A Comparability Study of the C1000 Touch 96–Deep Well and PTC Tempo Deepwell Thermal Cyclers for Use in Droplet Digital™ PCR Workflow

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Abstract

This study demonstrates the thermal performance between the PTC Tempo Deepwell Thermal Cycler and the C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module (C1000 Touch 96–Deep Well Thermal Cycler) for amplification of input DNA prior to Droplet Dgital PCR (ddPCR™) applications on the QX200™ and QX600™ Droplet Digital PCR Systems. The Bio-Rad QX600 ddPCR System delivers best-in-class performance with the addition of six-color multiplexing. A previous study determined thermal efficiency across a gradient, ramp rate, accuracy, and gradient performance, and demonstrated comparable thermal performance of the PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers (bulletin 3483). In this evaluation, the downstream amplification products provided by the PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers were measured for their use in a ddPCR workflow performed on the QX200 and QX600 ddPCR Systems, to determine the resulting accuracy and precision of the two formats. This comparability study demonstrates that the accuracy and precision of measurement of the DNA amplification products generated are comparable between the PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers. The PTC Tempo Deepwell Thermal Cycler is validated to support the Bio-Rad ddPCR workflow, specifically the QX200 and QX600 Droplet Digital PCR Systems.

Introduction

For this evaluation of compatibility with Droplet Digital PCR, the PTC Tempo Deepwell Thermal Cycler is used to amplify the DNA target to endpoint within each droplet partition. After droplet generation and PCR, each partition is read using a droplet reader (either with the QX200 or QX600 Droplet Reader) to determine the fraction of positive partitions that includes the target for calculation of target DNA concentration.

To ensure robust amplification, PTC Tempo Thermal Cyclers were designed to include precise temperature control in a flexible thermal gradient configuration. For DNA amplification in Droplet Digital PCR, the PTC Tempo Deepwell Thermal Cycler is preferred as it accommodates larger volumes and provides a greater amplification yield. Specifically, the deepwell reaction module offers a block height that ensures homogenous heating of the droplet emulsion during Droplet Digital PCR.

The PTC Tempo Deepwell Thermal Cycler builds on the performance of its predecessor, the C1000 Touch 96–Deep Well Thermal Cycler, and now includes user-friendly features such as an automatic lid capable of autosensing plates and tubes, an LED light display, audible status notifications, and connectivity options that include the Bio-Rad BR.io cloud platform, WiFi, USB, and Ethernet connections. Moreover, the PTC Tempo Deepwell Thermal Cycler maintains the same user access controls for user management with the addition of enhanced security and reporting features.

This study evaluates the consistency in DNA concentration measurements of samples obtained on the QX200 and QX600 ddPCR Systems using the PTC Tempo Deepwell Thermal Cycler and the C1000 Touch 96–Deep Well Thermal Cycler. The study shows its ability to provide high accuracy and precision of a DNA target in ddPCR applications.

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Experimental Design

As shown in Figure 1, the experimental design was intended to demonstrate the comparability of amplified input DNA source material for detection by Droplet Digital PCR on the QX200 and the QX600 ddPCR Systems. The PTC Tempo Deepwell Thermal Cycler was compared to the C1000 Touch 96–Deep Well Thermal Cycler.

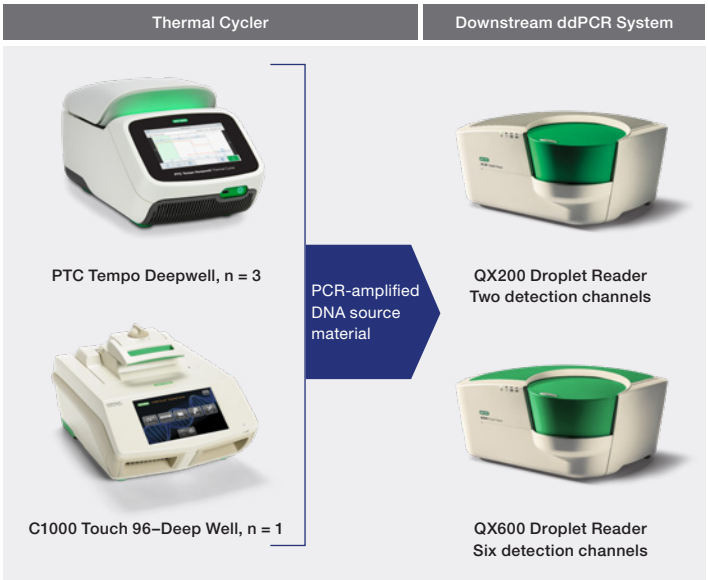

Fig. 1. Comparability study experimental workflow for DNA amplification followed by Droplet Digital PCR performed on QX600 and QX200 Droplet Readers.

