Supporting Information

Rapid Cytometric Antibiotic Susceptibility Testing Utilizing Adaptive Multidimensional Statistical Metrics

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This supporting information includes: (1) The complete set of flow cytometry data for the laboratory strain *E. coli* (ATCC33456), *P. aeruginosa* (ATCC27853) and the clinically isolated *E. coli* (Mu14S) with all the tested antibiotics. (2) Phase contrast images support the antibiotic-induced morphology changes in *E. coli* (ATCC33456). (3) The performance comparison of PB-sQF and PB-χ^2.

All the flow cytometry data presented here were labeled with MH-IR786. For the scatter 2D plots, the pseudocolor plots are the paired-control, the no-antibiotic data, for each antibiotic-strain. The contours are the antibiotic-treated data with the antibiotic concentration indicated otherwise.

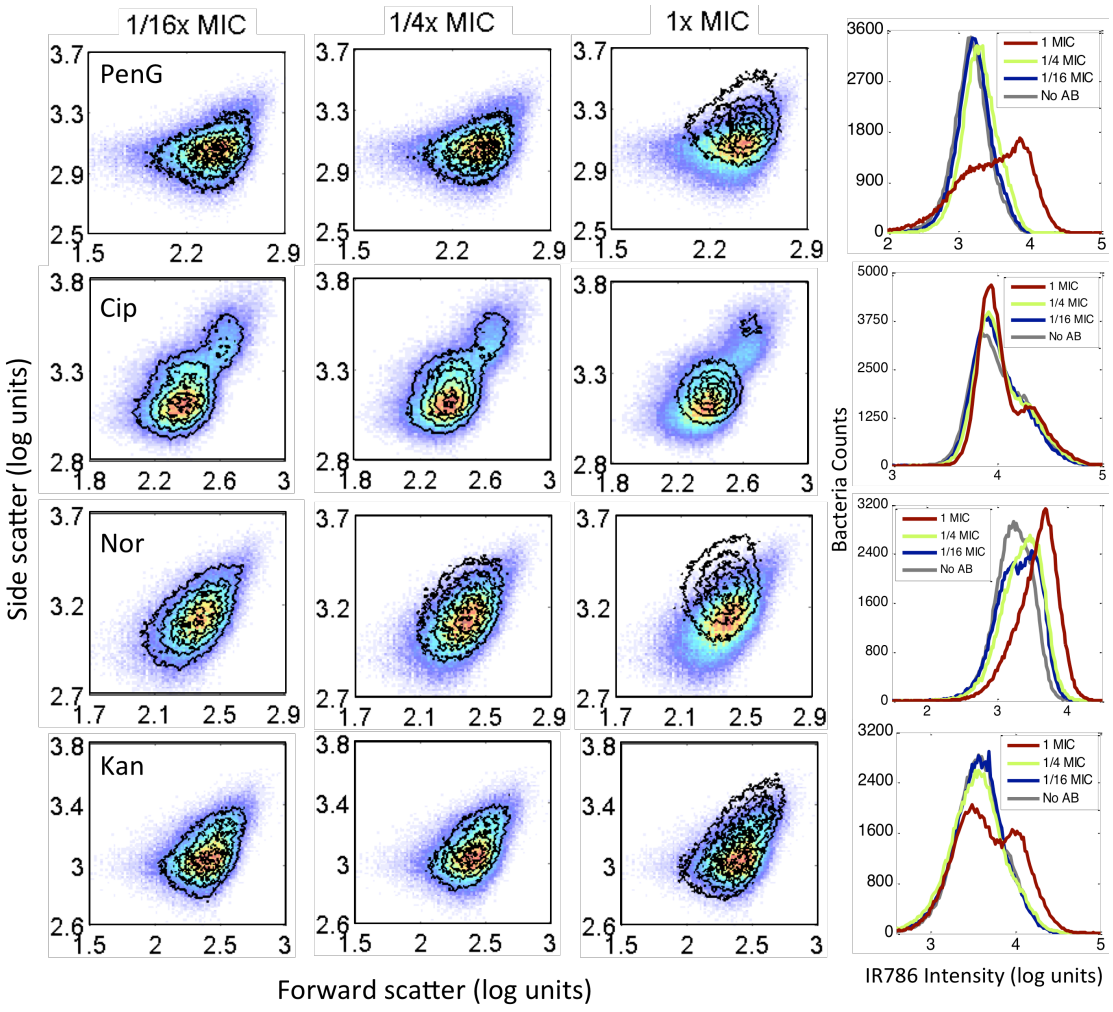

