ORIGINAL ARTICLE

A New Quantum Dot-Based Lateral Flow Immunoassay for the Rapid Detection of Influenza Viruses

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Abstract

Rapid and accurate diagnosis of influenza is crucial to contain influenza virus outbreaks. In clinical settings, lateral flow immunoassays (LFIAs) are widely used for rapid influenza antigen detection. The choice of label plays an important role in determining the sensitivity of the LFIA. Quantum dots are one of the most promising fluorescent reporters. Here, we evaluated a novel quantum dot-based assay, QuantumPACK Easy Influenza A + B (QuantumPACK Easy; BioSquare Inc., Korea). A total of 394 nasopharyngeal swab samples, including 94 influenza A virus-positive, 98 influenza B virus-positive, 175 influenza A and B virus-negative, and 27 other respiratory pathogen-positive samples, were collected. Samples were tested with QuantumPACK Easy, Allplex RP real-time RT-PCR assay (Allplex RP; Seegene, Korea), and Sofia Influenza A + B FIA (Sofia; Quidel, CA, USA). The sensitivity and specificity of QuantumPACK Easy was analyzed using the Allplex RP assay. The agreement between QuantumPACK Easy and Sofia assays was also analyzed. The sensitivity of QuantumPACK Easy for influenza A and B was 80.9% and 83.7%, respectively. The specificity of QuantumPACK Easy was 100%. Cross-reactivity with other respiratory pathogens was not observed. Total agreement between QuantumPACK Easy and Sofia was 89.6% (kappa 0.783). The sensitivity of the Sofia assay was 66.0% for influenza A virus and 61.2% for influenza B virus. QuantumPACK Easy had acceptable performance, with better sensitivity than a commercially available antigen detection assay, possibly due to the characteristics of the quantum dot.

Keywords Quantum dot · Influenza · Rapid antigen test · Lateral flow immunoassay

1 Introduction

Influenza virus infections are a leading cause of acute respiratory infection. Before the coronavirus disease 2019 (COVID-19) pandemic, there were an estimated one billion influenza cases and 290,000–650,000 influenza-related deaths worldwide, every year [1]. During the COVID-19 pandemic, global influenza activity remained at a lower level than in previous years. In the United States, between October 2020 and July 2021, 2136 samples were reported to the Centers for Disease Control and Prevention as influenza positive, and 748 deaths were coded as influenza, which represented a sharp decrease compared to the 35 million cases of influenza-related illness and 20,000 influenza-related deaths estimated in 2019–2020 [2, 3]. Possible explanations for the reduced incidence of influenza in circulation include non-pharmaceutical interventions, reduced travel and population mixing, and possible virus–virus interactions [4]. It is, therefore, difficult to predict the influenza activity in the upcoming influenza season. Herd immunity against influenza virus has decreased for almost 2 years, and there are concerns of vulnerability to the influenza virus. Therefore, rapid and accurate diagnosis of influenza is of paramount importance to contain influenza virus outbreaks.

Rapid antigen detection assays and nucleic acid amplification tests are commonly used for viral detection in the clinical setting. Currently used rapid antigen detection assays are immunochromatography-based systems that can detect a target protein on a membrane within 15 min. The sensitivity of immunochromatography is determined by the label used for detection. Conventional immunochromatography uses gold or europium nanoparticles as the label, but recently developed

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quantum dots are promising alternative fluorescent reporters, with high stability and long fluorescence duration [5].

The newly developed QuantumPACK Easy Influenza A + B (QuantumPACK Easy; BioSquare, Hwasung, Korea) is a lateral flow immunochromatography assay that makes use of QuantumPACK as a fluorescent label. QuantumPACK comprises nanoparticles packed with quantum dots on the surface of a silica core. In the present study, we evaluated the performance of QuantumPACK Easy.

