

Direct detection and drug-resistance profiling of bacteremias using inertial microfluidics

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Supplementary information

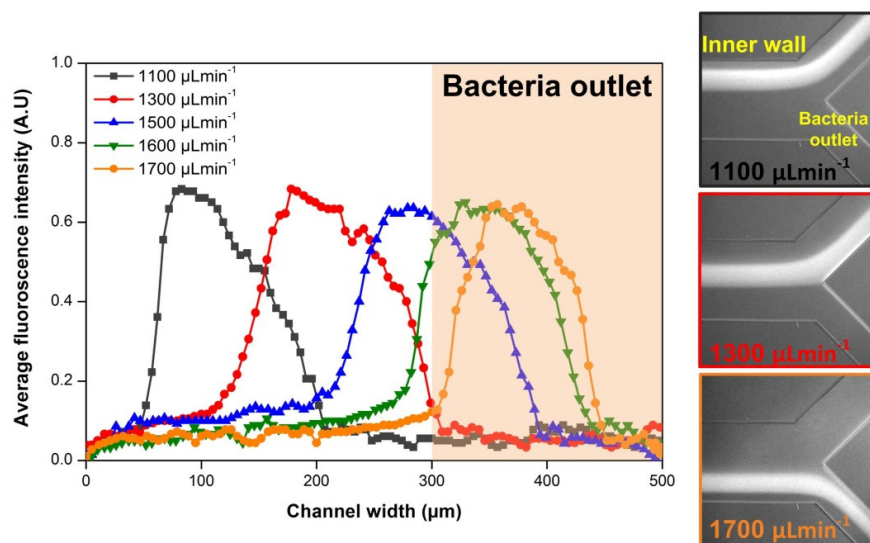


Figure S1 Average fluorescence intensity linescans indicating normalized distribution of FITC-conjugated *E. coli* bioparticles across the channel width (x-axis origin represents inner wall) prior the outlet bifurcation at different flow rates. Approximate position of the bacteria outlet (outer wall region) is shown on the plot. Corresponding average composite images illustrate efficient collection of bacteria into bottom (bacteria) outlet at a flow rate of 1700 μLmin^{-1} .

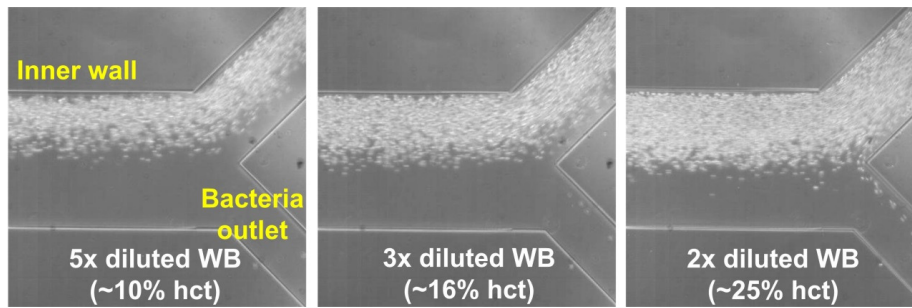


Figure S2 High speed images indicate broadening of RBCs occupied regions at the channel inner wall for increasing hematocrit (hct) prior outlet bifurcation at a sample flow rate of $150 \mu\text{Lmin}^{-1}$ ($1500 \mu\text{Lmin}^{-1}$ for sheath flow (1:10)). 3x diluted whole blood (WB) was chosen for subsequent experiments which resulted in minimal RBCs entering the bacteria outlet (bottom).

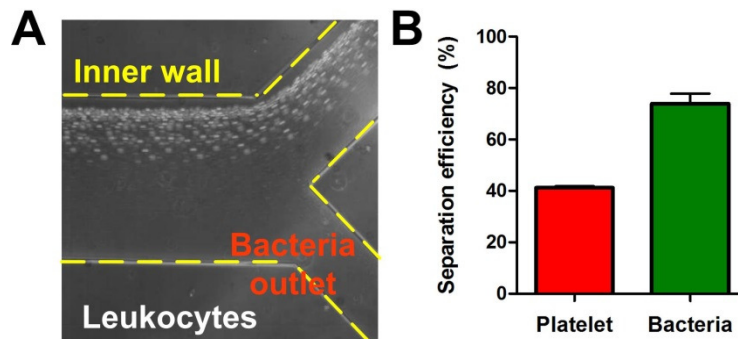


Figure S3 (A) Averaged composite high speed image represents the tight focusing of larger leukocytes at the inner wall which resulted in complete removal at the bacteria outlet. Yellow dashed lines indicate position of channel walls. (B) Histogram plot showing efficient bacteria recovery (>70%) with lower platelet recovery (~40%) in the sorted samples collected from the bacteria outlet.