Figure S1. Antibiotic-induced flow cytometry signal changes at different antibiotic concentrations for *E. coli* (ATCC 33456). The contours are the antibiotic-treated data from 1/16x MIC, 1/4x MIC, to 1x MIC as indicated at the top of each column. From the top to the bottom rows are data of penicillin g, ciprofloxacin, norfloxacin, and kanamycin. The right column contains the corresponding fluorescence data. Scattered light histograms correspond to the concentrations labeling the blue, green, and red curves in the fluorescence histograms.

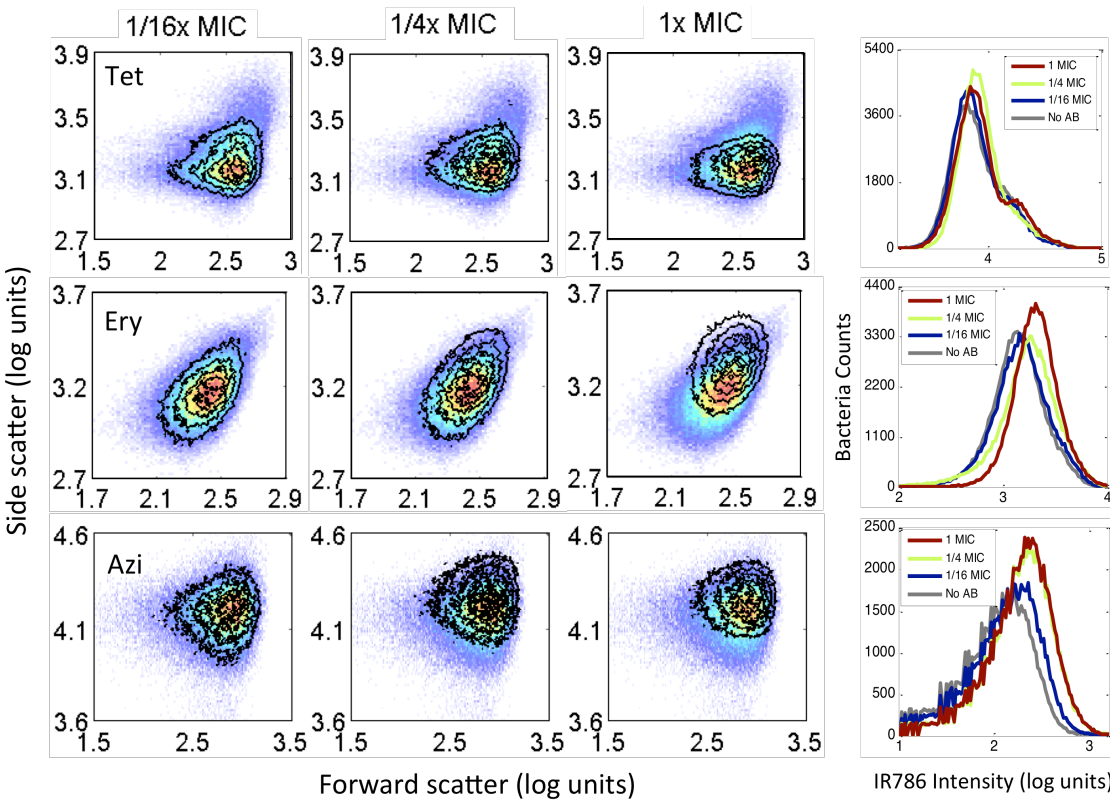

Figure S2. Flow cytometry data for bacteriostatic antibiotics. Analogous to data in Figure S1, from the top to the bottom rows are data of *E. coli* (ATCC 33456) exposed to tetracycline, erythromycin and azithromycin. Both bactericidal and bacteriostatic antibiotics give gradually increasing scattered light and fluorescence signal shifts from 1/16x MIC to 1x MIC.

