Nanoplate Digital PCR for Wastewater Surveillance

DHS/NIST Workshop: Standards to Support an Enduring Capability in Wastewater Surveillance for Public Health

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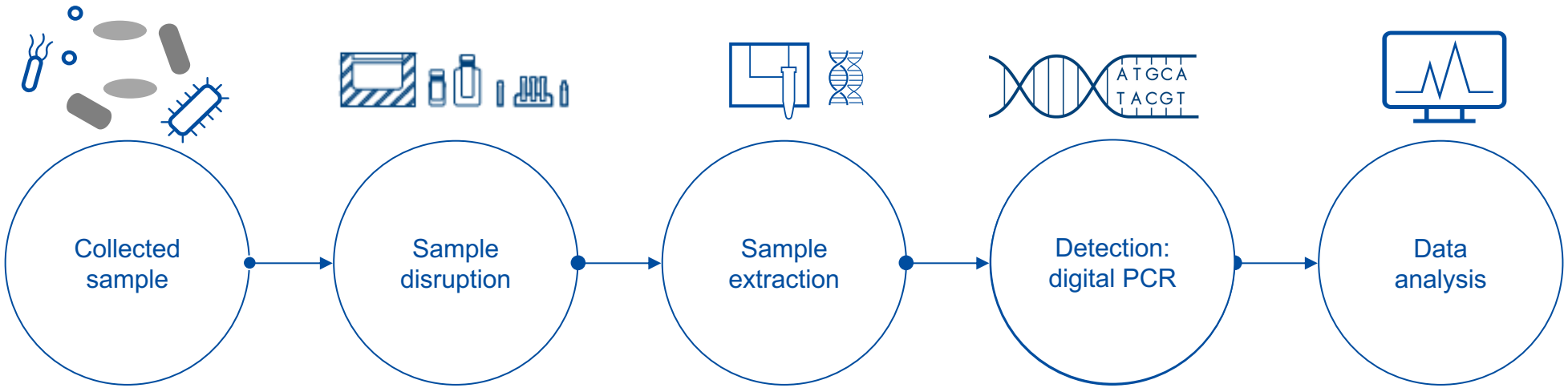
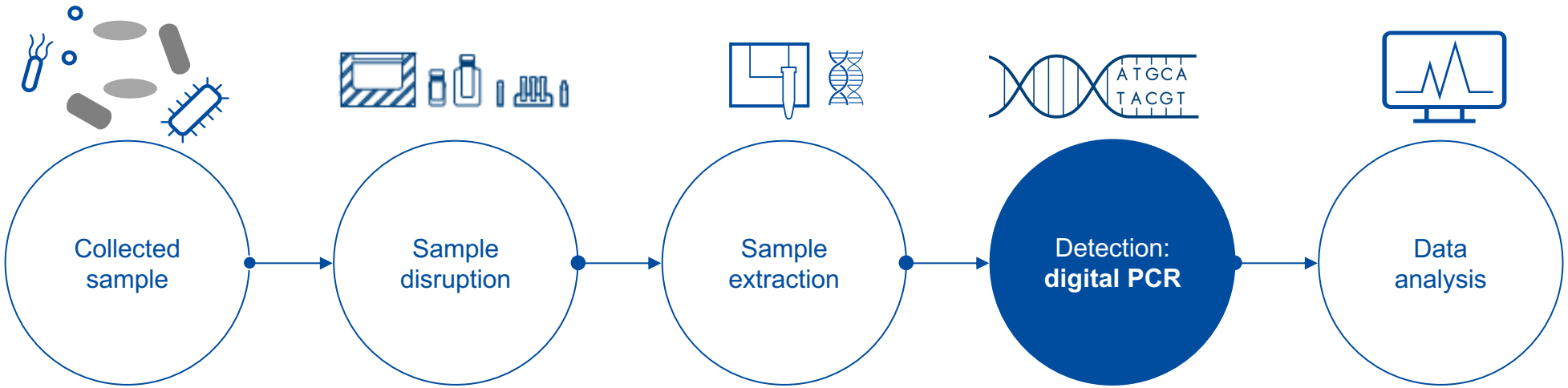
Sources of variability in wastewater testing



