PCR

Performance evaluation of the QuantStudio 5 Dx Real-Time PCR System and the QuantStudio 7 Pro Dx Real-Time PCR System

Abstract

The performance of the Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR System and the QuantStudio™ 7 Pro Dx Real-Time PCR System to a respective predicate IVD PCR system is demonstrated. We report the results for reproducibility, repeatability, and method comparison studies using nucleic acid of influenza A and influenza B as the representative targets, detected with a clinically relevant assay to show substantial performance equivalence.

Introduction

Molecular diagnostics (MDx) tests have become indispensable tools in the diagnostic landscape. For decades, PCR assays have proven to be a powerful technology for diagnostic applications, such as oncology and infectious diseases [1-4]. PCR has high analytical sensitivity, offering the ability to detect a target with only a single copy present, and specificity, allowing the ability to detect and differentiate between even closely related pathogens [5-7]. Modern PCR technology and chemistry offer robust and fast performance, enabling the detection of multiple targets in the same reaction (multiplex).

With high analytical sensitivity and specificity combined with fast sample-to-result turnaround times, PCR-based assays for the detection of SARS-CoV-2 are widely considered the "gold standard" for SARS-CoV-2 testing [8-10].

Thermo Fisher Scientific has over 25 years of qPCR instrument and over 10 years of clinical instrument manufacturing experience, continually developing systems that meet specific needs of MDx and assay developers. With the increased security and compliance requirements for *in vitro* diagnostic (IVD) devices in mind, Thermo Fisher has developed high-performance qPCR instruments for clinical laboratories including the QuantStudio 5 Dx Real-Time PCR System and the QuantStudio 7 Pro Dx Real-Time PCR System (Table 1).

QuantStudio 5 Dx and 7 Pro Dx Real-Time PCR Systems

The QuantStudio 5 Dx Real-Time PCR System and the automated QuantStudio 7 Pro Dx Real-Time PCR System are *in vitro* diagnostic devices intended to perform fluorescence-based PCR to provide detection of nucleic acid sequences in human-derived specimens. Both systems are intended to be used by trained professionals in combination with *in vitro* diagnostic assays.

The QuantStudio 5 Dx Real-Time PCR System is a compact, flexible system designed to simplify workflows and minimize training needs. With the ability to differentiate target quantities as small as 1.5-fold in a singleplex reaction, while offering 10 orders of magnitude of linear dynamic range, the QuantStudio 5 Dx Real-Time PCR System provides outstanding reliability, sensitivity, and accuracy. Multiplexing of up to six targets is supported, and the Applied Biosystems™ VeriFlex™ Block technology allows up to six independent temperature zones. The security, auditing, and e-signature (SAE) module supports multiple clients operating in a secure environment, and the intuitive and simple-to-use touchscreen interface allows users to easily operate the system. The QuantStudio 5 Dx Real-Time PCR System provides users with confidence in performance and supports both development* and IVD modes.

The QuantStudio 7 Pro Dx Real-Time PCR System combines modern hardware and software in a compact footprint to efficiently utilize bench space, while offering interchangeable 96-well and 384-well** block options for maximum flexibility and increased throughput. The "plug-and-run" design promotes compatibility with automated workflow solutions. The instrument supports assays of different regulatory status (Research Use Only (RUO), Investigational Use Only (IUO), *In Vitro* Diagnostic (IVD)) with the same intuitive workflow supporting assay development. The system's software supports the use of an assay definition file (ADF), allowing users to configure and lock in run, analysis, and report settings to reduce the risk of manual errors and improve efficiency. The QuantStudio 7 Pro Dx Real-Time PCR System

* Test development mode is for Research Use Only.
** The performance evaluation for the 384-well block is pending.

Table 1. Overview of Applied Biosystems™ real-time PCR instruments for clinical laboratories.

