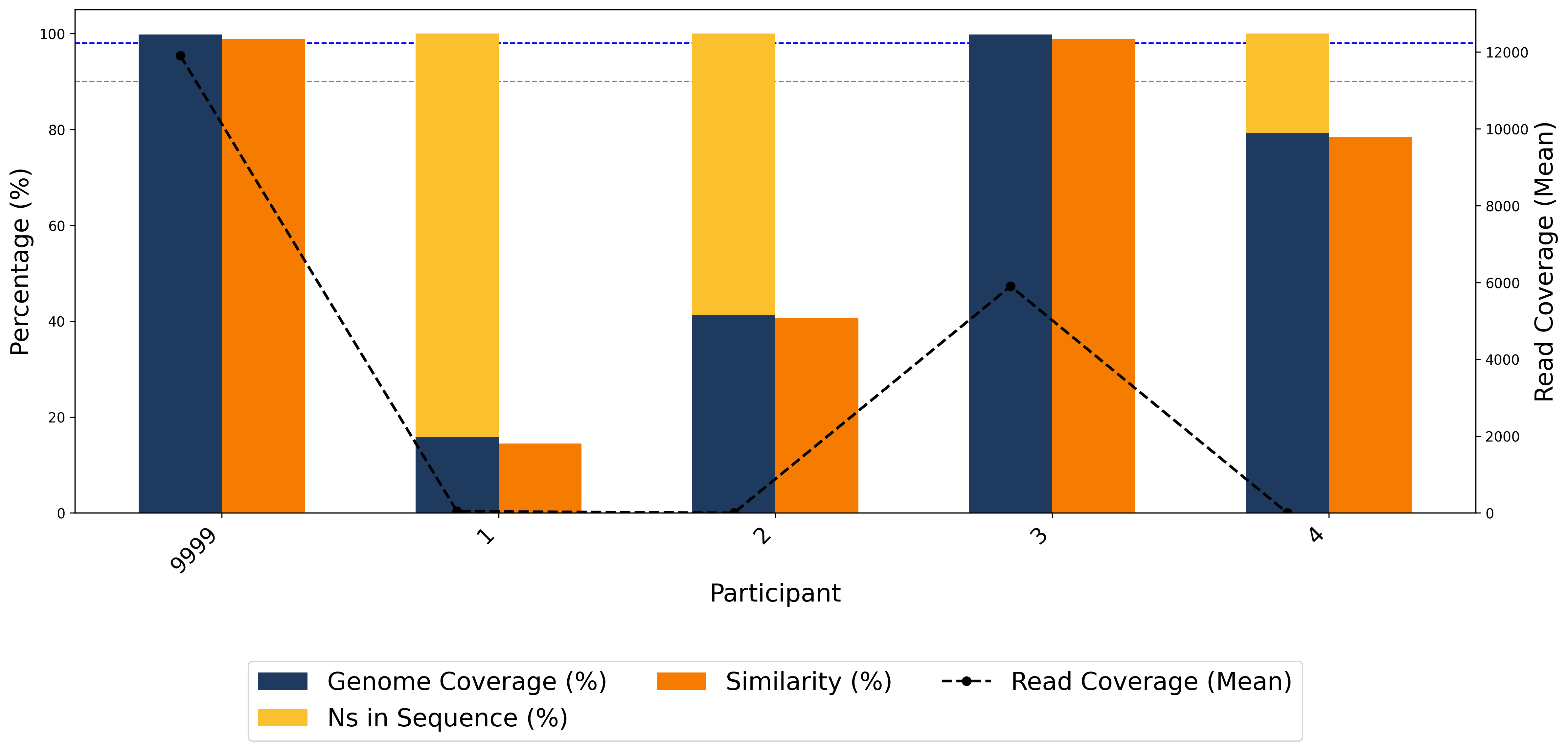
* Some participants' clade assignments differed from the reference lab.

