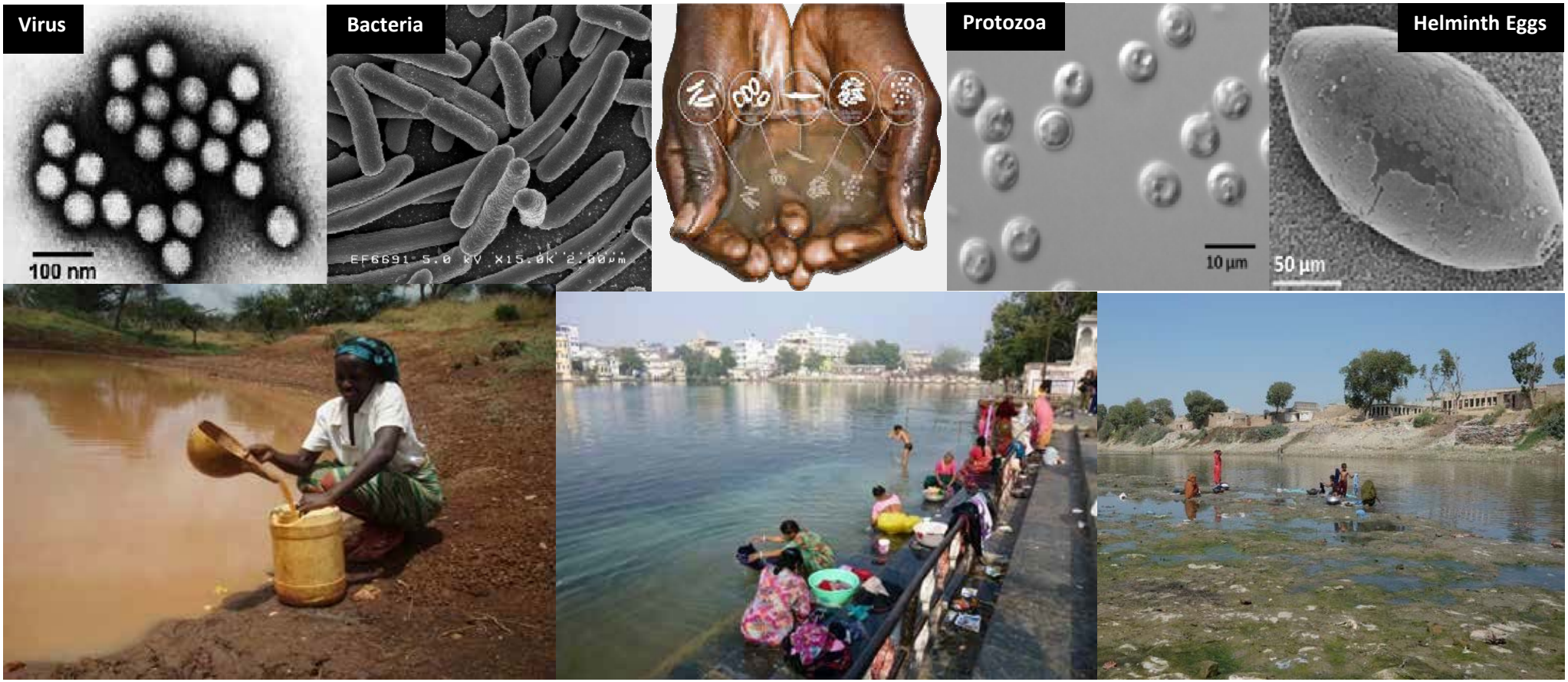
Low-Cost Microbial Quantification Methods for Water Quality Control in the Developing World

Prof. Michael R. Hoffmann
Engineering and Applied Science
California Institute of Technology

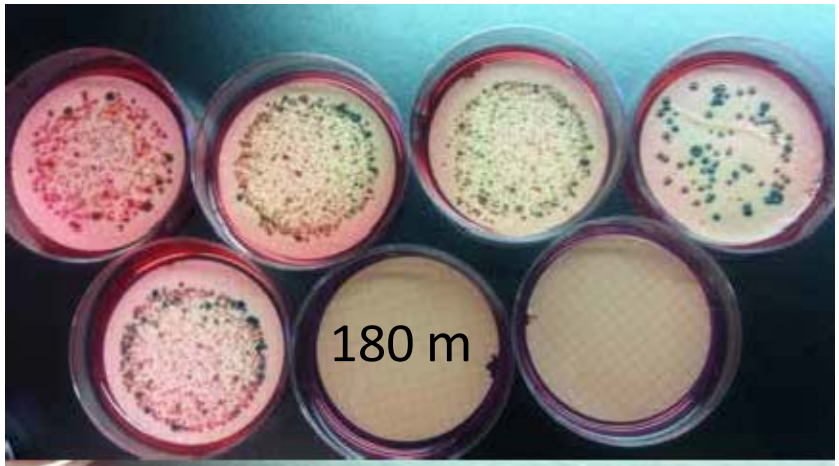


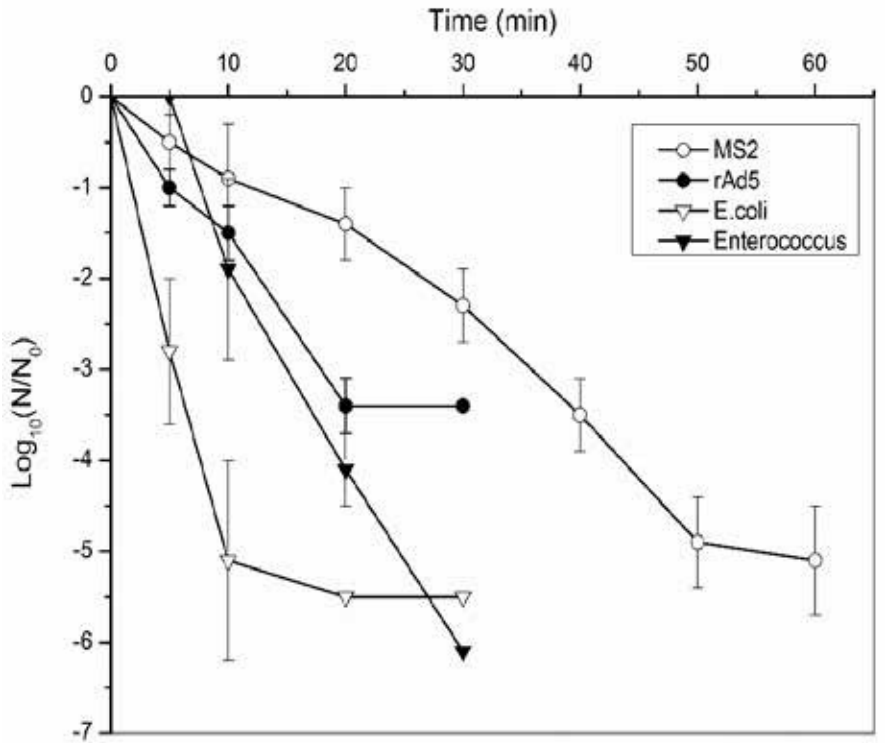
Sustainable Human Waste Treatment: Disinfection & Recycling

