Supplementary Materials for

Digital Dipstick: Miniaturized bacteria detection and digital quantification for the point-of-care

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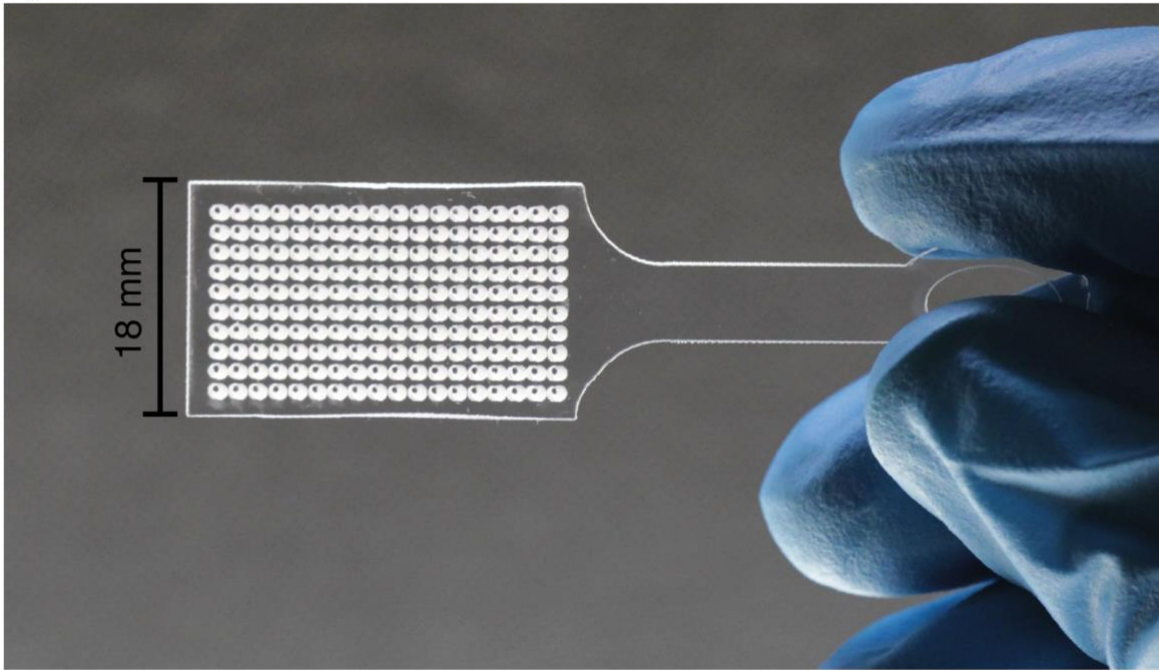* Email: iseri@kth.se; wouter@kth.se

This file includes:

