Supplementary Information for

Fluid-Screen - as a Real Time Dielectrophoretic Method for Universal Microbial Capture

Robert Emanuel Weber[1], Janusz Jurand Petkowski[2], Brandye Michaels[3], Kamil Wisniewski[4], Anna Piela[4], Slawomir Antoszczyk[1], Monika Urszula Weber[1]*

Affiliations:

[1] Fluid-Screen, Inc. 100 Cummings Center, Suite 243-C, Beverly, MA 01915, USA
[2] Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, 77 Mass. Ave., Cambridge, MA 02139, USA
[3] Pfizer, 1 Burtt Rd, Andover, MA 01810, USA
[4] Hener, Wrocław Technology Park, BETA building, room 104, Klecińska 125, 54-413 Wrocław, Poland

* Correspondence to: monika.weber@fluid-screen.com

1. Online Methods:

1.1 Engineering Design and Operation of Fluid-Screen System

Figure S1 illustrates a schematic diagram of the Fluid-Screen chip for capturing bacteria from a sample using system in Figure 1 of the main text. As shown, in the presence of an electric field generated using electrode (yellow), bacteria (green) are attracted to the electrode by a positive DEP force (arrows) acting on the bacteria in the sample. Sample components are introduced to the Fluid-Screen chip from an influent sample. The sample flows past the electrode in the microfluidic chip at a predetermined flow rate. Sample components not captured by the electrode flow to the effluent sample.

Figure S2 illustrates a schematic diagram of capturing bacteria from a sample using system in Figure 1 of the main text. The bacteria captured on the electrodes are imaged using the optical system to perform a direct on-chip quantification. Influent sample and effluent sample are plated on agar plates for PCM.

The Fluid Screen electrode system is the ring structure that consists of concentric rings (Figure 1 in main text). Every second ring is connected to the same potential. The outer radius of the inner ring is 50 µm and the outer radius of the outer ring is 250 µm. The ring structure captured 100%

of bacteria according to PCM method (Table 1 in main text; Table S1) which is much higher than previously reported capture efficiency results for the similar technology[1].

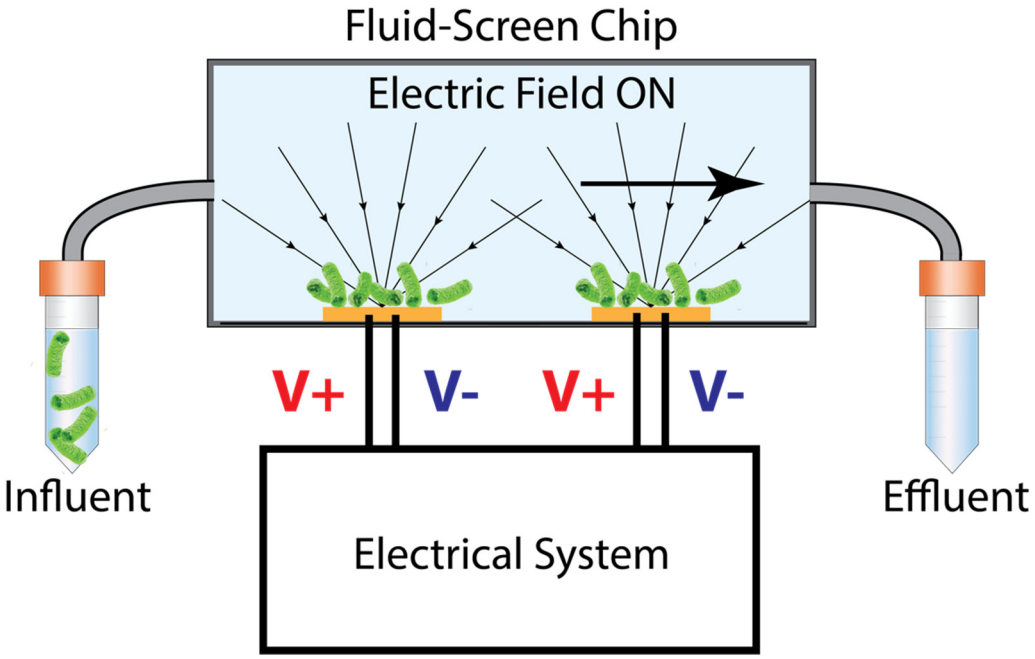

Figure S1. Schematic of the Fluid Screen chip in operation: microchannel, electrodes on the bottom (yellow), influent and effluent samples are connected via tubing. The Electrical System applies alternating voltage V+, V- to the electrodes generating electric field inside the microfluidic channel. The main arrow shows the direction of fluid flow through the chip from inlet (left) to the outlet (right). Arrows pointing to the electrodes show the directions of the dielectrophoretic force while the electric field is turned on.