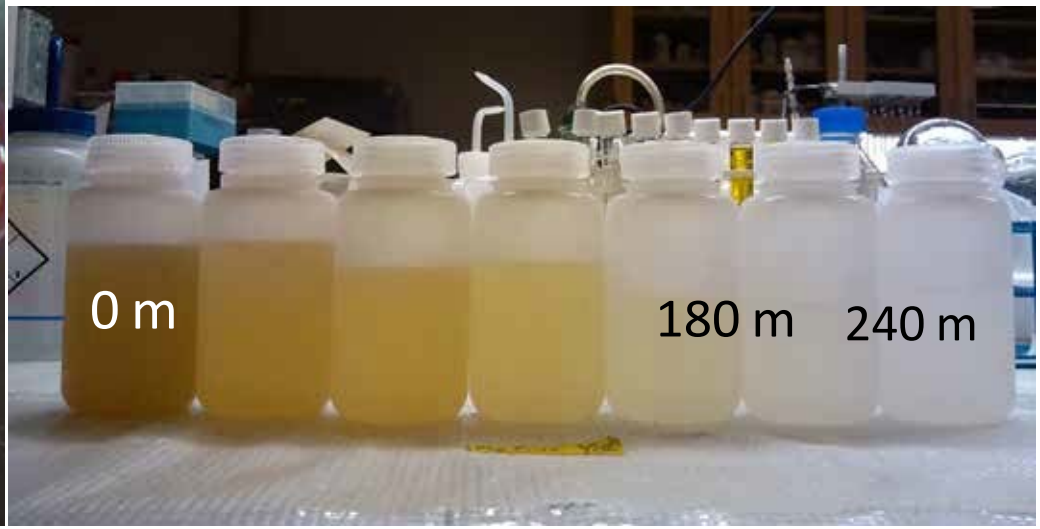
Caltech

Disinfection Achieved within 2-3 Hours

Fecal Coliform Counts vs. Time







Human Health Parameters (Protozoa, Clostridium) Spiked Samples Introduced into the EC Reactors Banana City, Durban, South Africa

		E.coli Log/100ml			
Date		S1	S2	S3	Log Red
Wed, 20/6/2018	8:00				
	10:00				
Thu, 21/6/2018	8:00	5.07	7.37	0	7.37
	10:00	5.1	7.72	0.22	7.5
	12:00	5.3	7.68	0.22	7.46
	14:00	5.41	7.74	0	7.74
Fri, 22/6/2018	8:00				
	10:00				
Mon, 25/6/2018	8:00	5.03	7.51	0	7.51
	10:00				
	12:00	5.26	7.34	0	7.34
	14:00				
Tue, 26/6/2018	8:00	5.13	7.47	0	7.47
	10:00				
	12:00	5.33	7.38	0	7.38
	14:00				
Wed, 27/6/2018	8:00	5.2	7.54	0.3	7.24
	10:00				
	12:00	5.43	7.73	0.37	7.36
	14:00				

		Clostridium Log/100ml			
Date		S1	S2	S3	Log Red
Wed, 20/6/2018	8:00	4.43	6.29	0.82	5.47
	10:00	4.82	6.41	0.73	5.68
Thu, 21/6/2018	8:00				
	10:00				
	12:00				
	14:00				
Fri, 22/6/2018	8:00	4.07	6.54	0.22	6.32
	10:00	4.61	6.54	0.7	5.84
Mon, 25/6/2018	8:00				
	10:00	4.02	6.64	0	6.64
	12:00				
	14:00	4.07	6.57	0	6.57
Tue, 26/6/2018	8:00				
	10:00	4.08	6.54	0	6.54
	12:00				
	14:00	4.37	6.56	0	6.56
Wed, 27/6/2018	8:00				
	10:00	4.2	6.63	0	6.63
	12:00				
	14:00	4.48	6.55	0	6.55

Environmental Science
Water Research & Technology

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Cite this: *Environ. Sci.: Water Res. Technol.*, 2018, 4, 956

Propidium monoazide pretreatment on a 3D-printed microfluidic device for efficient PCR determination of 'live *versus* dead' microbial cells†

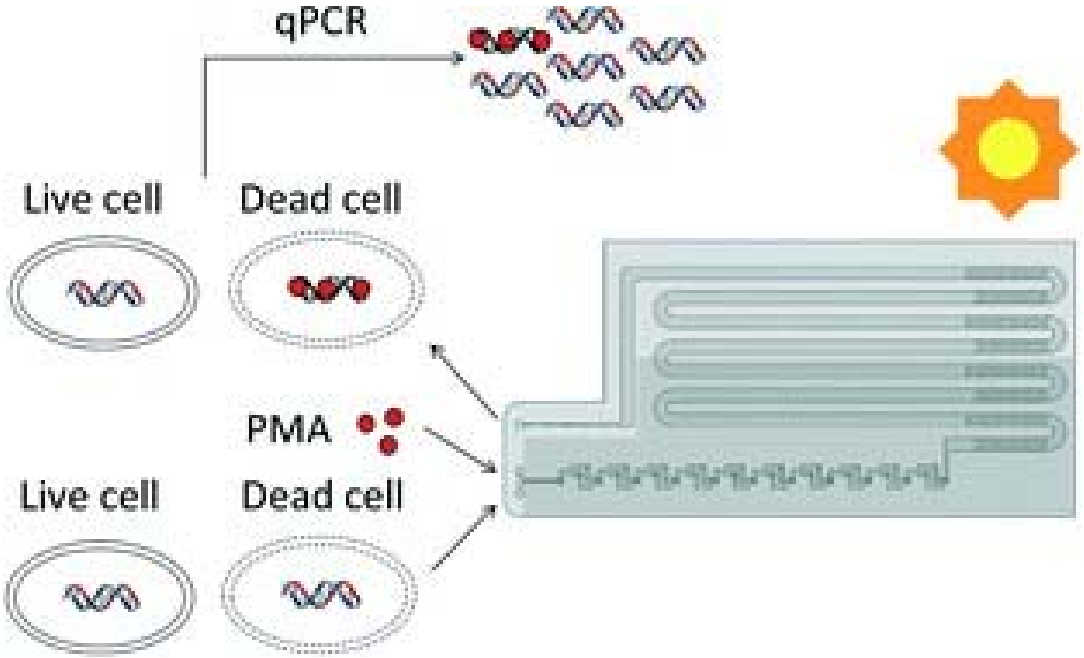
Yanzhe Zhu,[a] Xiao Huang,[a] Xing Xie,[ab] Janina Bahnemann,[ac] Xingyu Lin,[a] Xunyi Wu,[a] Siwen Wang[a] and Michael R. Hoffmann*[a]

