Accurate and sensitive detection of microbial DNA and RNA targets using nanoplate dPCR

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Introduction

Microorganisms are highly diverse and have occupied countless ecological niches throughout evolution. One such niche is the human body, upon which colonization can have both beneficial and harmful effects. This is often associated with the presence of health-threatening virulence and resistance to antimicrobial agents.

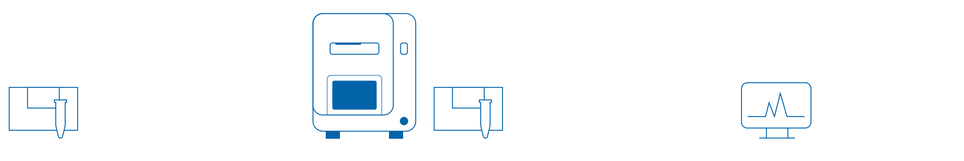
Therefore, the specific detection and quantification of microbes, virulence genes and antimicrobial resistance genes is of particular importance for human healthcare. Given this challenge, digital PCR in combination with specific detection methods is a powerful tool for accurate and sensitive quantification of human pathogenic microbes and their resistance and virulence genes.

QIAGEN's dPCR Microbial DNA Detection Assays are designed to test for the presence of microbes, resistance or virulence factor genes using digital PCR. The assays target over 685 bacterial, fungal, parasitic, viral, antibiotic resistance or virulence factor genes and can be run on the QIAcuity® in 2 hours with minimal hands-on time.

Simple workflow for the detection of microbes

Sample extraction is a critical step in the workflow where sample type-specific factors, such as inhibitor content, must be considered. QIAGEN offers a variety of solutions for microbial DNA and RNA extraction from challenging samples, such as stool or soil samples. The assay portfolio consists of 685 assays that mostly cover human disease-related microbes present in different sample types (e.g., blood, the gut, wastewater or soil).

Each assay is supplied in single tube format and contains freeze-dried primers and probes dissolved in a 20x mixture. Each assay can be ordered with any of the five dyes FAM™, HEX™, TAMRA™, ROX™ and Cy5® allowing to multiplex up to 5 targets in one reaction using a mix-and-match approach. For DNA targets, the assays are combined with the QIAcuity Probe PCR Mastermix. For RNA targets or RNA+DNA targets, the QIAcuity OneStep Advanced Probe Kit can be used. For the detection of low abundance microbial targets, the QIAcuity UCP Probe PCR Mastermix can be used. In this ultraclean production master mix, contaminating DNA background is depleted in a dedicated process.



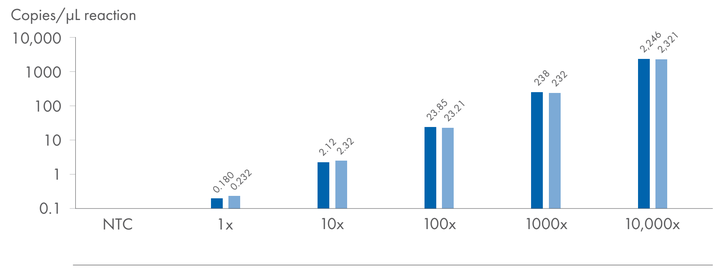
- DNeasy® PowerLyzer® Microbial Kit
- AllPrep® Bacterial/Fungal Kit
- DNeasy Plant Pro Kit
- AllPrep PowerViral® Kit
- RNeasy® PowerFecal ® Pro Kit
- And many more

- 685 dPCR Microbial DNA Detection Assays (cat. no. 250207) including 387 bacterial, 35 fungal, 46 viral, 202 virulence factor and AMR gene assays, and 15 parasite targeting assays
- QIAcuity Probe PCR Kit for DNA targets
- QIAcuity OneStep Advanced Probe Kit for RNA and DNA targets
- QIAcuity UCP Probe Kit for low abundance targets