***Table 5. Lineage assignments for sample 2526, including RSV subtype.***

|  |  |  |  |
| --- | --- | --- | --- |
| **Participant** | **RSV Subtype** | **Lineage** | **Legacy lineage** |
| *9999* | RSV-B | B.D.E.1 | GB5.0.5a |
| *1* | RSV-B | B.D.E.1 | GB5.0.5a |
| *2* | RSV-B | B.D.E.1 | GB5.0.5a |
| *3* | RSV-B | B.D.E.1 | GB5.0.5a |
| *4* | RSV-B | B.D.4.1.1 | GB5.0.5a |

## Sequencing quality

* Participants 1, 2, 4 failed to satisfy the threshold for genome coverage (90%).
* Participants 1, 2, 4 failed to satisfy the threshold for Ns in Sequence (greater than 2%).
* Participants 1, 2, 4 failed to satisfy the threshold for Similarity (98%).
* Participants 1, 2, 4 failed to satisfy the threshold for Read Coverage (Mean depth of 50).



***Figure 3. Quality metrics for sample 2526 for participants that submitted appropriate data files***

***Table 6. Quality metrics data for sample 2526***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Participant** | **Genome Coverage (%)** | **Ns in Sequence (%)** | **Similarity (%)** | **Read Coverage (Mean)** |
| *9999* | 99.8 | 0.0 | 98.9 | 11912.1 |
| *1* | 15.8 | 84.2 | 14.5 | 42.8 |
| *2* | 41.4 | 58.6 | 40.6 |  |
| *3* | 99.8 | 0.0 | 98.9 | 5903.4 |
| *4* | 79.3 | 20.7 | 78.4 |  |

**Appendix 1: Additional Information  
  
*Samples provided and testing required***Original samples distributed by UK NEQAS Microbiology were freeze dried.  
 ➢ Sequencing-only laboratories received samples directly and were instructed to reconstitute with 0.5mL of  
molecular grade water prior to testing.  
 ➢ Samples processed by a primary testing laboratory were forwarded on as RNA / lysate and handled and  
stored according to local procedures / policies.  
Sequencing was carried out according to the laboratory's normal procedure.  
  
***Data submission and analysis***Data collection, quality control (QC), storage and analysis to WHO defined standards and requirements was carried out by University of Cranfield in collaborations with UK NEQAS for Microbiology and Micropathology ltd.  
Participants were required to submit FASTA, FASTQ or BAM files which are used to assess quality metrics. FASTQ files are preferred over BAM files. If a FASTQ file is not provided but a BAM file is, it will realigned to reference sequence. Should a FASTA not be provided, a consensus sequence will be automatically generated from the FASTQ/BAM, otherwise the provided FASTA will be assumed to be the consensus sequence.  
  
***Validated results***The EQA cases were validated by a national RSV sequencing reference laboratory using Illumina sequencing with 99.9% of the genome covered at 20x. The quality of the reference laboratory sequences was classed as very high. Participants have only been assessed against regions successfully covered by the reference laboratory. Stated lineages were established using Nextclade and Nextstrain identifiers are reported. Participant submissions were compared against the reference sequences suggested by GISAID: EPI\_ISL\_412866 (RSV A) and EPI\_ISL\_1653999 (RSV B) for all regions successfully sequenced by the reference laboratory.  
  
***Quality Metrics***Where appropriate data files have been provided by the participant, the following quality metrics will be stated on the reports:  
➢ Genome Coverage (%) - The percentage of reference bases covered, with a threshold typically set at ≥90%. Computed using Nextclade.  
➢ Ns in Sequence (%) - The percentage of ambiguous bases (N's) in the sequence, with a threshold typically set at ≤2%. Computed using Nextclade.  
➢ Similarity (%) - The percentage of sequence similarity compared to the reference, with a threshold typically set at ≥98%. Computed using Nextclade.  
➢ Read Coverage (Mean Depth) - The average depth of sequencing reads, with a threshold typically set at ≥50. Computed using Qualimap2.  
  
***Participation and scoring submissions***xxxxxxxxxxxxxxxxxx  
  
***Scheme compliance***All participating laboratories complied with Scheme instructions.

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