	QuantStudio 7 Pro Dx system	QuantStudio 5 Dx system	QuantStudio Dx system
Formats	 96-well, 0.2 mL 384-well (user-interchangeable blocks)*	 96-well, 0.2 mL	 96-well, 0.1 mL
Colors	Up to 6 colors (21 filter combinations)	Up to 6 colors (21 filter combinations)	Up to 6 colors (21 filter combinations)
Touchscreen	21.5 cm (8.46 in.)	21.5 cm (8.46 in.)	26.4 cm (10.4 in.)
VeriFlex Blocks temperature control	Yes, 6 zones	Yes, 6 zones	No
Security, auditing, electronic signature	Yes	Yes	Yes
Dimensions (H x W x D)	55 x 34 x 53 cm (21.6 x 13.4 x 20.9 in.)	40 x 27 x 50 cm (15.7 x 10.6 x 19.7 in.)	75 x 53 x 70 cm (29.1 x 19.7 x 26.0 in.)
Weight	38 kg (83.8 lb)	26 kg (57.3 lb)	70 kg (154 lb)

* The 384-well block is under development.

is a true stand-alone system, as the graphical user interface (GUI) enables an end-to-end IVD workflow without requiring an accompanied computer and even supports hands-free operation with voice commands. The system comes with the Thermo Fisher™ Connect Platform supporting remote access. The simple but powerful software offers enhanced security features and meets the updated FDA cybersecurity requirements. The QuantStudio 7 Pro Dx Real-Time PCR System enables customers performing MDx tests to achieve high efficiency, enhanced productivity, and imporved accuracy.

Repeatability and reproducibility studies were conducted on the QuantStudio 5 Dx and QuantStudio 7 Pro Dx Real-Time PCR Systems to evaluate the precision of each system. Repeatability focuses on the closeness of agreement between successive and independent results obtained by the same method on identical test material under the same conditions (i.e., same instrument, users, laboratory), whereas reproducibility is the closeness of agreement between single-test results on identical test material using the same method, but obtained in different laboratories with different users using different equipment [11,12]. The Clinical and Laboratory Standards Institute (CLSI) has published a guidance for evaluating precision performance [13]. Repeatability and reproducibility studies define the precision of a real-time PCR system and determine the level of measurement variability attributed to the user, equipment, and environmental factors.

To evaluate performance equivalence, a method comparison study with an already established predicate IVD instrument was conducted. The performance of the QuantStudio 5 Dx Real-Time PCR System was compared to the QuantStudio Dx Real-Time PCR System, and the performance of the QuantStudio 7 Pro Dx Real-Time PCR System was compared to the QuantStudio 5 Dx Real-Time PCR System. The method comparison study determined the percent agreement between the QuantStudio 5 Dx and 7 Pro Dx systems and their respective predicate diagnostic PCR systems.

Performance results

The precision (repeatability and reproducibility) and method comparator studies were carried out using a representative, clinically relevant assay designed to detect RNA sequences of influenza A (flu A) and B (flu B) in human clinical or contrived nasopharyngeal swab samples. The Lyra™ Influenza A+B Assay (Quidel Corporation) was used in the precision and method comparison studies for the QuantStudio 5 Dx Real-Time PCR System. The Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B Combo Kit was used to evaluate the performance of the QuantStudio 7 Pro Dx Real-Time PCR System.

Precision studies

A panel of 20 samples was prepared with volumes sufficient for all tests, including both pooled clinical samples and contrived samples. To ensure equivalency of the samples between runs and test sites, frozen aliquots containing nucleic acid from the same extraction were used in all tests. (Extraction was performed using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with the Thermo Scientific™ KingFisher™ Flex magnetic particle processor.) In each run, all 20 samples of the panel were randomized and blinded prior to the run.

The precision sample panel for the QuantStudio 5 Dx Real-Time PCR System consisted of extracted nucleic acid derived from 14 clinical sample pools (6 negative pools, 4 flu A positive pools, 4 flu B positive pools) and six contrived sample pools (3 pools positive for flu A at either 3x limit of detection (LOD), 5x LOD, or 10x LOD; 3 pools positive for flu B at either 3x LOD, 5x LOD, or 10x LOD).

The precision sample panel for the QuantStudio 7 Pro Dx Real-Time PCR System consisted of extracted nucleic acid from 12 clinical sample pools (7 negative pools, 2 flu A positive pools, 2 flu B positive pools, 1 flu A and flu B positive pool) and eight contrived sample pools (3 pools positive for flu A at either 3x LOD, 5x LOD, or 10x LOD; 3 pools positive for flu B at either 3x LOD, 5x LOD, or 10x LOD; 2 pools positive for flu A and flu B at either 5x LOD or 10x LOD).

An overview of the samples included in the panels is presented in Tables 2 and 3.

Table 2. Sample panel for the precision study of the QuantStudio 5 Dx system.

