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Rapid Detection of Contaminant Bacteria in Platelet Concentrate Using Differential Impedance

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Abstract

BACKGROUND AND OBJECTIVES—Current FDA approved culture-based methods for the bacterial testing of platelet concentrate (PC) can yield false negative results attributed to Poisson-limited sampling errors incurred near the time of collection that result in undetectable bacterial concentrations. Testing PC at the point-of-issue (POI) extends the incubation period for any contaminant bacteria increasing the probability of detection. Data are presented from time-course experiments designed to simulate POI testing of bacterially contaminated PCs at different stages of growth using differential impedance sensing.

STUDY DESIGN AND METHODS—Whole blood-derived PCs were typically spiked with low numbers of bacteria (~100 CFU/mL) and incubated under standard PC storage conditions. Each infected unit was evaluated every two hours over a 12- hour period. All samples were treated with a chemical compound that induces stress in the bacterial cells only. The development of any bacterial stress was monitored by detecting changes in the dielectric properties of the PC using differential impedance.

RESULTS—Differential impedance measurements and corresponding cell counts at the different time points are presented for six organisms implicated in post-transfusion septic reactions. All infected PCs were detected once contaminant bacteria reached concentrations ranging between 0.6×10^3 and 6×10^3 CFU/mL irrespective of the phase of growth. Results were obtained within 30 minutes after the start of the assay and without the need for cell lysis or centrifugation.

CONCLUSION—Differential impedance sensing can detect bacterial contamination in PC rapidly at concentrations below clinical thresholds known to cause adverse effects.

Keywords

stress response; differential impedance sensing; dielectric permittivity; platelet contamination; bacterial detection

INTRODUCTION

Currently, one of two culture release test methods approved by the FDA, BacT/Alert or eBDS, are typically used at blood centers to detect contaminant bacteria in platelet concentrate (PC). While sensitive, these methods are limited by Poisson-sampling errors incurred near the time of collection that result in a significant number of false-negative outcomes.[1-4] Despite implementation of these preventative measures, bacterial contamination of PC continues to be a leading cause of transfusion related bacterial sepsis and improved detection methods are needed.

One alternative for overcoming the limitation of the current testing strategy is to test PC at the point-of-issue (POI). POI testing enables the detection of larger bacterial burdens from the longer incubation times.

In a previous publication, we described a new rapid method intended for detecting low concentrations of bacteria growing in PCs at the point-of-issue that does not require lysis or concentration steps.[5] Contaminant bacteria were identified in PC in real-time by monitoring the development of cellular stress of intentionally stressed viable bacteria using differential impedance sensing. The development of cellular stress has been demonstrated to reduce the polarizability of the stressed cells compared with unstressed cells using differential impedance.[6] In our earlier investigation, proof-of-principle experiments were performed by individually spiking the PC with bacteria at pre-determined concentrations followed by immediate data collection. Consequently, all detected organisms were in the same physiological state irrespective of the population size. In this report, we present the results from a series of time-course experiments designed to test bacterial contaminated PCs at different physiological stages of growth using differential impedance sensing and simulate the POI testing in a clinical setting.

MATERIALS AND METHODS

Platelet concentrates

A total of 32 in-dated whole blood derived platelet concentrates (WBDPC) were obtained from the Rhode Island Blood Center (Providence, RI) for examining the expansion of low-level bacterial loads ($\sim 10^2$ CFU/mL) in donor platelets. In-dated PC units received were typically two days old and were stored at 20-24°C with constant rotation (Orbitron Rotator II, Boekel Scientific, Feasterville, PA). Upon receipt, each PC unit was screened for bacterial contamination using culture-based methods. Tubes containing 5mL of bacterial culture broth were inoculated with 25 μ L of each unit and 1 mL from each unit was plated onto solid growth medium. A unit was considered **sterile** if these cultures yielded no visible microbial growth after a 48 hour incubation period at 37°C. Two WBD PC units of the same blood type were pooled together to provide adequate volume (150 mL) for each series of experiments.

