Whole-genome sequencing of Human Respiratory Syncytial Virus using Oxford Nanopore technology

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Introduction

Human respiratory syncytial virus (HRSV) is a leading cause of severe lower respiratory illness in young children and the elderly. HRSV is classified within the Orthopneumovirus genus of the Pneumoviridae family and is divided in two major antigenic groups HRSV-A and HRSV-B. Based on whole-genome phylogeny and signature amino acids, HRSV sequences are currently classified into 24 HRSV-A and 16 HRSV-B lineages. Accurate and rapid characterization of HRSV genomes is essential for large-scale genomic surveillance and monitoring drug resistance mutations associated with novel antiviral therapies.

Materials & Methods

The analytical performance of the HRSV whole-genome assay was assessed on ATCC strains (n=5), nasal wash (NW) isolates (n=20), virus strain dilutions (n=36), and nasopharyngeal (NP) swabs (n=30).

The HRSV whole-genome amplification approach and assay workflow (wet lab and dry lab) are presented in Figure 1.

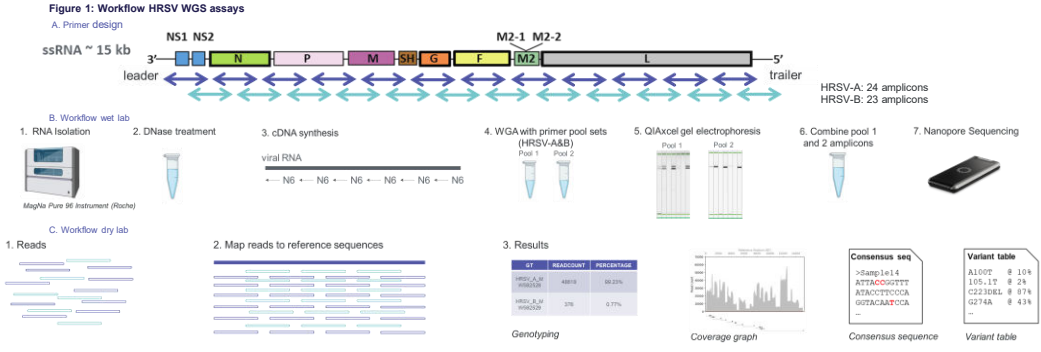
A. HRSV-A and HRSV-B whole genome amplification (WGA) was performed with subgroup specific primers using a 2-primer-pool multiplex PCR-tiling approach, covering the full-length viral genome in respectively 24 and 23 overlapping amplicons ranging from 560 to 1170 bp.

B. Viral RNA extraction was performed with the MagNA Pure 96 instrument and HRSV detection, subgroup identification and viral RNA quantification was performed with an in-house validated real-time one-step duplex RT-qPCR targeting highly conserved regions in the N-gene.

C. Whole genome amplification was performed with the respective HRSV subgroup specific primer pool set after DNase pre-treatment and cDNA synthesis using random hexamers. Successful amplification was confirmed by gel electrophoresis and the combined sample pools were sequenced with V14 kit on a MinION sequencing instrument.

D. Data analysis was performed using Cerba Research's NL in-house developed bio-informatics pipeline.

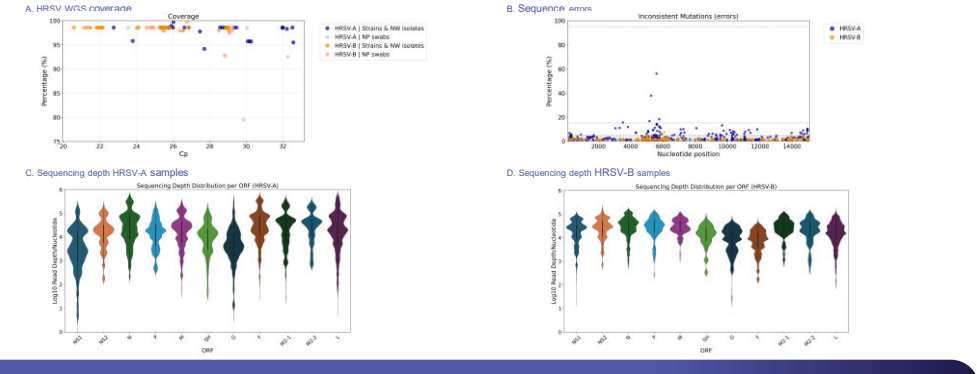
Figure 1: Workflow HRSV WGS assays



Results

- All HRSV-A/B ATCC strains, NW isolates, and strain dilution replicates (4.6–7.5 $\log_{10}$ copies/mL) were successfully amplified, yielding ≥94% complete genome coverage (Figure 2A).
- For HRSV strain dilutions most sequence errors occurred at frequencies ≤4%. A limited number of errors (n=5) with a frequency of ≥15% were detected in the HRSV-A mid (5.7 log10 copies/mL) and low (4.7 log10 copies/mL) viral load samples (Figure 2B).
- 29 of the 30 NP swabs were confirmed positive for HRSV by RT-qPCR.
  - Amplification results were obtained for 4/6 (67%) HRSV-A, 13/24 (54%) HRSV-B samples, including one HRSV-A/B co-infected NP swab. Viral loads ranging from 4.60 log10 copies/mL to 7.21 log10 copies/mL.
  - WGS achieved >92% coverage for all amplified samples except the co-infected swab (~5.0 $\log_{10}$ copies/mL), which yielded 80% coverage for HRSV-A (Figure 2A).
- The median sequencing depth per nucleotide position across all 11 ORFs ranged between 3.5 -4.6 log10 reads (Figure 2C and 2D).
- Full-length F-gene sequences were obtained for all samples, apart from the co-infected NP swab, which exhibited 92% F-gene coverage for HRSV-A.

Figure 2: The HRSV WGS coverage for ATCC strains, NW isolates, strain dilutions and NP swabs (A). Sequencing errors for HRSV-A and HRSV-B (B). The violin plots summarize the read depth distribution per nucleotide position of all HRSV-A (C) and HRSV-B (D) samples.



Conclusion

HRSV-A/B WGS assays demonstrate robust performance and high sensitivity, suitable to be applied directly on respiratory samples for large-scale viral genomic surveillance studies and investigating drug resistance mutations of new antiviral therapies.

Contact & Disclosures

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Authors are all Cerba Research NL employees with no conflict of interest.