2 Materials and Methods

2.1 Sample Collection and Workflow Analysis

A total of 394 nasopharyngeal swab samples, which included 94 influenza A virus-positive, 98 influenza B virus-positive, 175 influenza A and B virus-negative, and 27 other respiratory pathogen-positive samples (five respiratory syncytial virus (RSV) A and RSV B-positive samples and samples positive for adenovirus (ADV), human coronavirus (CoV)-229E, CoV-OC43, CoV-NL63, CoV-HKU1, rhinovirus, enterovirus, parainfluenza virus (PIV) 1, PIV 2, PIV 3, PIV 4, human metapneumovirus (hMPV), bocavirus, *Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila,* and *Pneumocystis jirovecii*), were collected from patients at the Asan Medical Center in Korea in 2019–2020. *P. jirovecii*-positive specimens were obtained by bronchoalveolar lavage, and the other specimens were all obtained using nasopharyngeal swabs. The samples were transported in 3 mL universal transport media (UTM; Copán, Brescia, Italy) and stored at − 15 ℃. Samples were tested using the Allplex Respiratory Panel 1, 2, 3 real-time RT-PCR assay (Allplex RP; Seegene, Seoul, Korea), Sofia Influenza A + B Fluorescence Immunoassay (Sofia; Quidel, San Diego, CA), and QuantumPACK Easy assays. This study was approved by the Institutional Review Board of the Asan Medical Center (IRB No. 2021–0123).

2.2 QuantumPACK Easy Influenza A + B

QuantumPACK Easy makes use of nanoparticles packed with quantum dots on the surface of a silica core [6]. Quantum dots are spherical semiconductors with unique optical and electrical properties. Unlike other nanoparticles, quantum dots have a quantum confinement effect when they become smaller than the Bohr exciton radius and can emit light at the desired wavelength by controlling the particle size (Fig. 1) [7]. QuantumPACK-conjugated antibodies are applied to the conjugation pad of the diagnostic strip for specific detection of target proteins. Nasopharyngeal specimens in UTM (400 μL) were added to the provided extraction tubes and mixed. Three drops of the extracted sample were dropped vertically onto the sample pad to expose the viral antigens to the influenza-specific QuantumPACK-conjugated antibodies on the pad and thus complete the lateral flow reaction (Fig. 2). Results were analyzed using a QuantumP-ACK Easy Reader (BioSquare) in 10 min (Fig. 3).

2.3 Allplex Respiratory Panel Real-Time RT-PCR Assay

Five to seven respiratory viruses can be detected using the Allplex respiratory panel (RP). Influenza viruses are included in panel 1, which can detect influenza A, influenza A-H1, influenza A-H1pdm09, influenza A-H3, influenza B virus, RSV A, and RSV B. Nucleic acids were extracted using NucliSENS eMAG (bioMérieux, Boxtel, The Netherlands) according to the manufacturer's instructions. Real-time RT-PCR was performed on a CFX96 instrument (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Data were analyzed with Seegene Viewer software. Samples showing a well-defined exponential fluorescence curve with Ct values below 42 for each target were considered positive for that virus.

2.4 Sofia Influenza A + B Fluorescence Immunoassay

Sofia employs immunofluorescence to detect influenza A and influenza B viral nucleoprotein antigens with a lateral flow design. Nasopharyngeal specimens in UTM (250 μL) were added to the reagent tube and mixed according to the manufacturer's instructions. Reaction mixtures (120 μL) were added to the test cassette sample wells. Results were read using a Sofia 2 analyzer (Quidel) in 15 min.

2.5 Statistical Analysis

We analyzed the sensitivity and specificity of each tested assay based on the results of the Allplex RP assay. The Ct values determined by Allplex RP for influenza A or B virus-positive samples were stratified into 5 groups, and the sensitivity of the QuantumPACK Easy assay for these samples was analyzed according to these groups. The results of the QuantumPACK Easy assay were compared with the results of the Sofia assay. Percent agreement and kappa index between assays were evaluated. Two-sided $P < 0.05$ was considered statistically significant. Excel 2016 (Microsoft Corporation, Redmond, WA, USA) and SPSS Statistics for Windows version 19.0 (IBM Corp., Armonk, NY, USA) were used for statistical analysis.