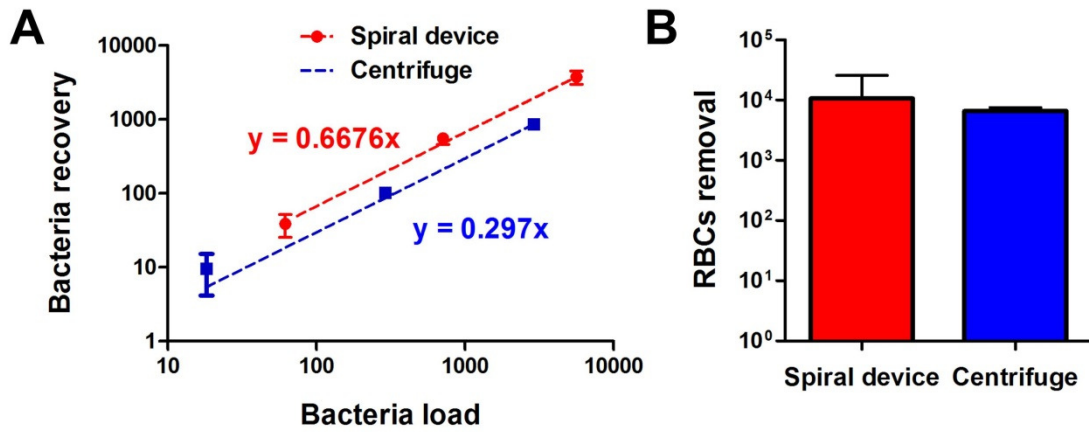


Figure S4 Separation performance comparison between spiral device and centrifuge. **(A)** Higher bacteria recovery was achieved with the spiral device over a range of bacterial loads (*E. coli*) spiked in 1 mL of whole blood. **(B)** Histogram plot indicating similar RBCs removal efficiency ($\sim 10^4$ -fold) for both spiral device and centrifuge.

Figure S5. rRNA probes for specific detection of *P. aeruginosa* (PsA), *E. coli* (Ec), and *K. pneumoniae* (Kp). Probe targets are highlighted in multiple sequence alignments of (A) 16S and (B) 23S rRNA subunits from these three species. Green = PsA-specific probe; Blue = Ec-specific probe; Red = Kp-specific probe; Purple = dual-specificity probe for either Ec or Kp but not PsA. Mismatches in other species corresponding to the region recognized by each probe are shown in lowercase letters; asterisks below the alignment correspond to regions of conservation across the three species. Specific probe sequences designed to target these regions are shown in supplementary Table S1.

A.

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PsA_16S -----AACGAGAGGTTGCTGTAGAATGCGCGCCTC
Ec_16S  AGAAAAATCCTGAAATTCAGGGTTGACTCTGAAAGAGGAAAGCGTAATATACGCCACCTC
Kp_16S  -AGAAAAATCCTGAAAAACGGGTTGACTCTGAAAGAGGATAGCGTAATATACGCCACCTC
                *****      ***  **  ***  ****

PsA_16S  GGT--GAGACGAAAGCCTTGACCAACTGCTCTTTAACAAGTCGAATCAAGCAATTCGT
Ec_16S  GGCAGAGTGCCTAAAGCGCGTCGCAACTGCTCTTTAACAATTTATCA--GACAATCTGT
Kp_16S  GGCAGAGAGCGCTAAAGCGCGTCGCAACTGCTCTTTAACAATTTATCA--GACAATCTGT
                *      *  *****      ***** *      ****  **

PsA_16S  GTGGGTGCTTGTGATGTAAGACTGGTGATCGCAAGATTATCAGCAACGCAAGTAACACTC
Ec_16S  GTGGGCACTCGAAG---ATAC-----GGATTCTTAACG-----T-
Kp_16S  GTGGGCACTCAAAGTG--ACAT-----GGATTCTTAACG-----T-
                *****  **      *  *      ***** *  *  *      *

PsA_16S  GTGAATTCGAGAGTTTTATCTCTTTTAAAGAGAATGCGATTGCTGAGCCAAGTTTAGGGT
Ec_16S  CGCAAGACGAAAAATGAATACCAAGTCTCAAGAGTGAACACGTAATTCATTACGAAGCTT
Kp_16S  CCTCGGACGAAAAATGAATACCAAGTCTCAAGAGTGAACACGTAATTCATTACGAAGTTT
                *** *  *  ** *  *      *** **      *      *      ** *

PsA_16S  TTTCTCAAACCCAAGCAGTATTGAACTGAAGAGTTTGATCATGGCTCAGATTGAACGCT
Ec_16S  AATCTTTGAGCATCAAACCTTTAAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCT
Kp_16S  AATCTTTGAGCATCAAACCT--TAAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCT
                *      *  *      *  *  *  *****

PsA_16S  GGCGGCAGGCCTAACACATGCAAGTCGAgCGGatgaAGGgAGCtt-----GCTcCtgga
Ec_16S  GGCGGCAGGCCTAACACATGCAAGTCGAAGTCGAAGGTAACAGGAAGCAGCTTGCTGCTTCGCTG
Kp_16S  GGCGGCAGGCCTAACACATGCAAGTCGAgCGGTAgCAcagAGagc--TtgctCTcgGgTG
                ***** *****  ***      *      **      **

PsA_16S  accAGcGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCC
Ec_16S  ACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACT
Kp_16S  ACGAGcGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACT
                ** ***** ** ***** ***** *  * ***** *

PsA_16S  GGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGATCTTCGGACCTC
Ec_16S  GGAAACGGTAGCTAATACCGCATAAcgTCgcAAGaccAAAGaGGGGGAcCTTCGGgCCTC
Kp_16S  GGAAACGGTAGCTAATACCGCATAAatgTCgcAAGaccAAAGTGGGGGAcCTTCGGgCCTC
                ***** ***** *  *  *      *** ***** ***** ***