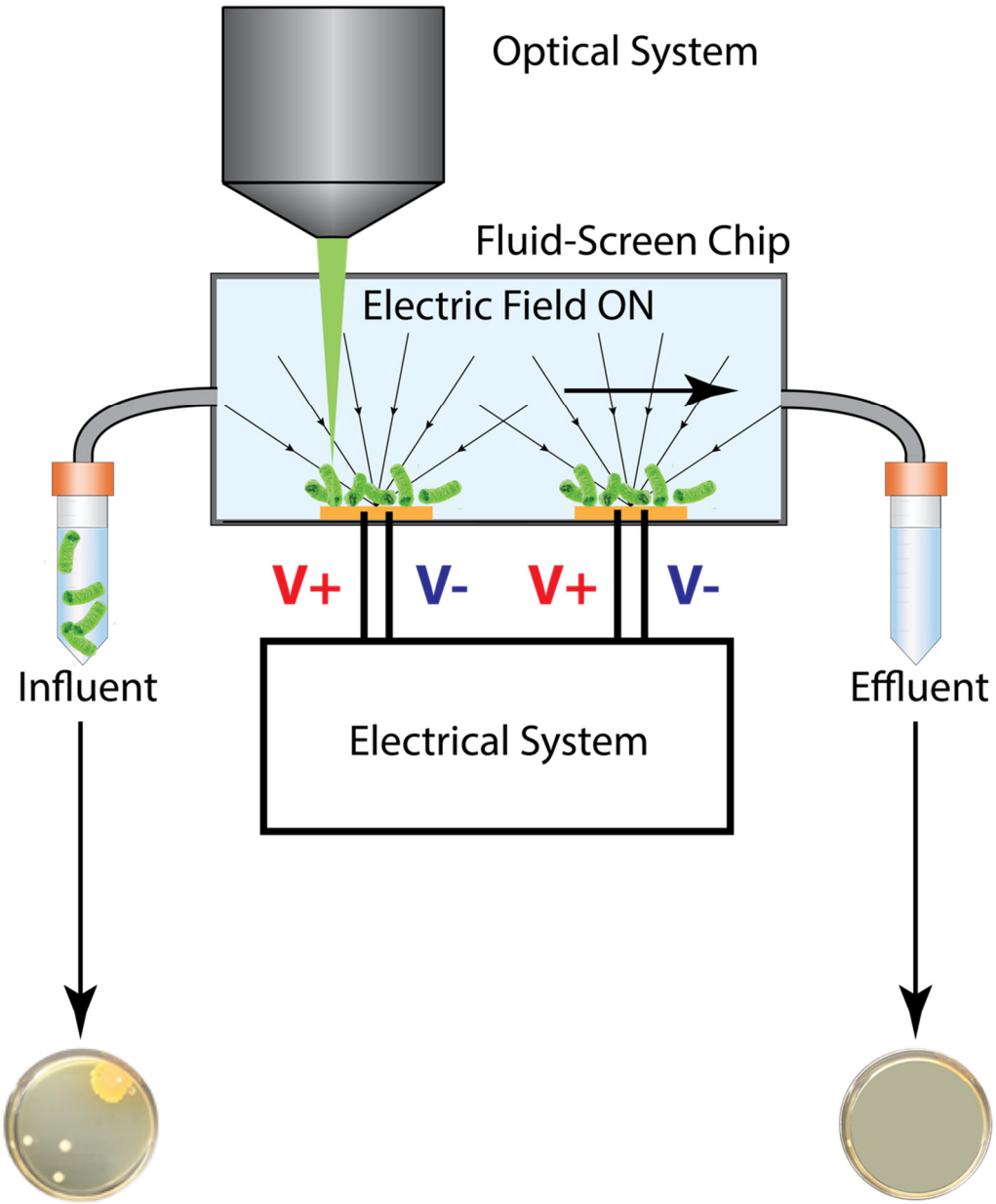

Figure S2. Schematic of the Fluid Screen experimental setup: microchannel, electrode on the bottom with +V and -V contacts, electrodes are connected to a function generator and an oscilloscope, bacterial motion is observed with a microscope and registered with a camera. Arrows pointing to the electrodes show the directions of the dielectrophoretic force while the electric field is turned on.

The experimental setup (Figure 1 in the main text) consists of the fabricated chip with the electrode structure located on the bottom of the microfluidic channel with fluid flow above the electrode. Both contacts are connected to opposite polarities with Fluid-Screen custom made interface, SMA cables, Fluid-Screen custom made amplifier and function generator (Siglent SDG5162, USA) bacterial capture is observed with a fluorescent microscope (Olympus BX63, USA).

Flow was controlled by Elvesys' microfluidics system and kept constant throughout the entirety of the experiment. During the experiment the measurement apparatus were controlled by the LabView System Controller and the scan image was obtained using Olympus CellSens Software.

1.2 Microfabrication

The procedure for fabrication of Polydimethylsiloxane (PDMS) devices is very similar to what is described in the literature[2] regarding soft lithographic techniques.

The steps of PDMS chip fabrication and assembly are the following:

1. Preparation of PDMS mixture with 1:10 curing agent: PDMS mixed in the fume hood, placed in the desiccator for 30 minutes to remove bubbles

2. PDMS mixture poured onto a wafer mold wrapped in aluminium foil and placed in the desiccator for 30 minutes to remove bubbles

3. Si wafer mold with PDMS layer placed on the hotplate at 80 °C for 5 hours

4. Cooled PDMS peeled off the wafer mold and punched holes with the hole puncher 5. Glass substrate and PDMS exposed to oxygen plasma at 350 mTorr at 200W for 30s

6. Microfluidic device assembled and bonded for 5 hours at 60 °C

Figure S3 shows the process flow to fabricate circular microelectrodes using a bilayer lift-off process. Note that PDMS device is used only in experiments of separation of *E. coli* from red blood cells (as shown in section 2.1.3 of the main text). All other microbial capture experiments presented in this paper were enabled by commercial chip fabrication.