2.6 Data Availability

The data set can be found in the Figshare repository with https://figshare.com/articles/dataset/Quantom_dot_evaluation/17060072.
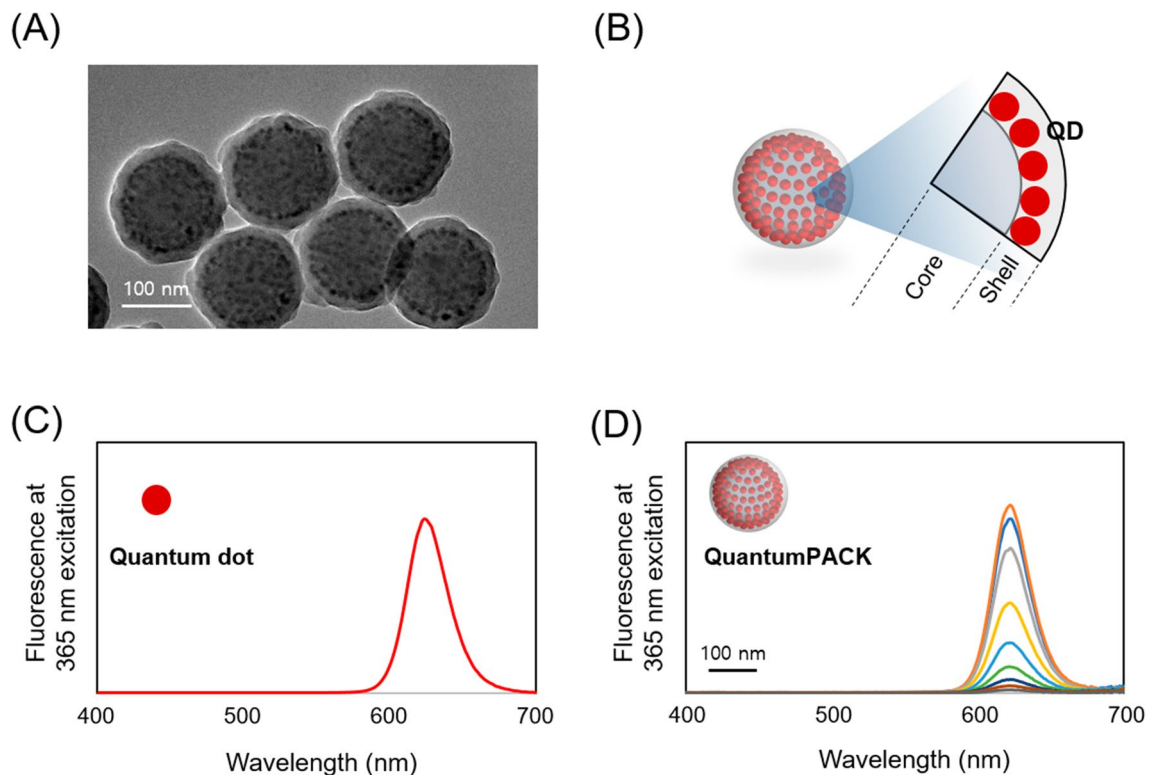
Fig. 1 Structure of QuantumPACK and fluorescence characteristics of quantum dots and QuantumPACK. **A, B** QuantumPACK uses nanoparticles packed with quantum dots on the surface of a silica core. **C** When a quantum dot is exposed to light, it emits fluorescence at a specific wavelength. **D** With QuantumPACK, each quantum dot is excited and can emit amplified light. Different sizes of quantum dots emit fluorescence at different wavelengths, enabling the detection of multiple target analytes

3 Results

3.1 Performance of QuantumPACK Easy Influenza A + B

The sensitivity of QuantumPACK Easy for influenza A and influenza B was 80.9% (76/94) and 83.7% (82/98), respectively (Table 1). Comparison of assay results according to Allplex RP results are described in Table 2. The median Ct values of influenza A- and influenza B-positive samples were 28.80 (17.84–37.79) and 24.84 (16.51–38.76), respectively. We analyzed QuantumPACK Easy sensitivity according to five Ct groups (Ct of 15.0–19.9, 20.0–24.9, 25.0–29.9, 30.0–34.9, and 35.0–39.9) and found that sensitivity decreased with increasing Ct values (Fig. 4). The Ct values of QuantumPACK Easy Influenza A-positive and -negative specimens were significantly different (28.07 vs. 32.92, $P < 0.001$). The Ct values of QuantumPACK Easy Influenza B-positive and -negative specimens were also significantly different (24.03 vs. 31.97, $P < 0.001$).