PsA_16S  ACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGC
Ec_16S  ttGCcATCGGATGtGCCcAGaTgGGATTAGCTtGTTGGTGGGGTAAcGGctcACCAAGGC
Kp_16S  AtGCcATCAGATGtGCCcAGaTgGGATTAGCTAGTAgGTGGGGTAAcGGctcACctAGGC
                ** *** ** ** ** * ***** ** ***** ** ** ** **

PsA_16S  GACGATCCGTAACCTGGTCTGAGAGGATGATCAGTCACACTGGAAGTGGAGACACGGTCCAG
Ec_16S  GACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAGTGGAGACACGGTCCAG
Kp_16S  GACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAGTGGAGACACGGTCCAG
                ***** ** ***** ***** ***** *****

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PsA_16S ACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCA
Ec_16S ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCA
Kp_16S ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCA

PsA_16S TGCCCGGTGTGTGAAGAAGGCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCA
Ec_16S TGCCCGGTGTGTGAAGAAGGCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAG
Kp_16S TGCCCGGTGTGTGAAGAAGGCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGT

PsA_16S GTAAGTTAATAACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACC GGCTAACTTCGTG
Ec_16S TAAAGTTAATAACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACC GGCTAACTCCGTG
Kp_16S TAAGTTAATAACCTTGCGGCTTACGTTACCCGCAGAAGAAGCACC GGCTAACTCCGTG
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Kp_16S CCAGCAGCCGGTAATACGAAGGTTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCG

PsA_16S CGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATC
Ec_16S CACGCAGGCGGTTTGTAAAGTCAAGTGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATC
Kp_16S CACGCAGGCGGTTCTGTCAAGTCGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATT
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PsA_16S CAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAAT
Ec_16S TGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGTAGAATTCAGGTGTAGCGGTGAAAT
Kp_16S CGAAACTGGCAGGCTAGAGTCTGTAGAGGGGGTAGAATTCAGGTGTAGCGGTGAAAT
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PsA_16S GCGTAGATATAGGAAGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACT
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Kp_16S GCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGGACAAAGACTGACGCT

PsA_16S GAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCGTAAAC
Ec_16S CAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCGTAAAC
Kp_16S CAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCGTAAAC

PsA_16S GATGTGCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGAGCTAACGCGATAAGTGC
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Kp_16S GATGTGCGATTTGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAATGC

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Ec_16S ACCGCTGGGGAGTACGGCCGCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCGCACA
Kp_16S ACCGCTGGGGAGTACGGCCGCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCGCACA

PsA_16S AGCGGTGGAGCATGTGGTTTTAATTCGAaGCAACGCGAAGAACCCTTACCTGGcCTTGACAT
Ec_16S **AGCGGTGGAGCATGTGGTTTTAATTCGATGCAACGCGAAGAACCCTTACCTGGTCTTGACAT**
Kp_16S AGCGGTGGAGCATGTGGTTTTAATTCGATGCAACGCGAAGAACCCTTACCTGGTCTTGACAT

PsA_16S gCtgaGAACCTTcCAGAGATGgattgGTGCCTTCGGGAACCTCAGACACAGGTGCTGCATG
Ec_16S **CCACGGAAGTTTTcCAGAGATGGAATGTGCCTTCGGGA**ACCGTgAGACAGGTGCTGCATG
Kp_16S CCACaGAACCTTagCAGAGATGctttgGTGCCTTCGGGAACCTGTGAGACAGGTGCTGCATG
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PsA_16S GCTGTGCTCAGCTCGTGTGCTGAGATGTTGGTTAAGTCCCCTAACGAGCGCAACCCTTG
Ec_16S GCTGTGCTCAGCTCGTGTGCTGAAATGTTGGTTAAGTCCCCTAACGAGCGCAACCCTTA
Kp_16S GCTGTGCTCAGCTCGTGTGCTGAAATGTTGGTTAAGTCCCCTAACGAGCGCAACCCTTA

PsA_16S TCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAG
Ec_16S TCCTTTGTTGCCAGCGGTCCGGCCGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAG
Kp_16S TCCTTTGTTGCCAGCGGTTCGGCCGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAG
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PsA_16S GAAGGTGGGGATGACGTCAA**GTATCATGGCCCTTACGGCCAGGGCTACACACGTGCTAC**
Ec_16S GAAGGTGGGGATGACGTCAAAGTCATCATGGCCCTTACGaCCAGGGCTACACACGTGCTAC
Kp_16S GAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGaCCAGGGCTACACACGTGCTAC

PsA_16S **AATGGTTCGGTACAAAGGTTGCCAAGCCGCGAGGTGGAGCTAATCCATAAAACCGATCG**
Ec_16S AATGGcgcataCAAAAGaGaaGCgAcctCGCGAGagcaAGCggAcCtCATAAAgtgCGTCG
Kp_16S AATGGcataTACAAAGaGaaGCgAcctCGCGAGagcaAGCggAcCtCATAAAgtatgTCG
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PsA_16S TAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAA
Ec_16S TAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGA
Kp_16S TAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGA
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PsA_16S TCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG
Ec_16S TCAGAATGCCACG**GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG**
Kp_16S TCAGAATGCTACG**GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG**
***** * * * * * ***** * * * * * ***** * * * * * *****