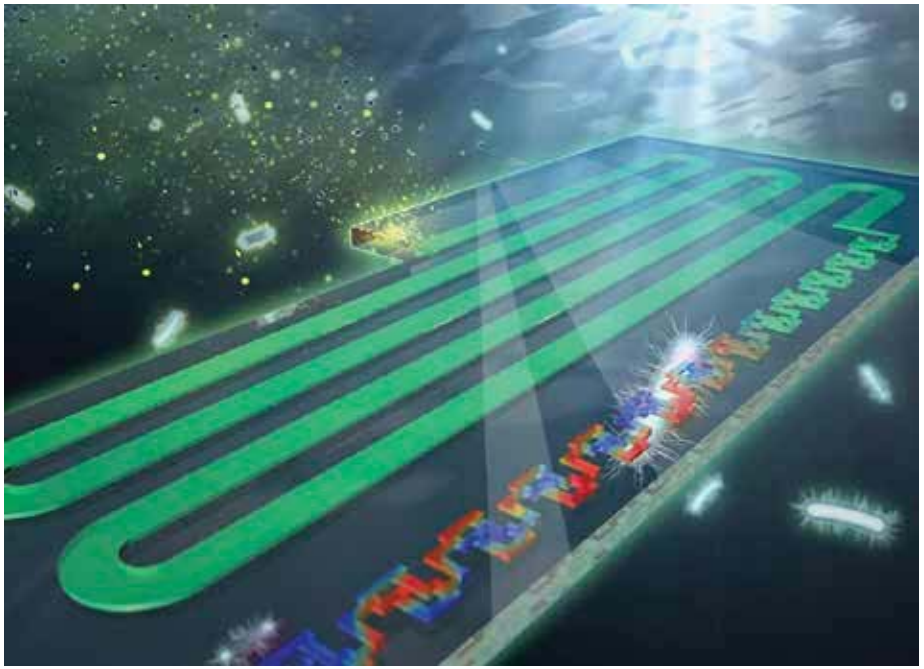
Waterborne microbial pathogen detection *via* nucleic acid analysis on portable microfluidic devices is a growing area of research, development, and application. Traditional polymerase chain reaction (PCR)-based nucleic acid analysis detects total extracted DNA, but cannot differentiate live and dead cells. A propidium monoazide (PMA) pretreatment step before PCR can effectively exclude DNA from nonviable cells, as PMA can selectively diffuse through compromised cell membranes and intercalate with DNA to form DNA–PMA complex upon light exposure. The complex strongly inhibits the amplification of the bound DNA in PCR, and thus, only cells with intact cell membranes are detected. Herein, this study reports the development of a microfluidic device to carry out PMA pretreatment 'on-chip'. Chip design was guided by computer simulations, and prototypes were fabricated using a high-resolution 3D printer. The optimized design utilizes split and recombine mixers for initial PMA-sample mixing and a serpentine flow channel containing herringbone structures for dark and light incubation. On-chip PMA pretreatment to differentiate live and dead bacterial cells in buffer and natural pond water samples was successfully demonstrated.

Received 31st January 2018,
Accepted 11th April 2018

DOI: 10.1039/c8ew00058a

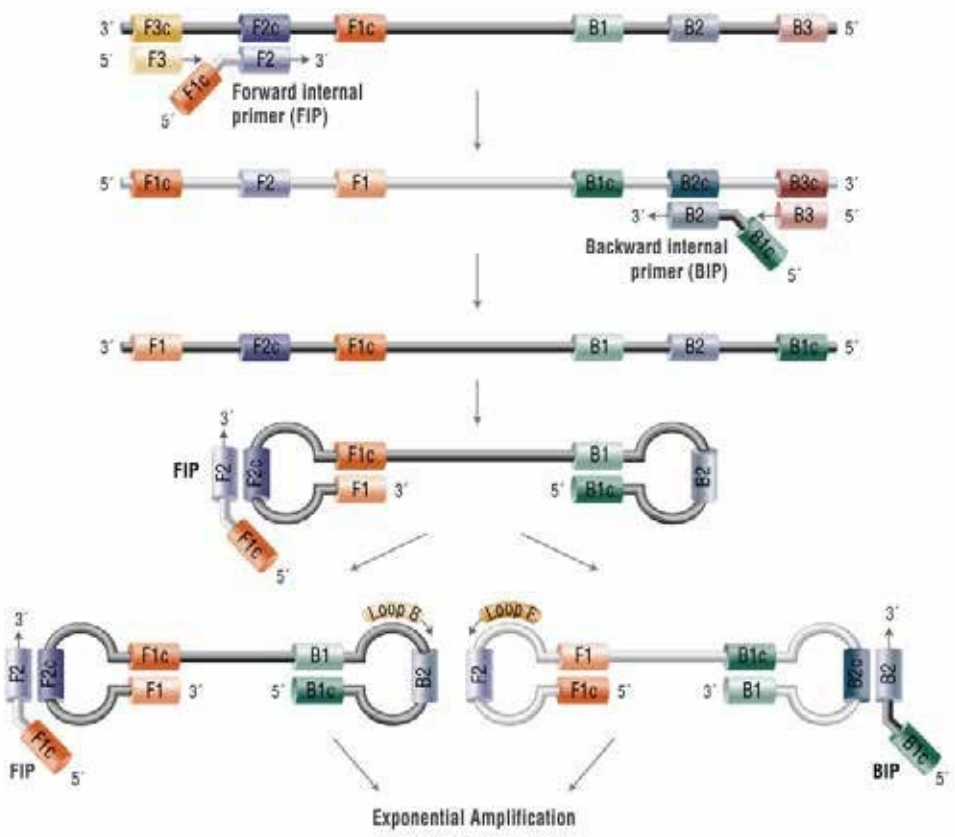
rsc.li/es-water


qPCR
Live cell
Dead cell
PMA
Live cell
Dead cell



Loop Mediated Isothermal Amplification (LAMP)

LAMP



PCR vs. LAMP

LAMP assays are much faster

	PCR	LAMP
Enzyme	Taq DNA Polymerases	*Bst* DNA Polymerase
Primers	2	4-6
Temperature	temperature cycling	Isothermal (60-65 °C)
Time	>2 hour	<1 hour
Yield	~ 0.2 µg/reaction	10-20 µg/reaction
Detection	Fluorescence	Fluorescence, turbidity
Inhibition	Sensitive to inhibitors	High tolerance
Multiplex	Yes	Difficult

Notomi et al, Nucleic Acids Res. 2000; 28(12): e63.

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Article

Cite This: *Environ. Sci. Technol.* 2018, 52, 6399–6407

pubs.acs.org/est

Smartphone-Based in-Gel Loop-Mediated Isothermal Amplification (gLAMP) System Enables Rapid Coliphage MS2 Quantification in Environmental Waters

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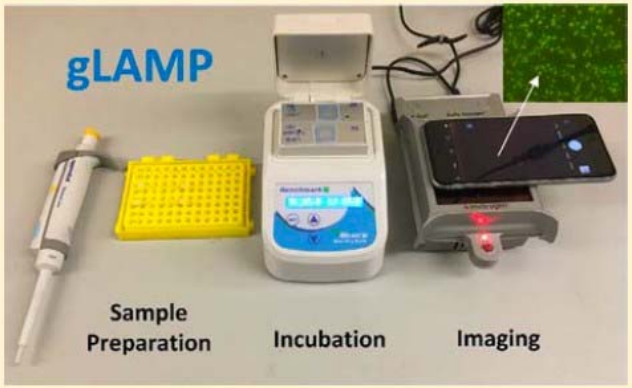
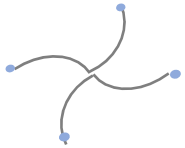
Supporting Information