Sample type	Number of samples	Sample origin	Sample concentration
Negative	6	6 clinical pools	0x LOD
Flu A	7	3 contrived pools	3x, 5x, and 10x LOD
		4 clinical pools	Unknown
Flu B	7	3 contrived pools	3x, 5x, and 10x LOD
		4 clinical pools	Unknown
Total	20		

Table 3. Sample panel for the precision study of the QuantStudio 7 Pro Dx system.

Sample type	Number of samples	Sample origin	Sample concentration
Negative	7	7 clinical pools	0x LOD
Flu A	5	3 contrived pools	3x, 5x, and 10x LOD
		2 clinical pools	Unknown
Flu B	5	3 contrived pools	3x, 5x, and 10x LOD
		2 clinical pools	Unknown
Flu A and flu B	3	2 contrived pools	5x and 10x LOD
		1 clinical pool	Unknown
Total	20		

Repeatability study

A summary of the repeatability study design is shown in Figure 1.

For the QuantStudio 5 Dx Real-Time PCR System, the repeatability study was conducted by two users in one laboratory, using three QuantStudio 5 Dx Real-Time PCR Systems. Each user performed two runs on each of the three instruments per day, and testing was conducted on 12 nonconsecutive days. In summary, a total of 144 runs were conducted for the repeatability study. Each run covered all 20 samples of the precision panel, and each sample covered two targets (influenza A and B), resulting in a total of 5,760 data points.

The repeatability study for the QuantStudio 7 Pro Dx Real-Time PCR System was carried out in one laboratory, using one instrument. The sample panel was tested on 12 nonconsecutive days; testing on each day was performed by two users, and each user performed two runs per day. A total of 48 runs, with each run consisting of the 20 panel samples and each sample including two targets (influenza A and B), were carried out for this repeatability study resulting in a total of 1,920 data points.

