

A Model qPCR System Comparability Study: CFX Touch and CFX Opus Real-Time PCR Platforms

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Abstract

A comparability study is a requirement in the pharmaceutical and biotechnology industries whenever a change is made to existing protocols and/or equipment. The goal of such a study, also called a bridging study, is to provide analytical evidence that the change will not adversely affect the previously approved process. Here, we present two model bridging studies to provide evidence that the new CFX Opus 96 and CFX Opus 384 Real-Time PCR Systems are comparable to the CFX96 and CFX384 Touch Real-Time PCR Systems, respectively, in terms of specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability.

Introduction

Bio-Rad recently commercialized its CFX Opus Real-Time PCR Systems. These systems are the next evolution of Bio-Rad's family of CFX Real-Time PCR Systems. Our previous generation, the family of CFX Touch Real-Time PCR Systems, has been robust and well adopted for over a decade.

Laboratories working under current good manufacturing practices (cGMP) must document and validate all changes to their existing protocols and equipment. With this in mind, we designed the following studies to serve as proof data and as examples for those wishing to set up their own comparability studies on the CFX Opus platforms.

We referred to the guidelines set out in the United States Pharmacopeia (USP) (2017) and the International Conference on Harmonisation (ICH) (2005) as we designed our comparability studies. Although there is other guidance available, we believe that basing a comparability or bridging study on these will provide an appropriate framework. The data presented highlight a specific application of the CFX Opus 96 and CFX Opus 384 Systems. They do not represent the complete capabilities of the CFX Opus Systems. Additional specifications for CFX Opus Systems can be found in the relevant product information sheets: bulletin 7299 (CFX Opus 96 System) and bulletin 7300 (CFX Opus 384 System).

Experimental Design

These studies were designed to compare the following characteristics that must be tested by labs transitioning from one real-time PCR system to another: specificity, linearity (R²), range, accuracy, precision, limit of detection, limit of quantification, robustness, and system suitability. Detailed definitions for each of these characteristics are provided in Appendix A.

Using the Zika, Dengue, and Chikungunya (ZDC) Real-Time PCR Assays with Reliance One-Step Multiplex RT-qPCR Supermix (as reported by Ma et al. 2019), we compared three CFX96 Touch Systems to three CFX Opus 96 Systems in the initial study. In the second study, we compared three CFX384 Touch Systems to three CFX Opus 384 Systems. For each study, a total of nine independent standard curves were made over 3 days; each standard curve was used to prepare replicate plates to be run on one CFX Touch System and one CFX Opus System. Testing was carried out such that each CFX96 Touch System was paired with a different CFX Opus 96 System. Similarly, each CFX384 Touch System was paired with a different CFX Opus 384 System. All data analysis was carried out using CFX Maestro Software.



Our predefined data acceptance criteria for both 96-well and 384-well studies were as follows:

- Efficiency between 90 and 110%, and linearity (R^2) greater than or equal to 0.99. These are even more stringent than the standards provided by Kibbey (2017), where linearity may be slightly relaxed to R^2 greater than or equal to 0.98, and the efficiency range is roughly 83–110%
- No template control (NTC) quantification cycle (Cq) greater than 40 and detection above the LOD in 95% of replicates. If any NTCs are positive, the Cq must not be less than the Cq of the lowest standard concentration. Additionally, the Cq value of the lowest standard must not be more than 38

For 96-well:

- For the last dilution to be included in linearity and efficiency calculations, all 3 replicates must cross the threshold with a standard deviation (SD) less than 0.6

For 384-well:

- For the last dilution to be included in linearity and efficiency calculations, a minimum of 7 out of 8 replicates must cross the threshold with a standard deviation (SD) less than 0.6

Combined, these acceptance criteria also define system suitability for this comparability study.

Materials and Methods

Individual primers and probes (proprietary sequences) for use in Droplet Digital™ PCR (ddPCR™) were purchased from Integrated DNA Technologies (IDT). Human genomic DNA was purchased from Takara and used at a final concentration of 10 ng per 20 μ l reaction. The ZDC Multiplex RT-PCR Assay (Bio-Rad Laboratories, Inc., catalog #12003818) was used with the Reliance One-Step Multiplex RT-qPCR Supermix (Bio-Rad, #12010220). Fivefold serial dilutions of ZDC Control RNA were made in Tris-EDTA buffer containing 5 ng/ml Yeast tRNA (Thermo Fisher Scientific Inc., #AM7119). A constant amount of internal positive control synthetic RNA template and human genomic DNA (gDNA) was added to each reaction.

All reverse transcription quantitative PCR (RT-qPCR) reactions were set up with Reliance One-Step Multiplex Supermix and used the 12.5x ZDC Multiplex PCR Assay Mix at 1x final concentration. This multiplex kit contains primers and probes for the detection of Zika virus (ZKV, FAM), chikungunya virus (CHK, HEX), dengue virus (DV, Texas Red), and internal positive control RNA template (IPC, Cy5). We used the optional RNase P Assay (Cy5.5) (Bio-Rad, #12004601) to detect gDNA in the 96-well reactions. For the 96-well plates, 20 μ l reactions were prepared, and for the 384-well plates, 10 μ l reactions were prepared. Reactions were run with three replicates on white-well Hard-Shell™ 96-Well PCR Plates (Bio-Rad, #HSP9655) or with eight replicates on white-well Hard-Shell 384-Well PCR Plates (Bio-Rad, #HSP3805) and sealed with Microseal™ 'B' PCR Plate Sealing Film (Bio-Rad, #MSB1001).

The following RT-qPCR protocol was run for all experiments: reverse transcription at 50°C for 10 min; reverse transcriptase inactivation and polymerase activation at 95°C for 10 min; and 45 cycles at 95°C for 10 sec and at 60°C for 30 sec with data capture at this step. Data analysis was performed using CFX Maestro Software (Bio-Rad). Efficiency and Cq values were obtained by setting the threshold for each fluorophore at 10% of the maximum number of relative fluorescence units (RFU) in that channel. Data were exported to Excel 2013 (Microsoft) and analyzed with Analyse-it Method Validation Edition Software version 5.81 (Analyse-it).

Droplet Digital PCR was used to determine copy number. Droplets were prepared using the Automated Droplet Generator (Bio-Rad, #1864101), and DNA was amplified using a 96-well T100 Thermal Cycler (Bio-Rad, #1861096). Droplets were analyzed with the QX200™ Droplet Digital PCR System (Bio-Rad, #1864001). The One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad, #1864022) was used for ddPCR reactions (20 μ l) with 900 nM primers and 250 nM probes. ZKV (FAM) and CHK (HEX) were assayed together in the same wells. DV (HEX) was assayed individually in separate wells.

Results and Discussion

As an initial test, we prepared serial dilutions of the positive control RNA that comes with the ZDC Assay and ran replicate plates on all qPCR systems. We also included a fixed concentration of internal positive control RNA and human gDNA in each reaction (Figure 1).