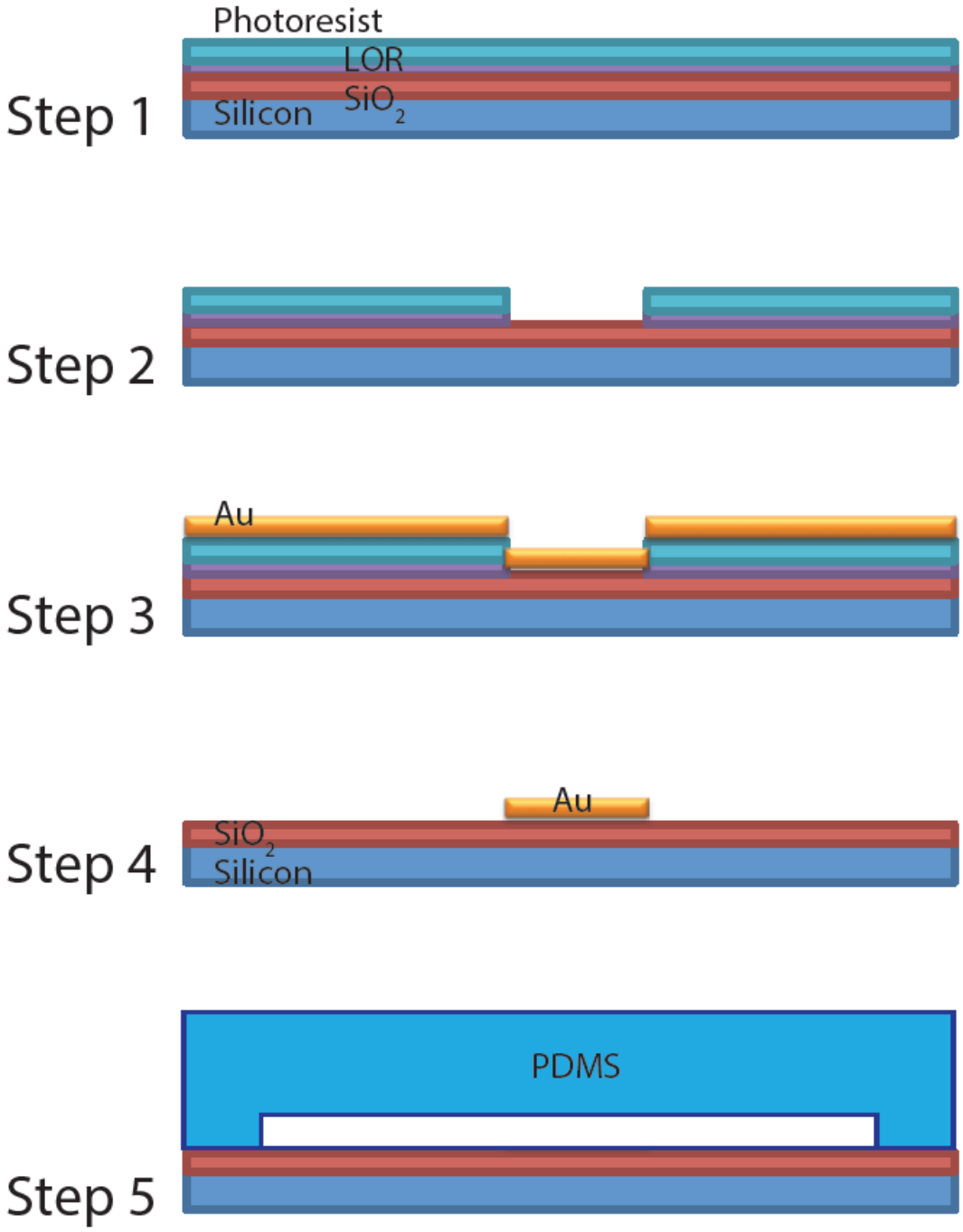

Figure S3. Electrode pattern fabrication using a bi-layer lift-o process, step 1: on a silicon wafer with 3µm SiO_2 spin *LOR*10*A* and *S*1808, step 2: expose and develop features with *MF*312 : *DI* 1 : 1, step 3: evaporate Ti 5 nm and Au 40 nm, step 4: lift o gold using 1165 Microposit remover, step 5: PDMS bonding to silicon substrate with oxygen plasma at 100 W for 30 seconds at 350 mTorr.

1.3 *E. coli* Bacterial Sample Preparation

1.3.1 Unstained *E. coli* Sample Preparation for Capture Efficiency Assessment

The *E. coli*-8739 strain was obtained from ATCC and cultured on Tryptic Soy Agar (TSA) and MacConkey (MC) agar plates at 37°C in aerobic conditions following the Harmonized USP/EP/JP.

The influent (input) sample the unstained *E. coli* capture experiment (Figure S9) was prepared in the following way. A day before the FS bacterial capture experiment on the FS system *E. coli*-8739 was re-streaked, using sterile inoculation loop, on TSA agar plates by progressive dilutions of an inoculum from a single colony on agar plates. From an overnight culture a large scoop of bacteria, avoiding taking bacteria from a biofilm area on the plates, was taken using sterile inoculation loop and suspended in 2 mL of PBS 1:1000 (Fluid Screen standard dilution testing buffer). A stock sample concentration of 10^7-10^8 bacteria/mL was determined using OD (optical density, CO8000 Cell Density Meter, Biowave) meter at 600 nm, and plated immediately after preparation in serial 10x dilutions (e. g. 10^{-4}, 10^{-5}, and 10^{-6}) and in triplicates on MAC agar plates to confirm bacteria concentration in cfu/mL. The stock sample was diluted by serial 10x dilution to the final bacterial concentration up to 250 cfu/mL. All experiments were conducted at room temperature. To prepare stock and experimental samples buffer and media were warmed up to room temperature prior to use.

To track any possible changes in bacteria concentration in influent, i.e. bacterial death over time, 100 µL aliquots of influent sample were plated. Each 100 µL influent aliquot was plated without and with single 10x dilution in triplicates on MAC agar plates to confirm bacteria concentration in cfu/mL.

1.3.2 Sybr Green I Stained *E. coli* Sample Preparation for Capture Efficiency Assessment

The *E. coli*-8739 was obtained from ATCC and cultured on Tryptic Soy Agar (TSA) or MacConkey (MAC) agar plates at 37°C in aerobic condition as recommended by the Harmonized USP/EP/JP.

The influent (input) sample for the stained *E. coli* capture experiment (Figure 3 in the main text) was prepared in the following way. A day before the experiment *E. coli*-8739 was re-streaked on TSA agar plates by the progressive dilution of an inoculum (from a single colony) on agar plates using sterile inoculation loop. From an overnight culture a large scoop of bacteria (avoid taking bacteria from a biofilm area on the plates) was taken using sterile inoculation loop and suspended in 2 mL of PBS 1:1000 (Fluid Screen standard dilution testing buffer). A stock sample concentration of 10^7-10^8 bacteria/mL were determined using OD meter at 600 nm, and plated (immediately after preparation) in serial 10x dilutions (e. g. 10^{-4}, 10^{-5}, and 10^{-6}) on MAC agar plate to confirm bacteria concentration in cfu/mL. The final concentration of influent up to 250 cfu/mL

was achieved by serial 10x dilution of stock sample. Meantime, standard cleaning procedure of FS system and chip was performed. To confirm that FS system is sterile before sample processing, unit was flushed with sterile DI UltraPure Water, 5 mL was collected and plated on agar media (TSA, MAC, MSA, SDA and CI), and 1 mL of Sybr Green I diluted 1:1000 in PBS was processed through the FS unit. The FS system was rinsed with DI UltraPure water to remove residue fluorescence dye, influent was processed through the FS system, and 1 mL of the effluent was collected and plated immediately on MAC agar for enumeration using PCM (to calculate the number of cfu). The electric field (EF) settings were determined based on a standard in-house calibration protocol and optimized for tested bacteria. Bacteria captured on FS system chip were stained with Sybr Green I and counted base on background subtraction. The [%] of capture efficiency was calculated based on formula presented in section 2.1.1 of the main draft. The experiment was carried out in 3 biological replicates with 3 technical replicates per each biological replicate (Figure 3 in the main text; see also Figure S9). All experiments were conducted at room temperature. To prepare stock and experimental samples buffer and media were warmed up to room temperature.

1.3.3 *E. coli* Sample Preparation for FS Repeatability Studies

The *E. coli*-8739 was obtained from ATCC and cultured on Tryptic Soy Agar (TSA) agar plates at 37°C in aerobic condition as recommended by the Harmonized USP/EP/JP.

