Validation and application of multiplex nanopore MinION sequencing for molecular typing of human adenoviruses in clinical samples: a cost effective sequencing strategy

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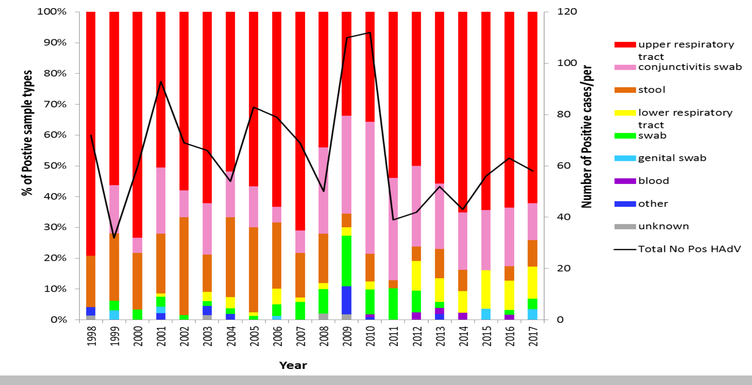
Background

Human adenovirus (HAdV) is a DNA virus associated with a wide range of illnesses ranging from benign colds to more serious conditions such as meningoencephalitis. It causes severe infections in immunocompromised hosts that may result in death. For HAdV identification and typing, Oxford Nanopore sequencing technology (ONT) could offer a promising tool for obtaining high-resolution genomic data that can be used to resolve viral taxonomy.

Aims

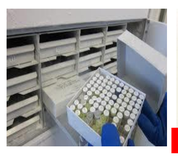
- To validate the sensitivity and specificity of ONT tailed universal PCR primers for HAdV typing
- To apply the multiplex nanopore MinION sequencing for identification and typing of HAdV in clinical samples
- To assess the suitability (accuracy, reproducibility, and cost effeciency) of ONT 1D PCR barcoding (96) amplicons for molecular typing of HAdV strains in comparision to the gold standard Sanger Sequencing.

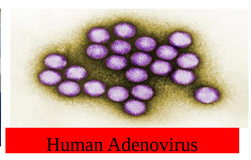
Figure 1: HAdV confirmed positive samples types of suspected cases / year referred to IFIK (Bern, Switzerland); 1998 -2017



METHODS

Wet lab



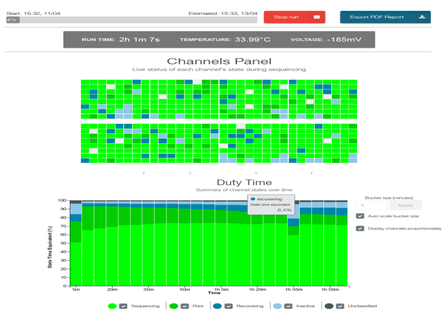


Human Adenovirus

Sample:Cell culture or original clinical material → Lysis and extraction •Automated (NucliSENS® easyMag®) → Conventional PCR • HVR-7 (Sarantis et al 2004) → Gel electrophoresis → Purification → Quantification → Sequencing (ONT vs Sanger)

Nanopore MinION sequencing • 1D PCR barcoding(96) amplicons/cDNA (SQK-LSK109)

Sanger



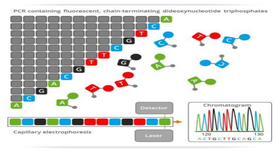


Figure 2: workflow for the wet lab

Method overview

- Validation was done using samples from years 2009 and 2010, the two years associated with an increased number of HAdV cases (Figure 1)
- Viral DNA was extracted from 200µL of the cell culture supertnant in a final elution volume of 50µL using the ***NUCLISENS***® easyMAG®.
- A volume of 10µL of eluted sample was amplified using a PCR procedure that targeted hypervariable regions 1-7 of the hexon gene (Sarantis et al 2004). Expected amplicons from this region ranged from 602 to 630bp in length
- Sequencing was performed in parallel with multiplex ONT MinION and Sanger sequencing.