Figures S1 to S5
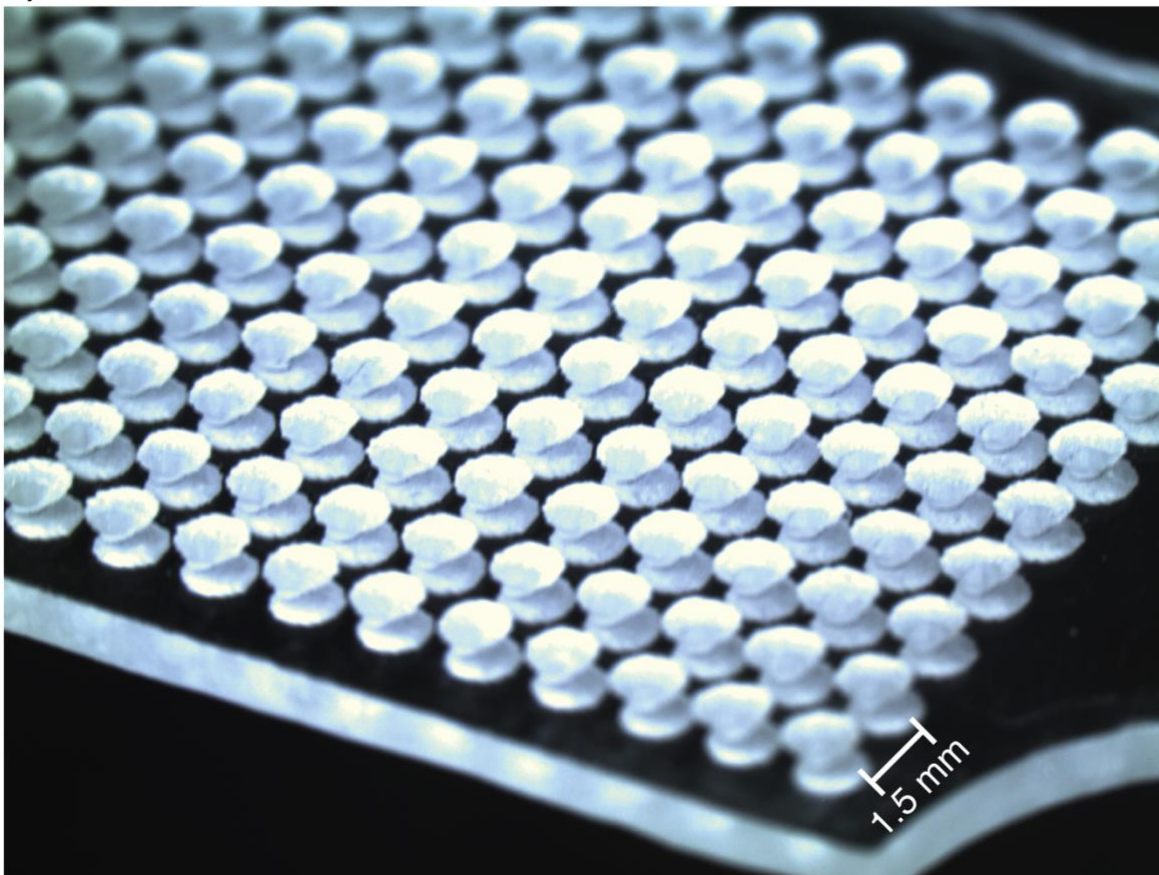Tables S1 and S3
Caption for Supplementary Video S1 and S2

Other Supplementary Materials for this manuscript include the following:

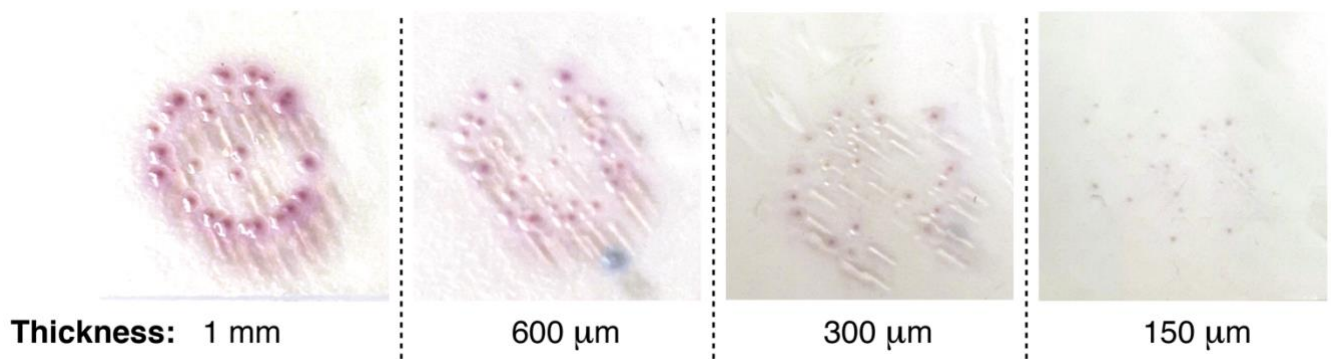Supplementary Video S1 and S2
Supplementary File S1

A)

B)

Fig S1 The Digital Dipstick frame after laser cut and drill. A) Photograph of the digital dipstick frame made after laser cutting and drilling of a 1 mm thick PMMA plate. **B)** A close-up perspective view of the digital dipstick frame shows the hourglass-shaped structure of wells.