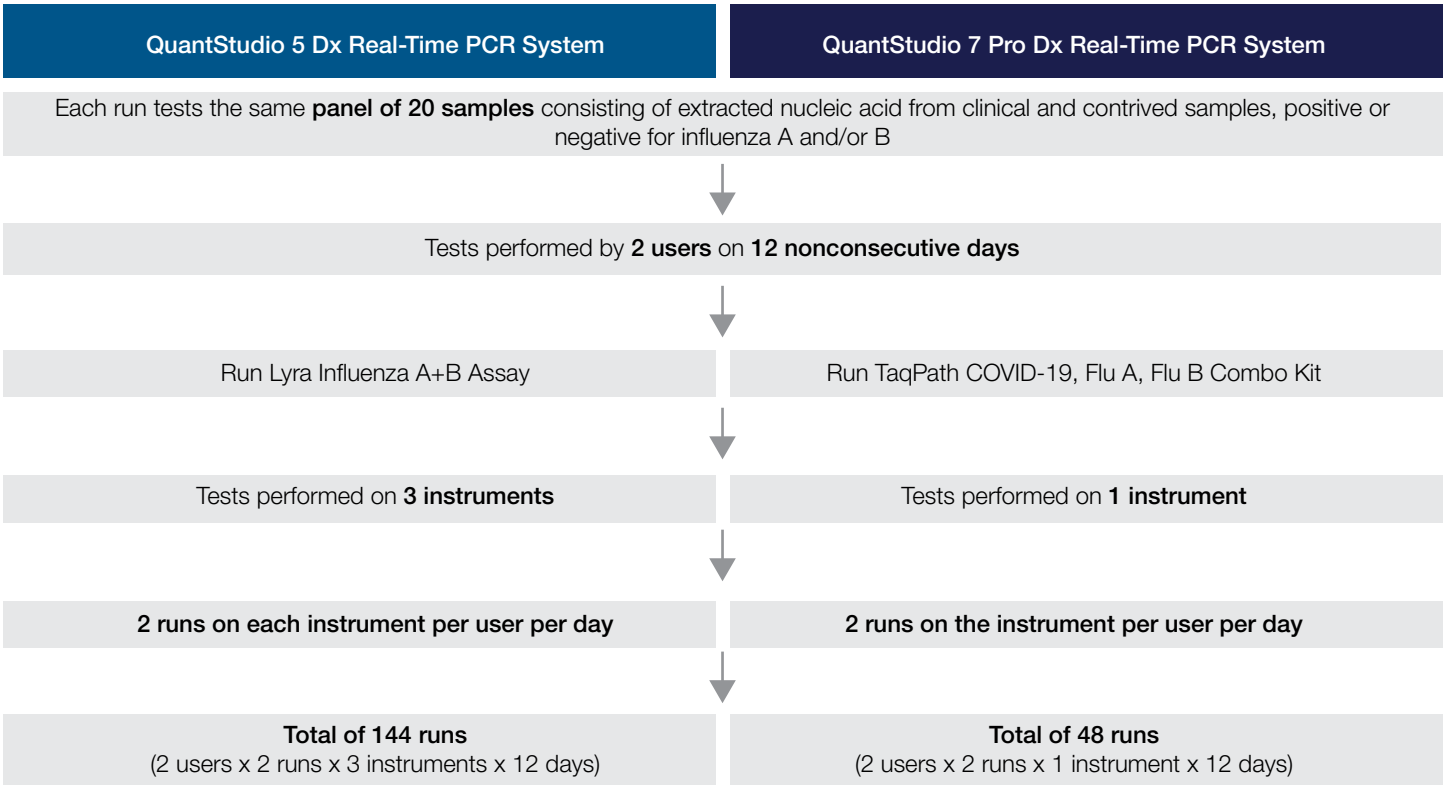

Figure 1. Study design for repeatability studies on the QuantStudio 5 Dx and 7 Pro Dx systems.

The run-to-run call concordance (repeatability for each tester on the same instrument) was calculated for each user and instrument combination using Equation 1, and the results can be seen in Table 4.

$$\frac{\text{Total number of blind samples per plate} \times \text{Total number of runs per instrument per user} - \text{Number of times each blind sample is called differently}}{\text{Total number of blind samples per plate} \times \text{Total number of runs per instrument per user}} \times 100\% \quad (1)$$

Table 4. Summary of repeatability results.

Instrument-to-user combination (Site A)		Run-to-run call concordance	95% confidence interval	
			Lower limit	Upper limit
QuantStudio 5 Dx Real-Time PCR System				
Instrument 1	User 1	97.71%	95.94%	98.85%
	User 2	96.46%	94.39%	97.92%
Instrument 2	User 1	98.76%	97.30%	99.54%
	User 2	98.33%	96.74%	99.28%
Instrument 3	User 1	98.96%	97.59%	99.66%
	User 2	98.85%	97.30%	99.54%
QuantStudio 7 Pro Dx Real-Time PCR System				
Instrument 1	User 1	99.17%	97.88%	99.77%
	User 2	99.17%	97.88%	99.77%

Reproducibility study

A summary of the reproducibility study design is shown in Figure 2.