These results serve as a visual guide for the assay that formed the basis of this comparability study. The three positive control RNAs were present at different starting amounts, resulting in two targets (ZKV and CHK) with six-point standard curves and the third target (DV) with a four-point standard curve.

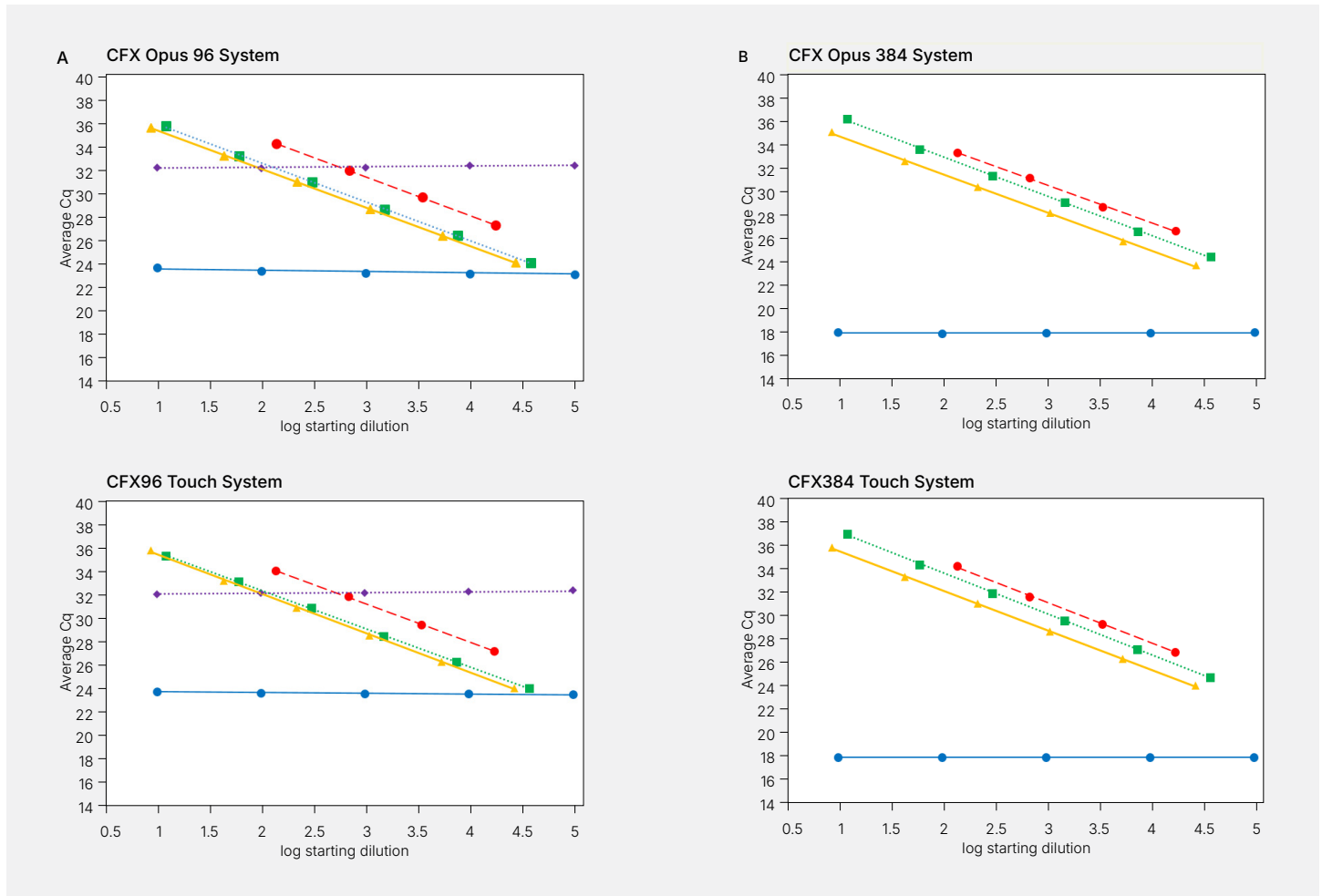


Fig. 1. Demonstration of five- and four-target multiplexing with (A) CFX Opus 96 and CFX96 Touch Systems and (B) CFX Opus 384 and CFX384 Touch Systems. The ZDC Multiplex RT-PCR Assay was used with Reliance One-Step Multiplex Supermix to demonstrate linearity of ZKV (■ FAM), CHK (▲ HEX), and DV (● Texas Red) RNA detection, while the concentration of internal positive control (IPC) synthetic RNA template (● Cy5) and gDNA (96-well only, ◆ Cy5.5, RNase P) remained constant. The results here represent one run on each system; they are shown for the purpose of demonstrating the experimental design. Cq, quantification cycle.

We investigated intrasystem equivalence to confirm that all three systems within each platform were performing comparably. The Bland-Altman test was used to demonstrate equivalence between systems of the same platform. Representative results are shown with the predicted 95% limit of agreement (LOA) between systems (Figure 2). Full datasets of Bland-Altman LOA plots for all intrasystem comparisons are provided in Appendix B. We also used Deming regression to calculate the intrasystem repeatability correlation coefficients for three variable and two fixed analytes. These data demonstrate the intrasystem equivalence in all detection channels (Table 1).

Table 1. Equivalence of three systems for each platform was demonstrated by Deming regression analysis of data in all five channels.

Real-Time PCR System	Intrasystem Comparison	Correlation Coefficient	Slope	95% CI	Intercept
CFX Opus 96	1 vs. 3	1.000	1.007	0.9902 to 1.023	0.1395
	1 vs. 2	1.000	1.015	0.9890 to 1.042	-0.1287
	2 vs. 3	1.000	0.992	0.9782 to 1.005	0.2680
CFX96 Touch	1 vs. 3	1.000	1.011	0.9845 to 1.037	-0.4630
	1 vs. 2	1.000	1.011	0.9886 to 1.034	-0.1566
	2 vs. 3	1.000	0.999	0.9880 to 1.011	-0.3065
CFX Opus 384	1 vs. 3	0.999	0.997	0.9706 to 1.023	0.0968
	1 vs. 2	0.998	1.006	0.9658 to 1.046	-0.3300
	2 vs. 3	1.000	1.010	0.9920 to 1.027	-0.4512
CFX384 Touch	1 vs. 3	0.996	0.985	0.9381 to 1.031	0.5969
	1 vs. 2	0.999	1.002	0.9887 to 1.016	-0.1267
	2 vs. 3	0.996	0.982	0.9381 to 1.027	0.7194

CI, confidence interval.

