NEXTGENPCR

APPLICATION PREVIEW

RAPID QPCR WORKFLOW FOR THE DETECTION OF INFLUENZA A VIRAL RNA USING THE NEXTGENPCR™ CYQLONE™ INSTRUMENT

HIGHLIGHTS

- Subtype-agnostic quantitative real-time fluorescence detection of IAV
- Ultra-fast analysis of 96 samples in ≤17 minutes using the groundbreaking cyQlone™ instrument
- Intelligent primer-probe design targeting a conserved part of the IAV genome
- Verified performance in clinical human and veterinary (including avian) RNA extracts
- Long reagent shelf life and high robustness by using a lyophilised in-house optimised RT-qPCR master mix

Influenza A virus (IAV) infections of the human respiratory system are a global health concern. IAV causes seasonal epidemics due to its high contagiousness and widespread prevalence. More concerning, however, is its capacity for uncontrolled antigenic drift and genetic reassortment, which creates a perpetual pandemic threat in the form of new influenza A viral strains [1]. This threat is becoming increasingly concerning with the rise of highly pathogenic influenza strains such as avian influenza (HPAI) that have been found in animals. Although animal reservoirs do not necessarily lead to crossover infections in humans, sporadic mutations may enable cross-species transmission. This was recently exemplified by the first reported human case of the H9N2 strain in Europe in March 2026 [2, 3]. Such reports underscore the importance of vigilant screening and surveillance for new pathogenic strains of this unpredictable virus.

Accurate and rapid detection of IAV in both human and animal nucleic acid isolates is critical for effective surveillance, outbreak control, and clinical and veterinary diagnostics. The NextGenPCR™ RT-qPCR IAV Detection Assay targets a conserved sequence of the IAV genome while accounting for common mutations, which enables the subtype-agnostic detection of IAV strains.
In combination with the cyQlone™ instrument, accurate results are achieved in under 17 minutes.

The assay uses a lyophilised, in-house optimised one-step RT-qPCR master mix, which supports more sustainable laboratory practices by enabling room-temperature shipping and storage. Long-term reagent stability of the lyophilised reagents makes the assay ideal for laboratories engaged in seasonal outbreak monitoring and pandemic preparedness initiatives. The formulation contains UDG and dUTP to effectively prevent carryover contamination, ensuring reliable and accurate results.

This application preview demonstrates the performance of the NextGenPCR™ RT-qPCR IAV Detection Assay on the ultrafast cyQlone™ instrument.

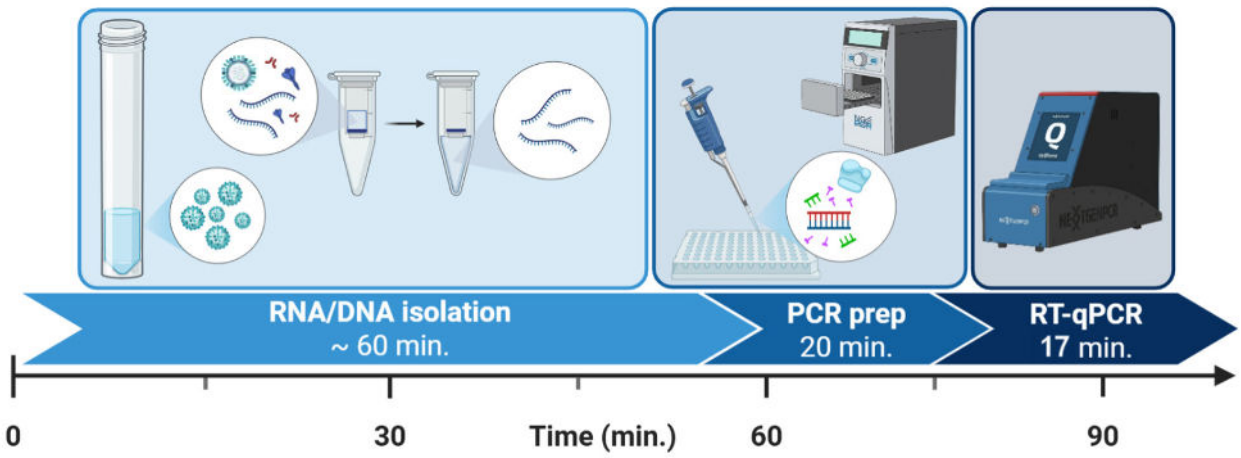

Figure 1. Workflow for the RT-qPCR-based detection of IAV with the cyQlone™ instrument.
Clinical samples, for example saliva, are first collected and subjected to nucleic acid extraction. Hereafter, the sample is added to the one-step RT-qPCR mix. Using the cyQlone™ instrument, cDNA-synthesis, real-time measurements of amplification and Cq-calculations are autonomously completed in just under 17 minutes.