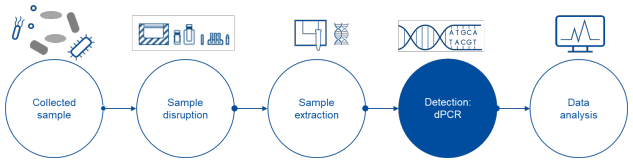
Sources of variability in wastewater testing



Variability in detection

- Process complexity
- Staff skill set
- Partition volume and count

Detection and absolute quantitation of pathogens in wastewater



Why digital PCR for wastewater testing?

- A standard curve is not required
- Better inter-laboratory comparability
- Higher precision due to absolute quantification
- Higher robustness for viral detection from complex samples

Why Nanoplate digital PCR?

- Fully integrated workflow with time-to-result ~2 h
- Easy operating and workflow like qPCR
- Flexible throughput and resolution options
- High multiplexing capability (up to 5plex)

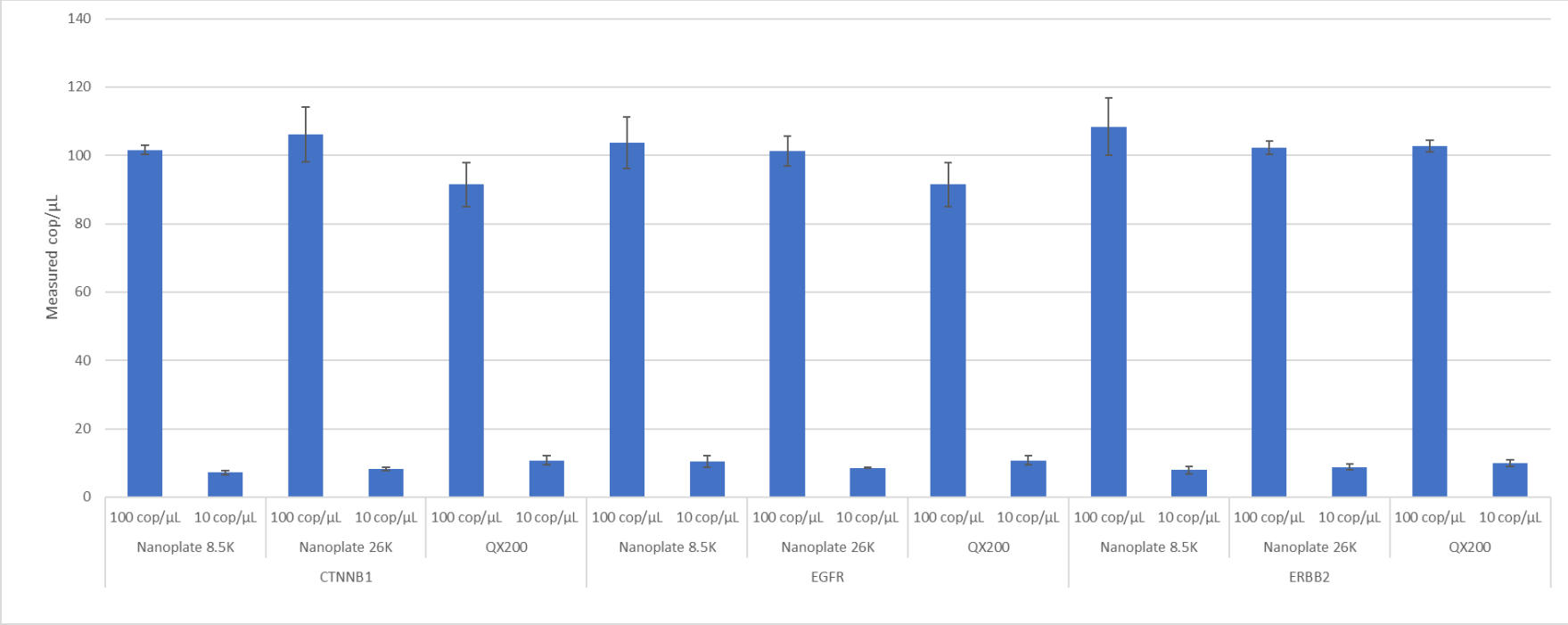
Less expertise and experience needed to run digital PCR experiments