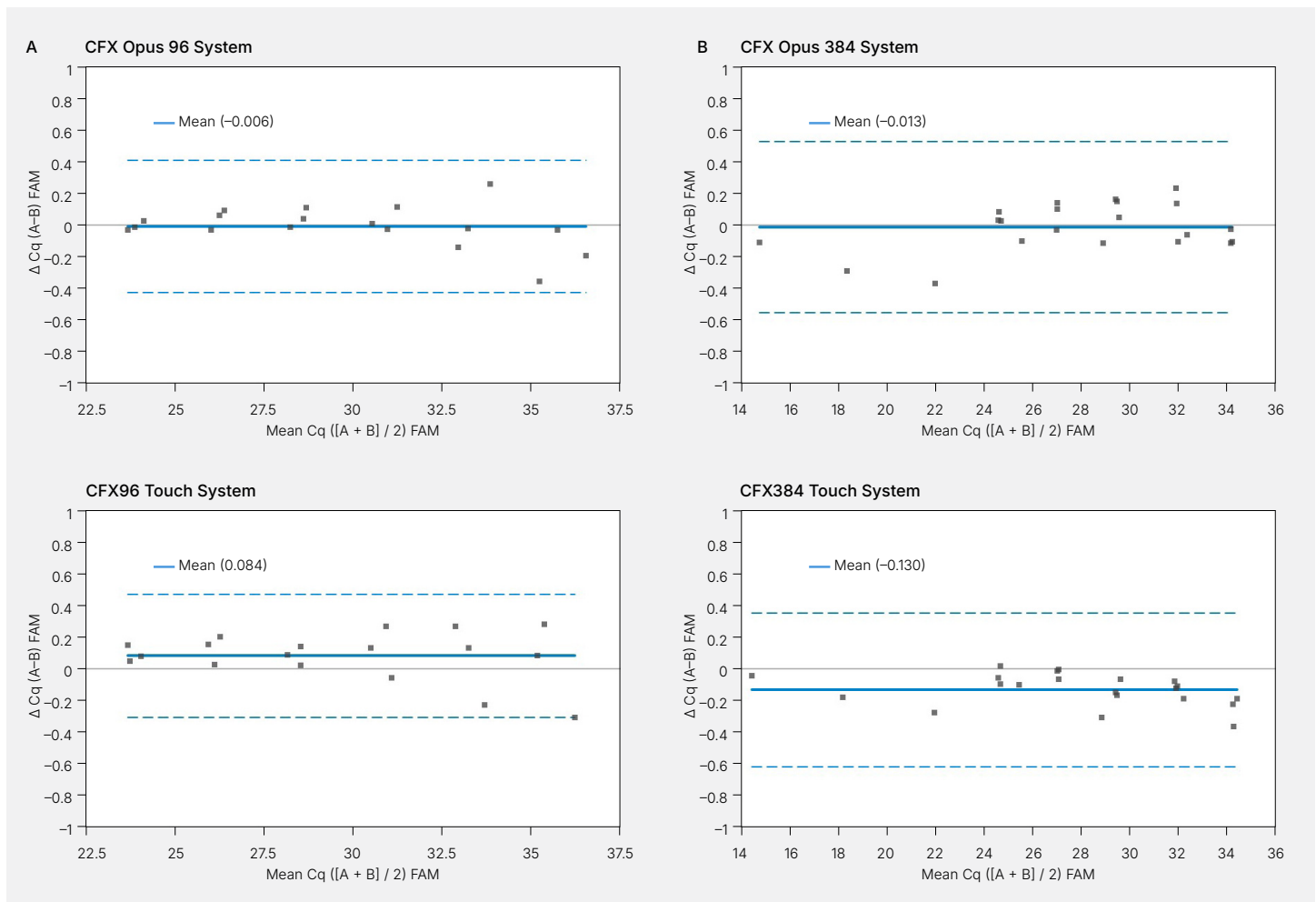


Fig. 2. Demonstration of platform equivalence in the FAM channel between (A) CFX Opus 96 and CFX96 Touch Systems and (B) CFX Opus 384 and CFX384 Touch Systems using Bland-Altman LOA plots. Representative plots of three pairwise system comparisons are shown; see Appendix B for the complete dataset. Dashed blue lines represent the 95% LOA between systems and the solid line represents the mean. The mean is an estimate of the average bias. Cq, quantification cycle; LOA, limit of agreement.

Our criteria for specificity were that NTCs must not cross the threshold with a Cq less than or equal to 40. This was achieved on all systems (CFX96 Touch, CFX Opus 96, CFX384 Touch, and CFX Opus 384) in our experiments. Robustness was demonstrated by including a small amount of human gDNA in all reactions on the CFX Touch and CFX Opus Real-Time Systems.

We report our findings on linearity, efficiency, range, LOQ, and LOD for the ZKV, CHK, and DV detection assays (Table 2). Notably, the range, LOQ, and LOD were determined to be equivalent between the CFX96 Touch and CFX Opus 96 Systems. Similar equivalency was seen between the CFX384 Touch and CFX Opus 384 Systems. The linearities and efficiencies of the three assays were well within accepted limits and almost identical between each platform.

A note about linearity for the ZDC Assay: we used fivefold serial dilutions of control RNAs (premixed) and determined that the LOQ was six dilution points for ZKV and CHK, and the LOD was seven dilution points for ZKV. However, the DV template was more dilute than the other two templates, such that only four dilution points were used for the LOQ determination and five dilution points were used for the LOD determination. It is important to note that the ICH guidelines recommend that five dilution points be used to establish linearity. For CHK on the 96-well systems and CHK and ZKV on the 384-well systems, the last dilution point yielded less than the required 95% detection of the total number of wells on each system, resulting in the LOD being the same as the LOQ. Therefore, additional testing with different dilutions would be needed to identify the true LOD. The results presented in Table 2 are virtually indistinguishable between the platforms.

Table 2. Summary of linearity, efficiency, LOQ, and LOD on CFX Opus 96, CFX96 Touch, CFX Opus 384, and CFX384 Touch Systems.

Parameter	Analyte	CFX Opus 96 System	CFX96 Touch System	CFX Opus 384 System	CFX384 Touch System
LOD	ZKV	2.17 copies	2.17 copies	2.8 copies	2.8 copies
	CHK	7.6 copies	7.6 copies	3.3 copies	3.3 copies
	DV	28.2 copies	28.2 copies	27.8 copies	27.8 copies
LOQ	ZKV	10.8 copies	10.8 copies	7.9 copies	7.9 copies
	CHK	7.6 copies	7.6 copies	6.4 copies	6.4 copies
	DV	141 copies	141 copies	111.1 copies	111.1 copies
Linear range	ZKV	33,880 to 10.8 copies	33,880 to 10.8 copies	36,067 to 7.9 copies	36,067 to 7.9 copies
	CHK	23,840 to 7.6 copies	23,840 to 7.6 copies	26,830 to 6.4 copies	26,830 to 6.4 copies
	DV	17,635 to 141 copies	17,635 to 141 copies	17,663 to 111.1 copies	17,663 to 111.1 copies
Linear range, Cq	ZKV	23.90 to 35.31	23.86 to 35.31	24.50 to 37.45	24.47 to 37.23
	CHK	23.97 to 35.68	24.06 to 35.75	23.67 to 36.39	23.70 to 36.38
	DV	27.23 to 36.29	27.12 to 36.38	26.67 to 35.04	26.75 to 35.07
Linearity, R ²	ZKV	0.999	0.999	0.998	0.998
	CHK	0.999	0.999	0.997	0.997
	DV	0.998	0.996	0.995	0.996
Efficiency, %	ZKV	101.73	101.60	96.85	97.23
	CHK	101.54	103.08	99.37	99.64
	DV	102.10	100.60	96.52	96.68

We investigated precision by carrying out our testing over several days and repeatability by testing three systems of each platform on each day (Table 3). Intermediate precision was incorporated into this study by including testing over multiple days, preparing three independent standard curves each day (to simulate user-user variability), and using all three of each of the tested systems.