PsA_16S AGTGGGTTGctccAGAAGTAGctAGtctAACCGcaGGgGgaCGgTTACCACgGAGTGAT
Ec_16S **AGTGGGTTGCAAAAGAAGTAGTACGTTAACCTTCGGGAGGGCGCTTACCACT**TTGTGAT
Kp_16S **AGTGGGTTGCAAAAGAAGTAGTACGTTAACCTTCGGGAGGGCGCTTACCACT**TTGTGAT
***** * * * * * ***** ** * * * * * ***** ** * * * *****

PsA_16S TCATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGGGAACCTGCGGCTGGATCACCT
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Ec_16S CCTTACCTTAAAGAAGCGTCTTTGAAGTGCTCACACAGATTGTCTGATGAAAATGAGAA
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PsA_16S CTGGTTAGACGATTGGGTCTGTAGCTCAGTTGGTTAGAGCGCACCCCTGATAAGGGTGAG
Ec_16S GT-AAAACCTCTACAGGCTTGTAGCTCAGTTGGTTAGAGCGCACCCCTGATAAGGGTGAG
Kp_16S GTAAAACCTCTACAGGCTTGTAGCTCAGTTGGTTAGAGCGCACCCCTGATAAGGGTGAG
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PsA_16S GTCGGCAGTTCGAATCTGCCAGACCCACCAATTGTTGGTGTGCTG-----
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Kp_16S GTCGGTGGTTCAGTCCACTCAGGCTACCAAATTTGCGAAGCAAATTTGAAGAGGTTGC
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Ec_16S AACTACATGTTATGGGGCTATAGCTCAGCTGGGAGAGCGCCTGCTTTGCACGCAGG----
Kp_16S AAA-----CGATGGGGCTATAGCTCAGCTGGGAGAGCGCCTGCTTTGCACGCAGGAGGT
* * * * * *****

PsA_16S CAGGAGTTCGATC
Ec_16S -----
Kp_16S CTGCGG-----

PsA_23S GACTGGAGGACCGAACCCACTCCCCTTAAAAAGGTAGGGGATGACTTGTGGATCGGAGTG
Ec_23S AACTGGAGGACCGAACCCACTAATGTTGAAAAATTAGCGGATGACTTGTGGCTGGGGGTG
Kp_23S AACTGGAGGACCGAACCCACTAATGTTGAAAAATTAGCGGATGACTTGTGGCTGGGGGTG
***** ** ***** **

PsA_23S AAAGGCTAATCAAGCTCGGAGATAGCTGGTTCTCCTCGAAAGCTATTTAGGTAGCGCCTC
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***** ** *****

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Kp_23S GTGAATTCATCTCCGGGGGTAGAGCACTGTTTCGGCTAGGGGGTCATCCCGACTTACCAA
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Kp_23S CCCGATGCAAACCTACGAATACCCGAAGAATG-TTATCACGGGAGACACACGGCGGGTGCTA
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PsA_23S ACGTCCGTCGTGAAAAGGGAAACAACCCAGACCAGCTAAGGTCCCAAAGTTGTGGTT
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Kp_23S ACGTCCGTCGTGAAGAGGGAAACAACCCAGACCAGCTAAGGTCCCAAAGTCATGGTT
***** ***** *****

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Kp_23S AAGTGGGAAACGATGTGGGAAGGCACAGACAGCCAGGATGTTGGCTTAGAAGCAGCCATC
***** ***** ***** ***** *****

PsA_23S CTTTAAAGAAAGCGTAATAGCTCACTAGTCGAGTCGGCCTGCGCGGAAGATGTAACGGGG
Ec_23S ATTTAAAGAAAGCGTAATAGCTCACTGGTCGAGTCGGCCTGCGCGGAAGATGTAACGGGG
Kp_23S ATTTAAAGAAAGCGTAATAGCTCACTGGTCGAGTCGGCCTGCGCGGAAGATGTAACGGGG
***** ***** *****

PsA_23S CTCAAACCACACACCGAAGC--TGCGGGTGTACGTAAGTGACGCGGTAGAGGAGCGTTC
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Kp_23S CTA AACCATGCACCGAAGCTGCGGCAGCGACACTATGTGTTGTTGGGTAGGGAGCGTTC
** ** * * * ** * ** *****

PsA_23S TGTAAGCCTGTGAAGGTGaGtTgAaGcTtGCTGAGGTATCAGAAGTGCGAATGCTGA
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***** ***** * ** ** *****

PsA_23S CATgAGTAACGAcAAtGgGtGTGAAAAaCaCcCaCGCCGaAAGACCAAGGGTTCCTGCGC
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Kp_23S CATAAGTAACGATAAAGCGGGTGAAAAGCCCGCTCGCCGGAAGACCAAGGGTTCCTGTCC
*** ***** ** * * ***** * * *****

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Kp_23S AACGTTAATCGGGGCAGGGTGTAGTCGACCCCTAAGGCGAGGCCGAAAGGCGTAGTCGATG
***** ***** ***** ***** *****