Bacterial species

Six species of bacteria associated with PC transfusion-septic reactions were used to obtain the final data in this study. All bacterial strains were obtained from the ATCC (Manassas,

VA). The bacteria used were the Gram-positive organisms *Staphylococcus aureus* (ATCC 29213), *Listeria monocytogenes* (ATCC 19115), *Staphylococcus epidermidis* (ATCC 700567) and Gram-negative organisms *Serratia marcescens* (ATCC 27143), *Klebsiella pneumoniae* (ATCC 13882), *Escherichia coli* (ATCC 25922). All bacteria were cultured in their recommended growth medium prior to PC adaptation.

PC adaptation and bacterial infection

Infecting bacteria were first adapted to PC to ensure that any identifying cellular stress detected was caused exclusively by our chemical stressing compound and not environmental conditions for ease in interpreting the results. To ensure successful bacterial growth in PC, the target organisms were spiked into aliquots of both PC and trypticase soy broth (growth reference) and allowed to grow to stationary phase (overnight) prior to enumeration by standard plate count procedure. Bacteria adapted to PC and companion growth reference cultures were then re-cultured and allowed to grow to stationary phase for spiking pooled PC bags for time-course experimental studies. The turbidity of the growth reference culture was then used to estimate the concentration of the PC adapted bacteria used to spike pooled bags. Pooled bags were spiked with a single PC adapted Gram-negative or Gram-positive organism to a target concentration of $\sim 10^2$ CFU/mL. The actual starting concentration of bacteria (CFU/mL) of the infected PC units was determined by plate counts the following day.

At the start of this investigation, bacteria were first adapted in a single PC unit and afterwards pooled with a second PC unit. This protocol resulted in partially adapted bacteria that produced no colony growth when plated and was used to obtain four experimental data sets (Figure 5A and Figure 5B). A modified protocol was used for all data sets other than those experiments: two WBD PC units were first combined followed by adapting the bacteria to the pooled PC unit.

Infected PC units were incubated under standard PC storage conditions, and sampled every two hours over a 12-hour period. All samples were immediately evaluated using the differential impedance platform and corresponding bacterial growth was enumerated in triplicate using standard plate counting methods.

Initial impedance measurements were made using bacteria adapted to one PC unit prior to pooling. The protocol was later changed to include adapting bacteria to pooled PC in order to closer mimic time-course experimental conditions and facilitated improved bacterial growth.

Impedance Sensing Hardware

All measurements of dielectric permittivities were obtained from differential impedance measurements. The dielectric permittivity is a measure of the polarizability of the biological sample. All impedance measurements were obtained with custom built hardware using standard electronic methods.[7] Specifically, each sample was subjected to a fixed sinusoidal voltage oscillating at 1000 Hz that exercised all charged particles in the sample.

The resulting polarizability was determined by comparing the corresponding measured current with the applied voltage to calculate the electronic capacitance of the material.

The custom-built instrumentation consisted of a 3"×3" cassette for holding the test samples and a table-top sized device into which the cassette was inserted for analysis (Figure 1). All cassettes used in these experiments contained four mechanically and thermally matched 100 μ L test chambers. Each test chamber was defined by a sub-millimeter electrode gap structure that encompassed a sample and measured its electrical properties. All electrodes were made from pure gold sputtered onto borosilicate glass substrates. All cassettes used in these experiments were hand-built and reusable.

The measuring device consisted of thermally controlled platens that surrounded the cassette and maintained a constant temperature with high precision. The magnitude and angle of the impedance vector for each detection chamber was continuously recorded over time with electronic circuitry developed in-house and stored in a computer.

The dielectric permittivity for the planar geometry used in the cassette design is directly proportional to the measured capacitance (obtained from the reactive component of the recorded impedance vector). For the planar geometry used in the cassette design, the dielectric permittivity was calculated according to the equation $C = \epsilon (A/d)$ where ϵ is the dielectric permittivity, A is the surface area of the electrodes, and d is the separation distance between the electrodes.

Protocol and analysis for differential impedance measurements

The dielectric changes of PC incubated with bacteria were measured as follows: Infected PCs were diluted 1:1 with a buffer solution (0.8% Glucose and 1mM EDTA) and the suspension was divided into two equal volumes. One portion was mixed with a sub-lethal concentration (60 μ g/mL) of the compound STR-103 described in our previous publication[5] that caused the development of stress in the bacteria but did not affect human cells; the other portion contained no stressor. One chamber of a test cassette was manually filled with infected PC containing the stressor while an adjacent reference chamber was filled with the same PC containing no stressor for direct comparison. The filled cassette was then inserted into a differential impedance analyzer set at 30°C and the impedance signals from each of the 100 μ L volume test chambers were recorded every two seconds over a 30-minute period.