The specificity of QuantumPACK Easy for influenza A and influenza B virus was 100%. Analytical specificity was evaluated with 19 potentially cross-reacting respiratory pathogens (RSV A, RSV B, ADV, CoV-229E, -OC43, -NL63, -HKU1, rhinovirus, enterovirus, PIV 1, PIV 2, PIV 3, PIV 4, hMPV, bocavirus, *M. pneumoniae, C. pneumoniae, L. pneumophila,* and *P. jirovecii*). No cross-reactivity was found. The resulting overall analytical specificity was 100%.

3.2 Comparator Analysis and Discrepancy Investigation

The total agreement for influenza A and B between QuantumPACK Easy and Sofia assays was 89.6% (329/367, kappa 0.783). The agreement of influenza A and influenza B was 94.8% (255/269, kappa 0.864) and 91.2% (249/273, kappa 0.773), respectively. Positive agreement for Influenza A and B was 100% (62/62) and 98.3% (59/60), respectively. Negative agreement for Influenza A and B was 93.2% (193/207) and 89.2% (190/213), respectively (Table 3).

The sensitivity of Sofia influenza A and influenza B was 66.0% (62/94) and 61.2% (60/98), respectively. The specificity of both Sofia influenza A and influenza B was 100%.

4 Discussion

The COVID-19 pandemic has placed an unprecedented strain on healthcare systems globally. Manpower and financial support shifted from other respiratory diseases to
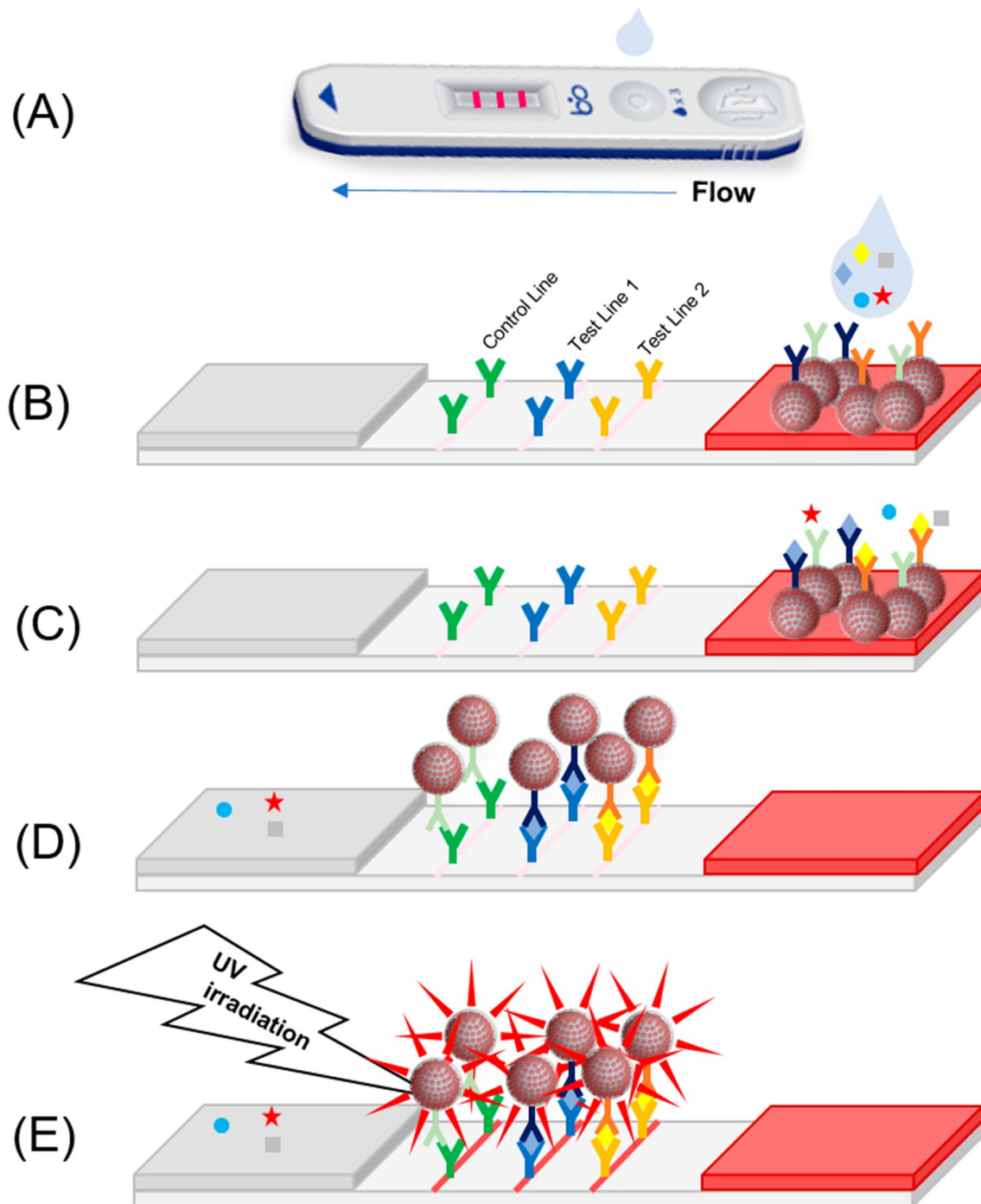
Fig. 2 Schematic diagram of the QuantumPACK Easy Influenza A+B assay. **A** The QuantumPACK Easy Influenza A+B strip and its flow. **B** The extracted sample is dropped onto the sample pad. **C** Viral antigen binds to antigen specific antibodies conjugated with QuantumPACK particles. **D** The antigen–antibody–QuantumPACK complex flows over the strip under capillary action and binds at the control line and the test line. **E** The strip is read using a QuantumPACK Easy Reader under UV irradiation

