IVDR compliance

The IVDR-compliant QuantStudio 5 Dx Real-Time PCR System

Molecular diagnostic (MDx) tests have become indispensable for clinical patient management. Real-time PCR systems are part of a comprehensive ecosystem for *in vitro* diagnostic (IVD) device manufacturers, in-house IVD test developers, and clinical testing laboratories. Thermo Fisher Scientific has over 25 years of experience providing customers with quantitative PCR (qPCR) systems, and we have more than a decade of clinical instrument manufacturing experience to meet the ongoing needs of MDx assay developers and clinical laboratories.

With implementation of the new *In Vitro* Diagnostic Regulation (IVDR) 2017/746 in the European Union (EU), many tests run on systems that comply with the EU *In Vitro* Diagnostic Directive (IVDD) will have to be migrated to IVDR-compliant systems. Even if there is an established workflow for a given assay, it can be challenging to migrate it to a new system because of intersystem variability. We designed the new IVDR-compliant Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR System to help customers navigate these challenges. You can rest assured knowing you can transition your test menu from the IVDD-compliant system to the IVDR-compliant system. You can do so expecting equivalent performance without re-optimizing your assay parameters.

The IVDR-compliant QuantStudio 5 Dx Real-Time PCR System is made with the same proven hardware as the IVDD-compliant Applied Biosystems™ QuantStudio™ 5 Dx Real-Time PCR System and includes updated firmware and software. The new software streamlines workflows to minimize hands-on time and improve efficiency. It offers a mode for assay development, and routine *in vitro* diagnostic tests can be run in IVD mode. This makes the IVDR-compliant QuantStudio 5 Dx system suitable for independent testing laboratories, hospitals, government and public health laboratories, and diagnostic test developers. The multimode software also supports network connectivity and has security, audit, and e-signature (SAE) features that satisfy IVDR criteria (Figure 1).

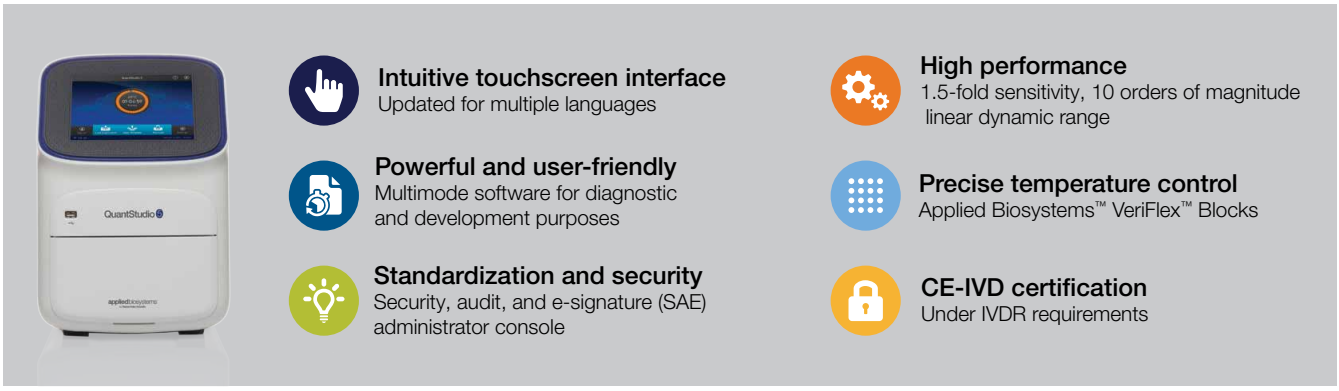

Figure 1. Features of the IVDR-compliant QuantStudio 5 Dx Real-Time PCR System.

With the new EU IVDR regulation, tests will need to be migrated to an IVDR-compliant system to maintain compliance. Transitioning existing workflows to a new system bears the risk of unexpected system-to-system variability. To demonstrate the equivalent performance of the IVDD-compliant and IVDR-compliant QuantStudio 5 Dx Real-Time PCR systems, the limit of detection (LOD), accuracy, and precision of an Applied Biosystems™ TaqMan™ assay targeting the Zika virus were evaluated on both systems.

Comparative studies and results

All studies were performed using Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix, CG (Cat. No. A15300), Applied Biosystems™ TaqMan™ amplification primers (Cat. No. 4304972), and MGB probes (Cat. No. 4316032) to amplify and detect Zika virus nucleic acid sequences and the peptidylprolyl isomerase A (*PPIA*) gene, used as an endogenous control. Samples were prepared by spiking water with known amounts of Amplirun™ Zika Virus RNA Control and Invitrogen™ Human Brain Total RNA (Cat. No. AM7962). PCR was run on the IVDD-compliant QuantStudio 5 Dx system, which has the same fundamental components for raw data acquisition as the IVDR-compliant system. The data were processed on both systems and compared (Figure 2). A C_t cutoff of 38 was applied for the Zika virus and *PPIA* targets to establish whether samples were positive or negative.

Limit of detection (LOD) and precision

The LOD study included runs on five different PCR instruments. Serial dilutions containing 20, 10, 5, or 2.5 copies of Zika virus RNA per reaction were tested on each instrument, and 20 replicates were prepared at each concentration. Each run also included positive and negative controls for Zika virus RNA and no-template controls that were negative for Zika virus and the *PPIA* endogenous control.