PsA_23S GGAAACAGGTTAATATTCTGTACTTCTGGTTACTGCGATGGAGGGACGGAGAAGGCTAG
Ec_23S GGAAACAGGTTAATATTCTGTACTTggtGTTACTGCGAaGgGGGACGGAGAAGGCTAT
Kp_23S GGAAACAGGTTAATATTCTGTACTTggtGTTACTGCGAaGgGGGACGGAGAAGGCTAT
***** ***** ** *****

PsA_23S GCCAGCTTGGCGTTGGTTGTCCAAGTTTAAAGTGGTAGGCTGAAATCTTAGGTAATCCG
Ec_23S GttGGCcgGGCGacGgTtGTCCcgGTTTAAGcgtGTAGGCTGgTtTtcTAGGCAAATCCG
Kp_23S GttAGCcgGGCGacGgTtGTCCcgGTTTAAGcatGTAGGCTGatGtCcAGGCAAATCCG
* ** ** ***** ***** ***** ***** *****

PsA_23S GGGTTTCAAGGCCGAGAGCTGATGACGAGTCGTCTTTTAGATGACGAAGTGTTGATGCC
Ec_23S GAAAATCAAGGCTGAGGCGTGATGACGAGGCA---CTACGGTGCTGAAGCAACAAATGCC
Kp_23S GATAATCAAGGCTGAGGTGTGATGACGAGGCA---CTACGGTGCTGAAGTAACAAATGCC
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Ec_23S CTGCTTCCAGGAAAAGCCTCTAAGCATCAGGTAACATCAAATCGTACCCCAACCGACAC
Kp_23S CTGCTTCCAGGAAAAGCCTCTAAGCATCAGGTAACATCAAATCGTACCCCAACCGACAC
***** ***** ***** ***** ** *****

PsA_23S AGGTGGTCGGGTAGAGAATACCAAGGCGCTTGAGAGAACTCGGGTGAAGGAACTAGGCAA
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Kp_23S AGGTGGTCAGGTAGAGAATACCAAGGCGCTTGAGAGAACTCGGGTGAAGGAACTAGGCAA

PsA_23S AATGGCACCGTAACTTCGGGAGAAGGTGCGCCGGCTAGGGTGAAG---GATTTACTCCGT
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Kp_23S AATGGTGCCGTAACCTTCGGGAGAAGGCACGCTGGTGTGTAGGTGAAGTCCCT-GCGGATG
***** ***** ** * * * * *

PsA_23S AAGCTCTGGCTGGTCAAGATACCAGGCCGTGCGACTGTTTATTA AAAACACAGCACTG
Ec_23S GAGCTGAAATCAGTCAAGATACCAGCTGGCTGCAACTGTTTATTA AAAACACAGCACTG
Kp_23S GAGCTGAGACCAGTCAAGATACCAGCTGGCTGCAACTGTTTATTA AAAACACAGCACTG
*** ***** ***** *****

PsA_23S TGCAAACACGAAAGTGGACGTATAGGGTGTGACGCCTGCCCGGTGCCGGAAGGTTAATTG
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Kp_23S TGCAAACACGAAAGTGGACGTATACGGTGTGACGCCTGCCCGGTGCCGGAAGGTTAATTG

PsA_23S ATGGGGTTAGCGC---AAGCGAAGCTCTTGATCGAAGCCCCGGTAAACGGCGGCCGTAAC
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Kp_23S ATGGGGTTATCCGTAAGGAGAAGCTCTTGATCGAAGCCCCGGTAAACGGCGGCCGTAAC
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Ec_23S ATAACGGTCCTAAGGTAGCGAAATTCCTTGTGCGGGTAAGTCCGACCTGCACGAATGGCG
Kp_23S ATAACGGTCCTAAGGTAGCGAAATTCCTTGTGCGGGTAAGTCCGACCTGCACGAATGGCG

PsA_23S TAACGATGGCGCGCTGTCTCCACCCGAGACTCAGTGA AATTGAAATCGCTGTGAAGATG
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Kp_23S TAATGATGGCCAGGCTGTCTCCACCCGAGACTCAGTGA AATTGAAATCGCTGTGAAGATG
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PsA_23S CAGTGTATCCGCGGCTAGACGGAAGACCCCGTGAACCTTTACTGTAGCTTTGCACTGGA
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Kp_23S CAGTGTACCCGCGCAAGACGGAAGACCCCGTGAACCTTTACTATAGCTTGACACTGAA
***** ***** ***** ***** *****

PsA_23S CTTT GAGCCTGTGTGTAGGATAGGTGGGAGGCTTTGAAGCGTGGACGCCAGTTTCGCGT
Ec_23S CATTGAGCCTGTGATGTGTAGGATAGGTGGGAGGCTTTGAAGTGTGGACGCCAGTTTCGCGT
Kp_23S CATTGAGCCTGTGATGTGTAGGATAGGTGGGAGGCTTTGAAGCGTGGACGCCAGTTTCGCGT
* ***** ***** ***** ** *

PsA_23S GGAGCCATCCTTGAAATACCACCCTGGCATGCTTGAGGTTCTAACTCTGGTCCGTAATCC
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Kp_23S GGAGCCAACCTTGAAATACCACCCTTTAATGTTTGTGATGTTCTAACGTTGGCCCTGACCC
***** ***** ** * ***** ** * * * *