Accuracy without complexity

Get accurate dPCR results with an easy-to-use and fast system

NIST Reference Material 2372a (human DNA quantification standard)

- Probe based detection (genomic targets are: CTNNB1, EGFR, ERBB2)
- NIST Reference Material was used to generate 10 and 100 copies/uL templates



Scalable and comparable

Reliable and reproducible results over Different Systems

- Three runs per instrument and plate types
- Input (expected concentration): 500 copies/µl
- Assay: QIAGEN dPCR Demo Assay (FAM)

Instrument type /Nanoplate 26K 24-well	Mean cp/µl	SD	CV%
QIAcuity One	480	16	3.3%
QIAcuity Four	484	15	3.1%
QIAcuity Eight	480	16	3.4%
Mean	481		
SD	3.83		
CV%	0.80%		

Instrument type /Nanoplate 8.5K 96-well	Mean cp/µl	SD	CV%
QIAcuity One	523	23	4.3%
QIAcuity Four	530	27	4.9%
QIAcuity Eight	521	27	5.1%
Mean	523		
SD	8.86		
CV%	1.69%		

Scalable and comparable

Reliable and reproducible results over Different Systems

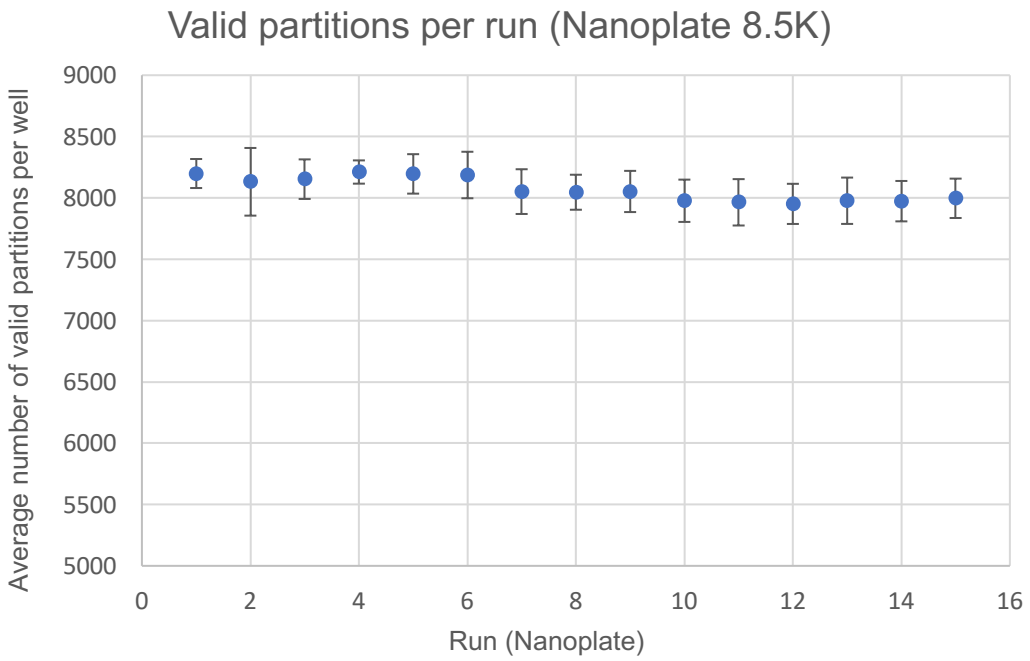
- Three runs per instrument and plate types
- Input (expected concentration): 500 copies/µl
- Assay: QIAGEN dPCR Demo Assay (FAM)