While other configurations are possible, we chose to use one chamber as the reference channel (PC alone) and three channels containing identical stressed sample (PC with STR-103). This enabled uncertainties to be calculated for each time point based on three independent measurements.

The Normalized Impedance Response (NIR) was formed by dividing the signal value for the chamber containing the stressor treated PC by the value for the untreated PC at each time point. Because the detection chambers are mechanically identical, this ratio is determined by the relative dielectric permittivities of the two chambers. Importantly, because the chambers are subjected to identical thermal/mechanical perturbations, signal contributions common to

both chambers are minimized. These contributions include interactions between organic compounds in the medium unrelated to any cellular processes. Thus, any changes in the NIR value over time reflect subtle changes in cellular metabolism caused exclusively by the stressor.

In order to characterize each NIR temporal profile as a single parameter, the Cumulative Normalized Impedance Response (cNIR) was defined by the equation $cNIR = \sum (NIR(t_i) - 1)$ where the sum includes all NIR values recorded during the 30 minute measurement. The cNIR is the effective area under the curve after rescaling and is designed such that a NIR profile having a constant value equal to one would have a cNIR value equal to zero.

Statistical analyses

Student's *t*-test was used to compare the measurements. P-values of less than 0.05 were considered to indicate statistically significant differences.

RESULTS

Overall, 32 in-dated WBD PC units were infected with a single organism and measured periodically to characterize the evolution of the bacterial growth and the corresponding change in dielectric permittivity. Bacterial growth in the PC was confirmed exclusively by plate count data sampled at each time point and enumerated the following day. Normalized Impedance Response (NIR) profiles from identical PC samples during exposure to proprietary stressor STR-103 were obtained in parallel to monitor any changes in dielectric permittivity caused by stressed bacteria in the samples. Stressor STR-103 is a proprietary bacterial membrane permeability modifying compound that acts on bacteria but not human cells **when used** at an optimal concentration. Differential measurement methods comparing PC with and without the stressor enable the bacterial response to be isolated.

Previously, all PC was tested using STR-103 at a concentration of 100 µg/mL with a purity of 60%. For the work reported here, the stressor used was of a higher purity (99.9%). Because of these chemical differences, the optimal concentration that produced a measureable response from bacteria but not human cells was 60 µg/mL and was used throughout these experiments.

To minimize the possibility of measurement bias, the dielectric permittivity data were obtained and analyzed by one experimenter and the bacterial enumeration data were independently measured and analyzed by a second experimenter. Typically, 24 hours after data taking was completed, both experimenters gathered to compare their respective analyzed data sets.

Typical NIR profiles obtained with our Differential Impedance Analyzer are shown in Figure 2 for WBD PC spiked with *S. aureus*. The NIR profile for sterile PC containing STR-103 is plotted as a negative control (**CTRL**). The corresponding values are constant demonstrating that the PC exposed to STR-103 at a concentration of 60 µg/mL does not produce a measurable response. A second curve shows the NIR profile for infected PC at the start of the experiment (**t=0**) when the bacterial population is small. Same as for the **CTRL**,

no response is seen because the bacterial population is below the detectable threshold. In contrast, the NIR profile for the infected PC obtained after 12 hours of incubation constantly decreases over time and is easily distinguished from the sterile and newly spiked PC by visual inspection. The latter profile corresponds to a viable bacterial population of 8.4×10^4 CFU/mL and is characteristic of profiles of detectable viable populations.

For data presented here on, the dielectric responses obtained are preferably expressed as Cumulative Normalized Impedance Response (cNIR) values. The cNIR value is the effective area under a NIR profile during the initial 30 minutes of measurement. A negative cNIR value reflects the development of cellular stress exclusively by bacteria growing in the PC. Progressively larger negative values correspond to more bacteria growing in the PC or the development of additional cellular stress.