PsA_23S GGATCGAGGACAGTGTATGGTGGGAGTTTACTGGGCGGTCTCCTCCTAAAGAGTAAC
Ec_23S GGGTTGCGGACAGTGTCTGGTGGGTAGTTTACTGGGCGGTCTCCTCCTAAAGAGTAAC
Kp_23S GGGTTGCGGACAGTGTCTGGTGGGTAGTTTACTGGGCGGTCTCCTCCTAAAGAGTAAC
** * * ***** ***** ***** ***** *****

PsA_23S GGAGGAGTACGAAGGTGCGCTCAGACCGGTCCGAAATCGGTTCGAGAGTATAAAGGCAAA
Ec_23S GGAGGAGCACGAAGGTTGGCTAATCTGGTCCGACATCAGGAGGTTAGTGCATGGCATA
Kp_23S GGAGGAGCACGAAGGTTAGCTAATCTGGTCCGACATCAGGAGGTTAGTGCATGGCATA

PsA_23S AGCGCGCTTACTGCGAGACAGACACGTCGAGCAGGTACGAAAGTAGGTCTTAGTGATCC
Ec_23S AGCCAGCTTACTGCGAGCGTGACGGCGCGAGGTGCGAAAGCAGGTTCATAGTGATCC
Kp_23S AGCTAGCTTACTGCGAGCGTGACGGCGCGAGCAGGTGCGAAAGCAGGTTCATAGTGATCC

PsA_23S GGTGGTTCTGTATGGAAGGGCCATCGCTCAACGGATAAAAGGTAAGTACTCCGGGGATAACAGG
Ec_23S GGTGGTTCTGAATGGAAGGGCCATCGCTCAACGGATAAAAGGTAAGTACTCCGGGGATAACAGG
Kp_23S GGTGGTTCTGAATGGAAGGGCCATCGCTCAACGGATAAAAGGTAAGTACTCCGGGGATAACAGG

PsA_23S CTGATACCGCCCAAGAGTTCATATCGACGGCGGTGTTTGGCACCTCGATGTCGGCTCATC
Ec_23S CTGATACCGCCCAAGAGTTCATATCGACGGCGGTGTTTGGCACCTCGATGTCGGCTCATC
Kp_23S CTGATACCGCCCAAGAGTTCATATCGACGGCGGTGTTTGGCACCTCGATGTCGGCTCATC

PsA_23S ACATCTGGGGCTGAAGCCGGTCCCAAGGGTATGGCTGTTCCGCAATTTAAAGTGGTACGC
Ec_23S ACATCTGGGGCTGAAGTAGGTCCCAAGGGTATGGCTGTTCCGCAATTTAAAGTGGTACGC
Kp_23S ACATCTGGGGCTGAAGTAGGTCCCAAGGGTATGGCTGTTCCGCAATTTAAAGTGGTACGC

PsA_23S GAGCTGGGTTTAGAACGTCGTGAGACAGTTCGGTCCCTATCTG**CCGTGGACGTTTGAGAT**
Ec_23S GAGCTGGGTTTAGAACGTCGTGAGACAGTTCGGTCCCTATCTGCCGTGGgCGcTgGAGAA
Kp_23S GAGCTGGGTTTAGAACGTCGTGAGACAGTTCGGTCCCTATCTGCCGTGGgCGcTgGAGAA

PsA_23S **TTGAGAGGGGCTGCTCCTAGTACGAGAGGACCGGAGTGGACGAACCTCTGGTGTTCGGT**
Ec_23S cTGAGgGGGGCTGCTCCTAGTACGAGAGGACCGGAGTGGACGcAtCaCTGGTGTTCgGGT
Kp_23S TTGAGgGGGGCTGCTCCTAGTACGAGAGGACCGGAGTGGACGcAtCaCTGGTGTTCgGGT

PsA_23S **TGTCACGCCAGTGGCATTGCCGG**GTAGCTATGTTCCGAAAAGATAACCGCTGAAAGCATC
Ec_23S TGTCAtGCCAaTGGCAcTGCCcGGTAGCTAAATGCGGAAGAGATAAGTGTGAAAGCATC
Kp_23S TGTCAtGCCAaTGGCAcTGCCcGGTAGCTAAATGCGGAAGAGATAAGTGTGAAAGCATC

PsA_23S TAAGCGGAAACTTGCCCTCAAGATGAGATCTCACTGGGAacTTgAtTCcCCTGAAGGgcC
Ec_23S TAAGCACGAAACTTGCCCCGAGATGAGTTCTCCCTGA**ctCcTTgAGagTCCTGAAGGAAC**
Kp_23S TAAGCACGAAACTTGCCCCGAGATGAGTTCTCCCTGA**GACTTTAAGTCTCCTGAAGGAAC**

PsA_23S GTcgAAGACtAcGACGTTGATAGGcTGGGTGTGTAAGCGttGTGAgGCGTTGAGCTAACC
Ec_23S **GTTgAAGACGAcGACGTTGATAGGCCGGGTGTGTAAGCGCAGCGATGCGTTGAGCTAACC**
Kp_23S **GTTAAAGACGATGACGTTGATAGGCCGGGTGTGTAAGCGCAGCGATGCGTTGAGCTAACC**