Materials and Methods

The ddPCR Six-Color System Check Kit* (Bio-Rad Laboratories, Inc., catalog #12016025) and QX200 Instrument Qualification Kit** (Bio-Rad, in development) assays were used in this study. Each kit contains proprietary DNA templates that are manufactured and quantitated by the National Measurement Institute of Australia (NMIA). Droplet generation was performed on the Automated Droplet Generator (Bio-Rad, #1864101) using DG32 Automated Droplet Generator Cartridges (Bio-Rad, #1864108), and Automated Droplet Generation Oil for Probes (Bio-Rad, #1864110). Detection was performed on the QX200 Droplet Reader (Bio-Rad, #1864003) and QX600 Droplet Reader (Bio-Rad, #12013328) using ddPCR Droplet Reader Oil (Bio-Rad, #1863004) with input DNA obtained from a total of four thermocyclers, three PTC Tempo Deepwell Thermal Cyclers (Bio-Rad, #12015392) and one C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module (Bio-Rad, #1851197), respectively. The thermal cycler conditions used for providing amplified DNA source material are listed in Table 2.

* The ddPCR Six-Color System Check Kit is a Bio-Rad service kit to test system performance to manufacturing specifications and is not available to order.

** The QX200 Instrument Qualification Kit is in development and will be a Bio-Rad service kit to test system performance to manufacturing specifications and is not available to order.

Table 1. Droplet Digital PCR assay metrics evaluated to compare amplified input DNA input from the PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers.

Droplet Reader	Channels	Metrics Evaluated
QX600 Droplet Reader	FAM, HEX, Cy5, Cy5.5, ROX, ATTO 590	▪ Mean concentration ▪ Accuracy, compared to target concentration ▪ Precision, % CV values for mean concentration in 6 detection channels ▪ Difference from control, PTC Tempo Deepwell vs. C1000 Touch 96–Deep Well Thermal Cycler ▪ Average droplet count
QX200 Droplet Reader	FAM, HEX	▪ Mean concentration ▪ Accuracy, compared to target concentration ▪ Precision, % CV values for mean concentration in 2 detection channels ▪ Difference from control, PTC Tempo Deepwell vs. C1000 Touch 96–Deep Well Thermal Cycler ▪ Average droplet count

CV, coefficient of variation.

Table 2. Thermal cycling protocol performed on the C1000 Touch 96–Deep Well and PTC Tempo Deepwell Thermal Cyclers.

Step	Temperature	Time	Ramp	Number of cycles
1	95ºC	10 min	2ºC/sec	1
2	94ºC	30 sec	2ºC/sec	40
3	58ºC	1 min	2ºC/sec	40
4	98ºC	10 min	2ºC/sec	1
5	4ºC	hold	2ºC/sec	1

Hold at 4ºC for at least 30 minutes prior to loading on QX200 or QX600 Droplet Reader.

QX600 Assay Procedure

The ddPCR Six-Color System Check Kit assay procedure uses a proprietary validated reference DNA standard from the NMIA. This NMIA reference DNA standard at 1,060 copies/µl was amplified on both the PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers, and the resulting amplified material was detected on the QX600 Droplet Reader to determine accuracy, precision, and absolute concentration.

QX200 Assay Procedure

For the QX200 ddPCR System, quantitation of the proprietary NMIA validated reference DNA standard at 1,060 copies/µl was amplified using either a PTC Tempo Deepwell or C1000 Touch 96–Deep Well Thermal Cycler and then detected on the QX200 Droplet Reader to determine the accuracy, precision, and absolute concentration.

Results

QX600 Droplet Reader Quantitation of NMIA Reference DNA Standard: Accuracy

The ddPCR Six-Color System Check assay was performed to determine accuracy and precision using the NMIA DNA reference standard on the C1000 Touch 96–Deep Well Thermal Cycler and the PTC Tempo Deepwell Thermal Cyclers (Table 3). Results exceeded all system specifications for minimum droplet count, variation from the NMIA standard reference concentration of 1,060 copies/µl, and concentration precision in each detection channel.

Accurate quantification was provided by DNA reference standard material and the ddPCR workflow with either thermal cycler platform, with no deviations greater than ±1% from the NMIA standard concentration (Table 3).