Fig. S2. *E. coli* **growth and signal generation on chromogenic agar (Chromagar Orientation) of different thickness.** The images show that the colony size and signal (salmon red) after 24 h of incubation deceases for decreasing thickness of agar.
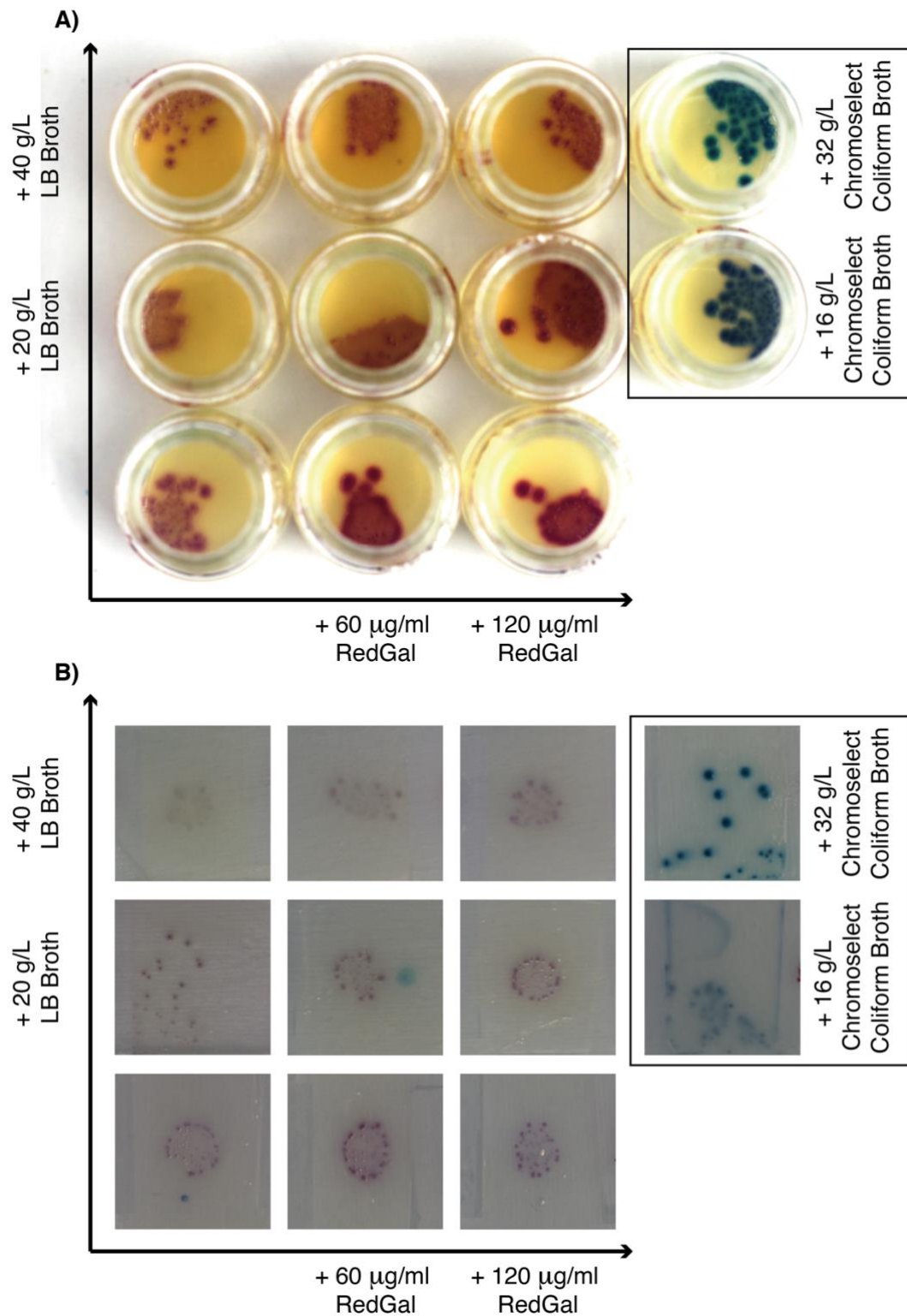
Fig. S3. Summary of modified chromogenic agar experiments in glass vials (A) and 300 μm thick agar samples (B). Bottom left samples are unmodified chromogenic agar (Chromagar Orient). For the x-axis of 3 by 3 matrix, 60 μg/ml and 120 μg/ml RedGal chromogenic substance are added in respected columns. For the y-axis of 3 by 3 matrix 20 g/L and 40 g/L LB broth are added in respected columns. Two samples on the most right is made by using Chromoselect Rapid Coliform Broth with single and double strength and this resulted in blue color change instead of red.