The *E. coli* samples for the repeatability experiments were prepared in similar fashion to the samples for the other experiments (see Section S1.3.1 and Section 1.3.2). A day before the experiment *E. coli*-8739 was re-streaked on TSA agar plates by the progressive dilution of an inoculum (from a single colony) on agar plates using sterile inoculation loop. From an overnight culture a large scoop of bacteria (avoiding taking bacteria from a biofilm area on the plates) was taken using sterile inoculation loop and suspended in 2 mL of PBS 1:1000. A concentration of tested sample 10^5-10^6 bacteria/mL were determined using OD meter at 600 nm and confirmed by plating (immediately after preparation) in serial 10x dilutions (e. g. 10^{-4}, 10^{-5}, and 10^{-6}) on TSA agar plate. To visualize bacterial response to electric field, 1 mL of the tested sample was stained with 1 µL of Sybr Green. Using a micropipette, 2 µL of stained sample were loaded to each channel of the chip under static conditions. When electric filed (EF) was ON bacteria were captured, and fluorescent image of entire spiral electrode was taken to quantify true number of bacteria. The EF settings were determined based on a standard in-house calibration protocol and optimized for tested bacteria. The experiment was carried out on in 2 biological samples with 12 technical replicates per each biological sample. Total repeatability was evaluated on eight chips, with 3 channels per chip. For null control image the whole spiral electrode from each chip (without bacteria, using the same fluorescent acquisitions), was used to analyze samples in FS chip channels.

All experiments were conducted at room temperature. To prepare stock and experimental samples buffer and media were warmed up to room temperature.

1.4 Other Microbial Samples Preparation

All microorganisms and adenovirus sample were bought from ATCC, in a freeze-dried format. Bacteria, yeast and fungus (black mold) culture were started and propagated at Fluid-Screen's laboratory following supplier's recommendation.

Growth conditions: *E. coli* (ATCC 8739), *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 9027), *B. subtilis* (ATCC 6633), *C. albicans* (ATCC 10231) and *A. brasiliensis* (ATCC 16404) are selected from USP <61> and <62>. Bacteria, yeast and fungus grow in aerobic condition between 20-37°C and are cultured no longer than 5 consecutive passages in the recommended culture conditions. All tested microorganisms are cultured on Tryptic Soy Agar (TSA) or MacConkey Agar (MAC), Mannitol Salt Agar (MSA) and Cetrimide Agar (CA) for bacteria and Sabouraud Dextraose Agar (SDA) for yeast and fungus as recommended by the Harmonized USP/EP/JP.

Samples for other microorganisms were prepared in an analogous way to the *E. coli* samples for the other experiments (see Section S1.3.1 and Section 1.3.2). A day before the experiment all microorganisms were re-streaked on appropriate agar plates, by the progressive dilution of an inoculum (from a single colony) using a sterile inoculation loop.

Analyzed samples ~10^7 microorganisms/mL (except *A. brasiliensis*) were prepared from an overnight culture in sterile PBS 1x (Life Science) diluted 1:1000 in DI UltraPure water (Life Science). *A. brasiliensis* sample was less concentrated (~10^3 spores/mL) and contained mix of spores (conidia), conidiophores and hyphae. Bacteria and yeast concentration were determined by OD meter at 600 nm. To evaluate that microorganisms, respond to electric field (EF), stock samples were spun down, supernatant discarded and suspended in CHO cell matrix diluted 1:100 in DI UltraPure water. For optical visualization microorganisms (except *A. brasiliensis*) were stained with Sybr Green I, working solution 1:1000 by adding the dye directly to the sample. Because Sybr Green I does not penetrate *A. brasiliensis* conidia and they are large enough, mold has been observed in a bright field. Control samples were prepared in the same way and microorganisms were suspended only in PBS diluted 1:1000 in DI UltraPure water and were stained with Sybr Green I. The 3 µL of sample was applied to the FS system under static condition. A video demonstrating that tested microorganisms respond to the EF were collected in triplicates. The EF settings were determined based on a standard in-house calibration protocol and optimized for tested microorganisms.

All experiments were conducted at room temperature. To prepare stock and experimental samples buffer and media were warmed up to room temperature.

For all FS experiments sterile conditions were verified and maintained. To verify agar media sterility, 3 plates from each batch were incubated at 37°C in aerobic conditions for 5 days. To verify aseptic sample preparation of test samples, 100 µL of the Fluid-Screen sample buffer used to prepare sample were plated on TSA, MAC, MSA, CA and SDA and incubated in 37°C in aerobic condition for 5 days. The sterility test of the FS system, media and other materials showed no growth.

Statistics and Calculations

For statistical analysis we used Microsoft Excel and GraphPad Prism. The acceptable growth and viability variance range of +/- 0.5 log recommended by the USP were calculated using the following example method: A value from plate enumeration or on-chip quantification was e.g. A, so for + 0.5 log value: Ax3.2=B, and for - 0.5 log value: A/3.2=C. All +/- 0.5 log values were calculated in the same way.

1.5. Red Blood Sample Preparation

Human blood sample was spun to separate RBCs (red blood cells). RBCs were fluorescently stained for visual clarity. Stained RBCs were added into the sample that was diluted 10 times with deionized water. *E. coli* expressing GFP was grown on LB broth, resuspended in testing buffer and added to the blood sample.

1.6 Fluid-Screen Bacterial Capture Experimental Procedure

E. coli-8739 stock sample was prepared in ~10^7cfu/mL in testing buffer (Fluid Screen standard dilution buffer) by measuring absorbance on OD meter at 600 nm. The final concentration of Influent, up to 250 cfu/mL was achieved by serial 10x dilution of stock sample.

The 1 mL of sample was applied to the FS system under flow conditions and electric field ON (voltage and frequency settings optimized for tested microorganism). Influent and effluent of each technical repeat were plated on MAC agar plates in triplicates to exclude non-lactose fermenters growth and false positive results, as well. Plates were enumerated based on PCM and results are presented in cfu/mL. The results are presented on Figure S9 and Table S1 in Extended Data Section.

