The impact of template addition volume on sensitivity in digital PCR

Miriam Hesse, Özlem Karalay, Ronny Kellner, Domenica Martorana, Colin Donohoe, Andreas Hecker, Daniel Heinz Löfgren, Francesca Di Pasquale, Andreas Missel

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Template addition volume and template analyzed volume

Digital PCR (dPCR) has become the method of choice for the detection of targets with minute copy numbers, using templates with ultra-low concentrations. There are several dPCR systems on the market, which differ in aspects such as type and number of partitions, the percentage of reaction that is analyzed and the template volume that can be added.

In this study, we compared three dPCR platforms and examined the impact of template addition volume and analyzed volume on sensitivity. We provide data that support the conclusion that the most important factors in determining the relative sensitivity of each system are template addition volume and template analyzed volume.

Reaction volumes for three commercially available dPCR technologies

	Nanoplate 26K	MAP 16 dPCR Plate	QX200 ddPCR
Reaction volume	40 µL	9 µL	22 µL
Maximum template addition volume (assuming 4x master mix and highly concentrated assays)	ca. 27 µL	ca. 6 µL	ca. 14 µL
Reaction volume analyzed = reaction volume – non-analyzed volume	21.6 µL	8.2 µL	11.5–12.6 µL*

*Based on mean droplet count of 16,000 and measured droplet volume of 0.718 to 0.786 nl.

Instruments and materials

Instruments

Instrument	Manufacturer
QIAcuity® Four dPCR System	QIAGEN GmbH
Bio-Rad® QX200™ with AutoDG	Bio-Rad
QuantStudio® Absolute Q™	Thermo Fisher Scientific

Templates

Templates	Manufacturer
Human genomic DNA from a male donor	Prepared in-house
QuantiNova® Internal Control DNA (synthetic dsDNA template)	QIAGEN

Assays

Target	Dye	Source
QN IC	HEX™	Custom
ERBB2	FAM™	Custom

Materials

Material	Manufacturer
Molecular grade water	Various
1x TE Buffer	Various
QIAcuity Probe PCR Kit	QIAGEN
Custom Primers/Probe mix for QIAcuity (0.8 µM primer and 0.4 µM probe)	Custom
Nanoplate 26k 24-well	QIAGEN
Bio-Rad ddPCR Supermix for Probes	Bio-Rad
Custom Primers/Probe mix for Bio-Rad and Thermo Fisher (0.9 µM primer and 0.25 µM probe)	Custom
Bio-Rad ddPCR consumables (various)	Bio-Rad
QuantStudio Absolute Q MAP16 Plate Kit	Thermo Fisher Scientific
Absolute Q DNA Digital PCR Master Mix	Thermo Fisher Scientific

dPCR reaction setup on three different platforms with maximal template input volume

We generated template solutions that represent the lower bounds of (d)dPCR technologies consisting of 10–40 total copies per 50 µL. We then generated dPCR/ddPCR reactions to assess three template addition volumes, 6 µL per 9 µL reaction, 27 µL per 40 µL and 10 µL per 22 µL reaction, each of them representing the upper volume limit of the platform.

Each resulting solution was tested in replicate of 12 (8; Absolute Q) on the respective system. Average concentration and CV were calculated. Additionally, the number of samples where no positive partitions were observed were counted and the percentage of negative samples was measured for each set of replicates at each concentration.

dPCR reaction setup

Starting template concentration		Template addition volume	dPCR reaction volume	dPCR System	Template analyzed volume	Expected concentration of dPCR reaction	Expected copies per dPCR analyzed
Total copies in 50 µL	Copies/µL	µL	µL	Name	µL	Copies/µL	Copies
40	0.8	6	9	Absolute Q	5.46	0.53	4.37
20	0.4	6	9	Absolute Q	5.46	0.27	2.18
10	0.2	6	9	Absolute Q	5.46	0.13	1.09
0	0	6	9	Absolute Q	5.46	0.00	0.00
40	0.8	27	40	QIAcuity	14.58	0.54	11.66
20	0.4	27	40	QIAcuity	14.58	0.27	5.83
10	0.2	27	40	QIAcuity	14.58	0.14	2.92
0	0	27	40	QIAcuity	14.58	0.00	0.00
40	0.8	10	22	QX200	5.2 to 5.7	0.36	4.14 to 4.53
20	0.4	10	22	QX200	5.2 to 5.7	0.18	2.07 to 2.26
10	0.2	10	22	QX200	5.2 to 5.7	0.09	1.03 to 1.13
0	0	10	22	QX200	5.2 to 5.7	0.00	0.0

Increased template addition volume provides higher sensitivity of detection

Results obtained from maximum template addition volume across various template concentrations on three dPCR platforms.

MAP16: 6 µL template volume input

		Measured copies/µl	CV	Negative samples, #	Negative samples, %
FAM	40 copies/50 µL	0.439	40%	0	0%
	20 copies/50 µL	0.341	60%	0	0%
	10 copies/50 µL	0.055	107%	4	50%
	NTC	0.030	–	–	–
HEX	40 copies/50 µL	0.496	39%	0	0%
	20 copies/50 µL	0.183	98%	3	38%
	10 copies/50 µL	0.069	83%	3	38%
	NTC	0.028	–	–	–

ddPCR: 10 µL template volume input

		Measured copies/µl	CV	Negative samples, #	Negative samples, %
FAM	40 copies/50 µL	0.272	46%	0	0%
	20 copies/50 µL	0.183	54%	1	8%
	10 copies/50 µL	0.095	83%	3	25%
	NTC	0.03	–	–	–
HEX	40 copies/50 µL	0.28	58%	0	0%
	20 copies/50 µL	0.15	52%	0	0%
	10 copies/50 µL	0.08	73%	3	25%
	NTC	0.00	–	–	–

QIAcuity: 27 µL template volume input

		Measured copies/µl	CV	Negative samples, #	Negative samples, %
FAM	40 copies/50 µL	0.615	26%	0	0%
	20 copies/50 µL	0.336	40%	0	0%
	10 copies/50 µL	0.112	56%	2	17%
	NTC	0.00	–	–	–
HEX	40 copies/50 µL	0.51	41%	0	0%
	20 copies/50 µL	0.27	44%	0	0%
	10 copies/50 µL	0.13	45%	0	0%
	NTC	0.00	–	–	–

Summary of results

- High template addition volumes (27 µL) result in accurate detection of copy numbers (with little-to-no variation between obtained and expected results) across samples with different initial template concentrations
- Low template addition volumes (6 µL and 10 µL), result in high deviation between the expected concentrations (in copies/µL) and the measured concentrations.
- At lower template addition volumes, we observed an increased uncertainty in copy number estimation, as reflected in higher coefficients of variation (%CV) values across replicates.
- The percentage of dPCR replicates with no amplification, referred to as negative samples (samples that contain no templates, thus show no positive signals) is significantly higher in samples with low template addition volumes.

Conclusions

- Digital PCR is a superior method to qPCR for the detection and absolute quantification of low concentration target templates.
- There are multiple digital PCR systems on the market that differ in numerous aspects including the amount of dead volume, i.e., the volume that is loaded but not analyzed by the instrument.
- The most important factors in determining the relative sensitivity of each system are template addition volume and template analyzed volume.
- Higher template addition volumes can overcome any limitations that dead volume may have on the sensitivity of a dPCR application.

Sample to Insight