# A qPCR Comparability Study to Demonstrate the Analytical Equivalence of CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems

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## Abstract

A comparability study is intended to provide analytical evidence that transitioning from one platform to a different one will not adversely affect an approved process. Prior bridging studies have shown comparability between the Bio-Rad Laboratories, Inc. research use only (RUO) CFX96 Touch and CFX Opus 96 Real-Time PCR Systems* (Woo et al. 2021). Here we designed studies to evaluate the newer CFX Opus Deepwell Dx Real-Time PCR System and its equivalency to the CFX96 Deep Well Dx Real-Time PCR System. Specific performance criteria were examined, including specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability for a quantitative PCR (qPCR) application. The purpose of this study is to guide users who plan to transition their process from the CFX96 Deep Well Dx System to the CFX Opus Deepwell Dx System.

## Introduction

The CFX Opus 96 Dx, CFX Opus 384 Dx, and CFX Opus Deepwell Dx Real-Time PCR Systems from Bio-Rad provide a robust and reliable open platform for the development and performance of in vitro diagnostic (IVD) assays. The family of CFX Opus Dx Systems offers exceptional thermal uniformity, providing sensitive and precise detection and quantification, and can perform multiplexed detection of up to five targets per well for 96-well formats and four targets per well for 384-well formats. The format of the CFX Opus Deepwell Dx System is designed for the detection of DNA and RNA using either standard 20 µl volume or larger volume applications, up to 125 µl. Unlike the previous CFX96 Dx and CFX96 Deep Well Dx Systems, which use CFX Manager Dx Software, the CFX Opus Deepwell Dx System uses the more recent CFX Maestro Dx SE Software. This updated software provides easy setup, run monitoring, data analysis, and report generation, including traceable audit-ready reports to ensure the integrity of patient results. The purpose of this comparability study is to demonstrate that the new CFX Opus Deepwell Dx System performs equivalently to the previous CFX96 Deep Well Dx System.

To perform this qPCR comparability study, the guidelines set out by the United States Pharmacopeia Convention (USP, 2017) and the U.S. Food and Drug Administration (FDA, 2021) were followed. In defining the acceptance criteria for transferring molecular assays to the newer CFX Opus Deepwell Dx System, we referred to Gurtler et al. (2018) and Bio-Rad's earlier bridging study (Woo et al. 2021). Experiments were conducted using serial dilutions of SARS-CoV-2 in vitro transcribed (IVT) RNAs and detection by a SARS-CoV-2 triplex diagnostic Emergency Use Authorization (EUA) assay. Data analysis was performed using the two different diagnostic software packages and the performance of the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems were shown to be comparable. This study is intended to guide diagnostic users who are interested in transitioning from the CFX96 Deep Well Dx System to the new CFX Opus Deepwell Dx System. Diagnostics users will need to use a 510(k) cleared kit on an instrument platform that is cleared by the FDA.

* Comparability studies also performed for CFX384 Touch and CFX Opus 384 Real-Time PCR Systems.



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## Experimental Design

The Bio-Rad Reliance SARS-CoV-2 RT-PCR Assay Kit, an EUA kit, has been validated for use on the Bio-Rad CFX96 Dx System.* This reverse transcription quantitative PCR (RT-qPCR) comparability study was designed (Figure 1), first, to show that the Reliance SARS-CoV-2 Assay Kit provides equivalent performance on the CFX96 Dx System and the CFX96 Deep Well Dx System. Second, the analytical equivalency of the CFX96 Deep Well Dx System with the newer CFX Opus Deepwell Dx System was compared. As in the recent comparability study of the CFX Opus 96 and CFX Opus 384 Systems (Woo et al. 2021), the key performance characteristics measured here for a total of nine systems were linearity ($R^2$), range, accuracy, precision, LOD, LOQ, robustness, and system suitability. The detailed definitions for each of these analytical characteristics are provided in Appendix A and the characteristics must be tested by diagnostic labs intending to add the CFX Opus Deepwell Dx System to existing SOPs approved for use with other real-time PCR systems.

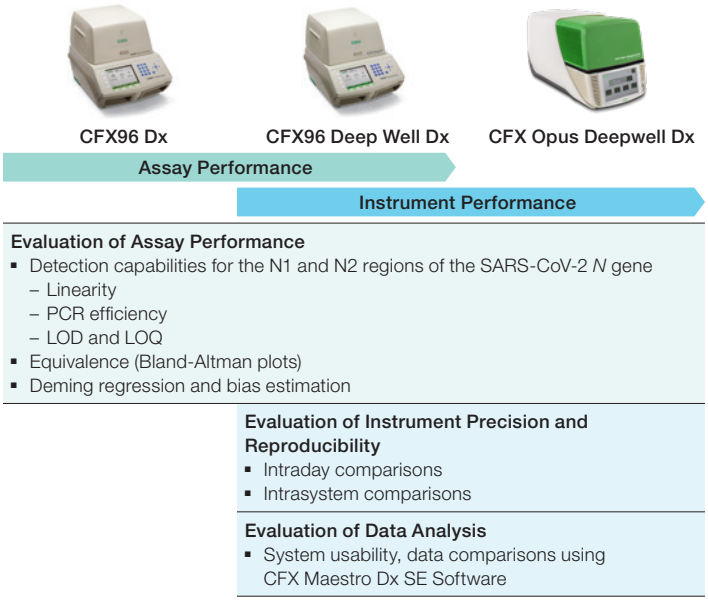


**Fig. 1. Schematic representation of experimental plan for determining assay and instrument performance of CFX96 Dx, CFX96 Deep Well Dx, and CFX Opus Deepwell Dx Systems.** For the Reliance SARS-CoV-2 RT-PCR Assay Kit, assay performance had already been validated for standard volume formats on the CFX96 Dx System but not on the CFX96 Deep Well Dx System. Therefore, the process of transferring the assay to a deep well format (CFX96 Deep Well Dx System) was evaluated first. Once assay performance had been demonstrated in the deep well format, the assay was used to evaluate comparability between the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems by measuring instrument precision and reproducibility. Finally, data were analyzed to demonstrate equivalence in the calculations provided by CFX Manager Dx and CFX Maestro Dx SE Software. The evaluations were performed across three days with three systems per platform. LOD, limit of detection; LOQ, limit of quantitation.

Assay performance between the standard volume and deep well assay format (Figure 1) was determined using three instruments each of the CFX96 Dx and CFX96 Deep Well Dx Systems, with three standard curves, each completed on three different days. The evaluation included replicate plates prepared and run each day.