2. Extended Data:

2.1 Additional Validation of the Fluid Screen Method

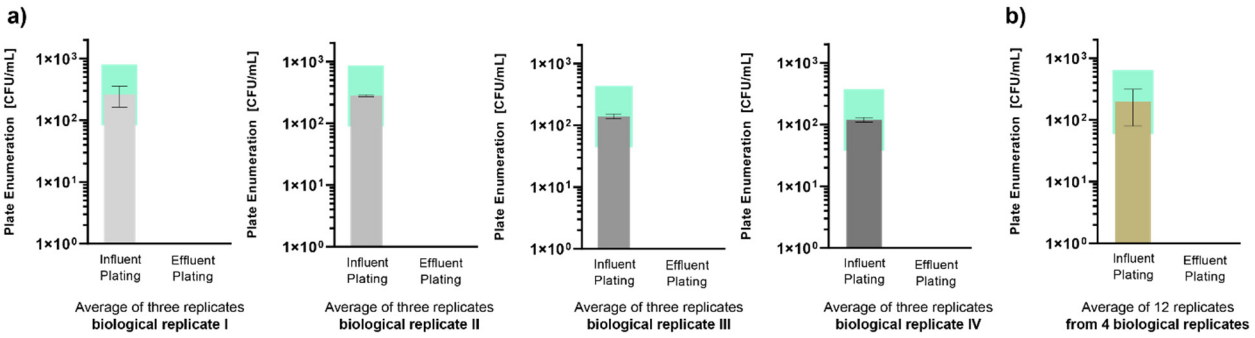

Figure S9. Results of capture efficiency experiments on the FS system. Plate Counting Method (PCM) quantification of bacteria in influent and effluent, after bacterial capture with the Fluid-Screen Chip. **(a)** data is presented as a mean and +/-SD from 3 technical replicates per each biological replicate; **(b)** data is presented as a mean and +/-SD from four biological replicates and their technical replicates, for a total of 12 tests. The turquoise bar represents the acceptable growth and viability variance range of +/- 0.5. log following the USP guidelines. In every experiment the FS system captured 100% of bacteria, as evidenced by zero PCM growth in effluent samples. The 100% capture efficiency is maintained in a broad range of bacteria concentrations, including as high as $4.2x10^2$.

Plate-Count Method [cfu/mL]												
	Biological Replicate 1			Biological Replicate 2			Biological Replicate 3			Biological Replicate 4		
	Tech Rep 1 [Ave cfu/mL]	Tech Rep 2 [Ave cfu/mL]	Tech Rep 3 [Ave cfu/mL]	Tech Rep 1 [Ave cfu/mL]	Tech Rep 2 [Ave cfu/mL]	Tech Rep 3 [Ave cfu/mL]	Tech Rep 1 [Ave cfu/mL]	Tech Rep 2 [Ave cfu/mL]	Tech Rep 3 [Ave cfu/mL]	Tech Rep 1 [Ave cfu/mL]	Tech Rep 2 [Ave cfu/mL]	Tech Rep 3 [Ave cfu/mL]
Neg_{CTRL}	0	0	0	0	0	0	0	0	0	0	0	0
Influent ≤250 cfu/mL	330	240	200	280	420	130	130	290	0	190	150	20
Effluent	0	0	0	0	0	0	0	0	0	0	0	0
Cap_{eff} [%]	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table S1. The bacterial capture efficiency as tested by the Plate Counting Method (PCM) quantification. In every experiment the FS system captured 100% of bacteria, as evidenced by zero PCM growth in effluent samples. The 100% capture efficiency is maintained in a broad range of bacteria concentrations, including as high as $4.2x10^2$.

In addition, to evaluate that Fluid-Screen chip is able to capture 100% of bacteria, *E. coli* stock sample was prepared in ~10^7cfu/mL in PBS. The final concentration of Influent, up to 250 cfu/mL was achieved by serial 10x dilution of stock sample. The 1 mL of sample was applied to the FS system under flow conditions and EF settings optimized for tested microorganism. When EF was still ON bacteria captured on FS system chip were stained with Sybr Green I for optical visualization and quantification (see Table 1 in the main text). Influent and Effluent of each

technical replicate were plated on MAC agar plates to exclude non-lactose fermenters growth and false positive results, as well. Plates were enumerated based on PCM and results are presented in cfu/mL. All 9 replications (3 biological and 3 technical) on FS system demonstrate reproducibility in 100% of bacteria capture. Results of these experiments is summarized in Figure 3 and Table 1, in the main text. Additionally, sterility tests of FS system, agar media and aseptic sample preparation, showed no growth of microorganisms at all.

2.2 Repeatability of bacterial quantification with the Fluid-Screen Method

Two biological samples with 12 technical repeats per each biological experiment on *E. coli*-8739 sample were performed to demonstrate the repeatability of the direct-on chip bacteria number quantification with the FS system. These results demonstrate the ability of the FS system to count the bacteria captured from a tested sample directly on the FS chip. The variance in results from the FS method measurement is caused by manual operation of the FS system. However, note that the FS bacterial counting error is low and the FS method is more accurate than the required +/- 0.5 log recommended by the USP for a new method validation (Figure S10).