Table 3. Comparable accuracy of reference DNA standard in the ddPCR workflow with PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers for the QX600 Droplet Reader. Both deep well thermal cyclers provided similar performance. The concentration calls for the NMIA standard were accurate on the QX600 Droplet Reader, with no deviations greater than ±1% from the NMIA standard concentration.

		PTC Tempo Deepwell		
Metric	C1000 Touch 96–Deep Well	Unit 1	Unit 2	Unit 3
Accuracy, detected copies/µl,* NMIA target 1,060 copies/µl	1,056	1,053	1,050	1,052
Difference from control**	—	–0.2%	–0.5%	–0.4%

NMIA, National Measurement Institute of Australia.

* Detected copies/µl is calculated as the average concentration across a plate, detected by each channel (FAM, HEX, Cy5, Cy5.5, ROX, ATTO 590).

** C1000 Touch 96–Deep Well Thermal Cycler.

QX600 Droplet Reader Quantitation of NMIA Reference DNA Standard: Precision and Droplet Count

As shown in Table 4, the PTC Tempo Deepwell and the C1000 Touch 96–Deep Well Thermal Cycler formats provided very similar results for ddPCR precision, with <2.5% CV values for the mean concentration across a plate in all six detection channels of the QX600 Droplet Reader. Droplet counts are also shown to be similar using either the C1000 Touch 96–Deep Well or PTC Tempo Thermal Cycler in the ddPCR workflow.

Table 4. Precision of reference DNA standard quantitation in the ddPCR workflow with PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers using the QX600 Droplet Reader. Concentrations were measured across all six detection channels (FAM, HEX, Cy5, Cy5.5, ROX, ATTO 590) with % CVs of <2.5%, and droplet counts were comparable for both deep well thermal cycler formats across an entire plate.

			PTC Tempo Deepwell		
Metric	Detection Channel	C1000 Touch 96–Deep Well	Unit 1	Unit 2	Unit 3
Precision, % CV, mean concentration	FAM	2.0%	1.7%	2.3%	1.9%
	HEX	2.2%	1.7%	2.4%	2.1%
	Cy5	2.1%	1.6%	2.3%	2.1%
	Cy5.5	2.1%	1.7%	2.4%	1.9%
	ROX	2.1%	1.7%	2.3%	1.9%
	ATTO 590	2.2%	1.9%	2.4%	1.9%
Droplet count, average across a plate		21,426	21,280	20,332	20,452

CV, coefficient of variation.

QX600 Droplet Reader Quantitation of NMIA Reference DNA Standard: Mean Concentration Plot

As shown in Figure 2, performance of the PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers are comparable, based on the consistent concentrations obtained following detection of NMIA reference DNA standard on all six QX600 channels.

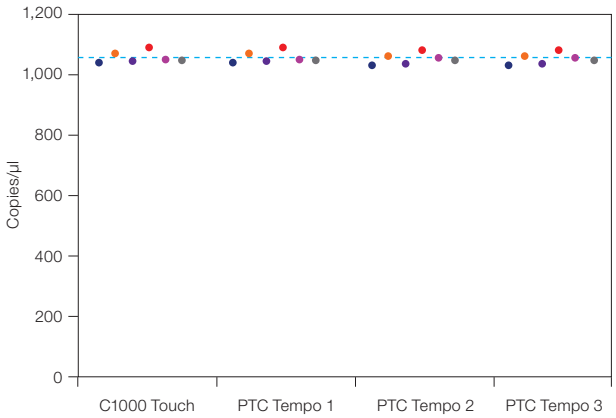

Fig. 2. Comparable performance of PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers for mean concentration of reference DNA standard across a plate. The ddPCR workflow with PTC Tempo Deepwell (three units) and C1000 Touch 96–Deep Well (one unit) Thermal Cyclers provided consistent concentration calls across all six detection channels of the QX600 Droplet Reader. NMIA standard target concentration of 1,060 copies/µl (- - -), measured average concentration across a plate FAM (●); HEX (●); Cy5 (●); Cy5.5 (●); ROX (●); ATTO 590 (●). NMIA, National Measurement Institute of Australia.

QX200 Droplet Reader Quantitation of NMIA Reference DNA Standard: Accuracy and Droplet Count

The NMIA reference DNA standard at 1,060 copies/µl was amplified by PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers and then detected on the QX200 Droplet Reader to determine absolute concentration.