For the bridging study (Figure 1) between the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems, on each of three days, standard curves were prepared and tested simultaneously on three units of each platform. Testing was carried out such that each day a different CFX96 Deep Well Dx System was paired with a different CFX Opus Deepwell Dx System (see Table 1).

CFX Manager Dx Software version 3.1 was used to run and analyze assay performance experiments on the CFX96 Dx and CFX96 Deep Well Dx units. For the CFX Opus Deepwell Dx bridging study, CFX Maestro Dx SE Software was used to operate the CFX Opus Deepwell Dx System, and to analyze both the CFX96 Deep Well Dx and CFX Opus Deepwell Dx data files. Regression analysis was also performed on the linear range of quantification cycles (Cq) values obtained for the CFX96 Deep Well Dx System in both software packages and a high correlation coefficient ($r = 1$) was obtained (Supplementary Figure 1).

The acceptance criteria for the runs in this study had to meet the following attributes and were aligned with those described in the prior comparability study between the CFX Touch and CFX Opus Real-Time PCR platforms (Woo et al. 2021):

- Efficiency between 90 and 110%, and linearity requirements used an $R^2$ greater than or equal to 0.99
- All 4 replicates of the last dilution must cross the threshold with a standard deviation (SD) less than 0.6 to be included in the linearity and efficiency calculations
- To calculate the LOD at 95%, the criteria specified that all 4 replicates of the last dilution, or 100% of all replicates, must cross the threshold
- Cq for no template controls (NTCs) must be at Cq greater than 40. However, if any NTCs are positive, the Cq must not be less than the Cq of the lowest standard concentration ($Cq < 36$)

Combined, these acceptance criteria also define system suitability.

## Materials and Methods

### Real-Time PCR Assay for the Comparability Study

The Reliance SARS-CoV-2 RT-PCR Assay Kit (Bio-Rad, catalog #12014115), a triplex EUA RT-qPCR assay, was used to 1) demonstrate assay performance on the CFX96 Deep Well Dx System, and 2) evaluate the analytical performance of the CFX Opus Deepwell Dx System compared to the CFX96 Deep Well Dx System. The SARS-CoV-2 RT-PCR Kit was designed for specific detection of the SARS-CoV-2 nucleocapsid gene at two regions, termed N1 and N2 using the same primer/probe sets reported by the Centers for Disease Control and Prevention (CDC, #2019-nCoVEUA-01). An additional primer/probe set was used to detect the human *RNase P* (RP) gene.

* Reliance SARS-CoV-2 RT-PCR Assay Kit also validated on CFX Opus 96, CFX96 Touch, CFX Opus 384, and CFX384 Touch Systems.

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Threefold serial dilutions of Exact Diagnostics SARS-CoV-2 IVT RNA (Bio-Rad, custom product) were made in Tris EDTA buffer (TE) containing 5 ng/ml yeast tRNA (Thermo Fisher Scientific Inc., #AM7119). A constant input of 5 ng human genomic DNA (gDNA) (Takara Bio USA, Inc., #636401) was added to each 20 µl reaction as an assay control that detects the human *RNase P* gene. All RT-qPCR reactions were performed in 20 µl, according to the SARS-CoV-2 RT-PCR Assay Kit instructions with the following exception: 10 µl of SARS-CoV-2 IVT RNA serial dilution, instead of extracted RNA sample, was added per well. For each individual experiment, reactions were run in quadruplicates on white well Hard-Shell™ 96-Well PCR Plates (Bio-Rad, #HSP9655) and sealed with Microseal™ 'B' PCR Plate Sealing Film (Bio-Rad, #MSB1001).

The following RT-qPCR protocol was run for all experiments: 50ºC for 10 min, followed by inactivation and activation at 95ºC for 10 min, followed by 45 cycles of 95ºC for 10 sec, 60ºC for 30 sec. Instrument operation and data analysis were performed using Bio-Rad CFX Manager Dx Software for the CFX96 Dx and CFX96 Deep Well Dx Systems, whereas CFX Maestro Dx SE Software was used for CFX Opus Deepwell Dx Systems. The PCR efficiency and Cq values were obtained by setting the threshold for each fluorophore at 10% of the maximum relative fluorescence units (RFU). The data were exported to Excel 2022 and analyzed with the Analyse-it Method Validation Edition version 6.15 (Analyse-it Software, Ltd.).

#### Droplet Digital™ PCR (ddPCR™) Assay for Confirming LOD/LOQ

The SARS-Cov-2 ddPCR Kit (Bio-Rad, #12013743) containing the 2019-nCoV CDC ddPCR Triplex Probe Assay (Bio-Rad, #12008202) was used to determine copy number for LOD, LOQ, and linear range. The oligonucleotide primers and probes for the SARS-CoV-2 ddPCR Assay were specific for detecting the N1 and N2 gene regions as reported by the CDC. An additional primer/probe set was used to detect the human *RNase P* gene in experimental and control samples. The droplets were prepared using the Automated Droplet Generator (Bio-Rad, #1864101), and amplification was on a C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module (Bio-Rad, #1851197). Reactions of 20 µl were set up according to the kit protocol. The Exact Diagnostics SARS-CoV-2 Standard (Bio-Rad, #COV019) and SARS-CoV-2 Negative (Bio-Rad, #COV000) with human genomic DNA background were used as controls for the assay. The following reverse transcription PCR (RT-PCR) protocol was used to run all ddPCR experiments: 50ºC for 10 min, followed by enzyme activation at 95ºC for 10 min, followed by 40 cycles of 94ºC for 30 sec, 55ºC for 60 sec, enzyme deactivation at 98ºC for 10 min, and droplet stabilization at 4ºC for 30 min. The droplets were read on the QX200™ Droplet Reader (Bio-Rad, #1864003). Copy number was determined using QuantaSoft™ Analysis Pro Software (Bio-Rad, download), refer to the Bio-Rad SARS-CoV-2 ddPCR Kit Instructions for Use 12013769 for more information.

## Results

For this study, because the Reliance SARS-CoV-2 RT-PCR Assay Kit was approved under EUA for the CFX96 Dx System, we first had to demonstrate that the kit showed comparable performance on the CFX96 Deep Well Dx System. Following that, we were able to use that assay to compare the CFX96 Deep Well Dx with the CFX Opus Deepwell Dx System.