Fluorescence and phase-contrast imaging

For imaging, a 2 µL aliquot of the *E. coli* sample was placed between two clean glass cover slips (Fisher Scientific). An inverted microscope (Olympus, IX-70) was used with a 100× oil immersion phase-contrast objective (Olympus, NA = 1.35) and CCD camera (Andor, Ixon). Data was collected and processed with Andor Solis software.

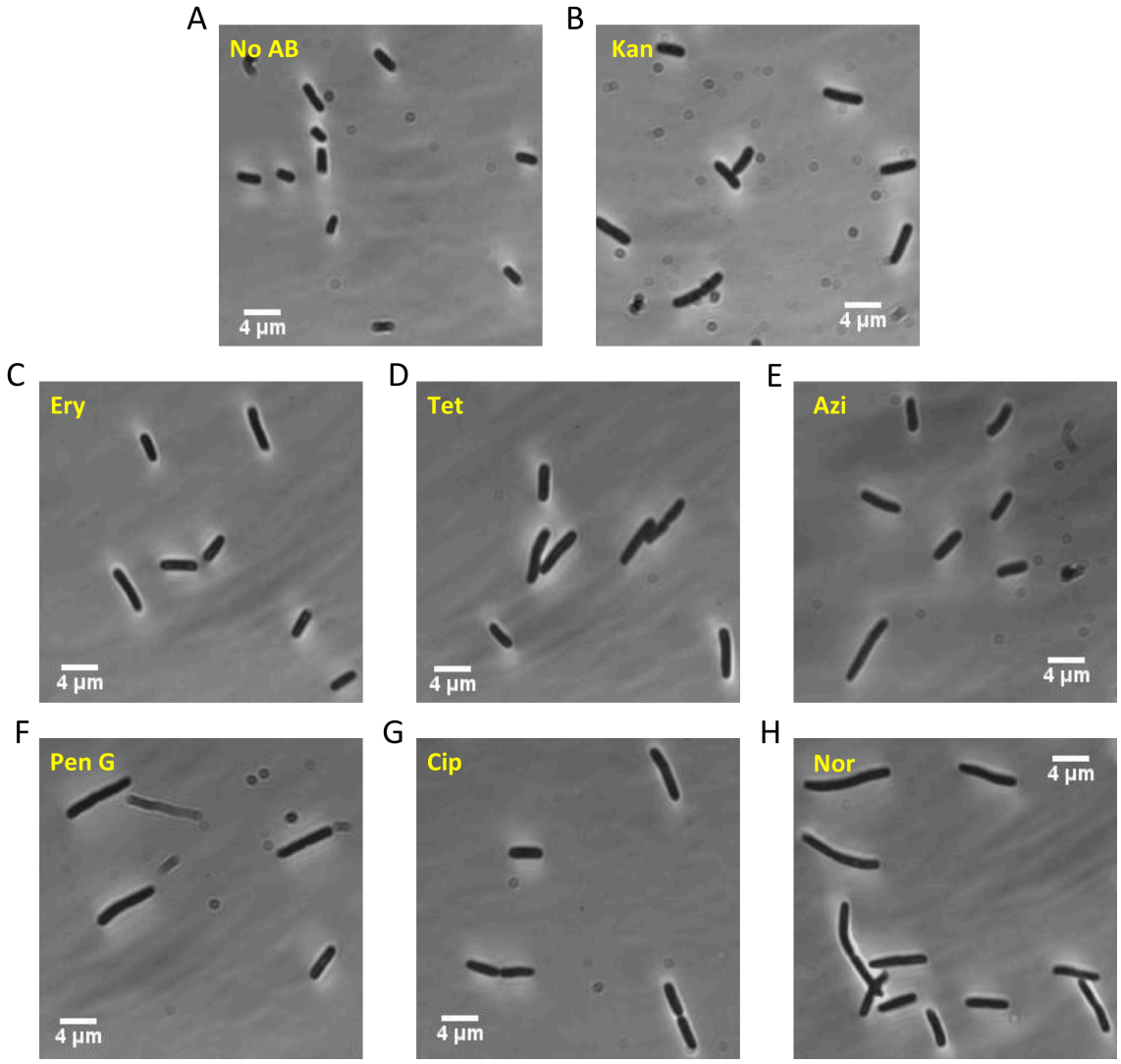

Figure S3. Morphology change of bacteria treated with 1x MIC of different antibiotics. (A) non-antibiotic control. (B) Kanamycin. (C) Erythromycin. (D) Tetracycline. (E) Azithromycin. (F) Penicillin G. (G) Ciprofloxacin (H) Norfloxacin. In general, antibiotic-induced filamentation was observed compared to the non-antibiotic control.