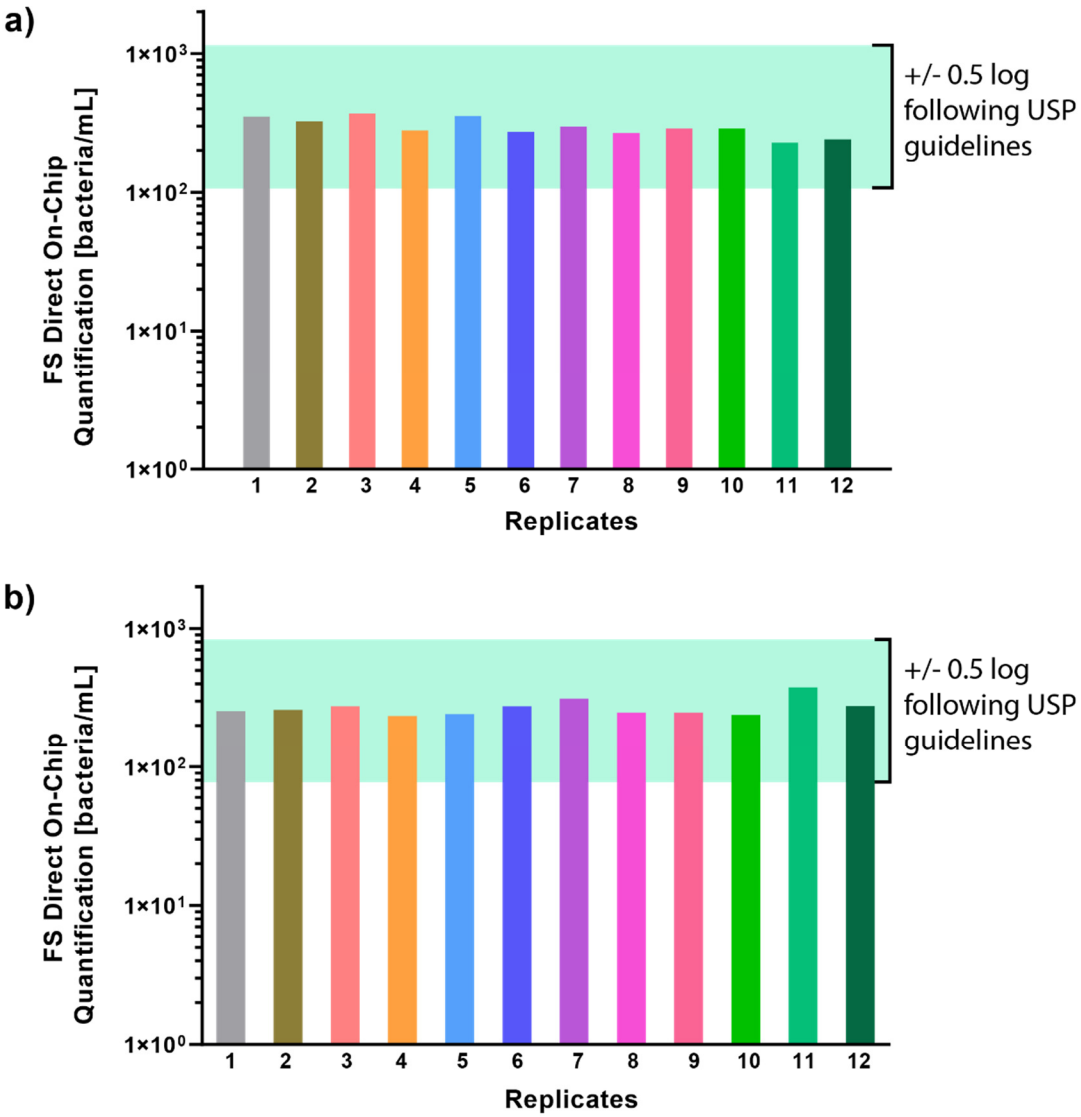

Figure S10. The repeatability of the bacterial capture and quantification on Fluid-Screen system. The Y axis: Total number of counted *E. coli*, X axis: repetitions of the Fluid-Screen capture experiment. The colored bars represent the number of bacteria captured in individual experiment. Fluid-Screen reliably captures bacterial cells, with a very high degree of repeatability. A sample of *E. coli*-8739 was analyzed 24 times in two sets of technical replicates, 12 technical each (a) and (b), demonstrating a high degree of system repeatability. The FS system results meet the +/- 0.5 log standards recommended by the USP for new methods. Note that +/- 0.5 log is calculated for the first grey bar repetition.

The repeatability in enumeration of the Fluid-Screen chips themselves is demonstrated on Figure S11. Eight different chips were used to directly quantify the number of captured *E. coli*-8739. There was no significant difference (ns) between biological replicates. Statistical analysis between the chips is summarized in Table S2. Again, the FS system demonstrated very high repeatability in the capture and quantification of bacteria.