To ensure the platforms were all performing as expected, the intrasystem repeatability of the CFX96 Dx, CFX96 Deep Well Dx, and CFX Opus Deepwell Dx instruments was studied. Deming regression analysis was performed on the Cq values obtained from the six-point dilutions curves for SARS-CoV-2 N1 (FAM channel) and N2 targets (HEX channel). Intrasystem equivalence was observed based on the correlation coefficients of r = 0.99 for both FAM and HEX channels in all CFX Opus Deepwell Dx, CFX96 Dx, and CFX96 Deep Well Dx Systems (Table 1, Supplementary Figures 2 and 3).

The Bland-Altman test was also used to demonstrate equivalence between systems of the same platform and estimate the bias. Representative results are shown with the predicted 95% limit of agreement (LOA) between systems (Figure 2), and full datasets of Bland-Altman LOA plots for all intrasystem comparisons are also provided (Supplementary Figures 4 and 5).

**Table 1. Equivalence of three systems for each platform.** Deming regression analysis of data from CFX96 Dx, CFX96 Deep Well Dx, and CFX Opus Deepwell Dx Systems in the FAM and HEX detection channels for the two targets, N1 and N2.

| Real-Time PCR System | Intrasystem Comparison | Fluorophore | Correlation, r | Slope | Confidence Interval | Intercept |
|---|---|---|---|---|---|---|
| CFX96 Dx | A vs. B | FAM | 0.997 | 1.016 | 0.968–1.064 | –0.584 |
| CFX96 Dx | B vs. C | FAM | 0.993 | 0.982 | 0.910–1.055 | 0.889 |
| CFX96 Dx | A vs. C | FAM | 0.987 | 0.997 | 0.897–1.096 | 0.382 |
| CFX96 Deep Well Dx | A vs. B | FAM | 0.999 | 0.999 | 0.969–1.029 | 0.023 |
| CFX96 Deep Well Dx | B vs. C | FAM | 0.999 | 1.012 | 0.985–1.039 | –0.289 |
| CFX96 Deep Well Dx | A vs. C | FAM | 0.999 | 1.012 | 0.984–1.039 | –0.265 |
| CFX Opus Deepwell Dx | A vs. B | FAM | 0.999 | 0.996 | 0.959–1.032 | 0.090 |
| CFX Opus Deepwell Dx | B vs. C | FAM | 1.000 | 1.007 | 0.990–1.023 | –0.241 |
| CFX Opus Deepwell Dx | A vs. C | FAM | 0.999 | 1.002 | 0.968–1.036 | –0.151 |
| CFX96 Dx | A vs. B | HEX | 0.995 | 1.019 | 0.966–1.071 | –0.718 |
| CFX96 Dx | B vs. C | HEX | 0.992 | 1.005 | 0.937–1.072 | 0.008 |
| CFX96 Dx | A vs. C | HEX | 0.995 | 0.986 | 0.917–1.053 | 0.805 |
| CFX96 Deep Well Dx | A vs. B | HEX | 0.998 | 1.014 | 0.973–1.054 | –0.342 |
| CFX96 Deep Well Dx | B vs. C | HEX | 0.998 | 0.998 | 0.966–1.028 | 0.179 |
| CFX96 Deep Well Dx | A vs C | HEX | 0.998 | 1.012 | 0.968–1.055 | –0.164 |
| CFX Opus Deepwell Dx | A vs. B | HEX | 0.998 | 1.025 | 0.981–1.069 | –0.642 |
| CFX Opus Deepwell Dx | B vs. C | HEX | 0.998 | 0.966 | 0.923–1.008 | 0.912 |
| CFX Opus Deepwell Dx | A vs. C | HEX | 0.999 | 0.990 | 0.965–1.015 | 0.301 |

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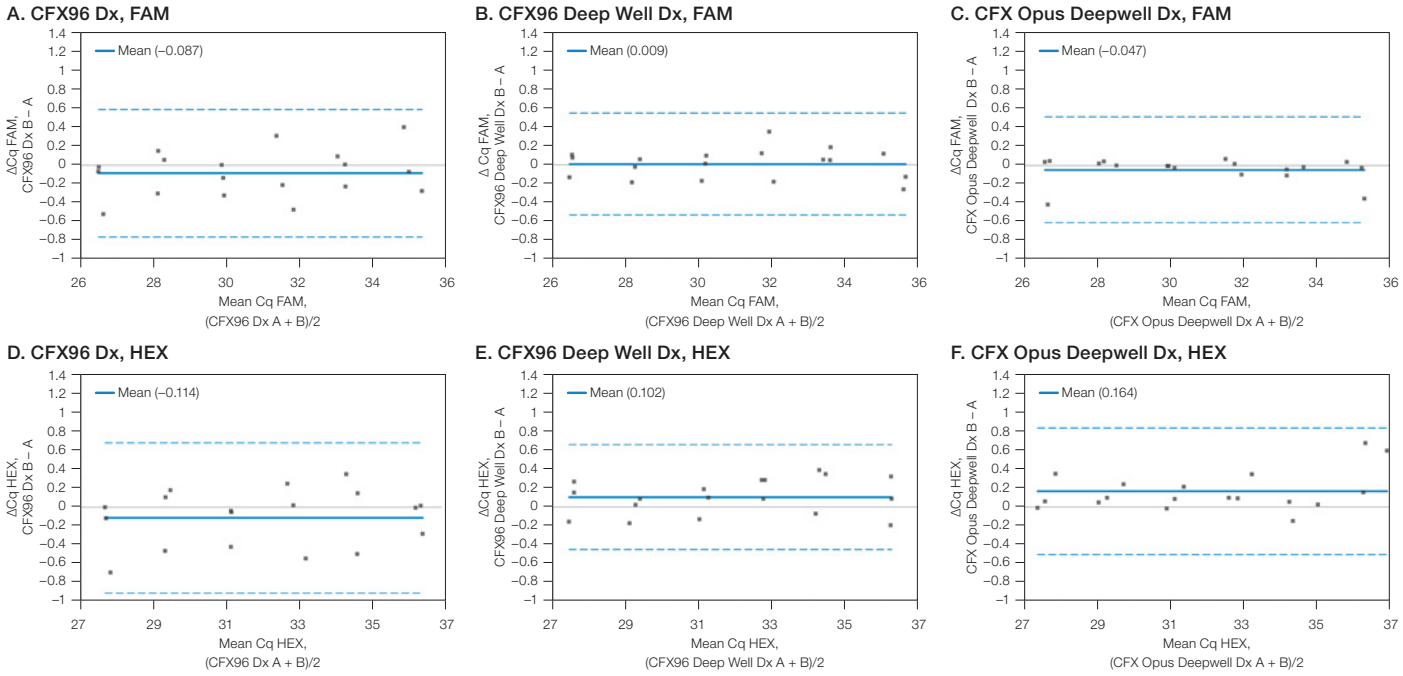


**Fig. 2. Demonstration of equivalence among systems of the same platform type.** Individual systems selected to represent each platform using Bland-Altman LOA plots for pairwise comparisons between two CFX96 Dx Systems **A**, FAM and **D**, HEX channels; two CFX96 Deep Welll Dx Systems **B**, FAM and **E**, HEX channels; two CFX Opus Deepwell Dx Systems **C**, FAM and **F**, HEX channels. Dashed blue lines represent the 95% LOA between units of each platform, and the solid line represents the mean. The mean is an estimate of the average bias between instruments. Cq, quantification cycle; LOA, limit of agreement.