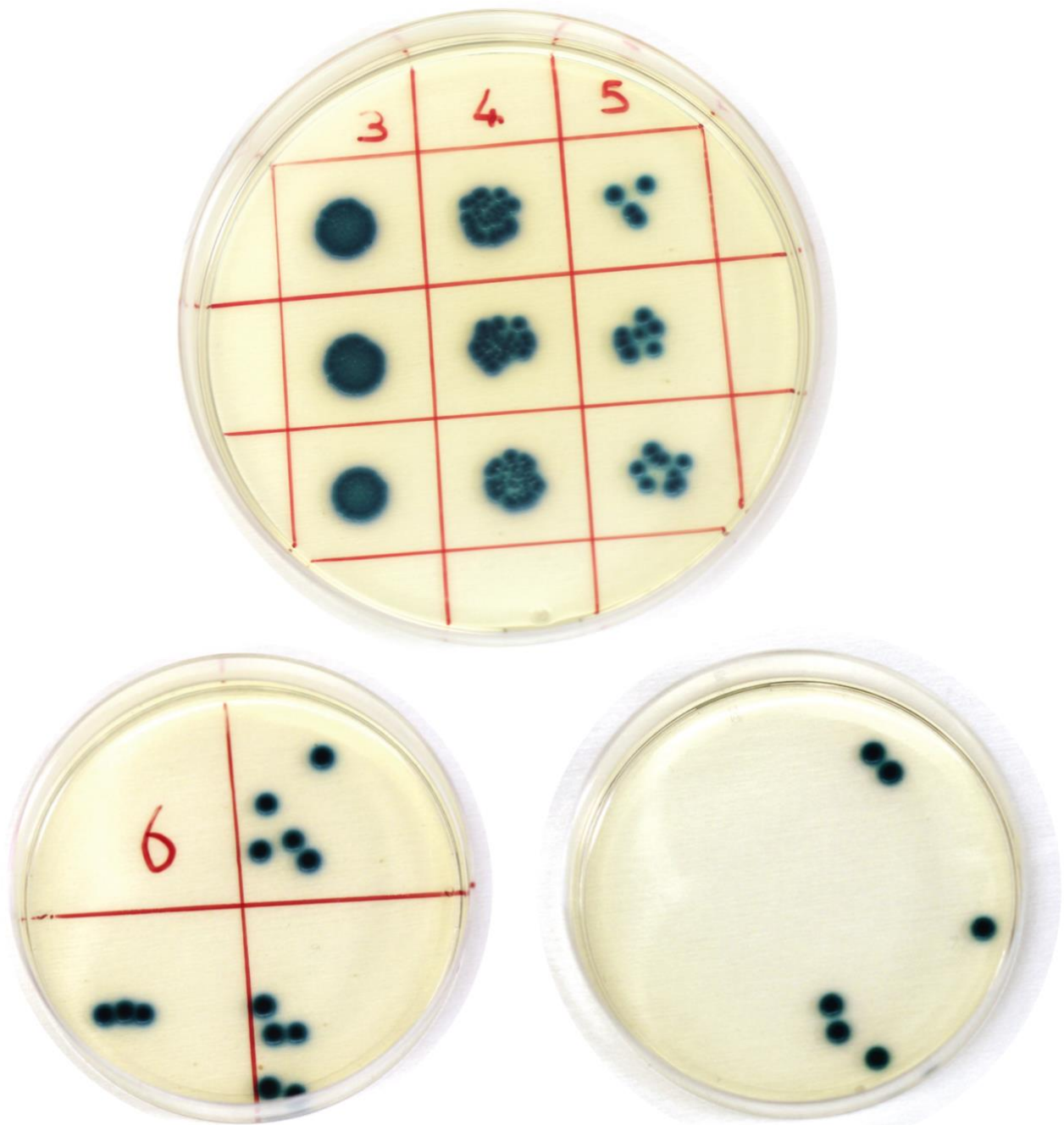
Fig. S4 Agar plate photographs after 24 hours of culturing to quantify different *E. coli* concentrations. Each segment of the top plate (90 mm diameter), separated by red lines had 1 μl of sample liquid with *E. coli* concentrations of 5×10^5 CFU/ml (dilution #3) (n=3), 5×10^4 CFU/ml (dilution #4) (n=3), and 5×10^3 CFU/ml (dilution #5) (n=3) for 3 columns. Each segment of the bottom left plate (55 mm diameter) had 10 μl of sample liquid with *E. coli* concentration of 5×10^2 CFU/ml (dilution #6) (n=3). Bottom right plate (55 mm diameter) had 100 μl of sample liquid 5×10^1 CFU/ml (dilution #7) (n=1).
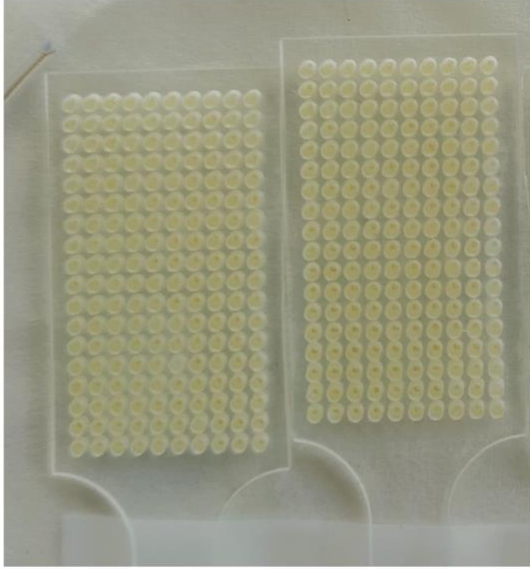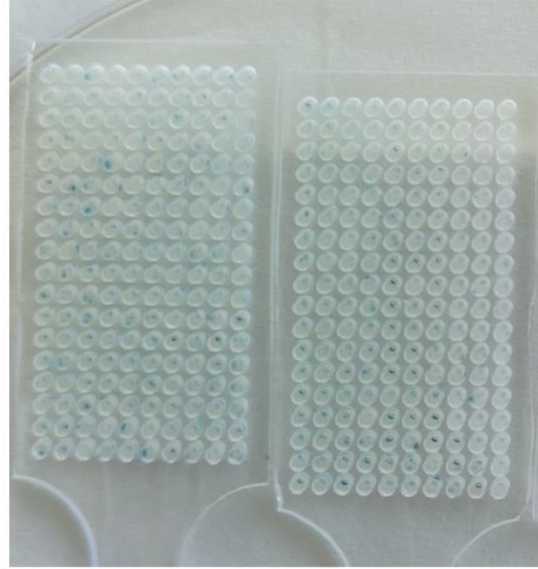
Fig. S5. Duplicate Digital Dipstick assay readouts for PBS samples with *Proteus mirabilis* (A), and *Klebsiella pneumoniae* (B).