COVID-19, and many countries have struggled to maintain influenza surveillance [8, 9]. According to the WHO, countries, areas, or territories reporting data to FluNet, the global influenza surveillance platform, decreased from 161 to 139 from September 2019–January 2020 to September 2020–January 2021 [2].

Fig. 3 Fluorescent image of the QuantumPACK Easy Influenza. **A** Influenza A positive and influenza B negative. **B** Influenza A negative and influenza B positive. **C** Influenza A and B positive. **D** Influenza A and B negative

Table 1 Performance summary of the rapid influenza antigen assay

Assay	Analyte	Sensitivity			Specificity		
		n	%	95% CI	n	%	95% CI
QuantumPACK Easy Influenza	Influenza A	76/94	80.9	71.4–88.2	273/273*	100.0	98.7–100.0
	Influenza B	82/98	83.7	74.8–90.4	269/269[†]	100.0	98.6–100.0
Sofia Influenza	Influenza A	62/94	66.0	55.5–75.4	273/273*	100.0	98.7–100.0
	Influenza B	60/98	61.2	50.9–70.9	269/269[†]	100.0	98.6–100.0

CI confidence interval

*175 influenza A- and B-negative samples and 98 influenza B-positive samples were analyzed

[†] 175 influenza A- and B-negative samples and 94 influenza A-positive samples were analyzed

Table 2 Comparison of QuantumPACK Easy Influenza A + B and Sofia Influenza A + B FIA assay results according to Allplex respiratory panel real-time RT-PCR assay

	Allplex respiratory panel real-time RT-PCR assay			
	Influenza A		Influenza B	
	Positive (n=94)	Negative (n=273)	Positive (n=98)	Negative (n=269)
QuantumPACK Easy Influenza				
Positive	76	0	82	0
Negative	18	273	16	269
Sofia Influenza				
Positive	62	0	60	0
Negative	32	273	38	269

Early diagnosis is crucial for infection control, enabling patient isolation and antiviral treatment. The turn-around time of influenza assays will be critical in the coming influenza season because of co-circulating influenza virus and SARS-CoV-2. Only testing can distinguish between influenza virus and SARS-CoV-2 infection and identify influenza virus and SARS-CoV-2 coinfection [10].