ABSTRACT: Model coliphages (e.g., ΦX174, MS2, and PRD1) have been widely used as surrogates to study the fate and transport of pathogenic viruses in the environment and during wastewater treatment. Two groups of coliphages (F-specific and somatic) are being explored as indicators of viral fecal pollution in ambient water. However, the detection and quantification of coliphages still largely rely on time-consuming culture-based plaque assays. In this study, we developed an in-gel loop-mediated isothermal amplification (gLAMP) system enabling coliphage MS2 quantification within 30 min using standard laboratory devices. Viral particles (MS2) were immobilized with LAMP reagents in polyethylene glycol hydrogel, and then viral RNAs were amplified through a LAMP reaction. Due to the restriction effect of the hydrogel matrix, one viral particle would only produce one amplicon dot. Therefore, the sample virus concentrations can be determined based on the number of fluorescent amplicon dots using a smartphone for imaging. The method was validated by using artificially spiked and naturally contaminated water samples. gLAMP results were shown to correlate well with plaque assay counts ($R^2 = 0.984$, $p < 0.05$) and achieved similar sensitivity to quantitative reverse-transcription polymerase chain reaction (RT-qPCR; 1 plaque-forming unit per reaction). Moreover, gLAMP demonstrated a high level of tolerance against inhibitors naturally present in wastewater, in which RT-qPCR was completely inhibited. Besides MS2, gLAMP can also be used for the quantification of other microbial targets (e.g., *Escherichia coli* and *Salmonella*). Considering its simplicity, sensitivity, rapidity, and versatility, gLAMP holds great potential for microbial water-quality analysis, especially in resource-limited settings.

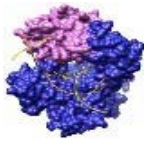




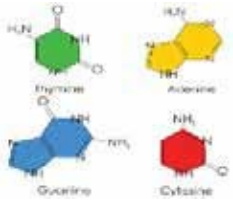
Monomer/**Macromer**

Crosslinker

MS2 Virus



DNA polymerase

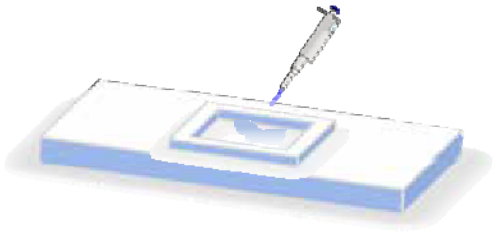


dNTP

Primers

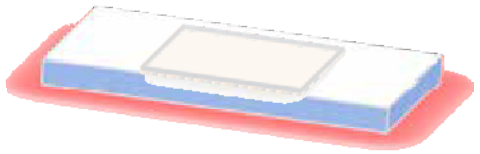
In-Gel LAMP or (gLAMP)

Gelation



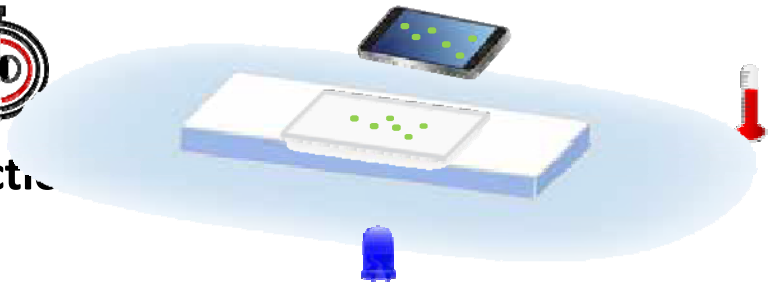
RT

Amplification



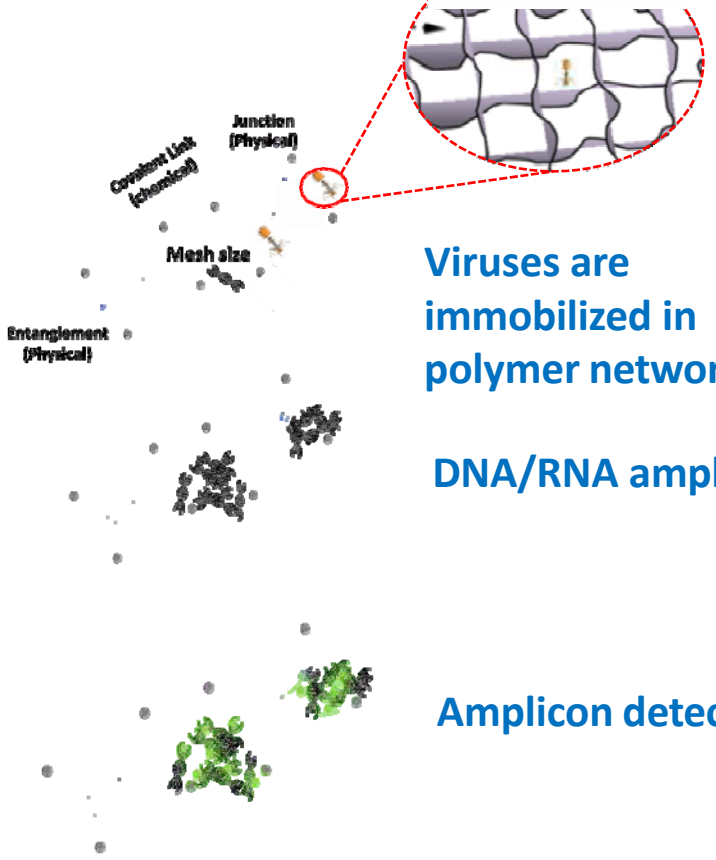
65 °C

Detecti



RT

H **x**



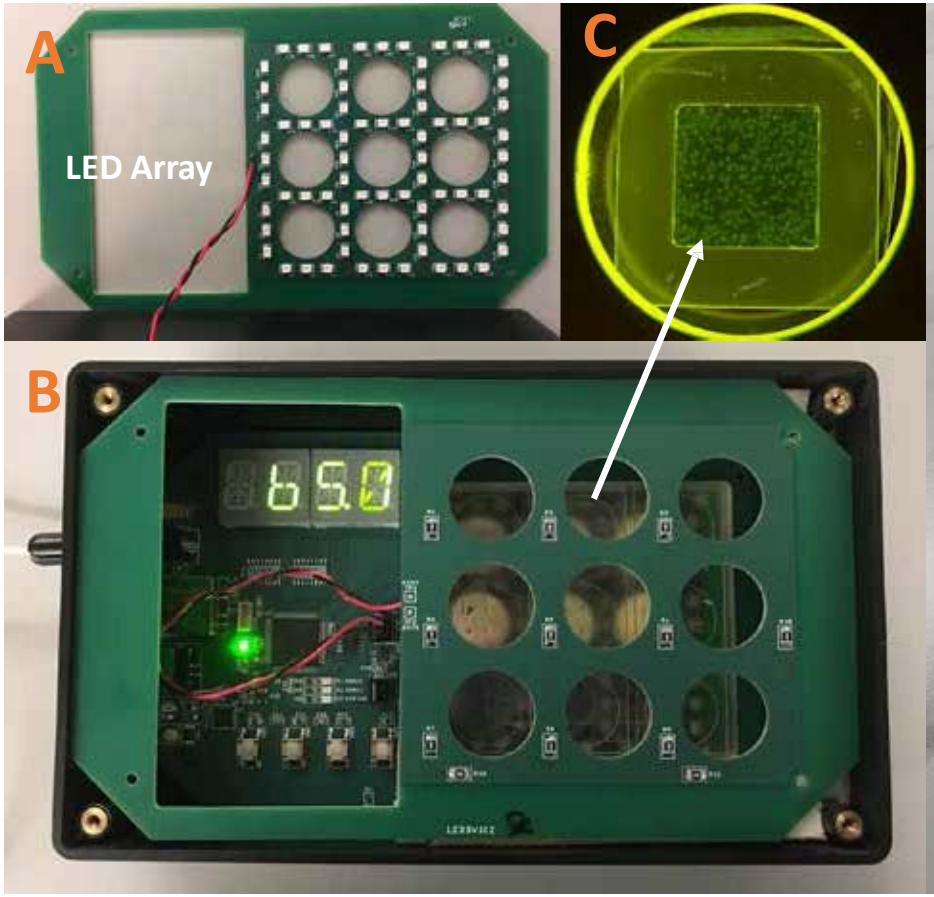
Viruses are immobilized in polymer networks