Table S1 Results of plate counting experiments for ten-fold serial dilutions of *E. coli* in PBS

#plate_sample	Dilution #4 (CFU/1ul)	Dilution #5 (CFU/1ul)	Dilution #6 (CFU/10ul)	Dilution #7 (CFU/100ul)
1_1	58	6	2	0
1_2	58	4	8	
1_3	60	7	3	
2_1	84	7	5	9
2_2	87	7	3	
2_3	59	6	2	
3_1	51	5	7	3
3_2	43	4	7	
3_3	54	5	6	
4_1	42	2	5	6
4_2	41	2	5	
4_3	46	1	3	
5_1	42	4	3	4
5_2	46	7	2	
5_3	54	9	0	
Average	55	5.066666667	4.066666667	4.4
Standard Deviation	14.09660547	2.250925735	2.282438128	3.361547263
	Dilution #4 (CFU/ml)	Dilution #5 (CFU/ml)	Dilution #6 (CFU/ml)	Dilution #7 (CFU/ml)
Average	5.50E+04	5.07E+03	4.07E+02	4.40E+01
Standard Deviation	1.41E+04	2.25E+03	2.28E+02	3.36E+01

Table S2 Positive hole fractions of Digital Dipstick triplicates at different time points (columns) calculated by Matlab script for different concentrations from 5×10^7 CFU/ml to 1×10^7 CFU/ml (C1 to C7 respectively).