Time-Course Data

Time-course data sets were obtained for 12 pooled PCs (24 units total) spiked with different bacteria where statistically significant cNIR values were measured in combination with significant bacterial growth. In two other pooled spiked PC units no cNIR impedance response was observed and no plate growth was found. In addition, two more data sets were obtained where cNIR values consistent with significant metabolic activity in response to the stressor were measured but final plate counts were all zero.

For all time-course experiments, the cNIR value for sterile stressor-treated PC was obtained as a negative control (CTRL). Figure 3 is a histogram of CTRL data containing 21 cNIR values from seven different PC units along with a Gaussian curve fitted to these data. The average cNIR value for all negative controls is 0.30 ± 0.50 . This value reflects the reproducibility of a typical measurement and includes both instrumental uncertainties and any variability from different PC units.

Representative cNIR responses for the six organisms tested are plotted together with their corresponding plate counts in Figure 4. Figures 4A-F are typical for all organisms having confirmed bacterial growth. Plate count data indicate bacterial populations increased in the PC after a several hour lag period following the initial spike. In these particular experiments, *L. monocytogenes*, *E. coli*, and *S. marcescens* had limited growth reaching a final concentration of only $\sim 10^4$ CFU/mL, *S. aureus*, and *S. epidermidis* eventually grew to greater than 10^5 CFU/mL, while *K. pneumoniae* exceeded 10^6 CFU/mL. Overall, corresponding cNIR values increased as the bacterial population increased. The cNIR values for *E. coli* (Figure 4F) peak at 8 hours and diminish thereafter in contrast to the other data sets plotted in the Figure 4.

cNIR values at each time point were compared with the corresponding negative control and a p-value was calculated for the pair. Statistically significant measurements ($p < 0.05$) are denoted by asterisks (“**”) under the datum.

Two data sets negative for growth and two data sets positive for growth were obtained for *S. marcescens*. A plot of the data from a representative negative growth experiment is shown in Figure 5A in comparison to the plot of *S. marcescens* with confirmed colony growth (Figure

4D). For the negative growth experiment, all cNIR values were consistent with the response for sterile PC (CTRL) and all corresponding final plate counts showed no colony growth over the entire 12 hour measurement period. In contrast, the cNIR values for the positive growth data set decrease over time as the corresponding plate counts increase before decreasing at 12 hours.

Figure 5B is a plot of one of two pooled PC spiked with *E. coli* that resulted in no colony growth for all time points despite progressively increasing cNIR values. The cNIR values obtained for both *E. coli* experiments are consistent with the response of the sterile CTRL data at measurement times 0, 2, and 4 hours followed by progressively decreasing cNIR values at measurement times 6, 8, 10, and 12 hours. These latter data points are similar to the *E. coli* experiment where metabolic activity was confirmed by colony growth (Figure 4F) along with all other data producing colony growth (Figures 4A-E). These latter cNIR values are also contrary to the *S. marcescens* data set in Figure 5A that also exhibit no colony growth but lack any statistically significant cNIR values.

The four data sets represented in Figure 5A and 5B obtained using PC spiked with partially adapted bacteria did not produce colony growth contrary to all other experiments using bacterial cells fully adapted to the pooled PC.

Detection Thresholds

To determine the lowest detectable bacterial level for each organism, the statistically significant cNIR value corresponding to the lowest CFU/mL was chosen for each time-course experiment. This value was usually the first statistically significant value measured with the exception of *E. coli* that showed a slight dip in detectable population prior to entering logarithmic growth (Figure 4F). All values obtained for the same organism were averaged together to obtain a minimum detectable CFU/mL level. These CFU/mL values are summarized in Table 1 and range between $5.60 \pm 1.1 \times 10^2$ CFU/mL for *E. coli* to $6.2 \pm 0.35 \times 10^3$ CFU/mL for *S. marcescens*.

The corresponding range of cNIR values varied between -1.74 ± 0.71 for *K. pneumoniae* to -2.52 ± 0.51 for *S. epidermidis*. While the cNIR value associated with the minimum detectable CFU/mL level for *E. coli* is -4.20 ± 0.13 , the first statistically significant value measured for *E. coli* was -1.31 ± 0.86 ($p=0.03$).

An overall threshold value for cNIR establishing statistical significance ($p=0.05$) was calculated using the average cNIR value for all CTRL runs (Figure 3) as a basis and equals -1.30 ± 0.87 . The uncertainty associated with the threshold value is the average error calculated from all experimental points irrespective of bacterial species obtained with infected PC.