Dry lab

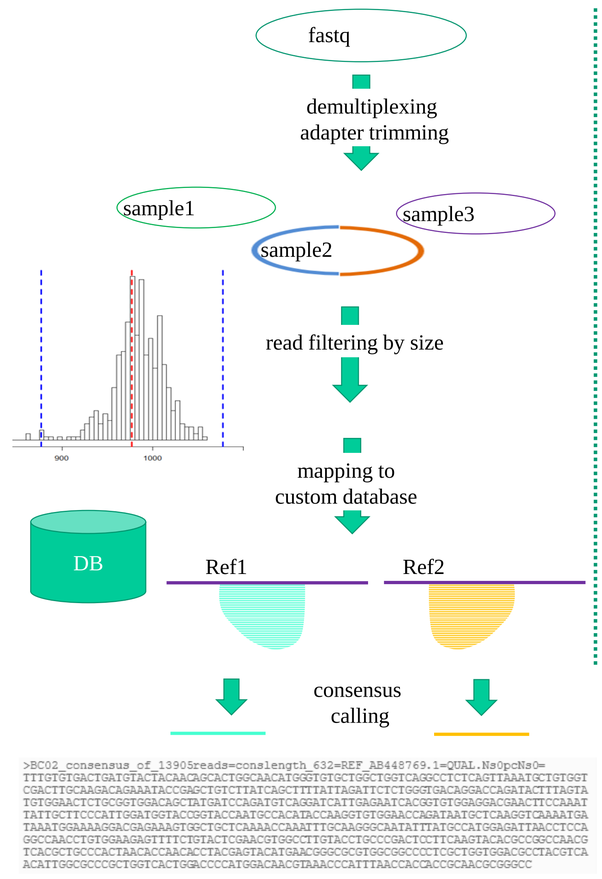
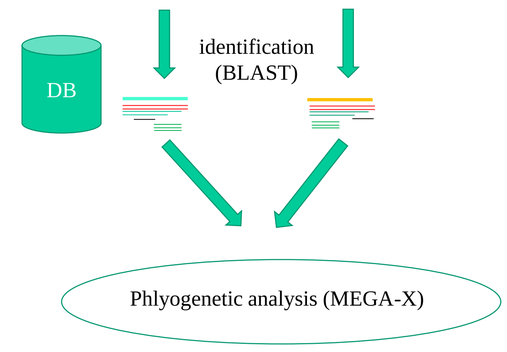


Figure 3: workflow of the bioinformatics LORCAN pipeline



Phylogenetic analysis performed to determine HAdV sequence relatedness

RESULTS

Figure 4: Validation of ONT tailed univeral PCR primers sensitivity and specificity for HAdV typing. The sensitivity of the assay was determined by performing PCR on 10-fold serial dillutions of HAdV8 with Ct values 13.5 to 31.1. Figure 4A and 4B show the first round PCR performed using Sarantis et al 2004 primers tailed with ONT universal sequences. Desalted primers (figure 4A) versus PAGE-Purified primers (figure 4B).

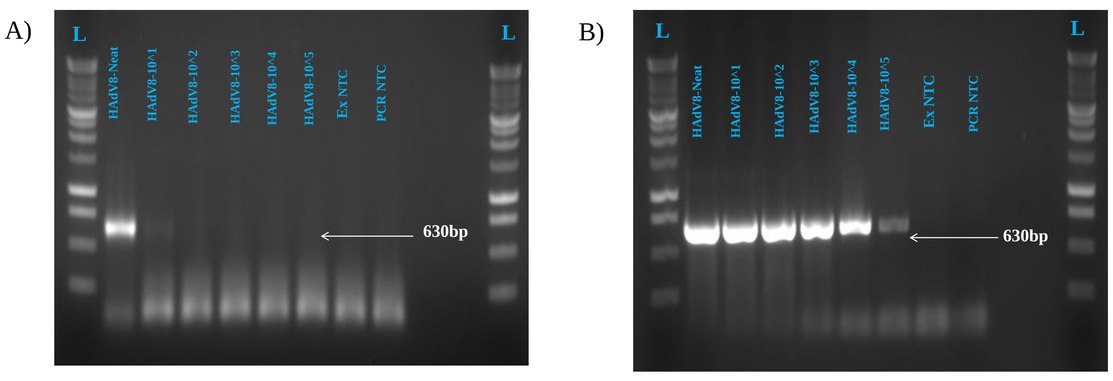


Table 1. Comparison of the accuracy of consensus sequences

- The average sequence identity to Sanger was 99.63% (LORCAN) and 99.59 % (LORCAN + Nanopolish)