The acceptance criteria for specificity required that NTCs must not cross the threshold, or must have a Cq value greater than or equal to 40; no NTCs crossed the threshold in any experiment within the CFX96 DX, CFX96 Deep Well Dx, or CFX Opus Deepwell Dx Systems tested.

Assay performance was first determined on the CFX96 Dx and CFX96 Deep Well Dx Systems using the Bio-Rad Reliance SARS-CoV-2 RT-Kit under EUA. As described in the experimental design, three units of each platform were tested over three days. Deming regression analysis was used to calculate the repeatability correlation coefficients between the two instrument platforms for all Cq measurements in the FAM and HEX detection channels (Figure 3). The correlation coefficients of r = 0.99 for the plots shown in Figure 4A and 4B suggest very similar assay performance on the CFX96 Dx and CFX96 Deep Well Dx Systems. Additional data are included in Supplementary Table 1.

Next, the analytical characteristics between the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems were evaluated for equivalency using a six-point standard curve, which resulted in Cq values of approximately 26–36 Cq on both platforms. Table 2 shows linearity, PCR efficiency, range, LOD, and LOQ for detection of the SARS-CoV-2 N1 and N2 targets in assays run on the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems. Linearities ($R^2$) and PCR efficiencies (%) were observed within acceptable limits ($R^2 \geq 0.99$ and 90–110% respectively) and were almost identical between platforms (Table 2).

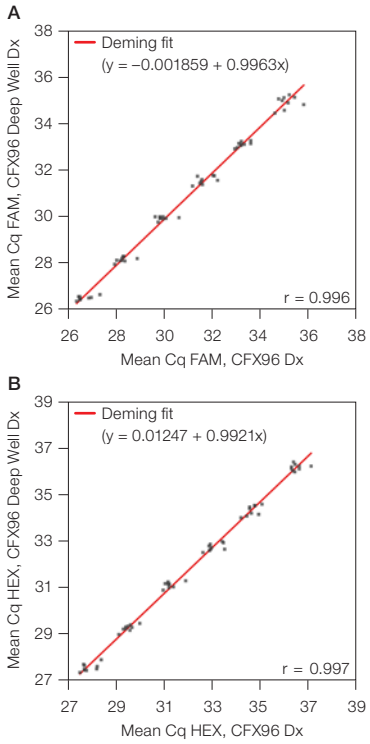


**Fig. 3. Deming regression analysis between CFX96 Dx and CFX96 Deep Well Dx Systems.** Similar assay performance for CFX96 Dx and CFX96 Deep Well Dx Systems based on correlation of mean Cq measurements. Triplicate measurements of three independent runs over three days on three systems of each platform were analyzed and the results plotted as mean Cq results for CFX96 Dx System (x-axis) versus CFX96 Deep Well Dx System (y-axis) for the FAM channel (**A**), the HEX channel (**B**), and Deming fit plotted (—) as shown. Cq, quantification cycle.

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**Table 2. Summary of analytical characteristics for CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems.** LOD, LOQ, linear range, linearity and efficiency on CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems, average of three systems on three days. LOD, limit of detection; LOQ, limit of quantification.

| Paramater | Analyte | Fluorophore | CFX96 Deep Well Dx | CFX Opus Deepwell Dx |
|---|---|---|---|---|
| LOD, copies | SARS-CoV-2 N1 | FAM | 3.81 | 3.81 |
| | SARS-CoV-2 N2 | HEX | 3.21 | 3.21 |
| LOQ, copies | SARS-CoV-2 N1 | FAM | 11.01 | 11.01 |
| | SARS-CoV-2 N2 | HEX | 6.11 | 6.11 |
| Linear range, molecules | SARS-CoV-2 N1 | FAM | 8,026–11.01 | 8,026–11.01 |
| | SARS-CoV-2 N2 | HEX | 4,454–6.11 | 4,454–6.11 |
| Linear range, Cq | SARS-CoV-2 N1 | FAM | 26.51–34.94 | 26.61–35.01 |
| | SARS-CoV-2 N2 | HEX | 27.58–36.20 | 27.59–36.33 |
| Linearity, $R^2$ | SARS-CoV-2 N1 | FAM | 0.996 | 0.997 |
| | SARS-CoV-2 N2 | HEX | 0.997 | 0.996 |
| Efficiency, % | SARS-CoV-2 N1 | FAM | 93.12 | 92.87 |
| | SARS-CoV-2 N2 | HEX | 90.66 | 90.35 |

Range, LOQ, and LOD for N1 (FAM) and N2 (HEX) targets were also determined to be equivalent between the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems (Table 2). These results support comparable analytical characteristics between the CFX96 Dx and CFX Opus Dx platforms.

Deming regression analysis was used to compare system performance. As shown in Figure 4, the correlation coefficient between mean Cq of CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems had an excellent correlation value (r = 0.99), demonstrating the equivalence of CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems.

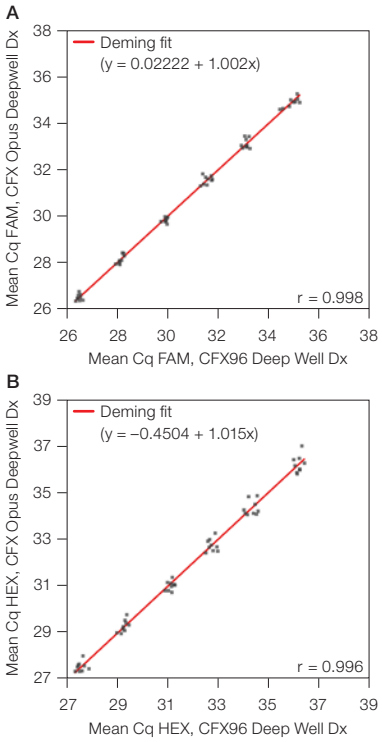


**Fig. 4. Deming regression analysis between CFX96 Dx Deep Well and CFX Opus Deepwell Dx Systems.** Quadruplicate measurements of three independent runs over three days and with three instruments of each platform were analyzed. Mean Cq for CFX96 Deep Well Dx Systems were plotted against CFX Opus Deepwell Dx Systems for FAM channel (**A**), HEX channel (**B**), and Deming fit plotted (—) as shown. Cq, quantification cycle.