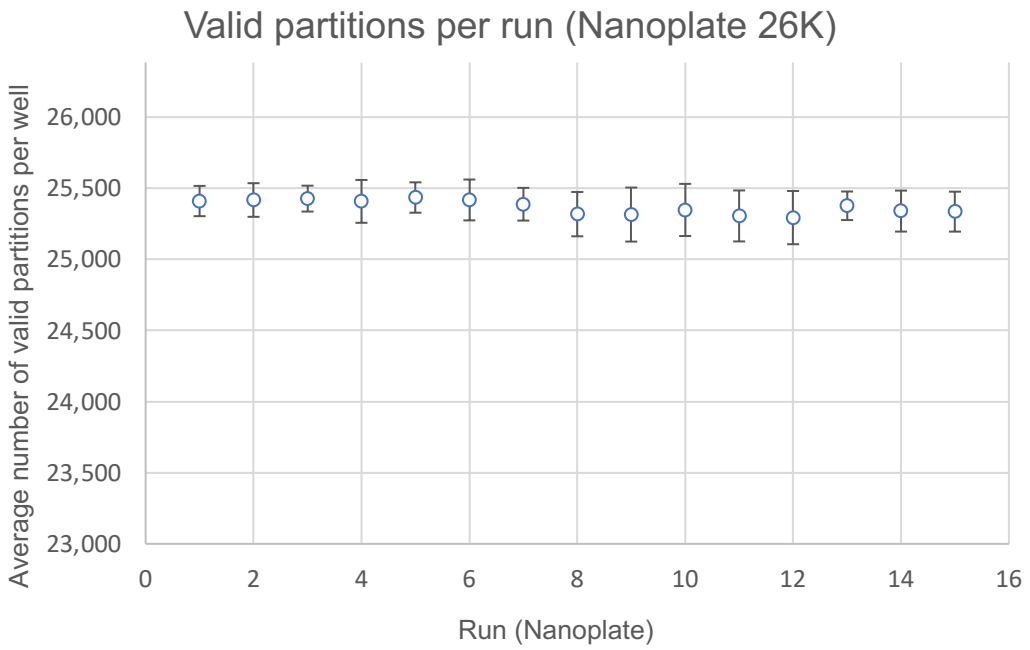
Instrument type – total	Mean cp/µl
QIAcuity One	499
QIAcuity Four	507
QIAcuity Eight	501
Mean	502
SD	22.23
CV%	4.43%

Predictable partition count

Robust quantification with high number of valid partitions

- Number of valid partitions per well for Nanoplate 26K 24-well and Nanoplate 8.5K 96-well