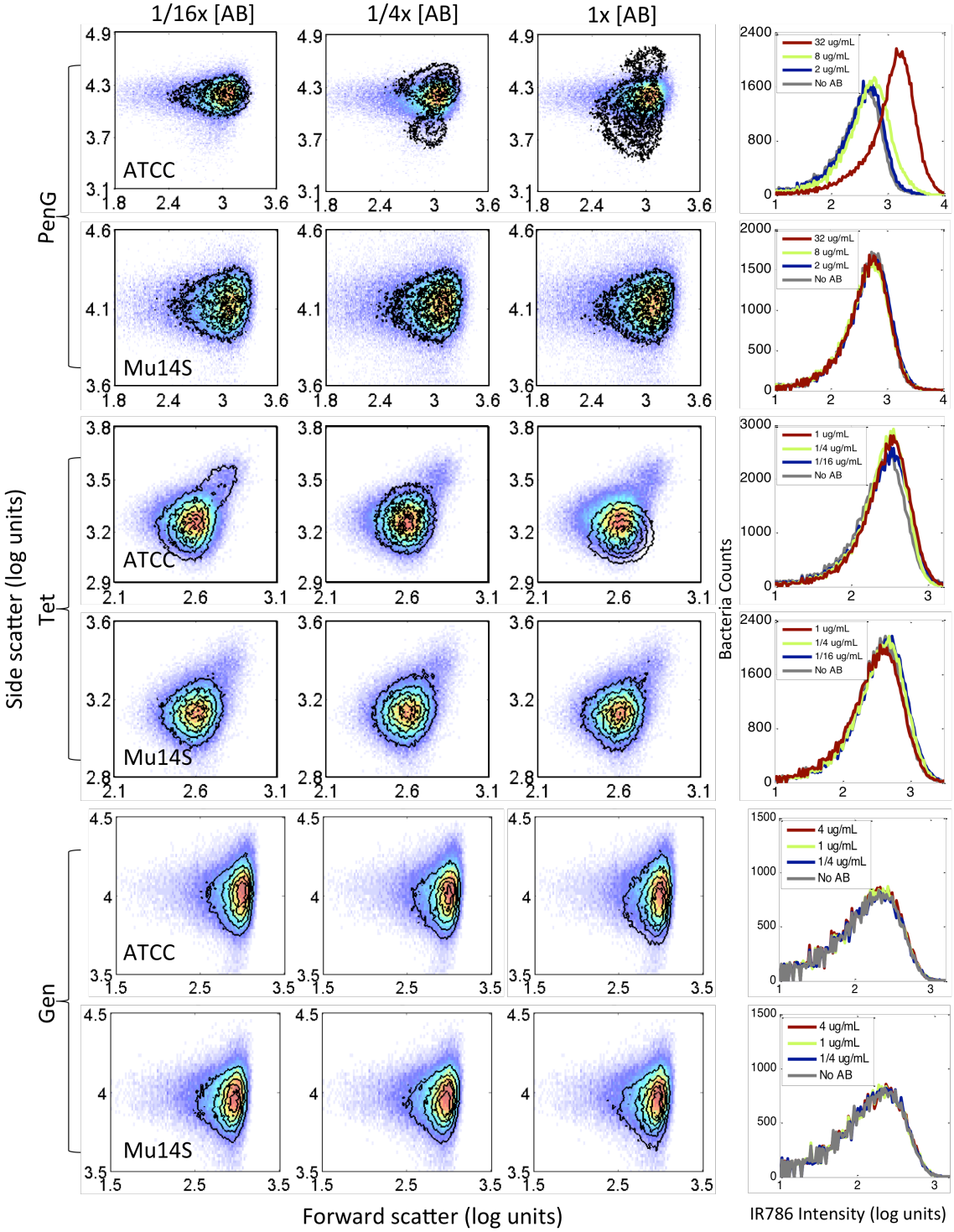

Figure S4. Flow cytometry data for lab strain *E. coli* (ATCC 33456) and clinically isolated resistant strain *E. coli* (Mu14S). The contours represent the antibiotic-treated data and the colored dot plots are the no-antibiotic control data. From left to right, the antibiotic concentrations correspond to those indicated by the blue, green, and red curves, respectively in the fluorescence histograms of column 4. For PenG and Tet data, both strains were treated at the MIC of ATCC. For Gen data, both strains were treated at the MIC of Mu14S.