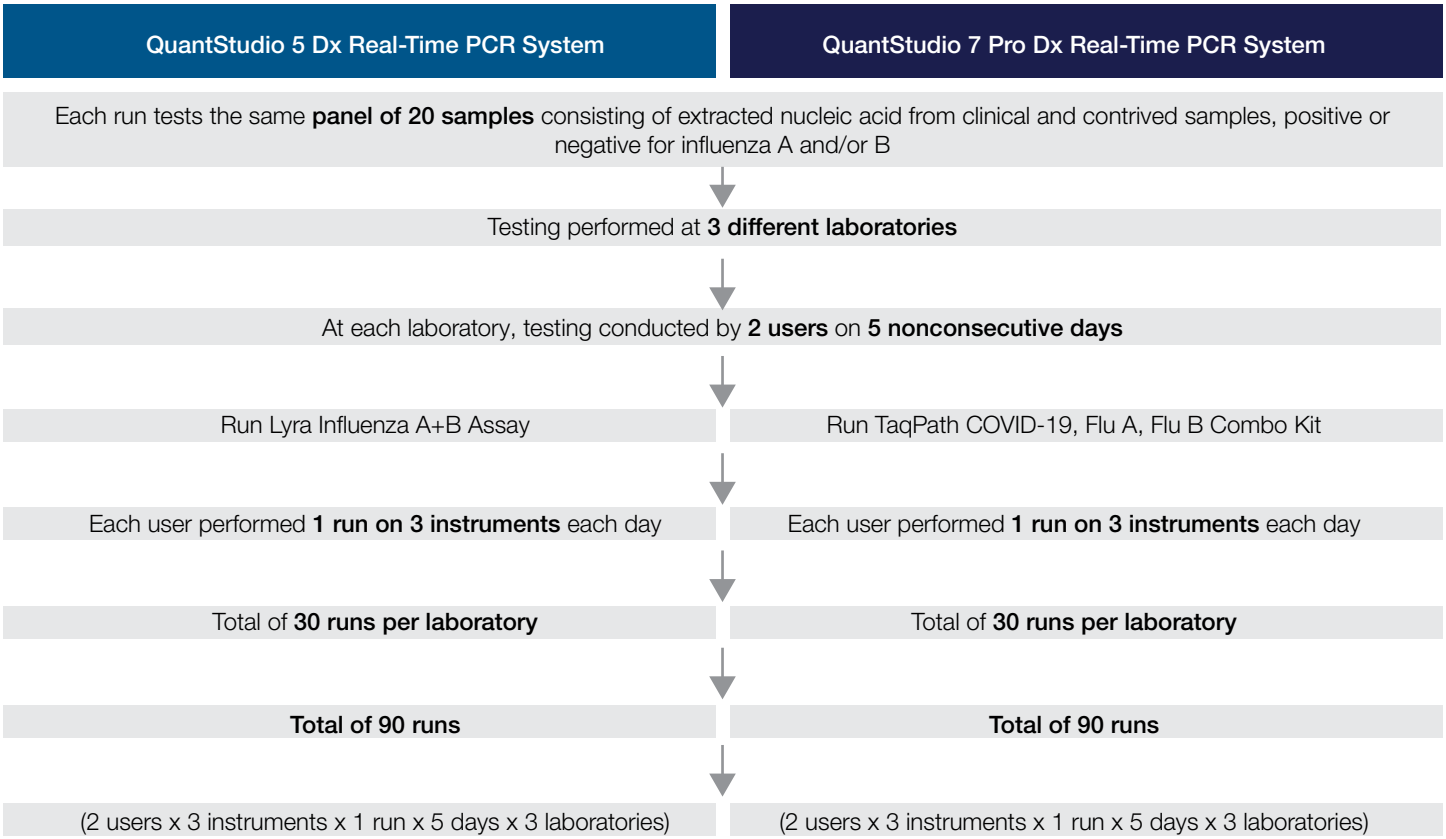

Figure 2. Study design for reproducibility tests on the QuantStudio 5 Dx and 7 Pro Dx systems.

The reproducibility studies for the QuantStudio 5 Dx and 7 Pro Dx Real-Time PCR Systems were each conducted at three different laboratories, each equipped with three of the respective PCR instruments. The sample panel was tested on five nonconsecutive days; testing on each day was performed by two users, and each user performed one run on each of the three instruments each day. For both systems, a total of 90 runs each (30 runs at each of the three laboratories) were performed. Each run consisted of the 20 panel samples and each panel sample included two targets (influenza A and B), resulting in a total of 3,600 data points for each system's reproducibility study.

Site-to-site concordance (reproducibility between test sites, encompassing data from all sites, instruments and users) was calculated using all runs from all sites (30 runs per site), using Equation 2.

The site-to-site call concordance, which summarizes the instrument-to-instrument and user-to-user reproducibility for all three laboratory sites, was 97.89% and 98.67% for the QuantStudio 5 Dx system and the QuantStudio 7 Pro Dx system, respectively (Table 5).

$$\frac{\text{Total number of blind samples per plate} \times \text{Total number of runs} - \text{Number of times each blind sample is called differently}}{\text{Total number of blind samples per plate} \times \text{Total number of runs}} \times 100\% \quad (2)$$

Table 5. Summary of site-to-site reproducibility results.

	Site-to-site call concordance	95% confidence interval	
		Lower limit	Upper limit
QuantStudio 5 Dx Real-Time PCR System	97.89%	97.11%	98.50%
QuantStudio 7 Pro Dx Real-Time PCR System	98.67%	98.02%	99.14%

Method comparison study

A summary of the method comparison study design is shown in Figure 3.