DNA/RNA amplified

Amplicon detection

Portable & Field-Ready gLAMP Microbial Quantification System

Total Cost = $500



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ACS NANO

Cite This: *ACS Nano* 2018, 12, 10281−10290

www.acsnano.org

Asymmetric Membrane for Digital Detection of Single Bacteria in Milliliters of Complex Water Samples

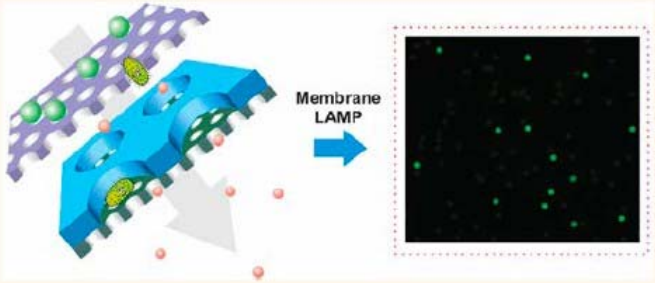
Xingyu Lin,[†] Xiao Huang,[†] Yanzhe Zhu,[†] Katharina Urmann,[†] Xing Xie,[†,‡] and Michael R. Hoffmann*[,†]

[†]Linde + Robinson Laboratories, California Institute of Technology, Pasadena, California 91125, United States

[‡]School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

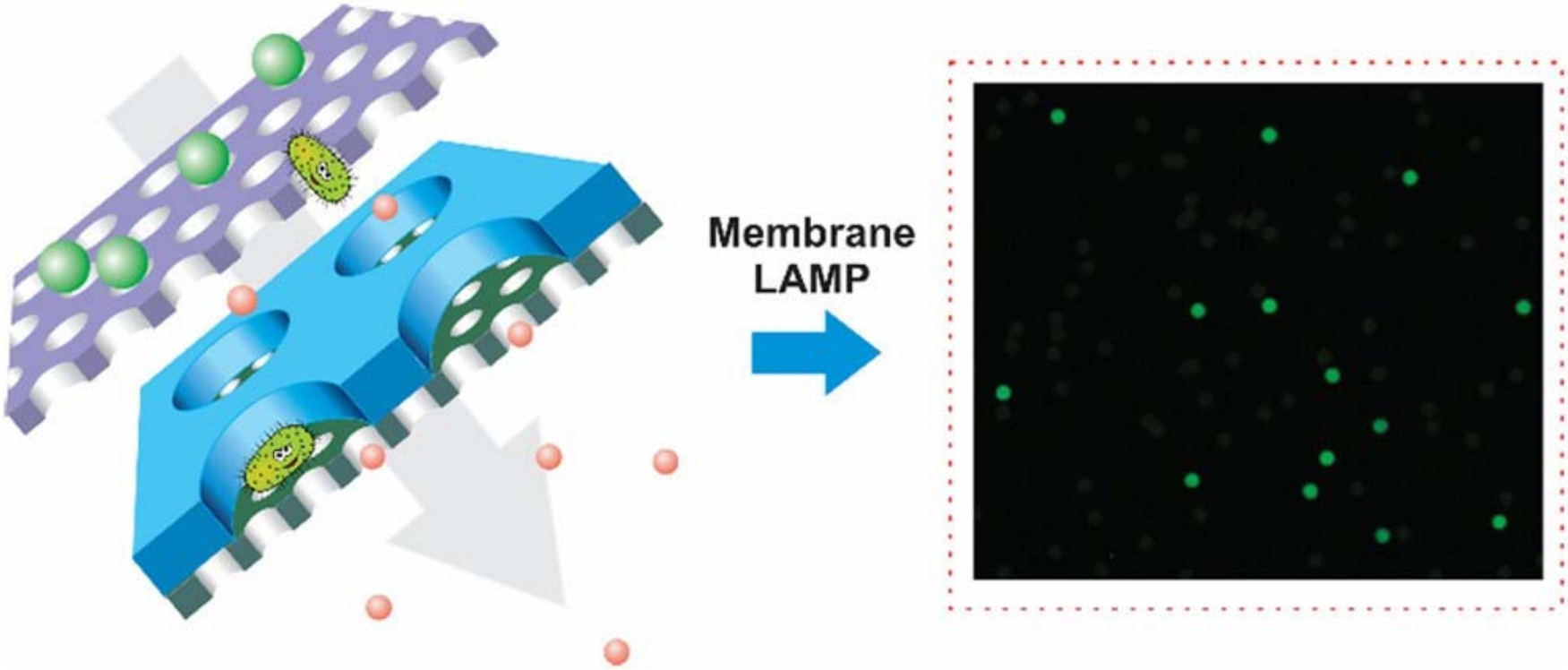
Supporting Information

ABSTRACT: In this work, we introduce an asymmetric membrane as a simple and robust nanofluidic platform for digital detection of single pathogenic bacteria directly in 10 mL of unprocessed environmental water samples. The asymmetric membrane, consisting of uniform micropores on one side and a high density of vertically aligned nanochannels on the other side, was prepared within 1 min by a facile method. The single membrane covers all the processing steps from sample concentration, purification, and partition to final digital loop-mediated isothermal amplification (LAMP). By simple filtration, bacteria were enriched and partitioned inside the micropores, while inhibitors typically found in the environmental samples (*i.e.*, proteins, heavy metals, and organics) were washed away through the nanochannels. Meanwhile, large particles, indigenous plankton, and positively charged pollutants in the samples were excluded by using a sacrificial membrane stacked on top. After initial filtration, modified LAMP reagents, including NaF and lysozyme, were loaded onto the membrane. Each pore in the asymmetric membrane functioned as an individual nanoreactor for selective, rapid, and efficient isothermal amplification of single bacteria, generating a bright fluorescence for direct counting. Even though high levels of inhibitors were present, absolute quantification of *Escherichia coli* and *Salmonella* directly in an unprocessed environmental sample (seawater and pond water) was achieved within 1 h, with sensitivity down to single cell and a dynamic range of 0.3−10000 cells/mL. The simple and low-cost analysis platform described herein has an enormous potential for the detection of pathogens, exosomes, stem cells, and viruses as well as single-cell heterogeneity analysis in environmental, food, and clinical research.