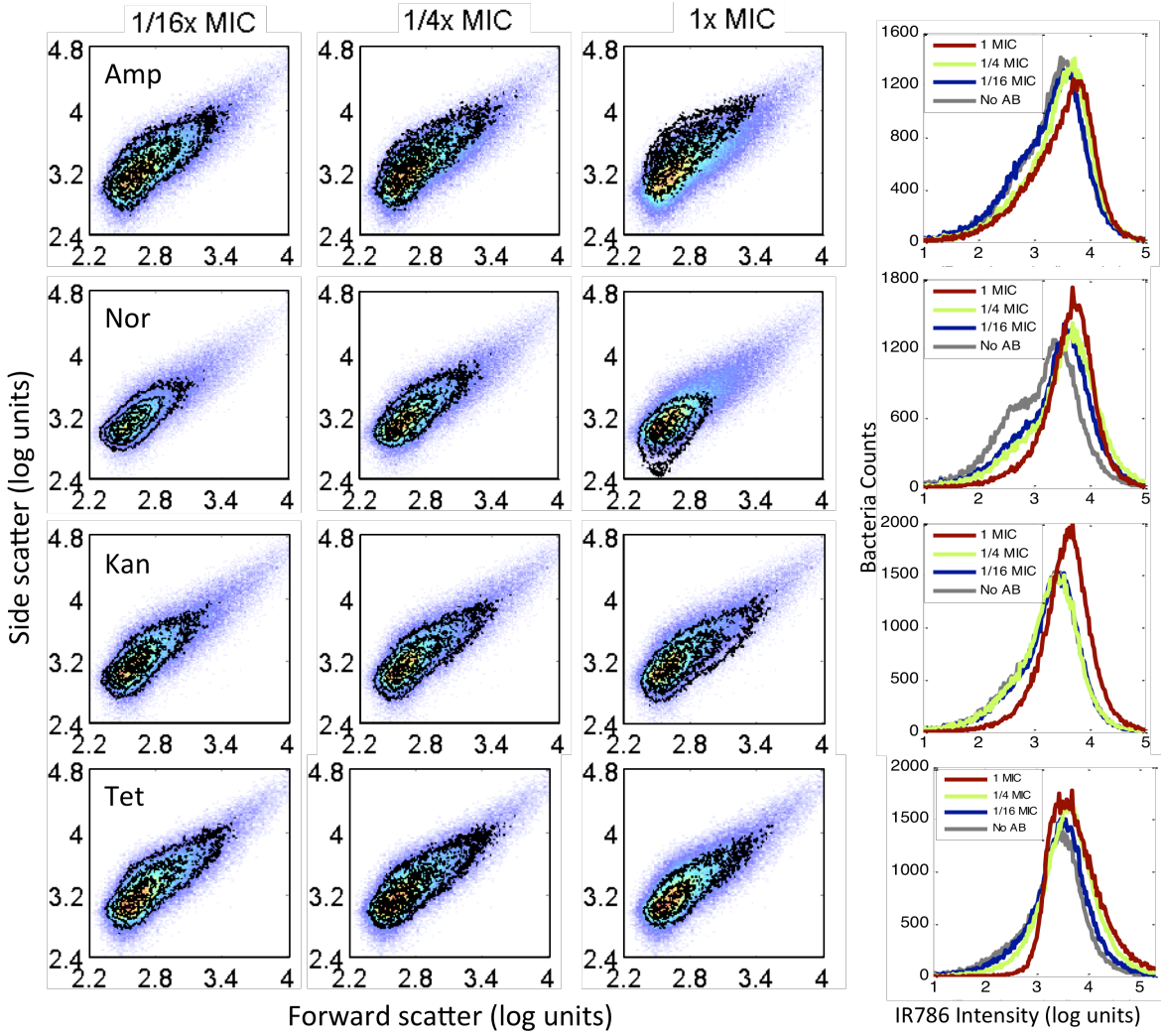

Figure S5. Antibiotic-induced scatter signal changes in *P. aeruginosa*. Scatter plots of *P. aeruginosa* treated with antibiotic from 1/16x MIC to 1x MIC. Actual antibiotic concentrations again correspond to those indicated in the fluorescence histograms for blue, green, and red curves. Scatter changes were most prominent at 1x MIC. The top to the bottom rows show data with ampicillin, norfloxacin, kanamycin, and tetracycline.

Convergence and Linearity

For both the test of convergence and test of linearity, the azithromycin-treated *E. coli* data and the paired, no antibiotic control were used to perform the tests. The test of convergence was exactly the same as the determination of confidence level as described in "Materials and Methods". 70 random sub-distributions of the no-antibiotic control and of the 1/16x MIC data, ranging from 500 to 8500 counts each were generated. A total of 125 bins in 3-dimensions were used and PB-χ^2 and PB-sQF were then applied respectively to calculate the test statistics between these sub-distributions. PB-χ^2 was performed as described by Roederer.[1]