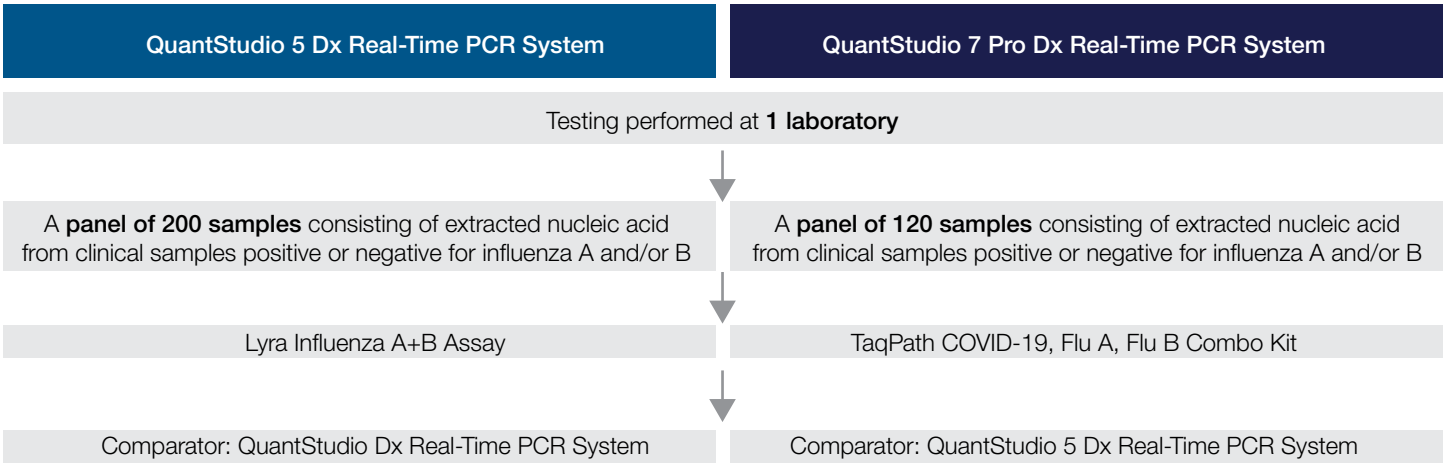

Figure 3. Study design for method comparison.

QuantStudio 5 Dx Real-Time PCR System

The method comparison study assessed agreement between the QuantStudio 5 Dx Real-Time PCR System and the predicate QuantStudio Dx Real-Time PCR System (FDA clearance under K123955 on March 08, 2013). The Lyra Influenza A+B Assay was used as a clinically relevant assay with a set of 200 extracted samples derived from individual clinical samples. The study was carried out at one site, using three QuantStudio 5 Dx Real-Time PCR Systems and one QuantStudio Dx Real-Time PCR System. All samples were randomized and blinded before being tested. The results are shown in Table 6.

Table 6. Results of the method comparison study for the QuantStudio 5 Dx Real-Time PCR System.

		Comparator: QuantStudio Dx Real-Time PCR System		
		Flu A positive	**Flu A negative**	**Total**
QuantStudio 5 Dx Real-Time PCR System	Flu A positive	17	0	17
	Flu A negative	1	182	183
	Total	18	182	200
		Flu B positive	**Flu B negative**	
QuantStudio 5 Dx Real-Time PCR System	Flu B positive	87	4	91
	Flu B negative	1	108	109
	Total	88	112	200

The positive percent agreement (PPA) and negative percent agreement (NPA) were calculated based on Equations 3 and 4 to determine the concordance between the QuantStudio 5 Dx Real-Time PCR System and the predicate QuantStudio Dx system. The QuantStudio 5 Dx Real-Time PCR System showed a PPA of 94.44% for influenza A and a PPA of 98.86% for influenza B detection. The NPA was 100% for influenza A and 96.43% for influenza B (Table 7).

$$PPA = \frac{\text{Number of positives by both evaluation method and its comparator}}{\text{Number of positives by comparator method}} \times 100\% \quad (3)$$

$$NPA = \frac{\text{Number of negatives by both evaluation method and its comparator}}{\text{Number of negatives by comparator method}} \times 100\% \quad (4)$$

Table 7. Concordance between the QuantStudio 5 Dx system and the predicate QuantStudio Dx system.

		Call concordance with the QuantStudio Dx system	95% confidence interval	
			Lower limit	**Upper limit**
Influenza A	PPA	94.44%	72.71%	99.86%
	NPA	100%	97.99%	100.00%
Influenza B	PPA	98.86%	93.83%	99.97%
	NPA	96.43%	91.11%	99.02%

QuantStudio 7 Pro Dx Real-Time PCR System

A total of 120 extracted nucleic acid samples from individual retrospective specimens were included in the method comparison study, and consisted of 12 influenza A positive samples, 32 influenza B positive samples, one influenza A and B positive sample, and 75 negative samples (Table 8). The study was carried out at one site, with one user and used the TaqPath COVID-19, Flu A, Flu B Combo Kit as a clinically relevant assay. All samples were randomized and blinded before being tested concurrently on three different QuantStudio 7 Pro Dx Real-Time PCR Systems and one QuantStudio 5 Dx Real-Time PCR System as comparator (FDA listed on March 25, 2021).