Point-of-care testing (POCT) enables rapid identification of influenza cases. Among POCT, lateral flow assay is mainly used. Lateral flow immunoassays (LFIAs) have various formats, bio-recognition molecules, labels, and detection systems [11]. Among them, labels play an important role in determining sensitivity. Colloidal gold nanoparticles are the most commonly used labels, since they have a high affinity to biomolecules and are easy to functionalize [11]. However, gold nanoparticles can only provide qualitative results that
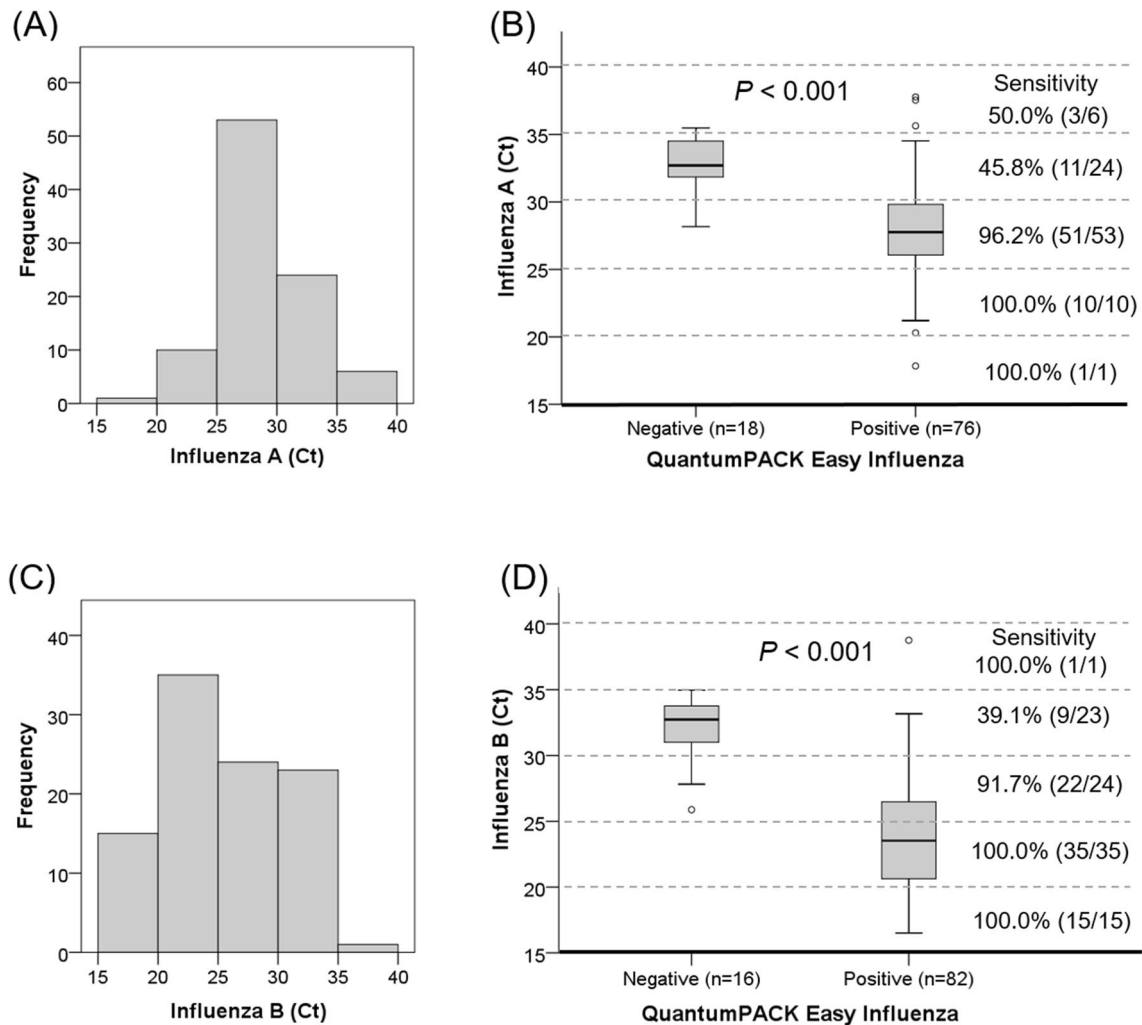