To evaluate the analytical equivalence of the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems, six serial dilutions of SARS-CoV-2 IVT RNA were prepared and run in replicate plates on three of each instrument type on each of three days. Consistent detection of six points was observed on the standard curve in both the FAM channel targeting SARS-CoV-2 N1 and the HEX channel targeting SARS-CoV-2 N2 on all systems tested in this study (Figure 5), demonstrating linearity. We conclude that the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems performed similarly to each other in this study.

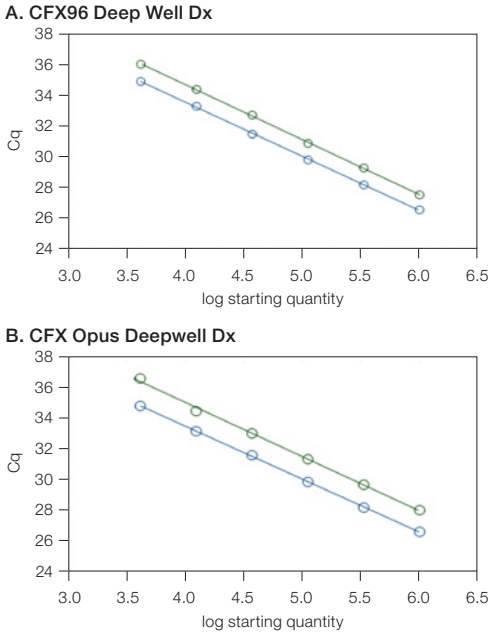


**Fig. 5. Demonstration of multiplex qPCR assay performance on A, CFX96 Deep Well Dx; B, CFX Opus Deepwell Dx Systems.** Linearity of detection for the SARS-CoV-2 nucleocapsid gene regions N1 [FAM (○)] and N2 [HEX (○)]. Cq, quantification cycle.

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**Table 3. Precision of CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems calculated from one input.** Standard deviation results are within the 95% CI of the inter-run and interday measurements.

| | | | Inter-Run | | Interday | | Intermediate |
|---|---|---|---|---|---|---|---|
| **Platform** | **Fluorophore** | **Analyte** | **SD** | **95% CI** | **SD** | **95% CI** | **SD** |
| CFX96 Deep Well Dx | FAM | SARS-CoV-2 N1 | 0.300 | 0.240–0.398 | 0.302 | 0.251–0.436 | 0.312 |
| | HEX | SARS-CoV-2 N2 | 0.146 | 0.116–0.199 | 0.165 | 0.135–0.267 | 0.183 |
| CFX Opus Deepwell Dx | FAM | SARS-CoV-2 N1 | 0.053 | 0.041–0.073 | 0.061 | 0.049–0.110 | 0.129 |
| | HEX | SARS-CoV-2 N2 | 0.081 | 0.064–0.110 | 0.111 | 0.087–0.201 | 0.241 |

CI, confidence interval; SD, standard deviation.

## Conclusions

Demonstrations of equivalent performance are critical to specific user environments. This series of comparability studies was conducted to show the analytical equivalence of the CFX Opus Deepwell Dx System with the CFX96 Deep Well Dx System and used performance characteristics described in the USP (2017) and International Conference on Harmonisation (ICH, 2005) guidelines. This study is intended to provide a guide to laboratories who wish to transfer assays from the CFX96 Deep Well Dx System to the CFX Opus Deepwell Dx System or use these two platforms together. If differences are seen when transferring protocols to new platforms, additional system optimization may be required.

The study results demonstrate comparable performance between the new CFX Opus Deepwell Dx System and the CFX96 Deep Well Dx System. An investigation of intrasystem variation between instruments of the same platform type (CFX96 Dx, CFX96 Deep Well Dx, and CFX Opus Deepwell Dx), conducted by both Deming regression analysis and Bland-Altman LOA plots, demonstrated that all units performed equivalently. To evaluate assay performance, a multiplexed RT-qPCR assay for the detection of SARS-CoV-2 was shown to perform similarly on both the CFX96 Dx and CFX96 Deep Well Dx Systems. Comparisons of the CFX96 Deep Well Dx and CFX Opus Deepwell Dx Systems for analytical characteristics determined that the PCR efficiency, linearity, LOD, LOQ, and linear range of Cq values are all equivalent between the two platforms. The study also included repeatability and precision analyses (Table 3) to investigate any variations occurring between PCR runs performed over the three consecutive days of this comparability study, and no differences were detected. Finally, the analysis of CFX96 Deep Well Dx data in CFX Maestro Dx SE Software and CFX Manager Dx Software yielded identical results (Supplementary Figure 1), demonstrating the ability to provide equivalent results.

Users of CFX96 Deep Well Dx Systems can be confident that when performing their own comparability study, they can expect to obtain similar results on the CFX Opus Deepwell Dx System.

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## 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.

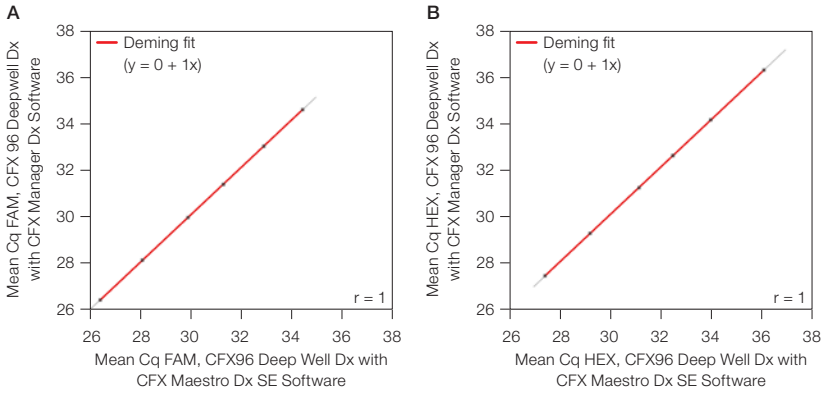
**Range** — the interval between the upper and lower concentration of analyte in a sample for which it has been demonstrated that the analytical procedure has an acceptable level of accuracy, precision, and linearity.