The RT-qPCR cycling conditions were as follows: one cycle of reverse transcription at 50 °C for 300 s, followed by enzyme inactivation/initial denaturation at 98 °C for 30 s. This was followed by 5 pre-cycles of denaturation at 98 °C for 4 s, annealing at 59 °C for 10 s, and extension at 69 °C for 3 s. Subsequently, 45 cycles were performed consisting of denaturation at 98 °C for 3 s, annealing at 59 °C for 5 s, and extension at 69 °C for 3 s.

RESULTS

The NextGenPCR™ RT-qPCR IAV Detection Assay was tested and validated using a variety of samples ranging from synthetic RNA to avian RNA extracts of which information is displayed in Table 1.

TABLE 1. OVERVIEW OF IAV SYNTHETIC AND AVIAN RNA STRAINS USED FOR THE ASSAY.

Sample	Origin	Subtype	Strain
Twist Synthetic Influenza H1N1 (2009)	Synthetic RNA	H1N1	A/California/07/2009(H1N1)
Twist Synthetic Influenza H3N2	Synthetic RNA	H3N2	A/New York/392/2004(H3N2)
Vircell Amplirun® Influenza A H5 RNA control	Synthetic RNA	H5N1	A/reassortant/NIBRG-14 (Viet Nam/ 1194/2004 x Puerto Rico/8/1934)(H5N1)
HPAI 104	Avian	H5N1	A/common_buzzard/Denmark/ 01104-1.02/ 2025(H5N1)
HPAI 506	Avian	H5N1	A/mute_swan/Denmark/ 01506-1.02/ 2025(H5N1)
HPAI 806	Avian	H5N1	A/barnacle_goose/Denmark/ 00806-1.02/ 2024(H5N1)
HPAI 973	Avian	H5N1	A/common_buzzard/Denmark/ 00973-1.02/2025(H5N1)

A 10-fold serial dilution of Twist Synthetic Influenza H3N2 was prepared, which was amplified using the assay with the cyQlone™. Concentrations ranged from 100,000 to 10 copies per reaction. Each reaction was duplexed with 100,000 copies human internal control to recreate a realistic background of host DNA. S-curves of relative fluorescence per cycle were generated from real-time fluorescence measurements by the cyQlone™ instrument (Figure 2). Samples with starting concentrations of 100,000 to 10 copies per reaction were all successfully amplified. High degree of linearity was observed between template concentrations ranging from 100,000 to 100 copies per reaction, as indicated by the R^2 value of 0.9963 (Figure 3).

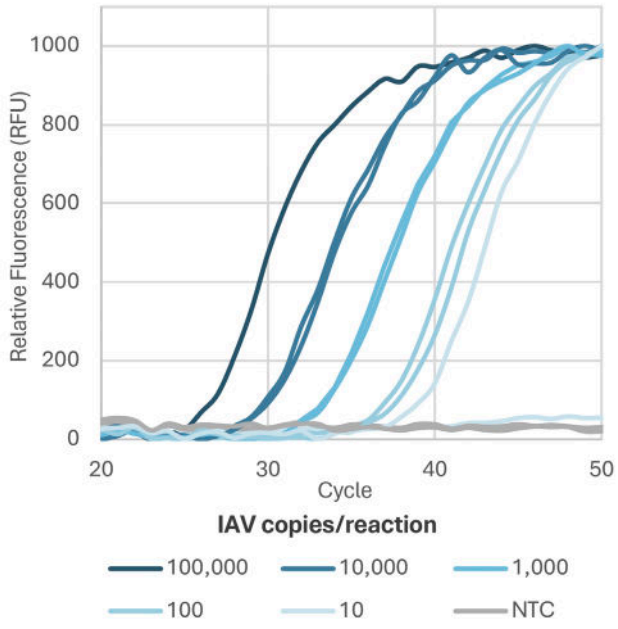

Figure 2. Amplification of IAV synthetic RNA 10-fold serial dilution, performed on the cyQlone™. *IAV dilution series ranging from 100,000 to 10 copies of template per reaction, amplified in duplicate.*