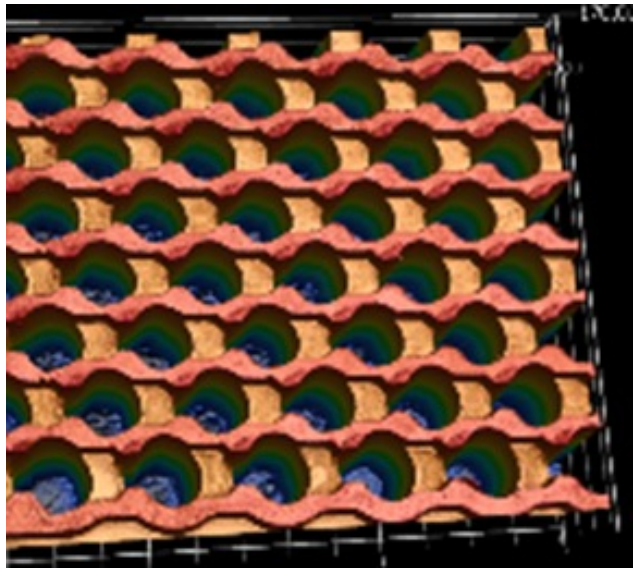


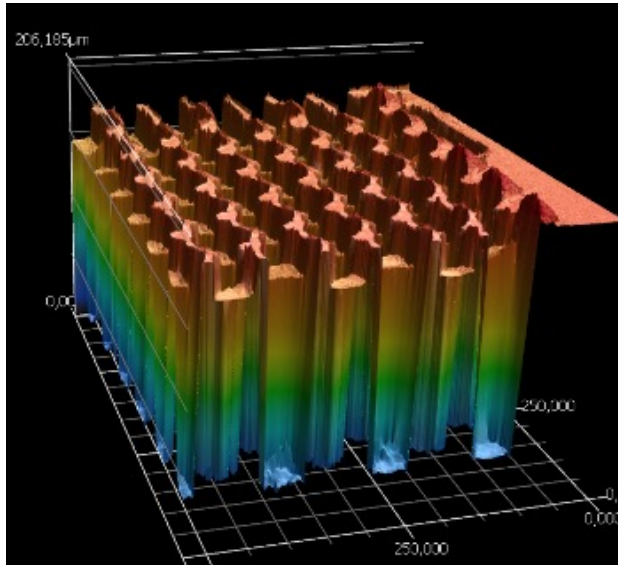


Accuracy in all measurements without being a dPCR expert

The VPF (Volume Precision Factor)

- Nanoplates and the VPF enable control of the partition volume





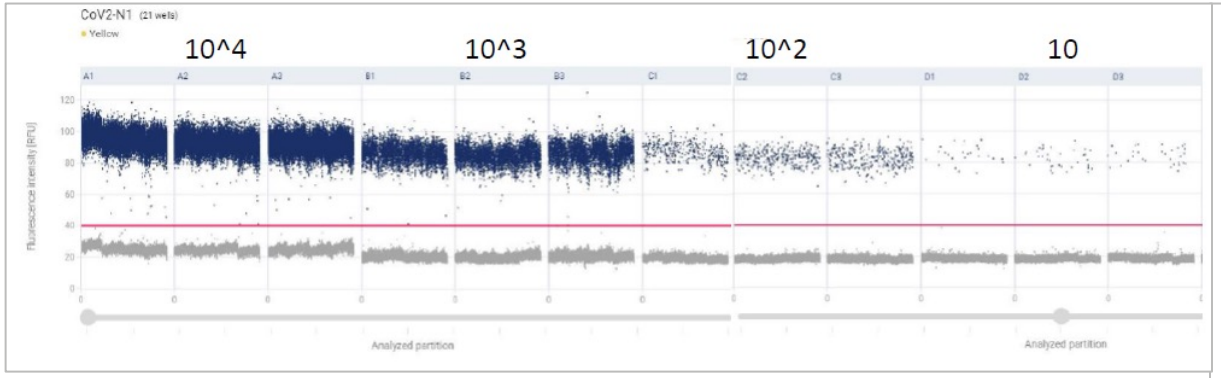
- Ensure precision of the quantitation
- Nanoplate batch & reaction/well specific calibration of the partition volume
- Automatically applied

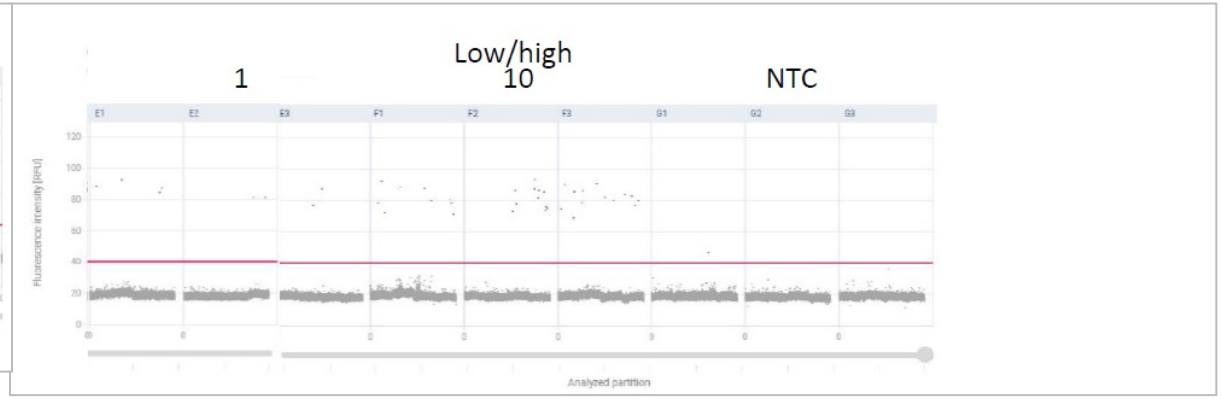
Example of results: SARS CoV-2 quantification