The goal of the internal positive control (IPC) is to confirm presence/absence calls, which does not require a highly precise Cq value. We did not calculate the intermediate precision of the IPC because its concentration varied across days. The variability we observed here does not affect the precision of the other analytes.

Table 3. Precision of CFX Opus 96, CFX96 Touch, CFX Opus 384, and CFX384 Touch Systems as calculated from one input. For each 96-well CFX Real-Time PCR System, N = 27; for each 384-well CFX Real-Time PCR System, N = 72.

Platform	Fluorophore	Analyte	Inter-Run		Interday		Intermediate
			SD	95% CI	SD	95% CI	SD
CFX Opus 96	FAM	ZKV	0.045	0.034 to 0.067	0.107	0.072 to 0.208	0.189
	HEX	CHK	0.020	0.015 to 0.030	0.105	0.068 to 0.225	0.131
	Texas Red	DV	0.057	0.043 to 0.085	0.166	0.110 to 0.336	0.166
	Cy5	IPC	0.246	0.223 to 0.275	0.305	0.249 to 0.392	N/A
	Cy5.5	gDNA	0.152	0.137 to 0.169	0.156	0.141 to 0.175	0.173
CFX96 Touch	FAM	ZKV	0.039	0.029 to 0.057	0.111	0.073 to 0.224	0.165
	HEX	CHK	0.041	0.031 to 0.060	0.224	0.145 to 0.481	0.224
	Texas Red	DV	0.042	0.032 to 0.062	0.093	0.063 to 0.179	0.149
	Cy5	IPC	0.237	0.215 to 0.264	0.404	0.293 to 0.649	N/A
	Cy5.5	gDNA	0.171	0.155 to 0.190	0.222	0.177 to 0.296	0.222
CFX Opus 384	FAM	ZKV	0.125	0.072 to 0.103	0.113	0.087 to 0.162	0.113
	HEX	CHK	0.106	0.085 to 0.121	0.137	0.105 to 0.198	0.145
	Texas Red	DV	0.151	0.128 to 0.183	0.263	0.188 to 0.439	0.337
	Cy5	IPC	0.147	0.076 to 0.108	0.095	0.080 to 0.117	N/A
CFX384 Touch	FAM	ZKV	0.125	0.106 to 0.151	0.085	0.107 to 0.150	0.139
	HEX	CHK	0.102	0.087 to 0.123	0.100	0.090 to 0.129	0.154
	Texas Red	DV	0.147	0.125 to 0.178	0.207	0.157 to 0.305	0.295
	Cy5	IPC	0.106	0.090 to 0.128	0.089	0.112 to 0.216	N/A

CI, confidence interval; SD, standard deviation.