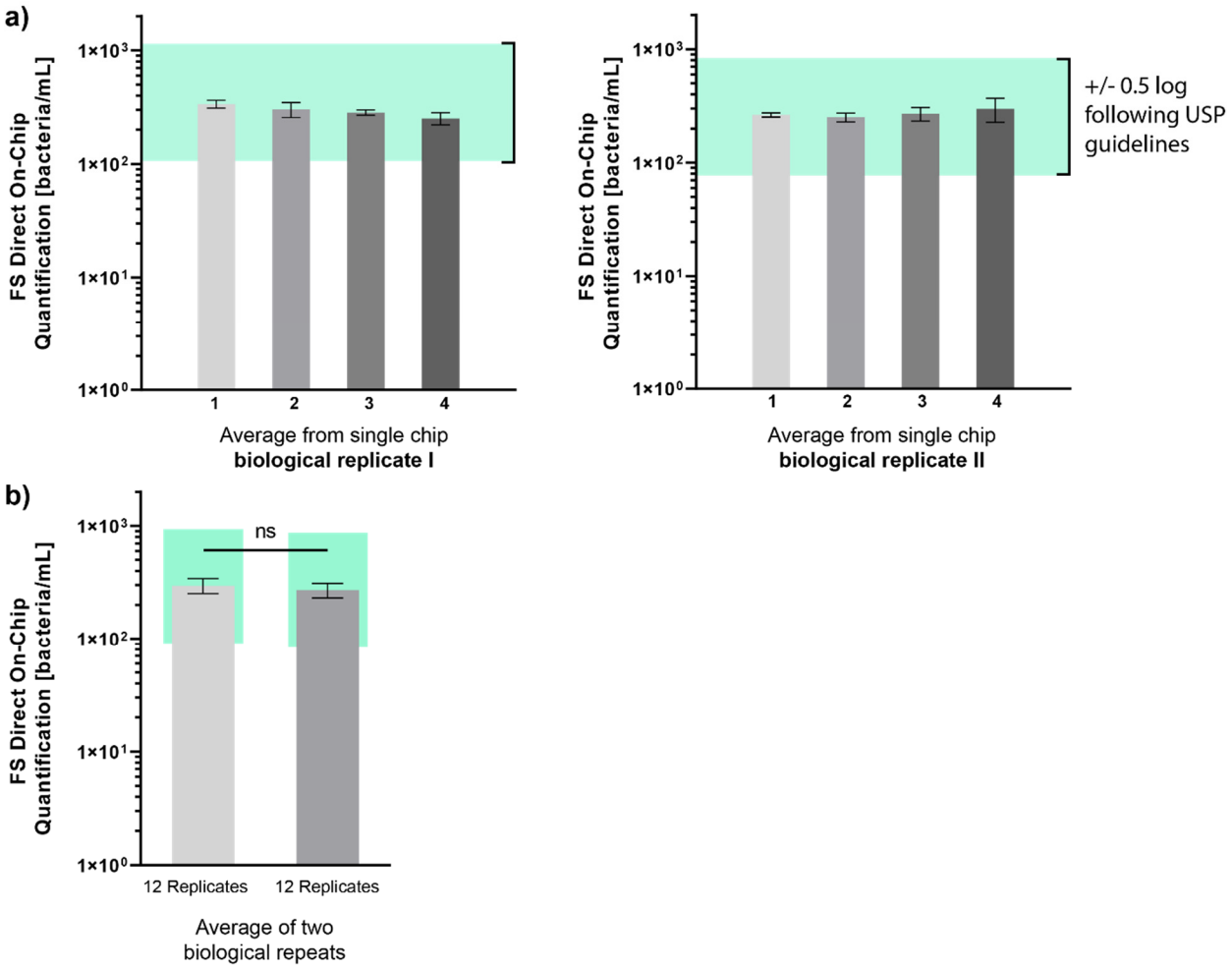

Figure S11. The directly FS-counted number of bacteria quantified with FS system on eight Fluid-Screen chips. **(a)** Each chip was used to count the bacteria three times (each chip has three channels), demonstrating a very high repeatability in chip capture and image processing performance. **(b)** Statistical comparison of two biological repeats, p=0.1438, unpaired t-test with Welch's correction. Turquoise bar represents acceptable growth and viability variance range of +/- 0.5. log recommend by the USP.

Biological replicate 1					
		True number of bacteria	Mean	SD	%CV
Chip 1	Channel 1	350	348.000	23.065	6.628
	Channel 2	324			
	Channel 3	370			
Chip 2	Channel 1	279	302.333	45.709	15.119
	Channel 2	355			
	Channel 3	273			
Chip 3	Channel 1	297	283.667	15.275	5.385
	Channel 2	267			
	Channel 3	287			

Chip 4	Channel 1	287	252.000	31.000	12.302
	Channel 2	228			
	Channel 3	241			
Biological replicate 2					
Chip 1	Channel 1	254	263.333	11.372	4.319
	Channel 2	260			
	Channel 3	276			
Chip 2	Channel 1	235	251.000	21.932	8.739
	Channel 2	242			
	Channel 3	276			
Chip 3	Channel 1	312	269.333	36.950	13.719
	Channel 2	248			
	Channel 3	248			
Chip 4	Channel 1	239	297.333	70.727	23.787
	Channel 2	376			
	Channel 3	277			

Table S2. The statistical analysis of the repeatability of the *E. coli* bacteria capture compared between several Fluid-Screen chips.

Example of microorganisms tested using Fluid-Screen technology

Bacteria (Gram negative)	Type of Respiration	Media	Significance	References/Comments
A. laidlawii	Aerobic	PBS Mammalian cell culture medium	Common contaminant of growth media for cell culture	ATCC-23206-TTR [3]
B. caccae	Anaerobic	PBS	Clinical pathogens	[4]
B. fragilis 25285				
B. thetaiotamicron 29148				
B. vulgatus 8482				
E. coli DH5-α	Aerobic	PBS Fecal	Testing microorganisms	ATCC-67879
E. coli-GFP S06	Aerobic	PBS Mammalian cell cultured medium	Testing microorganisms	ATCC-25922
E. coli	Aerobic	PBS Drug substance (concentrated protein solution)	Tests for microbial contamination	ATCC 8739 [5]
E. coli MC1060/pWTX594	Aerobic	Water from the Charles	Testing microorganisms	[6]

		River in Cambridge, MA		
E. coli-mCherry	Aerobic	PBS	Testing microorganisms	ATCC-MBA303 [6]
L. pneumophila 43109	Anaerobic	DI Ultra-Pure Water	Water Contamination	[7]
M. arginini	Aerobic	PBS	Contamination during manufacturing process (cell therapy, tissue engineered products, cell culture)	ATCC-23838-TTR [8,9]
P. aeruginosa	Aerobic	PBS Drug substance (concentrated protein solution) Mammalian cell culture medium	Tests for microbial contamination	ATCC-9027 [5,8,10]
Bacteria (Gram positive)				
A. kwangyangense	Aerobic	PBS or Water from the Charles River in Cambridge, MA	Isolated from a diesel contaminated costal sites	ATCC-700935 [11]
B. cereus 13061 (including endospores)	Facultative anaerobic	PBS	1. Foodborne pathogen producing toxins, causing two types of gastrointestinal illness: the emetic syndrome and the diarrhoeal syndrome 2. Skin infections – keratitis 3. Non-sterile processing 4. Manufacturing environmental contamination e.g. dust	[12–14]
B. circulans 9500	Aerobic	PBS	Pharmaceutical product contamination	[15]
B. coagulans BAA-738	Aerobic	PBS	Health supplement (probiotic)	www.fda.gov

B. megaterium 14581	Aerobic	PBS	Used as an alternative for high yield intra- and extracellular protein synthesis	ATCC-14581 [16]
B. oleronius 700005	Aerobic	PBS	Establishment of Sterilization Conditions	Guidance on the Manufacture of Sterile Pharmaceutical
B. subtilis AG 147	Aerobic	PBS Fecal	Pharmaceutical product contamination	ATCC [8,10,17,18] Guidance on the Manufacture of Sterile Pharmaceutical
B. subtilis 23857	Aerobic	PBS	Establishment of Sterilization Condition	
B. subtilis 6633	Aerobic	PBS	Establishment of Sterilization Condition	
B. subtilis 6051 (including endospores)	Aerobic	PBS	Pharmaceutical product contamination	
B. subtilis CAL1388	Aerobic	PBS	Testing microorganisms	[6]
C. bolteae BAA-613	Anaerobic	PBS	Microbiome research	ATCC
C. difficile 43598	Anaerobic	PBS	Causes life-threatening diarrhea	www.cdc.gov
C. perfringens 13124	Anaerobic	PBS	Pathogenic spore-forming bacteria, can be found on raw meat and poultry	
C. sporogenes 3584	Anaerobic	PBS	Testing organism Sterility assurance Testing Testing disinfectants Quality control of ENDO-SPOR™ hydrogen peroxide sterilization	1. Applied Biosystems, MicroSeq Pharmaceutical Validation Panel. 2. Supplemental Efficacy: Sterilizers. Washington, DC:Environmental Protection Agency;EPA EPA DIS/TSS-9. 3. Sterilization of single-use medical devices incorporating materials of animal origin--Validation and routine control of sterilization by liquid chemical sterilants. Geneva (Switzerland):International Organization for Standardization/ANSI;ISO ISO 14160:1998.