PsA_23S aGTACTAATtgcCCGTGAGGCTTGACCAT--
Ec_23S **GGTACTAATGAACCGTGAGGCTTAACCTT--**
Kp_23S **GGTACTAATGAACCGTGAGGCTTAACCTTAC**

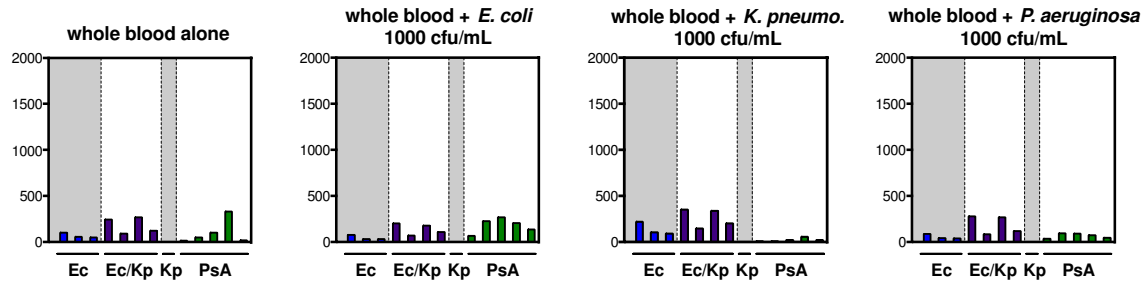
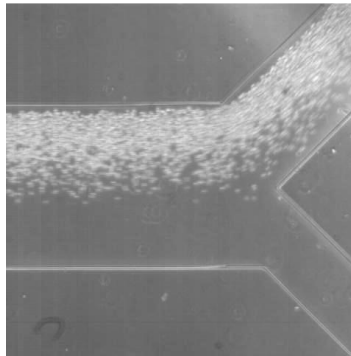


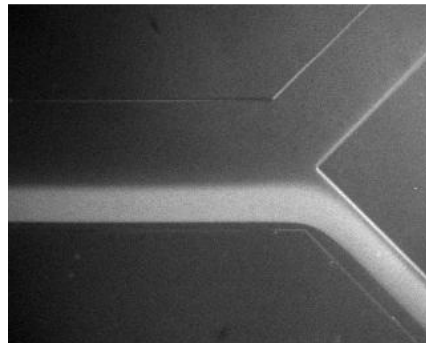
Figure S6 Similar background transcriptional signals from nCounter probes between whole blood and whole blood spiked with 1000 cfu/mL for different bacteria strains.

SI Movie Legends



Movie S1

High speed video (6400 fps) illustrating inertial focusing of RBCs (3x diluted whole blood, ~15% hct) at the inner channel wall at DC 1 and continuous collection into the waste outlet (upper).



Movie S2

Fluorescent video illustrating focusing of Alexa Fluor® 488 *E. coli* (K-12 strain) BioParticles® conjugates spiked in PBS at the outer wall at DC 1 and collection into the bacterial outlet (bottom).

Table S1. Sequences for rRNA probes targeting the 16S or 23S rRNA subunits from *E. coli*, *K. pneumoniae*, or *P. aeruginosa*. Nucleotide positions are relative to the annotated reference genome sequences of *E. coli* strain EcHS, *K. pneumoniae* strain MGH78578, and *P. aeruginosa* strain PAO1, respectively, from the NCBI database.

