Towards non-invasive real-time monitoring of avian influenza virus

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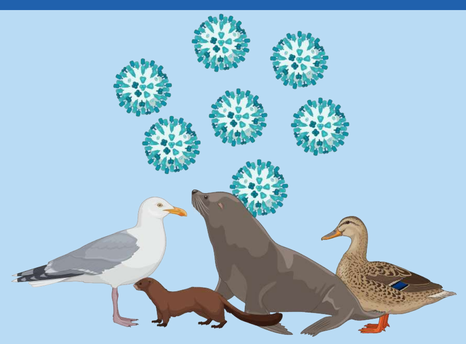
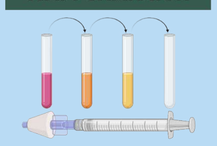
Non-invasive real-time monitoring



Avian influenza virus (AIV) is an RNA virus that can affect wild animals, livestock, and humans. Wild aquatic birds are the main reservoir of AIV and spread the virus around the globe, causing devastating outbreaks in wild and domestic populations. Early in situ detection and sequencing of AIV in wild populations is therefore essential to control transmission during an outbreak, understand global transmission patterns, and avoid devastating impacts on biodiversity, global health, and food security. Here, we present a pilot study to test direct RNA sequencing for developing a non-invasive real-time approach to rapidly and efficiently detect and sequence AIV through the East Atlantic flyway.

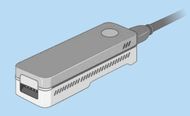
Collection of aerosol and faecal samples

Field-friendly RNA exraction



M1 Sample Prep® Cartridge Kit For RNA 2.0

Nanopore sequencing & bioinformatic analysis

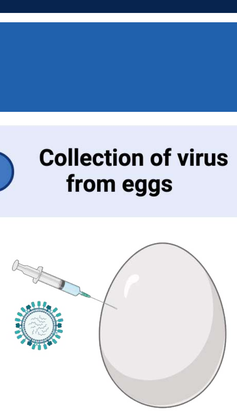


MinION

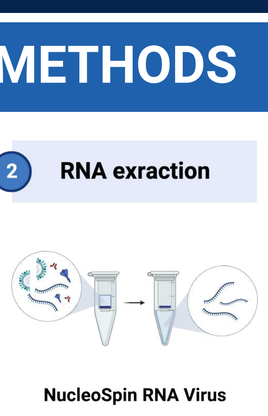
Direct RNA sequencing

METHODS

1 Collection of virus from eggs



2 RNA exraction

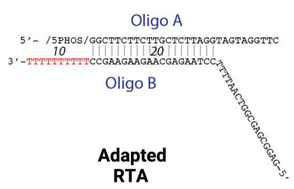


NucleoSpin RNA Virus

3 Viral quantificiation

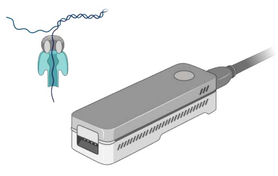
Portable Mic qPCR Cycler

4 RNA library preparation



Adapted RTA

5 Direct RNA sequencing

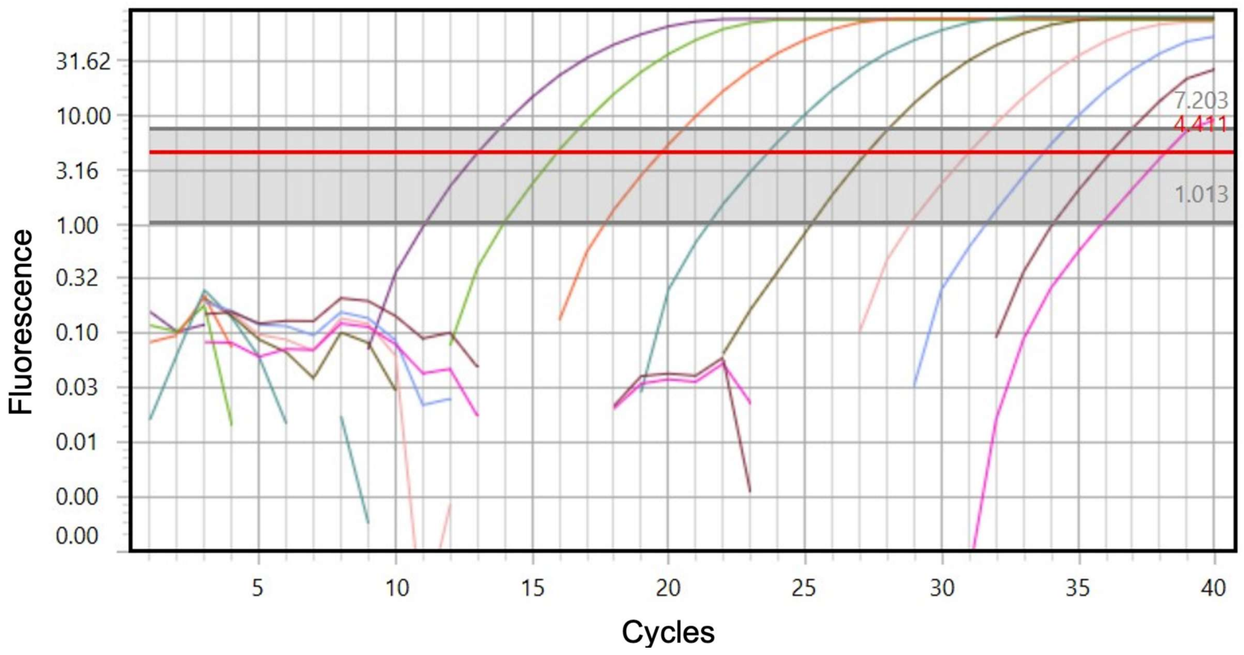


Direct RNA kit MinION

6 Bioinformatic analysis

Guppy + minimap2

RESULTS qPCR



Cycle thresholds (Ct) of an avian H1N1 serial dilution were analyzed with a rRT-PCR targeting the M segment of the virus using a portable Mic qPCR cycler. The original sample, with a Ct of 13, was direct RNA sequenced with the M1kc.

RESULTS NATIVE VIRAL RNA SEQUENCING

	Influenza A virus (A/duck/Italy/281904/2006(H1N1))
Reads	15,454
Mapped	10,029
% Mapped	64.9%
Consensus	99.61%

Name	Length	Mapped bases	Mean coverage
PB2	2308	1876669	813.1148
PB1	2310	2413382	1,044.7541
PA	2200	1225865	557.2114
HA	1741	166651	95.7214
NP	1530	223824	146.2902
NA	1424	168240	118.1461
M	989	342473	346.2821
NS	890	148519	166.8753

CONCLUSION

With direct RNA sequencing, we have been able to obtain good results (threshold of >10x coverage and >90% consensus identity). For our sample with low Ct, we have even been able to achieve >100x coverage and >99% consensus identity using kit 9 chemistry. This approach, together with the detection of the virus by rRT-PCR with portable Mic PCR, is a straightforward methodology for avian infuenza surveillance and sequencing in the feld. However, we expect to obtain significantly lower Ct values from environmental samples, so we will next test the sensitivity of direct RNA sequencing for our diluted samples. While cDNA sequencing after multi-segment amplifcation is likely to be more sensitive for such samples, we will test if the new nanopore chemistry can further improve direct RNA sequencing results.

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