Fig. 4 **A** Distribution of influenza A Ct values enrolled in the present study. **B** Clinical sensitivity of QuantumPACK Easy Influenza A according to Ct groups. **C** Distribution of influenza B Ct values enrolled in this study. **D** Clinical sensitivity of QuantumPACK Easy Influenza B according to Ct groups

Table 3 Comparison of QuantumPACK Easy Influenza A + B and Sofia Influenza A + B FIA assays

| | Sofia Influenza | | | | | |
| | Influenza A | | | Influenza B | | |
	Positive	Negative	Total	Positive	Negative	Total
QuantumPACK Easy Influenza						
Positive	62	14	76	59	23	82
Negative	0	193	193	1	190	191
Total	62	207	269*	60	213	273†

*94 influenza A-positive samples and 175 influenza A- and B-negative samples were analyzed

†98 influenza B-positive samples and 175 influenza A- and B-negative samples were analyzed

can be analyzed by the naked eye [5]. Alternative labels have been developed, including magnetic particles, color latex, and fluorescent reporters. Quantum dots are the most promising fluorescent reporters, as they have high quantum yields and high extinction coefficients, which leads to increased sensitivity [5]. Quantum dots have been applied in a tumor marker LFIA and in a toxin LFIA, which have shown promising results [12, 13]. Recently, a quantum dot-based LFIA for the detection of influenza virus antigen was developed and we evaluated QuantumPACK Easy Influenza A + B.

The sensitivity of the QuantumPACK Easy assay for influenza A and influenza B virus was 80.9% and 83.7%, respectively, while the sensitivity of the Sofia assay for influenza A and influenza B virus was 66.0% and 61.2%, respectively. This result is in agreement with a previous report that determined that the sensitivity of the Sofia assay for influenza A and influenza B was 68.8–85.4% and 55.8–86.1%, respectively [14]. False negative samples according to QuantumPACK Easy had significantly higher Ct values than true positive samples.

Rapid antigen detection tests are easy to use, have a rapid turn-around time, and have a lower cost than nucleic acid amplification tests. For these reasons, these tests are useful in Emergency Departments and outpatient settings, and many clinical laboratories employ rapid antigen detection assays as their first-line diagnostic tests for influenza virus infections [15]. However, rapid antigen detection tests have lower sensitivity than rapid nucleic acid amplification tests (53–85% vs. 92–95%) [14, 16]. In the present study, QuantumPACK Easy had increased sensitivity compared to a commercially available rapid antigen detection test. This new assay, therefore, preserves the advantages of a rapid antigen detection assay, while having increased sensitivity. The specificity of QuantumPACK Easy and Sofia assays was 100%, and we found no cross-reactivity with other respiratory pathogens.

In the present study, the QuantumPACK Easy assay was in good agreement with the Sofia assay for diagnosing influenza A and B (89.6%, kappa 0.783). All samples that disagreed for influenza A were QuantumPACK Easy positive and Sofia negative. Of the samples that disagreed

for influenza B, 95.8% (23/24) were QuantumPACK Easy positive and Sofia negative, and only one (4.2%, 1/24) sample was Sofia positive and QuantumPACK Easy negative. QuantumPACK Easy, therefore, had better sensitivity with samples containing a low viral load than a commercially available influenza antigen detection assay.

There are some limitations to the present study. Samples from an influenza B virus outbreak during March and May 2019 were included, and this resulted in a higher sensitivity and lower Ct value for influenza B virus than for influenza A virus. In addition, this study was performed retrospectively. Further prospective studies evaluating the clinical utility of the QuantumPACK Easy assay are required.

In conclusion, QuantumPACK Easy showed a clinically acceptable performance and had a good correlation with the Sofia assay for the diagnosis of influenza A and B. Using quantum dots as the label, QuantumPACK Easy has increased sensitivity compared to commercially available influenza antigen detection tests. With its high sensitivity, high specificity, ease of use, rapid turn-around time, and lower cost compared to nucleic acid amplification tests, QuantumPACK Easy is a promising point-of-care test for the diagnosis of influenza A and B infections.

Declarations

Conflict of interest The authors report no conflicts of interest.

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