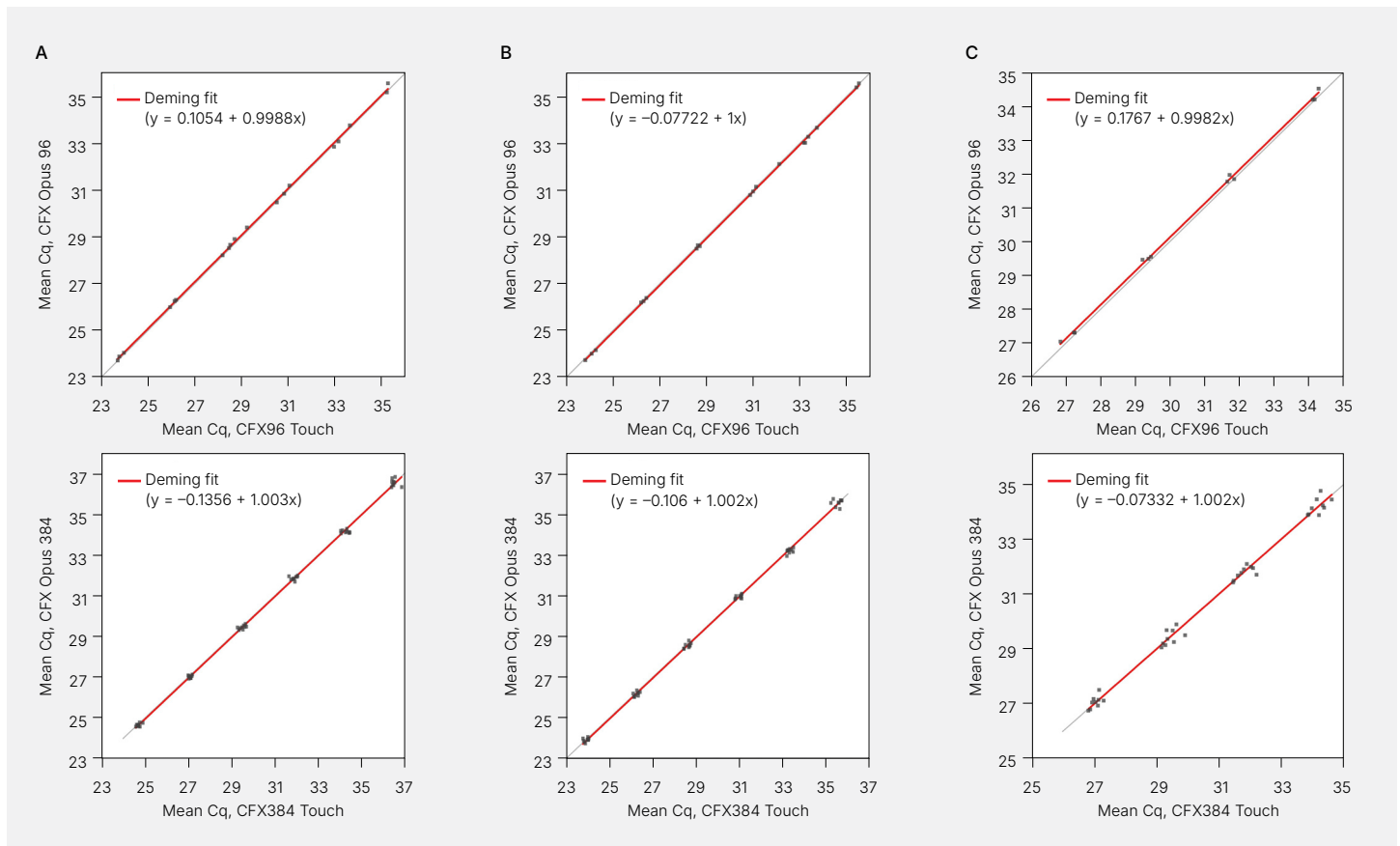


Fig. 3. Deming regression analysis of measurements in all channels. **A**, 96-well FAM channel: slope = 0.9988 (95% CI: 0.9800 to 1.018); 384-well FAM channel: slope = 1.003 (95% CI: 0.9902 to 1.015); **B**, 96-well HEX channel: slope = 1.000 (95% CI: 0.9923 to 1.008); 384-well HEX channel: slope = 1.002 (95% CI: 0.9901 to 1.014); **C**, 96-well Texas Red channel: slope = 0.9982 (95% CI: 0.9736 to 1.023); 384-well Texas Red channel: slope = 1.002 (95% CI: 0.9724 to 1.032). Three replicates for 96-well systems and eight replicates for 384-well systems in nine independent runs over 3 days on three systems of each platform were analyzed. Cq, quantification cycle.

The accuracy of the CFX Touch and the CFX Opus platforms, expressed as relative bias, was determined by measuring unknowns of known copy number by Droplet Digital PCR against the standard curve. The mean bias over the tested range was comparable between both 96-well systems and between both 384-well systems.

The regression analysis of all measurements over the course of this investigation in the FAM, HEX, and Texas Red channels shows resulting slopes with 95% confidence intervals (CIs) that indicate we can accept the hypothesis that the platforms are equivalent (Figure 3).

Conclusions

We conducted a set of comparability studies as a guide for labs that wish to replace or supplement their CFX Touch System with a CFX Opus System. These studies determine whether there are any differences between platforms, and this information is especially imperative within regulated environments. If differences are seen when transferring protocols to new platforms, then additional optimization may be required. We addressed instrument platform comparability by assessing performance characteristics described in the USP and ICH guidelines. The results of our model comparability studies demonstrate the equivalence between Bio-Rad's qPCR platforms.

References

Food and Drug Administration (FDA) Office of Regulatory Affairs (2020). Document ORA-LAB.5.4.5: Methods, Method Verification and Validation, Laboratory Manual Volume II. www.fda.gov/media/73920/download, accessed November 16, 2021.

Gurtler C et al. (2018). Transferring a quantitative molecular diagnostic test to multiple real-time quantitative PCR platforms. *J Mol Diagn* 20, 398–414.

International Conference on Harmonisation (2005). Q2(R1) Validation of Analytical Procedures: Text and Methodology.

Kibbey MC (2017). USP General Chapters and Reference Standards that Support the Development and Characterization of Biologics. *BioPharm Intl*. cdn.sanity.io/files/0vv8moc6/biopharm/567a2675cb84cf642712d04e99d5ddf20eb55334.pdf, accessed November 16, 2021.

Ma J et al. (2019). Sensitive codetection of viral-pathogen RNA and DNA using one-step multiplex RT-qPCR. *Bio-Rad Bulletin* 7196.

United States Pharmacopeial Convention (2017). U.S. Pharmacopeia <1225> Validation of Compendial Methods. www.uspbpep.com/usp29/v29240/usp29nf24s0_c1225.html, accessed November 16, 2021.

Appendix A: Definition of Characteristics

The following definitions were compiled from ICH (2005), USP (2017), and U.S. FDA (2020) guidelines.

Accuracy — the nearness of a result or the mean of a set of measurements to the true value.

Limit of detection (LOD) — the lowest concentration level that can be determined as statistically different from a blank at a specified level of confidence. It is determined from the analysis of sample blanks.

Limit of quantification (LOQ) — the level above which quantifiable results may be determined with acceptable accuracy and precision.

Linearity — the ability of a method to elicit results that are directly proportional to analyte concentration within a given range.

Precision — agreement between a set of replicate measurements. Precision does not necessarily refer to the true value. The precision of test results is described by statistical methods, such as a standard deviation or confidence limit. Repeatability expresses precision under the same operating conditions over a short period of time.

Intermediate precision expresses precision within laboratory variations, such as different days, different analysts, and different equipment. Reproducibility expresses the precision between laboratories.

Range — the interval between the upper and lower concentration of analyte in a sample for which it has been demonstrated that the analytical procedure has an acceptable level of accuracy, precision, and linearity.

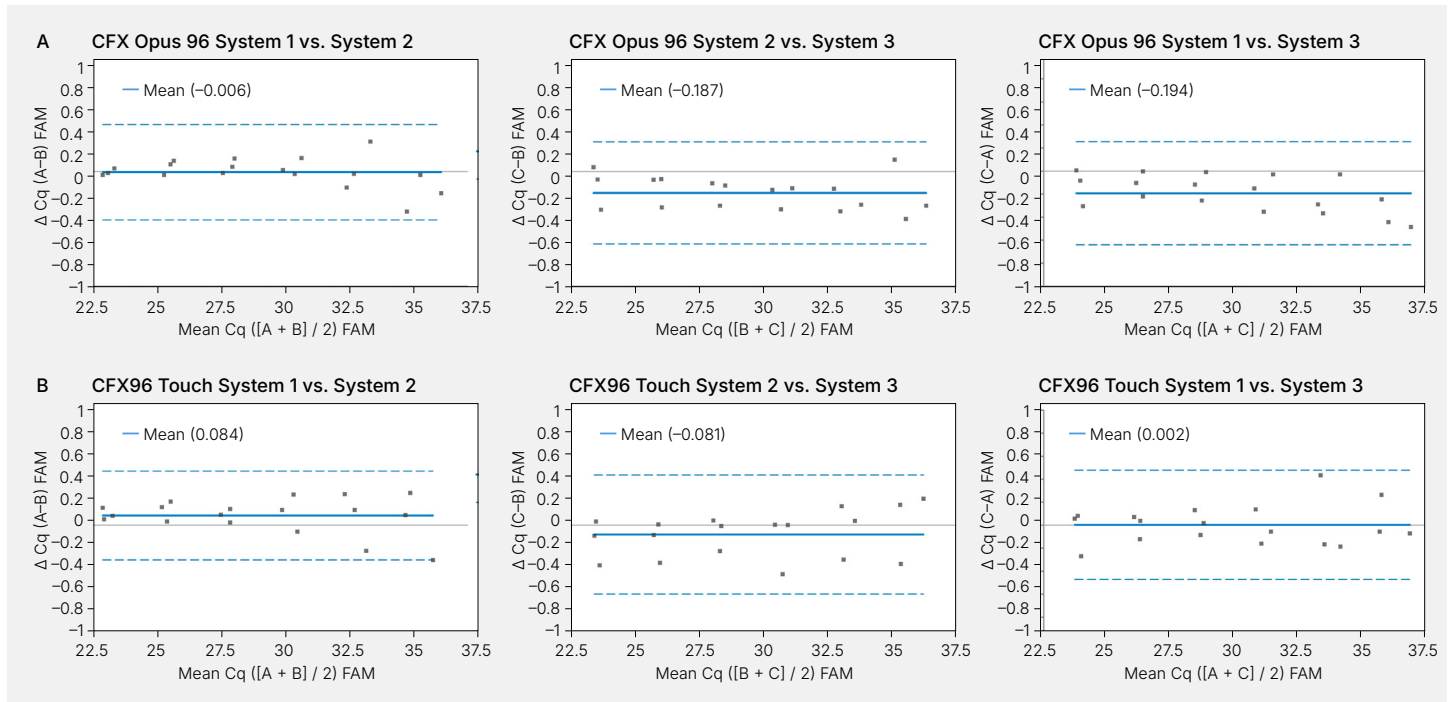
Robustness — an analytical procedure's capacity to remain unaffected by small but deliberate variations in method parameters. It provides an indication of the procedure's reliability during normal usage.