Table 8. Summary of samples included in the method comparison study.

Sample type	Status	Number of samples
Extracted nucleic acid from clinical specimen	Negative	75
	Flu A positive	12
	Flu B positive	32
	Flu A and B positive	1

All 13 samples positive for influenza A (12 samples positive for influenza A only and one sample positive for both influenza A and B) were correctly detected as positive for influenza A on all three QuantStudio 7 Pro Dx Real-Time PCR Systems, resulting in a PPA of 100% to the predicate device. For one sample, tests performed on two of the three QuantStudio 7 Pro Dx Real-Time PCR Systems reported an influenza A positive result, while the third QuantStudio 7 Pro Dx system and the comparator system did not detect influenza A in the specimen, resulting in a NPA of 99.07% for two and 100% for one of the three QuantStudio 7 Pro Dx Real-Time PCR Systems. The results are summarized in Table 9.

All three QuantStudio 7 Pro Dx Real-Time PCR Systems confirmed the presence of influenza B in all 33 specimens, resulting in a PPA of 100%. Instrument 2 reported influenza A and B in a sample negative for both targets, and instrument 3 detected influenza A and B in a specimen only positive for influenza A, leading to a NPA of 100% for instrument 1 and 98.85% for instruments 2 and 3 (Table 10).

Table 9. Summary of the method comparison study of the QuantStudio 7 Pro Dx system for flu A.

	QuantStudio 7 Pro Dx Real-Time PCR Systems			QuantStudio 5 Dx Real-Time PCR System
	Instrument 1	**Instrument 2**	**Instrument 3**	
Flu A positive	14	14	13	13
Flu A negative	106	106	107	107
Sample total	120	120	120	120
PPA (95% confidence interval)	**100%** (75.29–100%)	**100%** (75.29–100%)	**100%** (75.29–100%)	
NPA (95% confidence interval)	**99.07%** (94.90–99.98%)	**99.07%** (94.90–99.98%)	**100%** (96.61–100%)	

Table 10. Summary of the method comparison study of the QuantStudio 7 Pro Dx system for flu B.

	QuantStudio 7 Pro Dx Real-Time PCR Systems			QuantStudio 5 Dx Real-Time PCR System
	Instrument 1	**Instrument 2**	**Instrument 3**	
Flu B positive	33	34	34	33
Flu B negative	87	86	86	87
Sample total	120	120	120	120
PPA (95% confidence interval)	**100%** (89.42–100%)	**100%** (89.42–100%)	**100%** (89.42–100%)	
NPA (95% confidence interval)	**100%** (95.85–100%)	**98.85%** (93.76–99.97%)	**98.85%** (93.76–99.97%)	

Conclusions

In this performance evaluation study, both the QuantStudio 5 Dx Real-Time PCR System and the QuantStudio 7 Pro Dx Real-Time PCR System displayed excellent precision when a user performed testing on different days on the same instrument, or when testing was conducted with multiple users and different instruments at the same or different laboratories. The overall site-to-site call concordance for the QuantStudio 5 Dx Real-Time PCR System and QuantStudio 7 Pro Dx Real-Time PCR System was 97.89% and 98.67%, respectively.

The performance of the QuantStudio 5 Dx Real-Time PCR System was directly compared to the QuantStudio Dx Real-Time PCR System (FDA clearance under K123955 on March 08, 2013), and the QuantStudio 7 Pro Dx Real-Time PCR System used the QuantStudio 5 Dx Real-Time PCR System as comparator (510(k) exempt; device listing under FDA database is current). The method comparison study demonstrated that the QuantStudio 5 Dx Real-Time PCR System and the QuantStudio 7 Pro Dx Real-Time PCR System have equal instrument performance to their respective predicates.

The QuantStudio 5 Dx Real-Time PCR System is a compact, flexible system designed to seamlessly fit into established workflows. The QuantStudio 7 Pro Dx Real-Time PCR System provides a diagnostic platform for the future by combining modern hardware and software in a compact footprint. Both systems support MDx workflows by achieving maximal efficiency, enhanced productivity, and improved accuracy.

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