- QIAcuity Software Suite

Accurate quantification of microbial targets across a 4-log dynamic range

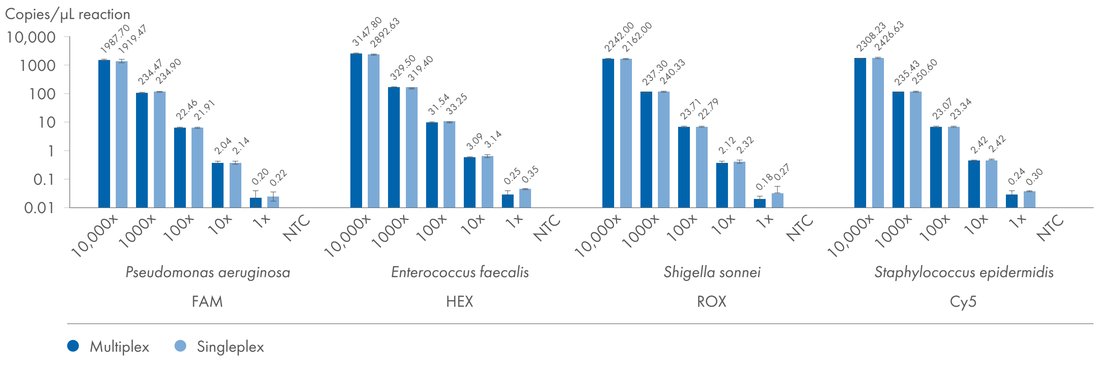
Accurate quantification is one of the core properties of digital PCR. To test the accuracy of absolute quantification of microbial targets, a gDNA reference standard from NIST, the National Institute of Standards and Technology in the USA, was quantified. This standard material is genomic DNA from the bacterium Shigella sonnei with an accurately quantified chromosome copy number per µL. The use of a single-copy gene assay from the dPCR Microbial DNA Detection Assay portfolio allowed comparison of the measured copies/µL concentration with the expected concentration. This showed strong concordance between measured and expected quantification, highlighting the high accuracy of dPCR over the entire dynamic range of 4 log levels.



Dilutions of *Shigella sonnei* gDNA from NIST were quantified using 26k 24-well Nanoplates and the QIAcuity Probe Kit on the QIAcuity Digital PCR System. Five template dilutions with 1x to 10,000x were used. The graph shows the mean measured concentrations (copies/µL reaction) of three replicates of each dilution with measured values above.

Multiplexing of up to five targets

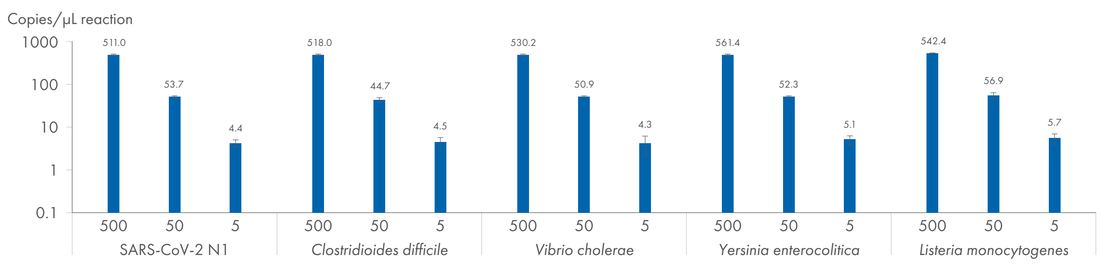
Multiplexing allows conservation of sample material, while detecting multiple targets in one reaction. To demonstrate that multiplexing does not compromise accurate quantification, we tested a series of four assays in singleplex and multiplex using the same input templates. The four bacterial targets were detected in four different channels (FAM, HEX, ROX and Cy5). As shown in the graph, quantification results are the same regardless of single- or multiplexing, even over a dynamic range of 4 log levels.



Singleplex versus multiplex setup quantifying four different bacterial targets Four assays were run in singleplex and 4-plex reactions using the same template genomic DNA material. In both setups, the same concentrations were observed for concentrations between 0.25 and 2500 copies/µL. dPCR, with 3 replicates for each condition, was performed using 26K 24-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

Parallel quantification of DNA and RNA targets

Health-related microbes also include RNA viruses such as SARS-CoV-2, whose detection by dPCR requires reverse transcription of RNA into DNA. For this purpose, microbial dPCR assays can be combined with the QIAcuity OneStep Advanced Probe Mix designed for one-step RT-dPCR reactions. Here, we performed a 5-plex detection of the viral RNA target SARS-CoV-2 together with four bacterial DNA targets normally found in wastewater samples. A mix of bacterial genomic DNAs and synthetic viral RNA were used as template inputs. The template dilution is accurately captured.



Multiplex detection of RNA and DNA targets. A mixture of four bacterial gDNAs *(Clostridioides difficile, Vibrio cholerae, Yersinia enterocolitica, Listeria monocytogenes)* and SARS-CoV-2 RNA was used as input. dPCR, with three replicates per condition, was run using 8.5k 96-well Nanoplates and the QIAcuity OneStep Advanced Probe Kit on the QIAcuity Digital PCR System. Three template dilutions with 500, 50 and 5 copies/µL were used. Bar chart shows the mean measured concentrations (copies/µL reaction) of three replicates each for each of the five targets.

Conclusions

Digital PCR on the QIAcuity combined with the dPCR Microbial DNA Detection assay portfolio offers four key features:

- **Coverage:** A portfolio of 685 microbial assays detecting bacteria, fungi, viruses, parasites, plus AMR and virulence genes
- **Accuracy:** Accurate linear quantification across a 4-log dynamic range in singleplex and multiplex reactions
- **Speed:** A fast workflow with a turnaround time of less than 2 hours
- **Multiplexing:** Mix-and-match option for up to five targets and, in combination with QIAcuity OneStep Advanced Probe Kit, the possibility for simultaneous detection of DNA and RNA targets

Sample to Insight