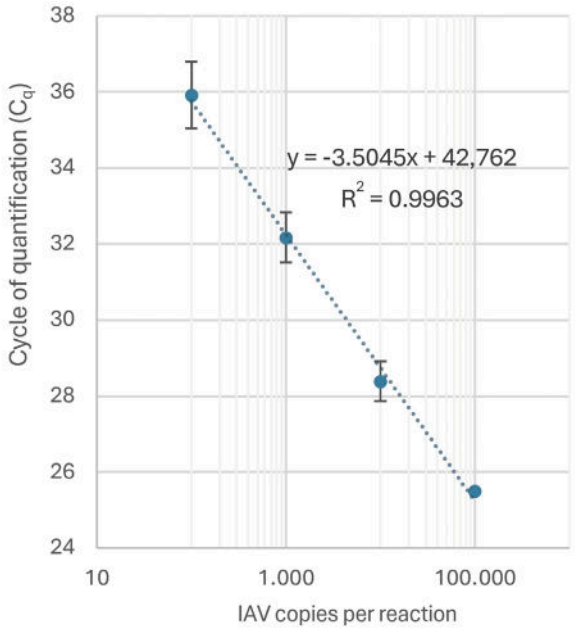

Figure 3. Linearity of the quantification of IAV synthetic RNA in a 10-fold serial dilution, performed on the cyQlone™. *A dilution range of 100,000 to 100 copies of starting template per reaction.*

Next, the assay was validated in a cohort of human nasopharyngeal swab RNA extracts positive for H1N1 or H3N2 (14/14 positive on cyQlone™ instrument) and a selection of H5N1-positive avian RNA extracts (4/4 positive on cyQlone™ instrument, Figure 4). This data shows that highly accurate influenza A detection can be achieved in varying sample types.

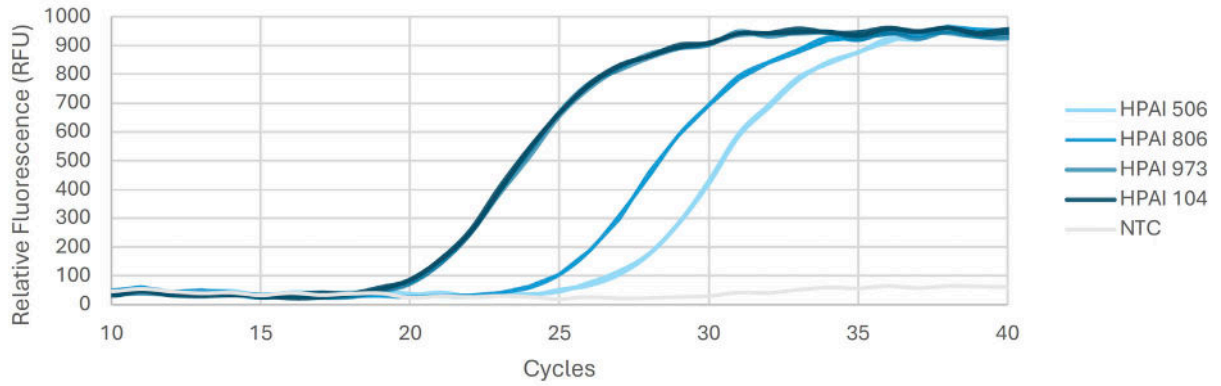

Figure 4. Amplification of avian RNA extracts obtained using the cyQlone™. *Results from real-time fluorescence measurements of avian RNA extracts, amplified in duplicate. C_q values ranging from 19 to 26.*

SUMMARY

The NextGenPCR™ RT-qPCR IAV Detection Assay is highly suitable for quick IAV detection and quantification when used in combination with the cyQlone™ instrument. This robust workflow is characterized by high accuracy of IAV detection that can be completed in an easy-to-use PCR protocol of ≤17 minutes. Importantly, the short cycling conditions are achieved without compromising sensitivity, reproducibility or robustness. Due to assay design, the protocol will work independently of IAV subtype as evidenced by excellent assay performance in different sample matrices.

For additional information or inquiries regarding this assay, the cyQlone™ instrument, or other components of the NextGenPCR™ technology, please contact sales@mbspcr.com.

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REFERENCES

[1] M. O. Altman, D. Angeletti, and J. W. Yewdell, "Antibody Immunodominance: The Key to Understanding Influenza Virus Antigenic Drift", Viral Immunology, Mar. 2018, doi: 10.1089/vim.2017.0129.

[2] E. Hutchinson, "First European case of H9N2 bird flu reported in Italy – What you need to know," The Conversation, Mar. 2026, doi: 10.64628/ab.hvqp63v56.

[3] European Centre for Disease Prevention and Control, "SURVEILLANCE REPORT Weekly Communicable Disease Threats Report, Week 13, 21–27 March 2026," Mar. 2026. [Online]. Available: https://www.ecdc.europa.eu/sites/default/files/documents/Communicable-disease-threats-report-week-13-2026.pdf

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