	0h	1h	2h	3h	4h	5h	6h	7h	8h	9h	10h	12h	24h
C1-1	0	0	0	0	0.044	0.95	1	1	1	0.98889	0.99444	1	0.98889
C1-2	0	0	0	0	0.039	0.98889	1	1	1	0.94444	1	1	0.95556
C1-3	0	0	0	0	0	0.83333	1	0.99444	1	0.99444	1	0.99444	0.99444
C2-1	0	0	0	0	0	0	0.85	0.98889	0.99444	1	1	1	1
C2-2	0	0.00556	0.00556	0	0	0	0.88889	0.98889	0.99444	1	0.98889	1	0.98889
C2-3	0	0	0	0	0	0	0.69444	0.98889	0.98889	1	0.98889	1	0.97222
C3-1	0	0	0	0	0	0	0	0.82222	0.98333	0.98889	0.96111	0.99444	0.98333
C3-2	0	0	0	0	0	0	0	0.13889	0.89444	0.98889	0.98889	1	0.99444
C3-3	0	0	0	0	0	0	0	0.72222	0.98889	1	1	1	1
C4-1	0	0	0	0	0	0	0	0	0.13333	0.70556	0.92778	0.98889	0.97222
C4-2	0	0	0	0	0	0	0	0	0	0.42222	0.74444	0.91111	0.95556
C4-3	0	0	0	0	0	0	0	0	0.03889	0.66111	0.90556	1	0.99444
C5-1	0	0	0	0	0	0	0	0	0	0.16667	0.21111	0.36111	0.52222
C5-2	0	0	0	0	0	0	0	0	0	0.01111	0.15	0.28889	0.38889
C5-3	0	0	0	0	0	0	0	0	0	0.11667	0.37778	0.47222	0.22222
C6-1	0	0	0	0	0	0	0	0	0	0.01111	0.02222	0.05	0.08889
C6-2	0	0	0	0	0	0	0	0	0	0.01111	0.01667	0.05556	0.08333
C6-3	0	0	0	0	0	0	0	0	0	0	0.02778	0.07222	0.09444
C7-1	0	0	0	0	0	0	0	0	0	0	0	0	0
C7-2	0	0	0	0	0	0	0	0	0	0	0	0	0
C7-3	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3 Results for Digital Dipstick assay validation with spiked urine samples.

Dipstick #	Concentration (CFU/ml)	# of negative wells	# of empty wells	# of positive wells	Positive hole fraction
1	1×10^7	0	0	180	1
2	1×10^6	0	7	173	1
3	1×10^6	0	0	180	1
4	1×10^6	0	0	180	1
5	1×10^5	10	1	169	0.944134078
6	1×10^5	7	5	168	0.96
7	1×10^5	1	0	179	0.994444444
8	1×10^4	88	1	91	0.508379888
9	1×10^4	103	15	62	0.375757576
10	1×10^4	81	1	98	0.547486034
11	1×10^3	166	0	14	0.077777778
12	1×10^3	173	0	7	0.038888889
13	1×10^3	175	0	5	0.027777778
14	1×10^2	179	0	1	0.005555556
15	1×10^2	171	9	0	0
16	1×10^2	180	0	0	0
17	1×10^1	180	0	0	0
18	1×10^1	180	0	0	0
19	1×10^1	180	0	0	0

Supplementary Video S1.

Demonstration of Digital Dipstick operation.

Supplementary Video S2.

Swiping off the excess sample liquid (blue dyed DI water) from the dipstick surface by inserting it in the incubation box.