PROBE	SPECIES RECOGNIZED	GENE	NUCLEOTIDES	PROBE 1 SEQUENCE	PROBE 2 SEQUENCE
<i>E. coli</i>	Ec_1	<i>E. coli</i>	16S rRNA	5-105	TGTTAGGCGCTGCCCGCAGCGTTCAATCTGAGCCATGATCAAACTCTTCCACTCGTACGGAAACACAGCAAGCTGGCTTCCTGTTCACCGTTTCGACTTGCA
	Ec_2	<i>E. coli</i>	16S rRNA	935-1035	GGTAAAGGTTCTTTCGCGTTGCAFCGAATTAACCCACATGCTCCACCGGCTTCCCGAAGGCACATTCTCATCTCTGAAACATCCCGTGGATGTCAAGACCA
	Ec_3	<i>E. coli</i>	23S rRNA	254-354	ACGCTTCCACTAACACACACACTGATTCAGGCTCTGGGCTGCCCGTGTGTCATTTTTGTGTACGGGGCTGTACCCCTGTATCGCACGCCCTTTCCAG
<i>Klebs. pneumoniae</i>	Ec/Kp_1	<i>E. coli</i> , <i>Klebs. pneumoniae</i>	16S rRNA	1370-1470	ACTCCCATGGTGTGACGGGGCGGTGTGTACAAAGGCCCGGAACTGATTCAGTGGTAAAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCC
	Ec/Kp_2	<i>E. coli</i> , <i>Klebs. pneumoniae</i>	23S rRNA	5-105	CTTTCATCGCCTCTGACTGCCAGGGCATCCACCGTGTACGCTTAGTCGGCTGTAAACGGTTCATATCACCTTACCGACGCTTTTCGACAGATTAGCAGT
	Ec/Kp_3	<i>E. coli</i> , <i>Klebs. pneumoniae</i>	23S rRNA	1210-1310	ATGTACAGCAATCCGACTTCTGTATACCTCCAGCATGCCCTCACACACACAAACCCCTTGGTCTTCCGGGAGCGGGCTTTTACCCGCTTTATTCGTTACTTT
	Ec/Kp_4	<i>E. coli</i> , <i>Klebs. pneumoniae</i>	23S rRNA	2790-2890	CGGCCTATCAACGTCGTCGCTCTTCAAGTTCTTCAGGACTTAAAGTACAGGTTCAATAGTACCCGGTTAGCTCAACGGCATCGCTGGCTTACACACC
<i>P. aeruginosa</i>	Kp_1	<i>Klebs. pneumoniae</i>	23S rRNA	260-360	TTCCAGACCGTTCCACTAACACACAAAGCTGATTCAGACTCTGGGCTGCTACAGCCCTGTCATTTTTGGTGTAGGGGACTATCACCCCTGTACCCGTCGGACT
	PsA_1	<i>P. aeruginosa</i>	16S rRNA	165-265	CATCTGATAGCGTGAGGTCGGAAGAATCCCCACATTTCTCCCTCAGGACCGTCCCTTGGTAGGCGCTTTACCCACCAACTAGCTAAATCCGACCTAGGCT
	PsA_2	<i>P. aeruginosa</i>	16S rRNA	1190-1290	ACCGACCAATTGTAGCACGCTGTGTAGCCCTGGCCGTAAGGGCCATGATGACGATCGGTTTTTATGGGATTAGCTCCACCTCGGGCTTGGCAACCCCTTTGT
	PsA_3	<i>P. aeruginosa</i>	23S rRNA	525-625	CGCTGACCCATTATACAAAAGGTACGCAGTCAACCTAAACAAGTAGGCTCGCTTTCGCTACGCCCTACCCCTATACGGTTAAGCTTCCCACTGAATATAAGT
	PsA_4	<i>P. aeruginosa</i>	23S rRNA	1375-1475	AGCCTTCTCGTCCCTCCATCGCAGTAAACCAAGTACAGGAATATTAAAGATTTACGCTAACACCTTAAACTTGGACAACCAACGGCAAGCTGGCT
PsA_5	<i>P. aeruginosa</i>	23S rRNA	2605-2705	CGGTCTCTCGTACTAGGAGCAGCCCTCTCAAAATCTCAAAACGTCCACCGGCAATGCCACTGGCTGTGACAAACCGGAAACACCAGAGGTTCTGTCCACTC	

Table S2. Sequences for mRNA probes targeting ciprofloxacin-responsive ("R_") and control ("C_") transcripts from *E. coli*. Nucleotide position is relative to the annotated reference genome sequence of *E. coli* strain EchS from the NCBI database.

PROBE	GENE	NUCLEOTIDES	PROBE 1 SEQUENCE	PROBE 2 SEQUENCE
R_1	<i>phoB</i>	50-150	CTTCCGCTTCGACCCGGGTGAAAGCCATTTTGTTCGAGCACGAAGCAGACAAATTAAATCCGGCCAGGGTTCATTCAGTTGATTCACAGCACTGTCAATAAT	AGGGACCTTTCGTGATGATTCACCCGCATGTTTCACGATCGCTAACCCCTAA
R_2	<i>phoR</i>	1114-1214	TCCGCTACCGCCGGTTTGCCGGGAACGGCTTTATCAACGCGATAAAAAATAGACAACCTTCACTGATAAGCCCGCTGTAGTGTTCCTGTAGAGACA	GTAACAATGGCAACAGTAGAAGTTGCCTCATCATGGGCTGATCTTCGGCGA
R_3	<i>sulA</i>	63-163	GACATTTTCTCTTCGCCCTCACGATGACTACGTATTTAAGCTTCAACCC	CCGTATCAAGCATTTTGTCAATAGCATCGGCTTCCCTTTTGGCTGGTAAAC
R_4	<i>yebG</i>	3-103	ATGGAACGGTCTTCAACCAGGCGCATGATGGAGCCTTTACCAAAATGTT	CAAGCCGATATCCAGTGAAGCCGAACCCGGTAGAGATGGTTTCCACATCC
R_5	<i>recA</i>	57-157	GTTCAGTTCTTCAAATGGTGATTTCTGTAAAAGCCAGTCGGCTCTCCAGC	AGATGATCGCGCAGTTTTCGCCATCTCCATTTCAITGAGCGGTCACCCGTGAC
R_6	<i>EchS_A3544</i>	34-134	GAAAATGGCGTTGATGTACTTGATTAAGGTAAATGCCATCGCCCGTACTTA	AGAAATCGCCGGGTGTTGTTCATGGTCAGAACAAATAATCGACAGGCTTGG
C_1	<i>rcsB</i>	220-320	GTTCCGGCGCTTCGAAAGAGATAACCTTGGTAAFAAATAACGTTGGATCTGC	AGATCAAGTTTAACTTCTGGTTGCCAATCTTTCITGGAAAAACGTGCGGGCGC
C_2	<i>secB</i>	31-131	TTTCACGTCACAGGCGCAATTCAGAACCGACACGGTACAGAGAACTTA	GCGTCAACGTACTTCGAGGGTTCAGACGACACCGGAAATCGGTGACGATGTC
C_3	<i>hflC</i>	361-461		