				4. Sterilization of single-use medical devices incorporating materials of animal origin --- Validation and routine control of sterilization by liquid chemical sterilants, Annex A. London, UK:British Standards Institution;British Standard BS EN ISO 14160:1998.
E. faecalis 19433	Aerobic	PBS Fecal	Testing organism Media testing Quality control strain Reference material	ATCC
E. faecalis 47077	Aerobic	PBS	Testing organism	ATCC
P. acnes 11827 B	Aerotolerant anaerobic	PBS	Human skin commensal, can be involved in the pathogenesis of acne	[19]
S. aureus	Aerobic	PBS Drug substance (concentrated protein solution) Mammalian cell culture medium	Tests for microbial contamination	ATCC-6538 [5,10]
Fungus/Yeast/Molds				
A. brasiliensis	Aerobic	PBS Drug substance (concentrated protein solution) CHO cultured media	Tests for microbial contamination	ATCC-16404 [8,10]
C. albicans	Aerobic		Tests for microbial contamination	ATCC-10231 [5,8,10]
S. cerevisiae	Aerobic	PBS	Essential to winemaking Foodborne spoilage microorganisms in commercial and fresh fruit juices	[20,21]
Virus				
Human adenovirus 5 VR-5/Adenoid 75	N/A	PBS Mammalian cells cultured medium	Viruscide testing Respiratory research	ATCC-VR-5_70010153

Mammalian cells				
Chinese Hamster Ovary (CHO)	5% CO_2	PBS Mammalian cell cultured medium	Mammalian cells for biopharmaceutical production (e.g. antibody)	[22,23]
Human cells				
Red blood cells	N/A	PBS	Medical testing	

Table S3. The diversity of selected microorganisms captured by Fluid-Screen system.

3. Supplementary References

1. Choi J-W, Rosset S, Niklaus M, Adleman JR, Shea H, Psaltis D. 3-dimensional electrode patterning within a microfluidic channel using metal ion implantation. *Lab Chip*. 2010;10(6):783-788.

2. McDonald JC, Duffy DC, Anderson JR, et al. Fabrication of microfluidic systems in poly (dimethylsiloxane). *Electrophor An Int J*. 2000;21(1):27-40.

3. Pharmacopeia US. 63—Mycoplasma Tests: A New Regulation for Mycoplasma Testing. USP 33/NF 28. In: *Usp Pharmacists' Pharmacopeia; United States Pharmacopeial Convention: Rockville, MD, USA*. ; 2010:S3.

4. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev*. 2007;20(4):593-621.

5. Pharmacopeia US. 62—Microbiological Examination of Nonsterile Products: Tests For Specified Microorganisms. USP 32. In: *Usp Pharmacists' Pharmacopeia; United States Pharmacopeial Convention: Rockville, MD, USA*. ; 2009:S3.

6. Weber M, Markewich H, Mallick E, Schiltz M, Simon X. Broad-Range Bacterial Capture from Fluid-Samples: Implications for Amplification-Free Contamination Detection. *Sensors & Transducers*. 2016;203(8):40.

7. Oliva G, Sahr T, Buchrieser C. The life cycle of L. pneumophila: cellular differentiation is linked to virulence and metabolism. *Front Cell Infect Microbiol*. 2018;8:3.

8. Pharmacopeia US. 71—Sterility Tests / Microbiological Tests. USP 35. In: *Usp Pharmacists' Pharmacopeia; United States Pharmacopeial Convention: Rockville, MD, USA*. ; 2009:S3.

9. Duguid J, Kielpinski G, du Moulin GC, Seymour B. Application of a Risk-Based Approach to Optimize a Rapid Mycoplasma Test for Cell Therapy and Tissue-Engineered Products. In: *AAPS Annual Meeting and Exposition*. Atlanta, GA; 2008.

10. Pharmacopeia US. 61—Microbiological examination of nonsterile products: Microbial enumeration tests. USP 31/FN26. In: *Usp Pharmacists' Pharmacopeia; United States Pharmacopeial Convention: Rockville, MD, USA*. ; 2009:S3.

11. Kuczenski RS, Chang H-C, Revzin A. Dielectrophoretic microfluidic device for the continuous sorting of Escherichia coli from blood cells. *Biomicrofluidics*. 2011;5(3):32005.

12. Asaeda G, Caicedow G, Swanson C. Fried rice syndrome. *JEMS a J Emerg Med Serv*. 2005;30(12):30.

13. Pinna A, Sechi LA, Zanetti S, et al. Bacillus cereus keratitis associated with contact lens wear. *Ophthalmology*. 2001;108(10):1830-1834.

14. Payne DN. Microbial ecology of the production process. *Guid to Microbiol Control Pharm Med Devices*. 2006;482.

15. FDA. Kingston Pharma, LLC RECALLS " DG™/Health NATURALS Baby Cough Syrup + Mucus" Because of Possible Health Risk. Center for Drug Evaluation and Research. https://tinyurl.com/yyupko3t. Published 2019.

16. Stammen S, Müller BK, Korneli C, et al. High-yield intra-and extracellular protein production using Bacillus megaterium. *Appl Environ Microbiol*. 2010;76(12):4037-4046.

17. PDA (Parenteral Drug Association). Technical Report No. 67: exclusion of objectionable microorganisms from nonsterile pharmaceuticals, medical devices, and cosmetics. 2014.

18. Cundell T. Exclusion of Objectionable Microorganisms from Non-sterile Pharmaceutical Drug Products. *Pharm Microbiol Qual Assur Control Pract Guid Non-Sterile Manuf*. 2019:371-400.

19. McLaughlin J, Watterson S, Layton AM, Bjourson AJ, Barnard E, McDowell A. Propionibacterium acnes and acne vulgaris: new insights from the integration of population genetic, multi-omic, biochemical and host-microbe studies. *Microorganisms*. 2019;7(5):128.

20. Mendoza LM, Fernandez de Ullivarri M, Raya RR. Saccharomyces cerevisiae: A key yeast for the wine-making process. 2018.

21. Brice C, Cubillos FA, Dequin S, Camarasa C, Martinez C. Adaptability of the Saccharomyces cerevisiae yeasts to wine fermentation conditions relies on their strong ability to consume nitrogen. *PLoS One*. 2018;13(2):e0192383.

22. Shin SW, Lee JS. CHO Cell Line Development and Engineering via Site-specific Integration: Challenges and Opportunities. *Biotechnol Bioprocess Eng*. 2020:1-13.

23. Scarcelli JJ, Shang TQ, Iskra T, Allen MJ, Zhang L. Strategic deployment of C HO expression platforms to deliver Pfizer's Monoclonal Antibody Portfolio. *Biotechnol Prog*. 2017;33(6):1463-1467.