Specificity — the ability to assess unequivocally an analyte in the presence of impurities, degradation products, or other components that may be present.

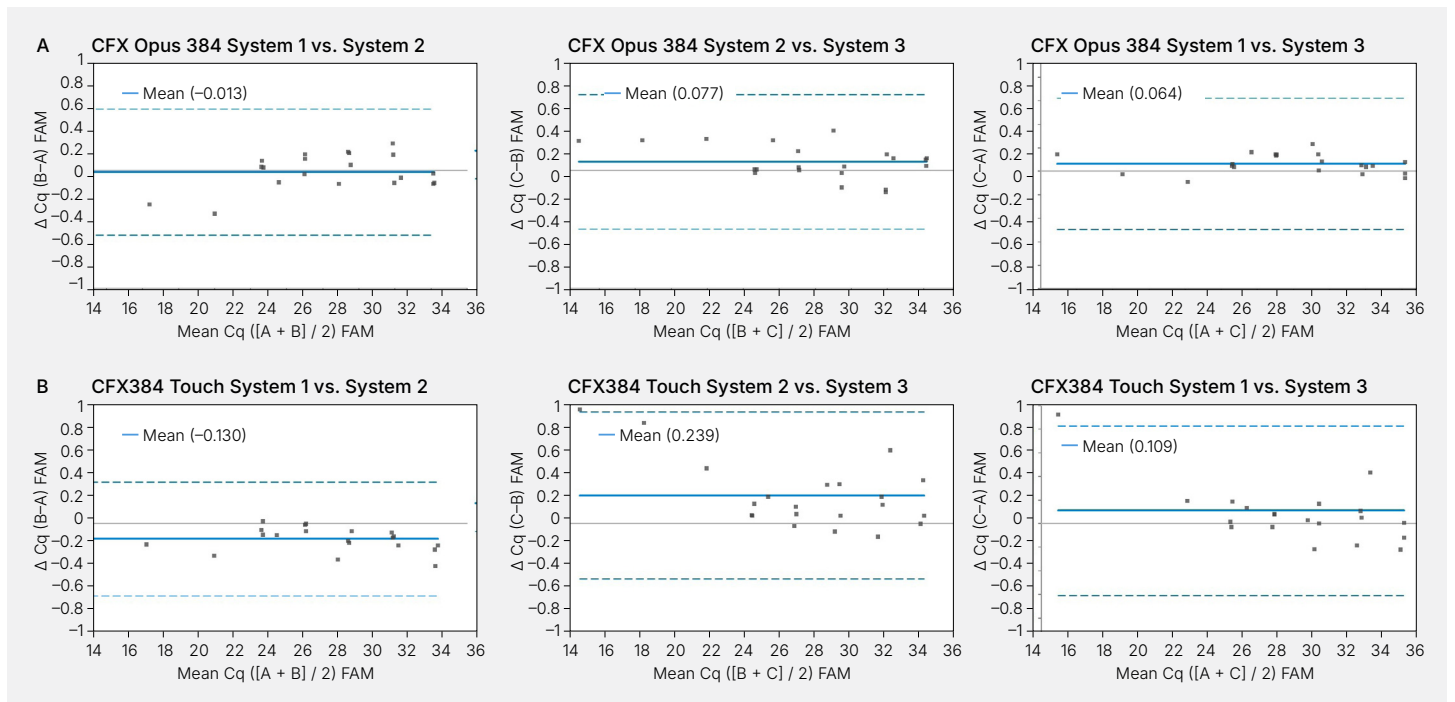
System suitability — system suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. See pharmacopeias for additional information.