**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




**Suppl. Fig 1. System usability.** Comparison of CFX96 Deep Well Dx System data using CFX Manager Dx and CFX Maestro Dx SE Software. Cq, quantification cycle.

**Suppl. Table 1. Summary of RT-qPCR performance.** LOD, LOQ, linear range, linearity, and efficiency on the CFX96 Dx System.

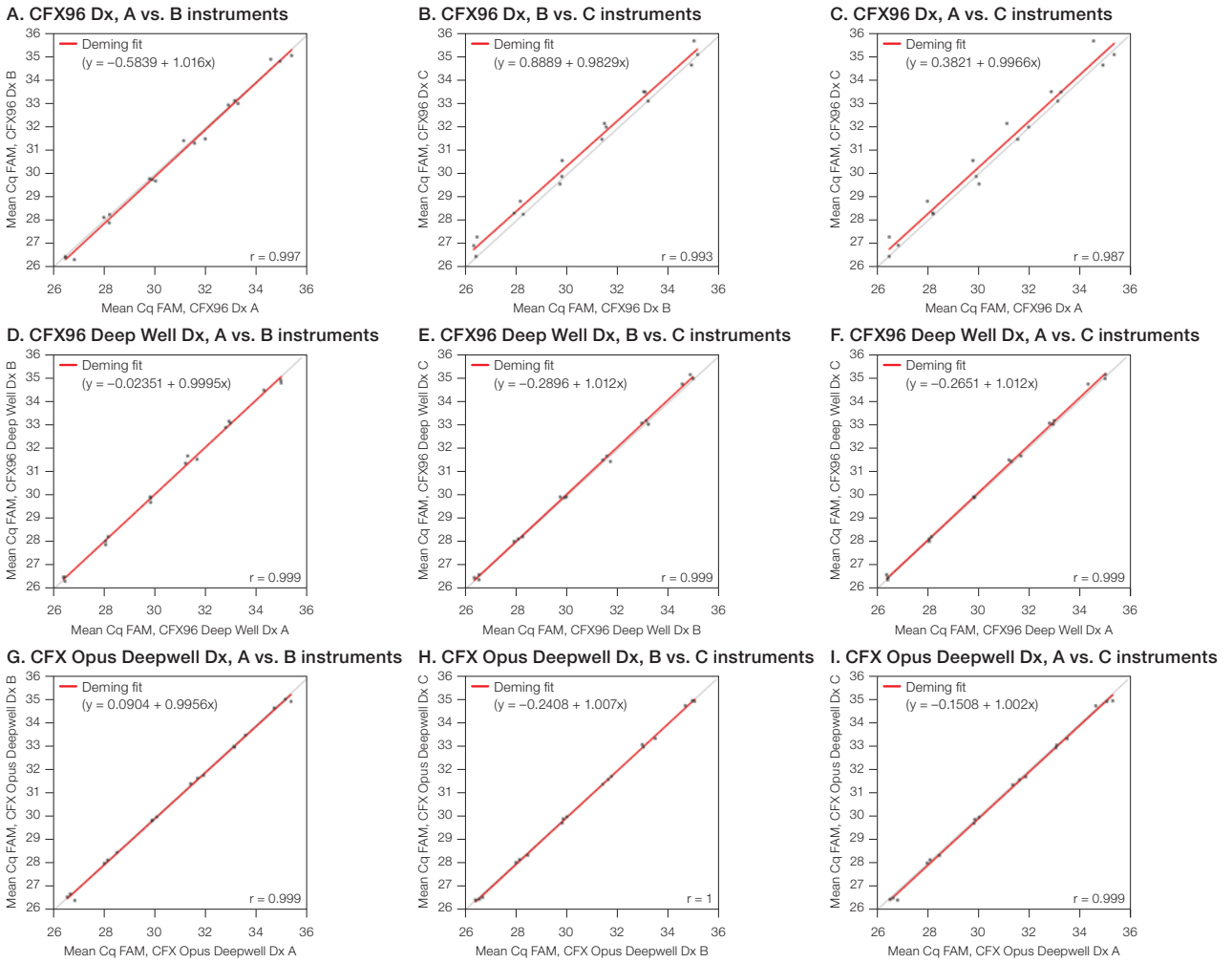
| Paramater | Analyte | Fluorophore | CFX96 Dx |
|---|---|---|---|
| LOD, copies | SARS-CoV-2 N1 | FAM | 3.81* |
| | SARS-CoV-2 N2 | HEX | 3.21 |
| LOQ, copies | SARS-CoV-2 N1 | FAM | 11.01 |
| | SARS-CoV-2 N2 | HEX | 6.11 |
| Linear range, molecules | SARS-CoV-2 N1 | FAM | 8,026–11.01 |
| | SARS-CoV-2 N2 | HEX | 4,454–6.11 |
| Linear range, Cq | SARS-CoV-2 N1 | FAM | 26.67–35.09 |
| | SARS-CoV-2 N2 | HEX | 27.86–36.48 |
| Linearity, $R^2$ | SARS-CoV-2 N1 | FAM | 0.995 |
| | SARS-CoV-2 N2 | HEX | 0.995 |
| Efficiency, % | SARS-CoV-2 N1 | FAM | 93.4 |
| | SARS-CoV-2 N2 | HEX | 90.47 |

* The LOD for FAM channel was detected between 3.81 and 9.02 copies/µl, within the acceptable threefold limit.
Cq, quantification cycle; LOD, limit of detection; LOQ, limit of quantification.