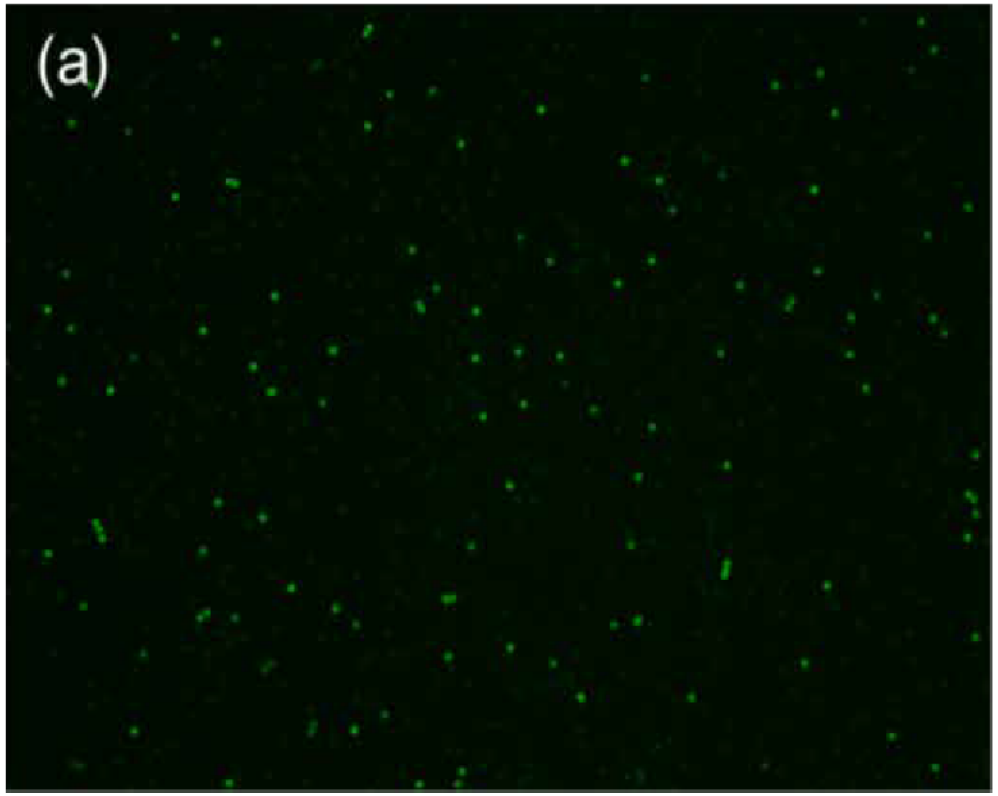


KEYWORDS: *asymmetric membrane, nanofluidics, pathogen detection, digital LAMP, single-molecule counting*

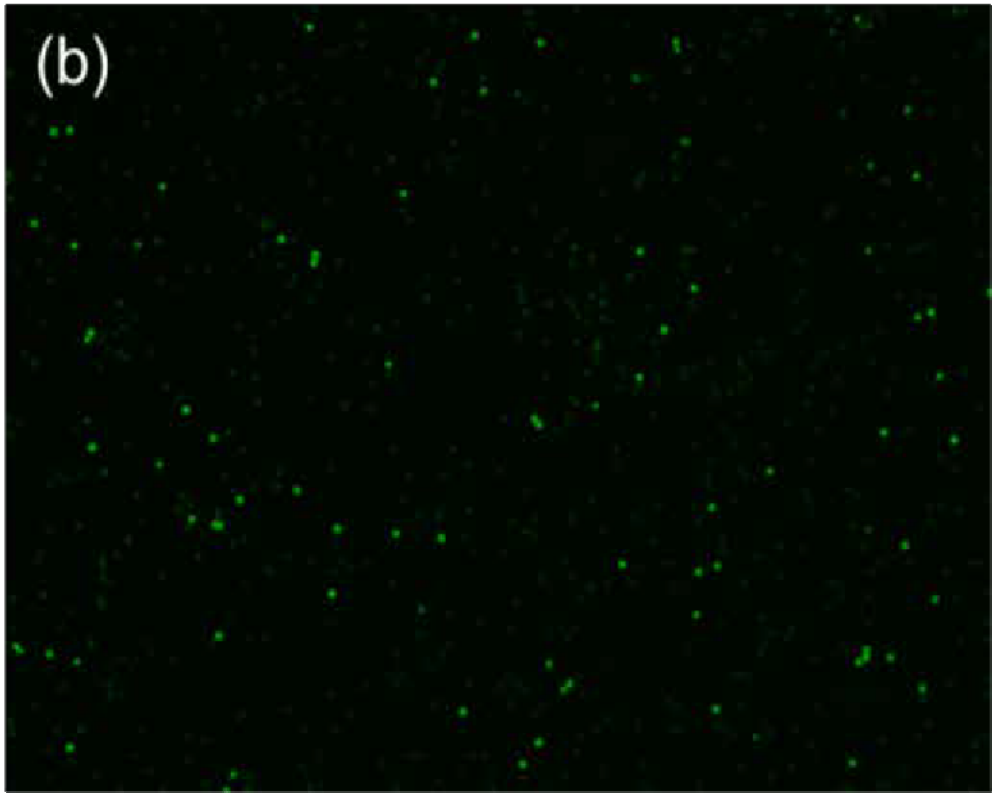
Asymmetric Membrane for Digital Detection of Single Bacteria in Milliliters of Complex Water Samples



Extracted DNA Amplification



Salmonella Typhi



Enterococcus faecalis

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ACS SENSORS

Article

Cite This: ACS Sens. 2019, 4, 242−249

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Digital Loop-Mediated Isothermal Amplification on a Commercial Membrane

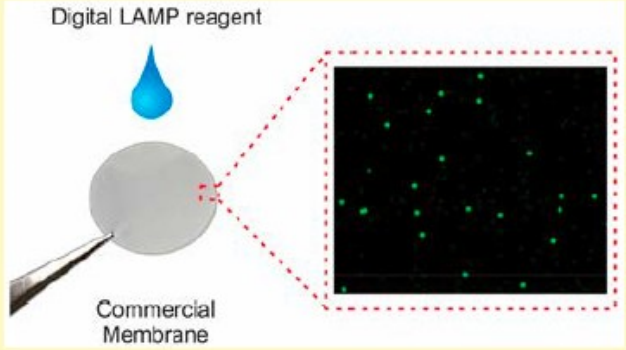
Xingyu Lin,[†] Xiao Huang,[†] Katharina Urmann,[†] Xing Xie,[†,§] and Michael R. Hoffmann*[,†]

[†]Linde + Robinson Laboratories, California Institute of Technology, Pasadena, California 91125, United States