The data were processed on the IVDD- and IVDR-compliant QuantStudio 5 Dx systems. The average C_t value for each set of Zika virus RNA replicates at each concentration on each PCR system is shown in Table 1. With only five exceptions, the average C_t value recorded for a given set of replicates was the same on the IVDD- and IVDR-compliant QuantStudio 5 Dx systems. Among the five exceptions with differing C_t values, none of the differences were statistically significant in a Student's *t*-test. In four cases, the average C_t values differed by 0.001. In the remaining case, the average C_t value differed by 0.004. There were no differences between the average C_t values and standard deviations for the endogenous *PPIA* control (data not shown). The performance of the analysis algorithm was equivalent, and we were able to detect 5 copies per reaction in ≥95% of the replicates tested at this concentration.

Accuracy

A panel of 20 samples was prepared by spiking water with Zika virus RNA at concentrations ranging from 0 copies per reaction to 20 times the LOD. Testing also included positive, negative, and no-template controls. All samples in the panel were tested in quadruplicate in each run, and all tests were run blind. Testing was conducted by three independent operators. Each operator performed three runs on three different instruments for a total of 27 runs with 2,160 blind sample replicates.

The results from all runs were analyzed on the IVDD- and IVDR-compliant QuantStudio 5 Dx systems. The numbers of samples that tested positive or negative for Zika virus on each system were then compared. Calls for all samples and controls run on both systems were concordant, including calls for the endogenous *PPIA* control (Table 2).

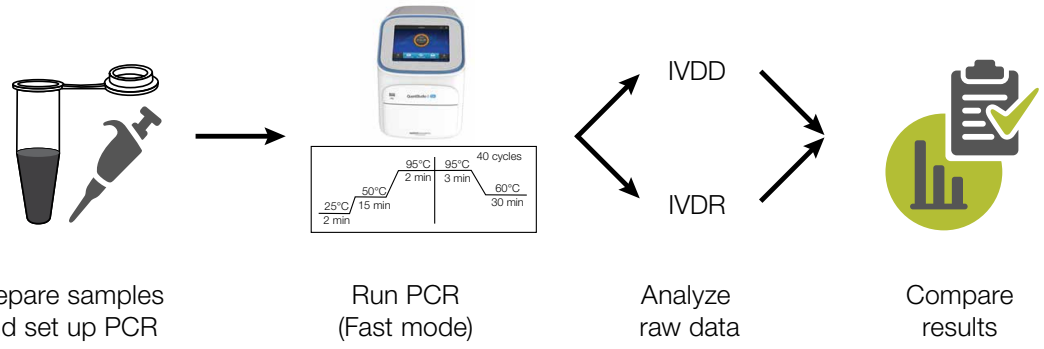

Figure 2. Experimental workflow for comparative studies of IVDD- and IVDR-compliant QuantStudio 5 Dx systems.

Table 1. Results of LOD determination and precision of IVDD- and IVDR-compliant QuantStudio 5 Dx systems.

Zika virus RNA	Instrument	Average C_t	Standard deviation (SD)	Positive rate
20 copies per reaction	Instrument 1	33.770	0.214	100%
	Instrument 2	33.809	0.269	
	Instrument 3	33.705	0.227	
	Instrument 4	33.752	0.295	
	Instrument 5	33.529	0.297	
10 copies per reaction	Instrument 1	34.560 (IVDD), 34.561 (IVDR)	0.424	100%
	Instrument 2	34.586 (IVDD), 34.587 (IVDR)	0.376	
	Instrument 3	34.892	0.465	
	Instrument 4	34.746	0.428	
	Instrument 5	34.576	0.390	
5 copies per reaction	Instrument 1	35.789 (IVDD), 35.793 (IVDR)	0.604 (IVDD), 0.610 (IVDR)	100%
	Instrument 2	35.856 (IVDD), 35.857 (IVDR)	0.757 (IVDD), 0.759 (IVDR)	100%
	Instrument 3	35.836	0.590	100%
	Instrument 4	36.063	0.957	95%
	Instrument 5	35.825	0.809	95%
2.5 copies per reaction	Instrument 1	36.728 (IVDD), 36.729 (IVDR)	0.827	85%
	Instrument 2	36.588	0.708	85%
	Instrument 3	36.844	0.999	80%
	Instrument 4	36.473	0.759	90%
	Instrument 5	36.545	0.725	90%

Note: All positive, negative, and no-template controls were valid on each system.

Table 2. Equivalent performance observed on the IVDD- and IVDR-compliant QuantStudio 5 Dx systems.

	Blind samples (n = 2,160)	Controls*
Positive and negative call rate concordance	100%	100%
Equivalent samples with different C_t values	7**	0

* A total of 108 positive controls, 108 negative controls, and 108 no-template controls were analyzed.

** Samples had the same positive and negative call rates for the respective targets.

Conclusion

The new QuantStudio 5 Dx Real-Time PCR System is compliant with the European Union *In Vitro* Diagnostic Regulation. Our LOD, precision, and accuracy data confirm that its performance is equivalent to that of the IVDD-compliant QuantStudio 5 Dx system. With the new EU regulation, many tests will need to be migrated to an IVDR-compliant system to maintain compliance. However, once a workflow is established, it is challenging to move a test menu to a new system. Applied Biosystems recognizes these challenges and has designed the new IVDR-compliant QuantStudio 5 Dx system with equivalent performance to the IVDD-compliant QuantStudio 5 Dx system. You can rest assured knowing that transitioning your test menu from IVDD to IVDR will demonstrate equivalent performance and not require additional time to optimize your assay parameters. Maintain the efficiency you have come to expect with the QuantStudio 5 Dx system.

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