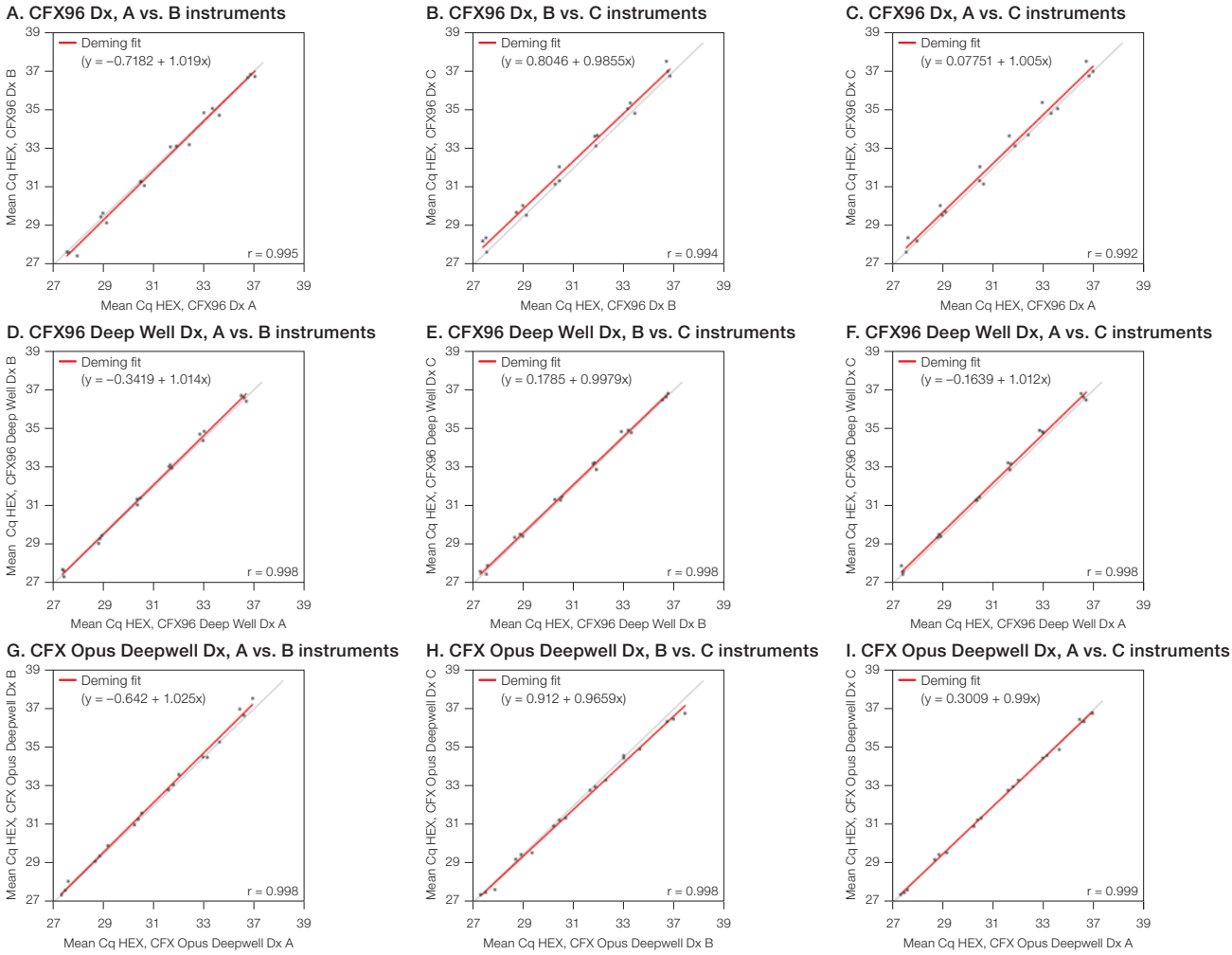
**Suppl. Table 2. Precision of CFX96 Dx Systems calculated from one input.**

| | | | Inter-Run | | Interday | | Intermediate |
|---|---|---|---|---|---|---|---|
| Platform | Fluorophore | Analyte | SD | 95% CI | SD | 95% CI | SD |
| CFX96 Dx | FAM | SARS-CoV-2 N1 | 0.317 | 0.251 to 0.431 | 0.405 | 0.324 to 0.715 | 0.405 |
| | HEX | SARS-CoV-2 N2 | 0.308 | 0.244 to 0.419 | 0.381 | 0.307 to 0.659 | 0.381 |

CI, confidence interval; SD, standard deviation.




**Suppl. Fig 2. Deming regression analysis of FAM channel measurements for the CFX96 Dx, CFX96 Deep Well Dx, and CFX Opus Deepwell Dx Systems.** Three replicates for each system in three independent runs over three days on three systems of each platform were analyzed. Results were plotted using mean Cq for the FAM channel and pairwise comparisons were made within each platform. Cq, quantification cycle.




**Suppl. Fig 3. Deming regression analysis of HEX channel measurements for the CFX96 Dx, CFX96 Deep Well Dx, and CFX Opus Deepwell Dx Systems.** Three replicates for each system in three independent runs over three days on three systems of each platform were analyzed. Results were plotted using mean Cq for the HEX channel and pairwise comparisons were made within each platform. Cq, quantification cycle.