Appendix B: Supplementary Data

FAM Channel

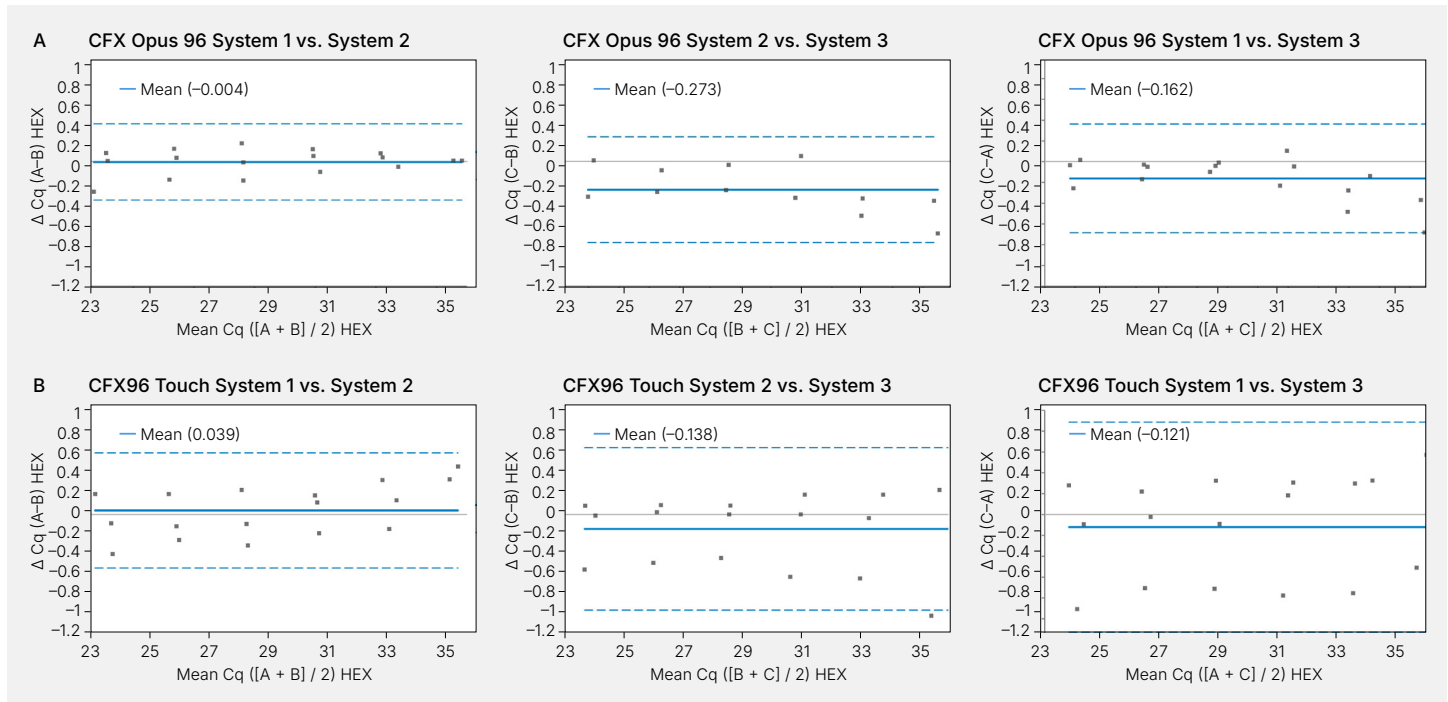


Suppl. Fig. 1. Demonstration of platform equivalence in the FAM channel between three (A) CFX Opus 96 Systems and (B) CFX96 Touch Systems using Bland-Altman LOA plots. Dashed lines represent the 95% LOA between systems, and the solid line represents the mean. The mean is an estimate of the average bias. Cq, quantification cycle; LOA, limit of agreement.

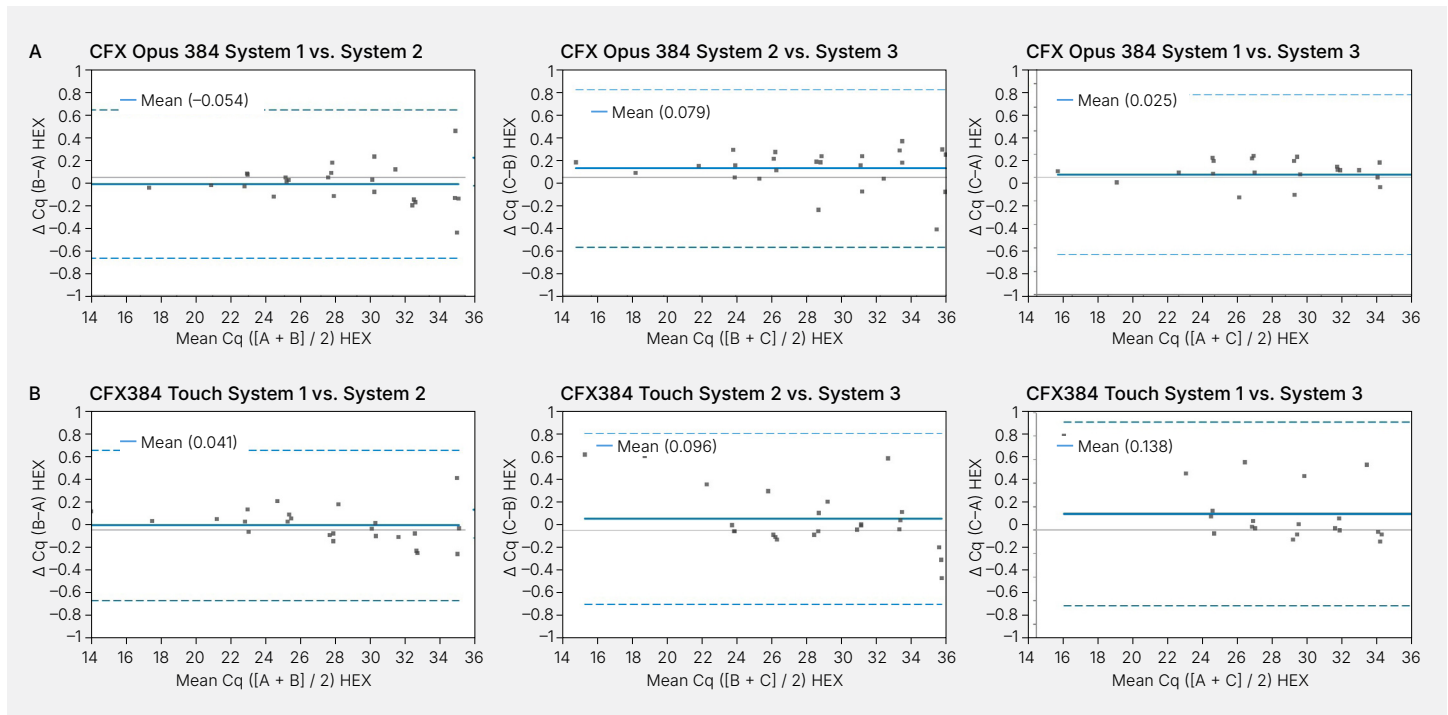


Suppl. Fig. 2. Demonstration of platform equivalence in the FAM channel between three (A) CFX Opus 384 Systems and (B) CFX384 Touch Systems using Bland-Altman LOA plots. Dashed lines represent the 95% LOA between systems, and the solid line represents the mean. The mean is an estimate of the average bias. Cq, quantification cycle; LOA, limit of agreement.