Sample I.D	Assigned HAdV genotype			Gaps		Identity to Sanger consensus (%)	
	Sanger	LORCAN	LORCAN + Nanopolish	LORCAN	LORCAN + Nanopolish	LORCAN	LORCAN + Nanopolish
1810454	HAdV53	HAdV53	HAdV22	1	0	99.84	95.76
1746835	HAdV8	HAdV8	HAdV8	2	0	99.68	100.00
1809358	HAdV8	HAdV8	HAdV8	2	0	99.68	100.00
1744471	HAdV8	HAdV8	HAdV8	2	0	99.68	100.00
1809213	HAdV8	HAdV8	HAdV8	2	0	99.68	100.00
1746913	HAdV8	HAdV8	HAdV8	1	0	99.68	99.84
1806792	HAdV8	HAdV8	HAdV8	1	0	99.68	99.84
1809341	HAdV8	HAdV8	HAdV8	1	0	99.68	99.84
1805919	HAdV8	HAdV8	HAdV8	2	0	99.67	100.00
1810526	HAdV8	HAdV8	HAdV8	2	0	99.52	99.84
1746276	HAdV8	HAdV8	HAdV8	2	0	99.52	99.84
1745354	HAdV8	HAdV8	HAdV8	1	0	99.52	99.68
1812202	HAdV8	HAdV8	HAdV8	2	0	99.51	99.84
1808326	HAdV8	HAdV8	HAdV8	2	0	99.50	99.83

Phylogenetic analysis

The evolutionary history inferred by Maximum Likelihood method based on Jukes-Cantor conducted with bootstrap method of 1000 replicates in MEGA-X

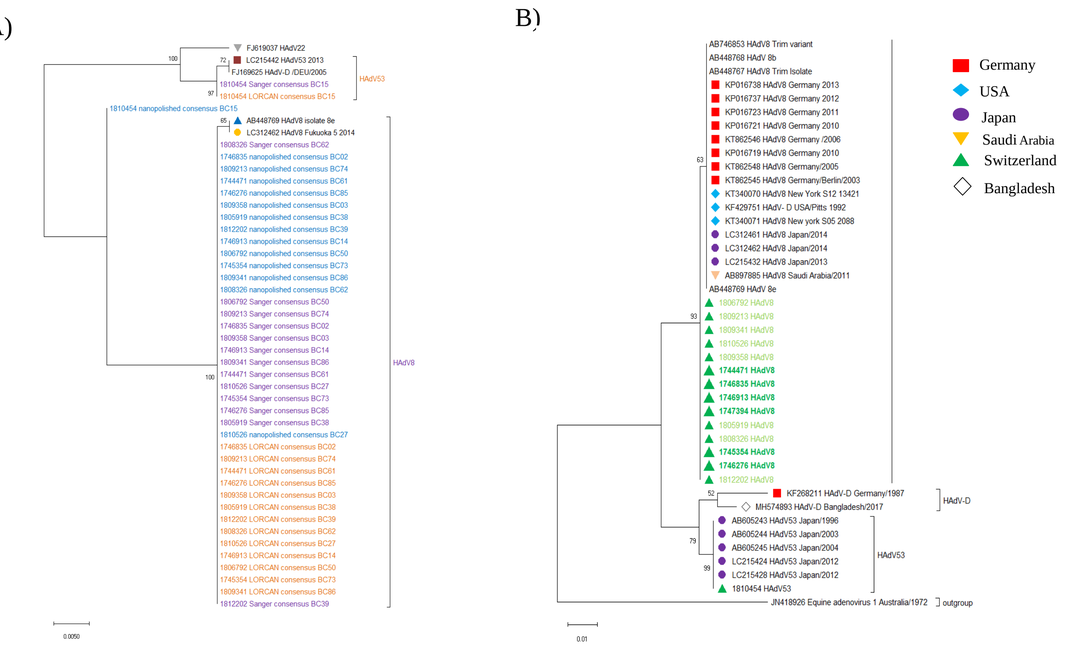


Figure 5: Validation of genotypes by phylogenetic analysis. The percentage of the trees in which the associated taxa clustered together is shown next to the branches. Figure 5A- phylogenetic comparison of MinION consensus sequences (LORCAN, and Nanopolish highlighted in orange and blue respectively) to Sanger (highligted in purple). The prototype strains shown by different symbols. The branch length measures the number of substitutions per site (0.0050). Figure 5B- phylogenetic analysis showing HAdV8 strains and HAdV53 identified from the biobank of clinical samples at IFIK (green triangle symbol) clustering with other HAdV8 and HAdV53 international isolates. Branch length measures the number of substitutions per site (0.01)

DISCUSSION

Phylogenetic analysis of sequences derived from both MinION and Sanger were similar (99.6%), identifying HAdV8 as the predominant type. Despite the high error rate of ONT sequencing compared with other sequencing methods, our results demonstrated that it is possible to generate consensus amplicon sequences of comparable accuracy to that obtained by Sanger sequencing, and that several tens of samples can be multiplexed in parallel in a time and cost efficient manner.

CONCLUSION & OUTLOOK

The results of this study provide support for the use of ONT amplicon sequencing for HAdV species identification and genotyping. ONT-based genotyping and comparison to strains involved in international outbreaks is well suited for epidemiological surveillance in the future

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