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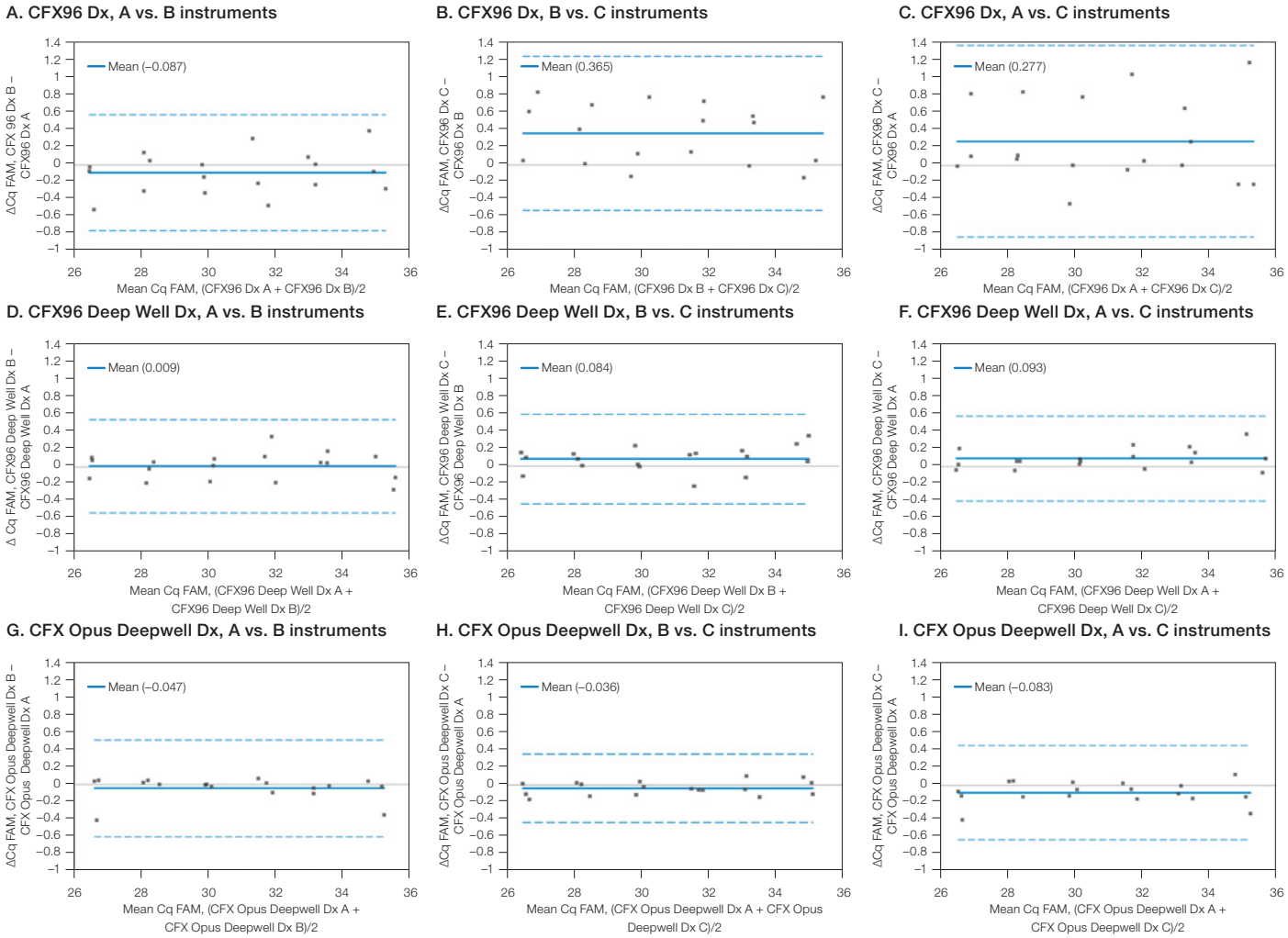
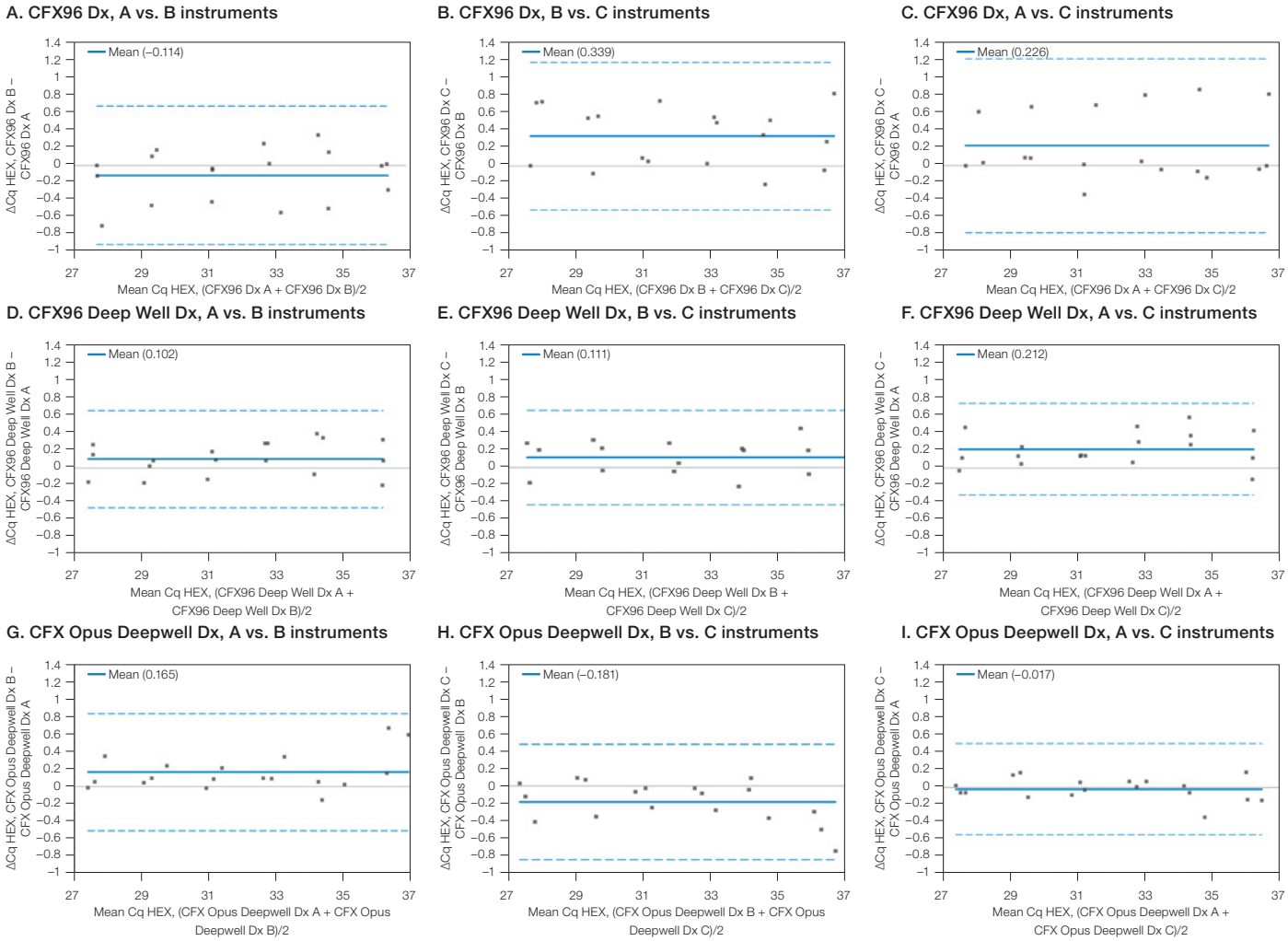



**Suppl. Fig 4. The Bland-Altman test demonstrates equivalence between systems of the same platform in limits of agreement (LOA) plots.** Representative results are shown using pairwise comparisons between three CFX96 Dx, three CFX96 Deep Well Dx, and three CFX Opus Deepwell Dx Systems in the FAM channel.




**Suppl. Fig 5. The Bland-Altman test demonstrates equivalence between systems of the same platform in limits of agreement (LOA) plots.** Representative results are shown using pairwise comparisons between three CFX96 Dx, three CFX96 Deep Well Dx, and three CFX Opus Deepwell Dx Systems in the HEX channel.
Cq, quantification cycle.

Visit **bio-rad.com/CFXOpusDx** for more information.

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