Sample: 10-fold serial dilution of positive control ATCC

- Concentration range between 10^4 to 1 copy/µl
- Test all samples in triplicates, 4 µl template for both
- Panel 1_3-plex SARS CoV2 N1/N2/E (HEX/Texas Red/FAM)
- “Low/high 10” → 10 copies of SARS-CoV-2 target in the presence of 10^5 copies each of the fecal indicator targets MS2, HF183 and crAssphage

N1 (HEX)





Reference:

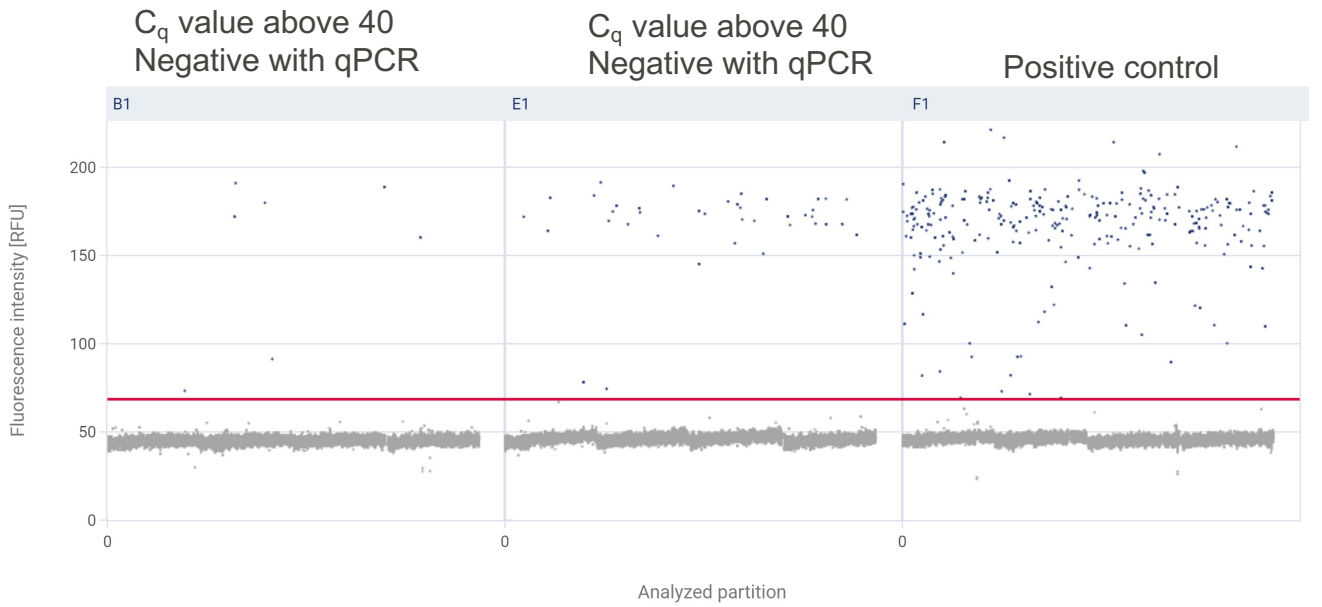
Taniuchi, Mami; Associate Professor, Medicine: Infectious Diseases and International Health; University of Virginia (Field tester of the QIAcuity One-Step Viral RT-PCR Kit, cus)

Example of results: Norovirus G1 quantification

Experimental approach

- Samples – water from swimming pools
- Sample prep – QIAamp Viral RNA Mini kit

Consistent fluorescence intensity in reference channel is a good indicator of uniform partitioning across a nanoplate



	Reaction Mix	Target	Sample/NTC/Control	Concentration copies/µL	CI (95%)	Partitions valid	positive	negative	Threshold
B1	A	Noro g1	23 E2 Janv	0.349	79%	25451	7	25444	60.18
E1	A	Noro g1	26 E1 Juin	1.9	32.6%	25467	37	25430	110.92
F1	A	Noro g1	27 E2 Juin	14.0	12%	24651	265	24386	68.85

Thank you for your attention.