Results shown in Table 5 confirm that both thermal cycler formats provided very similar uniform performance, based on the <1% variation in concentration following Droplet Digital PCR on the QX200 Droplet Reader.

Table 5. Comparable accuracy of amplified reference DNA standard from PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers for the QX200 Droplet Reader. Detection using the QX200 Droplet Reader is consistent in the ddPCR workflow using either PTC Tempo Deepwell (three units) or C1000 Touch 96–Deep Well (one unit) Thermal Cyclers in both FAM and HEX detection channels and shows <1% variation in concentration.

		PTC Tempo Deepwell		
Metric	C1000 Touch 96–Deep Well	Unit 1	Unit 2	Unit 3
Accuracy, detected copies/µl,* NMIA target 1,060 copies/µl	1,024	1,030	1,029	1,029
Difference from control**	—	+0.6%	+0.5%	+0.5%

NMIA, National Measurement Institute of Australia.

* Detected copies/µl was calculated as average of concentration across a plate detected by each channel (FAM, HEX).

** C1000 Touch 96–Deep Well Thermal Cycler.

QX200 Droplet Reader Quantitation of NMIA Reference DNA Standard: Precision and Droplet Count

As shown in Table 6, both ddPCR workflows using PTC Tempo Deepwell and C1000 96–Touch Deep Well Thermal Cycler formats provided very similar results for ddPCR precision with <2.4% CV values for the mean concentration across a plate detected in the FAM and HEX detection channels of the QX200 Droplet Reader. Droplet counts are also similar when using either C1000 Touch 96–Deep Well Thermal Cycler or PTC Tempo Deepwell Thermal Cycler for the ddPCR workflows.

Table 6. Precision of reference DNA standard DNA quantitation in the ddPCR workflow with PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers are similar using the QX200 Droplet Reader. <2.4% CV in concentration was measured for two detection channels (FAM, HEX), and droplet counts are comparable for both deep well thermal cycler formats. CV, coefficient of variation.

			PTC Tempo Deepwell		
Metric	Detection Channel	C1000 Touch 96–Deep Well	Unit 1	Unit 2	Unit 3
Precision, % CV, mean concentration	FAM	2.3%	1.8%	1.8%	1.9%
	HEX	2.1%	1.4%	1.6%	1.7%
Droplet count, average across a plate		18,822	19,407	19,359	19,739

QX200 Quantitation of NMIA Reference DNA Standard: Mean Concentration Plot

As shown in Figure 3, PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cycler performances are comparable, based on the consistent concentrations obtained following the detection of amplified input DNA on all QX200 Droplet Reader channels.

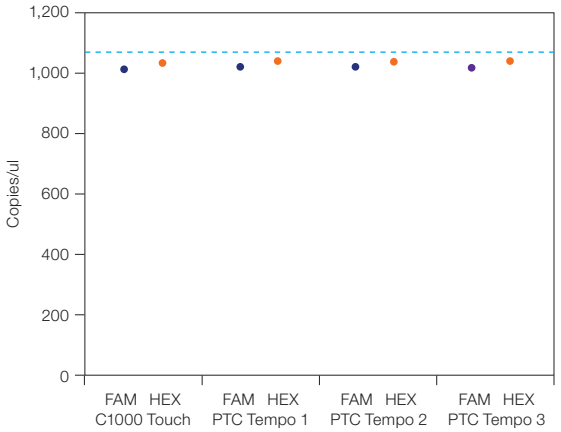

Fig. 3. PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cycler performances are comparable for mean concentration of reference DNA standard across a plate. The ddPCR workflow with the PTC Tempo Deepwell and C1000 Touch 96–Deep Well Thermal Cyclers provided very uniform concentration calls across all QX200 Droplet Reader detection channels, with similar concentrations. NMIA standard target concentration of 1,060 copies/µl (- - -), measured average concentration across a plate FAM (●); HEX (●). NMIA, National Measurement Institute of Australia.

Conclusions

The Bio-Rad QX200 and QX600 Droplet Digital PCR Systems provide precise and absolute quantification of nucleic acids, and high quality thermal cycling is an important part of the ddPCR worfklow.

This comparability study demonstrates that accurate and precise nucleic acid measurements can be achieved with Droplet Digital PCR using either the PTC Tempo Deepwell or the C1000 Touch 96–Deep Well Thermal Cycler and supports the adoption of the PTC Tempo Deepwell Thermal Cycler by QX200 and QX600 Droplet Digital PCR System users.

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