HEX Channel

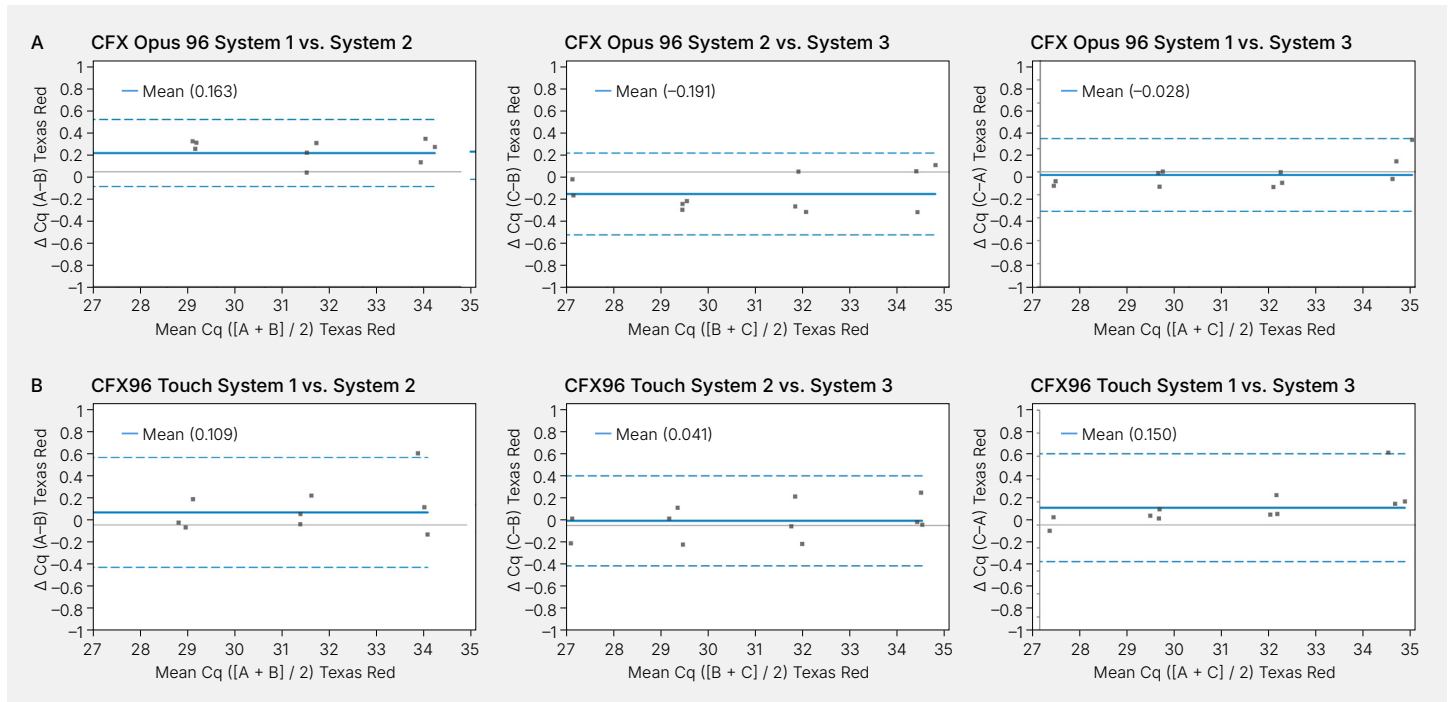


Suppl. Fig. 3. Demonstration of platform equivalence in the HEX channel between three (A) CFX Opus 96 Systems and (B) CFX96 Touch Systems using Bland-Altman LOA plots. Dashed lines represent the 95% LOA between systems, and the solid line represents the mean. The mean is an estimate of the average bias. Cq, quantification cycle; LOA, limit of agreement

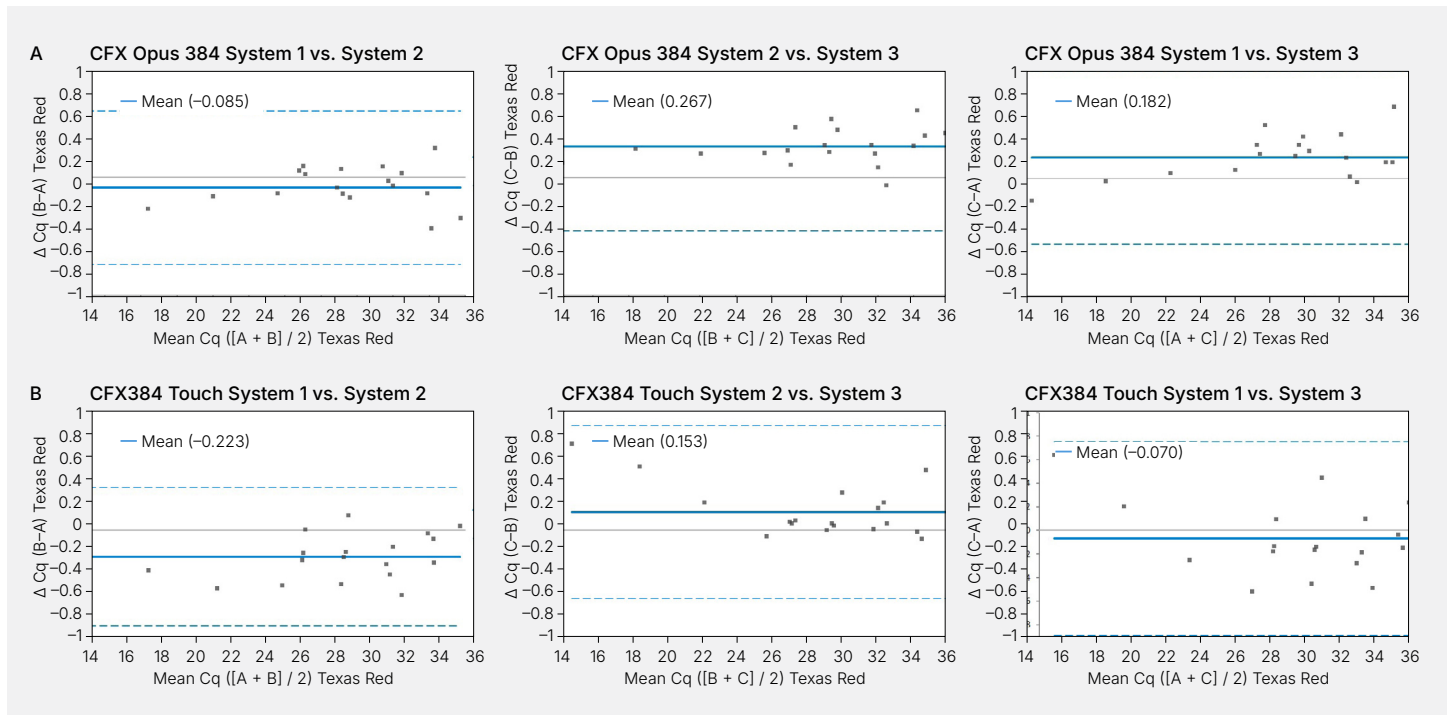


Suppl. Fig. 4. Demonstration of platform equivalence in the HEX channel between three (A) CFX Opus 384 Systems and (B) CFX384 Touch Systems using Bland-Altman LOA plots. Dashed lines represent the 95% LOA between systems, and the solid line represents the mean. The mean is an estimate of the average bias. Cq, quantification cycle; LOA, limit of agreement.

Texas Red Channel



Suppl. Fig. 5. Demonstration of platform equivalence in the Texas Red channel between three (A) CFX Opus 96 Systems and (B) CFX96 Touch Systems using Bland-Altman LOA plots. Dashed lines represent the 95% LOA between systems, and the solid line represents the mean. The mean is an estimate of the average bias. Cq, quantification cycle; LOA, limit of agreement.



Suppl. Fig. 6. Demonstration of platform equivalence in the Texas Red channel between three (A) CFX Opus 384 Systems and (B) CFX384 Touch Systems using Bland-Altman LOA plots. Dashed lines represent the 95% LOA between systems, and the solid line represents the mean. The mean is an estimate of the average bias. Cq, quantification cycle; LOA, limit of agreement.

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