[§]School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

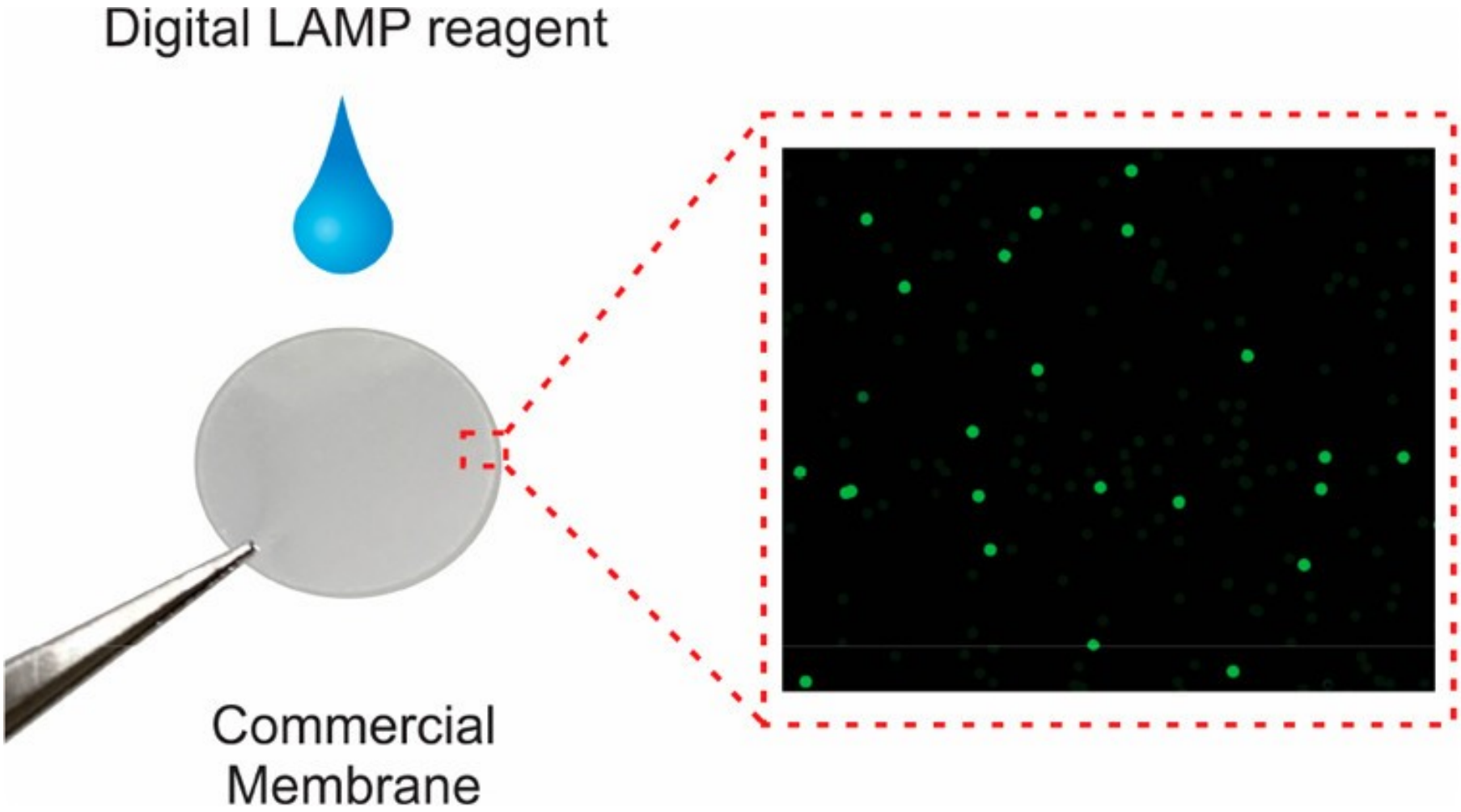
Supporting Information

ABSTRACT: In this work, we report digital loop-mediated isothermal amplification (LAMP) or reverse-transcription LAMP (RT-LAMP) on a commercial membrane, without the need for complex chip fabrication or use of specialized equipment. Due to the pore size distribution, the theoretical error for digital LAMP on these membranes was analyzed, using a combination of Random Distribution Model and Multivolume Theory. A facile peel-off process was developed for effective droplet formation on the commercial track-etched polycarbonate (PCTE) membrane. Each pore functions as an individual nanoreactor for single DNA amplification. Absolute quantification of bacteria genomic DNA was realized with a dynamic range from 11 to 1.1×10^5 copies/μL. One-step digital RT-LAMP was also successfully performed on the membrane for the quantification of MS2 virus in wastewater. With the introduction of new probes, the positive pores can be easily distinguished from negative ones with 100 times difference in fluorescence intensities. Finally, the cost of a disposable membrane is less than $0.10/piece, which, to the best of our knowledge, is the most inexpensive way to perform digital LAMP. The membrane system offers opportunities for point-of-care users or common laboratories to perform digital quantification, single cell analysis, or other bioassays in an inexpensive, flexible, and simplified way.



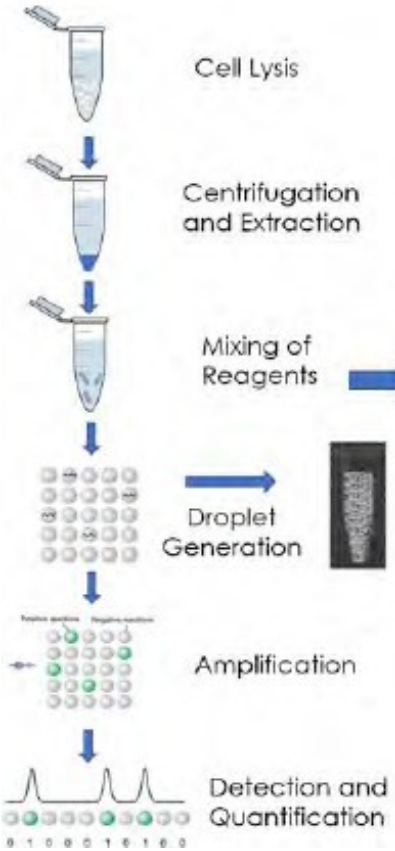
KEYWORDS: *digital LAMP, membrane, microfluidic, droplets, nucleic acid, paper-based analytical device, PCR*

Digital Loop-Mediated Isothermal Amplification on a Commercial Membrane

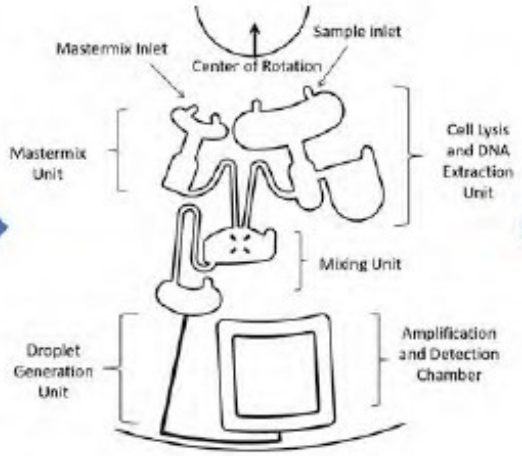


Portable Pathogen Analysis System (PPAS)