For the linearity test, the 1x MIC data were treated as 100% positive data while the no-antibiotic control data were viewed as 0%. Data points from the 100% positive data and the 0% positive data were then randomly selected and mixed into a spectrum of new fictional data set with sample size 100,000 range from 2.5% positive to 97.5% positive with 2.5% as increment step. Test statistics were then calculated between the fictional data and the control data by either PB-χ^2 or PB-sQF. The test statistics were then normalized by the test result between the 100% positive data and the control.

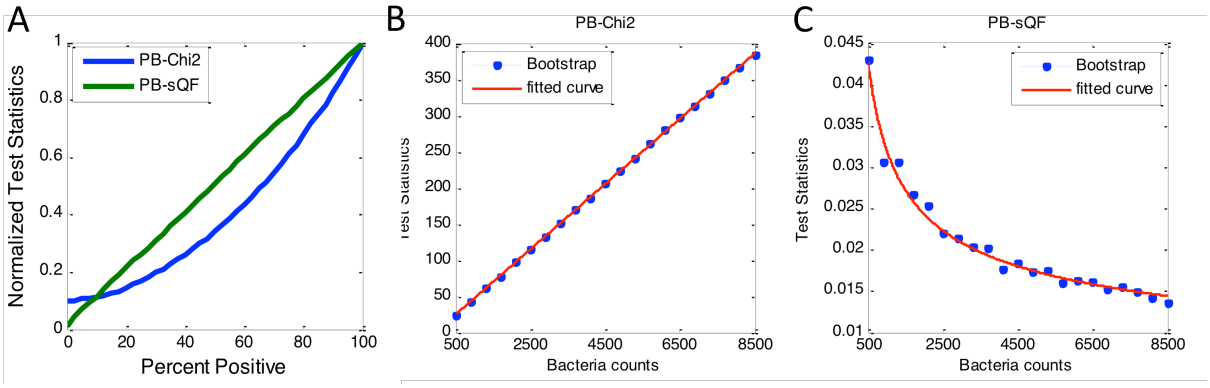

Figure S6. Linearity and convergence of PB-sQF and PB-χ^2. (A) The linearity of PB-sQF and PB-χ^2. PB-sQF showed a linear relation between the test results and the percent positive, while PB-χ^2, although increasing with percent difference, it does so in a nonlinear fashion. (B and C) The 99% confidence level of (B) PB-χ^2 and (C) PB-sQF. The confidence level of PB-χ^2 grows with bacteria counts while it reaches a limiting value in PB-sQF and could be fitted with an equation derived from the standard deviation of the mean.

Reference:

(1) Roederer, M.; Moore, W.; Treister, A.; Hardy, R. R.; Herzenberg, L. A. *Cytometry* **2001**, *45*, 47-55.