ddLAMP Assay

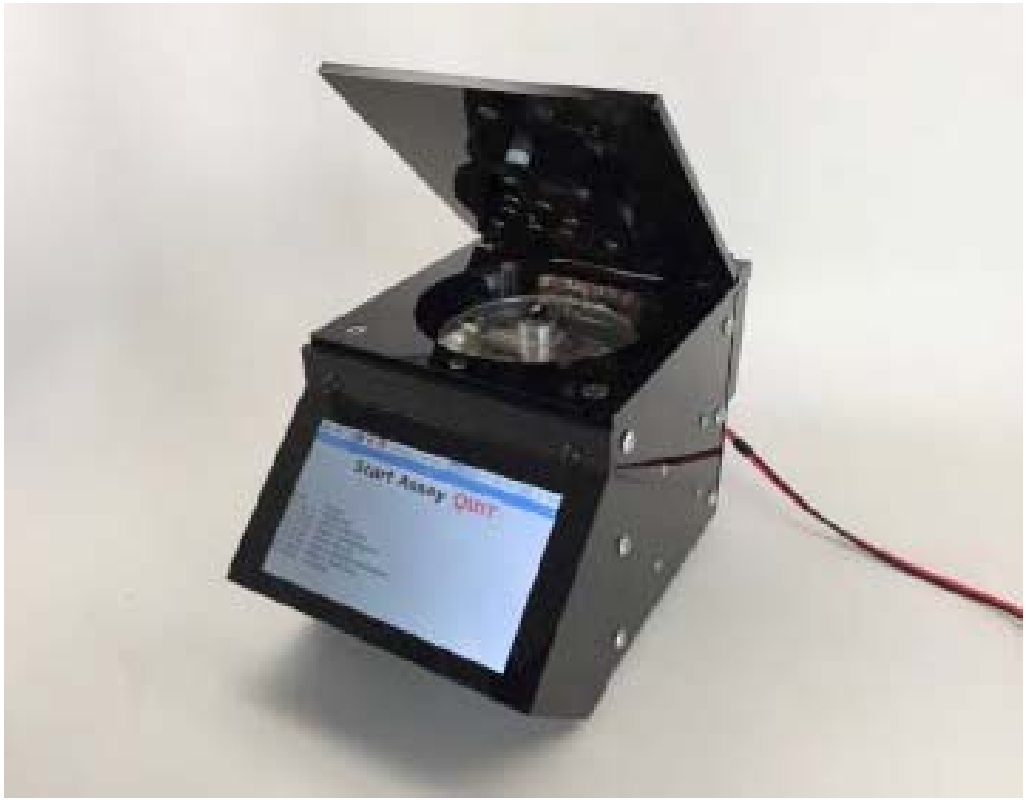


Design Integration of ddLAMP Assay for Disc

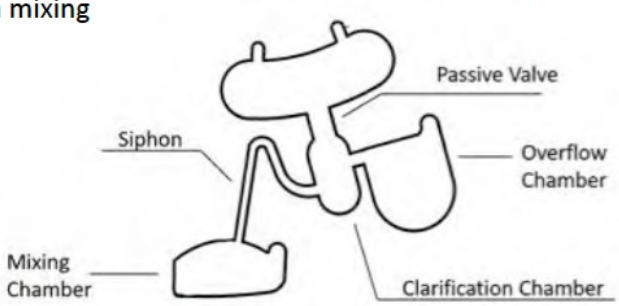


Assembly of Disc Layers

Final Disc



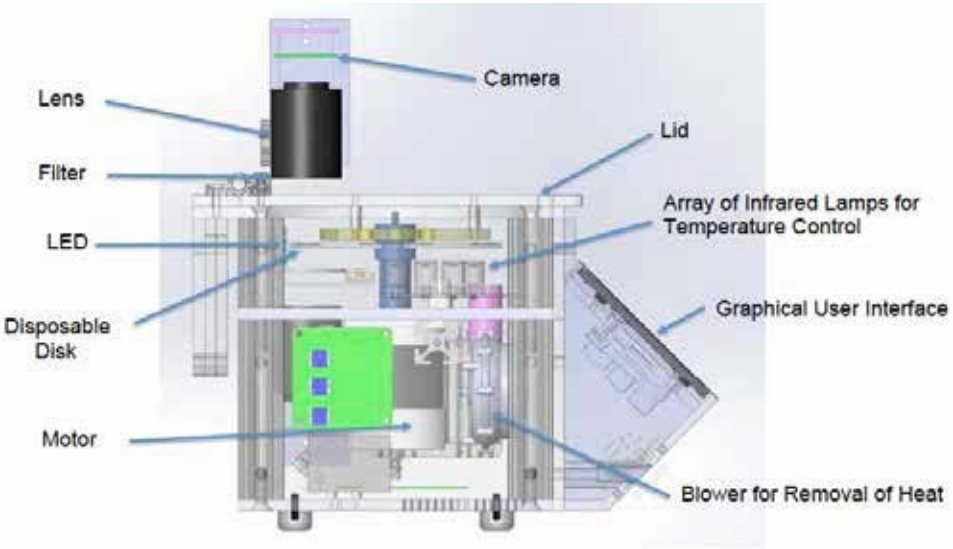
Lysed DNA is extracted, centrifuged, and metered to capture 2 uL for DNA for downstream mixing

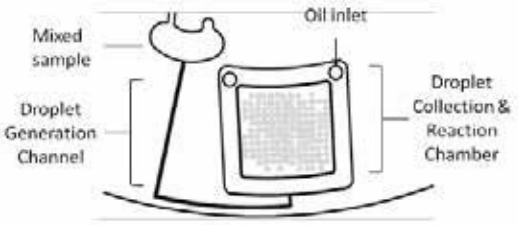


Centrifugation at 3000 RPM

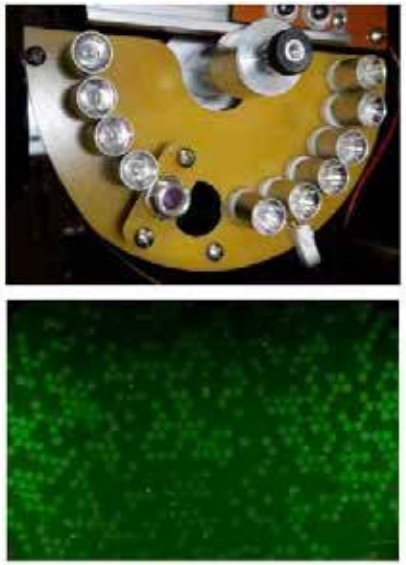
Siphon priming at 0 RPM

Metering at 500 RPM





Homogenous droplets (bottom) with less than +/- 10% variation are produced using the schematic (top).



On-Disc LAMP amplification is performed at 65 °C using heat provided by IR Lamps(top). Fluorescent images after amplifcation are shown (bottom) for *E. facealis*; Quantification is achieved by poisson distribution

PPAS Cube Microbial Detection System Cost Estimates

Estimated Cost per Reader Unit	
Component	**Price**
Camera	75
Lens	30
Filter	10
LED	20
Motor	75
Motor driver PCB	200
Heater Lamps	120
Blower	50
Heater Lamp PCB	20
Controller PCB	20
Power PCB	20
12VDC Power Supply	50
Enclosure (Injection molded ABS plastic)	150
Machined Metal Components	40
Graphical User Interface	100
TOTAL COST PER UNIT (USD):	**980**