Conservatively, if the smallest cNIR value measured for an individual CTRL run is substituted for the average value, the resulting threshold cNIR value for statistical significance equals -2.12 ± 0.87 ($p=0.05$). This threshold value was applied to the data for each organism tested and an effective measured CFU/mL level was extrapolated based on a simple linear calibration extracted for each organism (Table 1). The extrapolated levels for

all organisms were found to be nominally affected when compared with the actual measured minimum detectable levels with the exception of *E. coli* that showed a 0.5-fold decrease.

DISCUSSION

We have presented new data focused for a method previously described in an earlier publication simulating POI testing in a clinical setting.[5] Differential impedance sensing is used to monitor the development of cellular stress in intentionally stressed bacteria as a means to identify their presence in PC. Historically, impedance sensing has been used as an electronic analog of the Petri dish to monitor microbial proliferation.[8, 9] The approach described here does not use impedance to detect growth. Instead, impedance is used to detect subtle changes in the metabolic state of bacteria when exposed to adverse conditions. The discovery that compound STR-103 at an optimal concentration stresses bacteria but not human cells enables discrimination between the two cell types. When combined with differential sensing techniques, the approach is a powerful method for detecting low levels of bacteria ($\sim 10^3$ CFU/mL) surviving in a concentrated suspension of platelets ($>10^{10}$ platelets/mL) directly without the need for lysis or concentration.

In our earlier publication, proof-of-principal was demonstrated by detecting 17 different bacterial species implicated in transfusion related septic reactions in both WBD and aphaeresis PC. However, all organisms were spiked into PC at the desired final bacterial population and detected in logarithmic phase of growth only. In this study, pooled WBD PC units contaminated with low starting numbers of bacteria ($\sim 10^2$ CFU/mL) were sampled every two hours over a 12 hour incubation period to investigate if the different bacterial species could be detected in all physiological states of growth **and with a single detection threshold value**. In addition, the experiments were designed to simulate POI testing in a clinical environment.

For the six PC adopted organisms tested, growth typically occurred after two to four hours of incubation in PC. Spiked organisms occasionally remained in lag phase as long as six hours before population increases as seen in Figure 4E for *S. epidermidis*. The **rate of change** of the plate count data obtained for the different infected PCs (Figure 4 and other) indicate that the spiked bacteria pass from lag phase growth to logarithmic phase growth and in many cases plateau to the stationary phase of growth as seen in Figures 4C, 4D, and 4F. Statistically significant cNIR values were measured for all time points having bacterial growth above the detection threshold irrespective of growth phase of the bacteria. No significant correlation between cNIR value and growth phase could be determined.

Negative results were obtained from two experiments using pooled PC infected with partially adapted *S. marcescens*. In these data sets, the absence of plate counts at all time points was correlated with the absence of statistically significant cNIR values at those same time points. These data provide further confidence in the cNIR value as an indicator of contaminant bacteria in PC.

Limits of detection

The limit of detection for each organism was determined by the lowest bacterial population associated with a statistically significant cNIR value and ranged between 5.6×10^2 CFU/mL for *E. coli* to an upper limit of 6.2×10^3 CFU/mL for *S. marcescens* (Table 1).

The accuracy of these values is limited by the two-hour sampling frequency chosen for these experiments. Periodic sampling at shorter time intervals would lead to smaller and more representative detection limits. This is illustrated by the data sets for *S. marcescens* positive for growth. In one *S. marcescens* data set (Figure 5A) the cNIR value at 4h corresponding to 3.2×10^3 CFU/mL is just shy of statistical significance ($p=0.084$) while the time point measured two hours later corresponding to 1×10^4 CFU/mL has a very low p-value equal to 0.006. Consequently, the latter point was chosen as the limit of detection for this time-course experiment. For comparison, the limit of detection determined for a second *S. marcescens* experiment (Figure 4D) is 2×10^3 CFU/mL similar to all other organisms measured. When these two values are averaged together, an intermediate higher value is adopted as the final value. In general, the detection limit measured for all organisms is 2×10^3 CFU/mL or fewer, an order of magnitude less than the clinical threshold indicated for PC transfusion induced septic reactions.[10]

Detection Thresholds

The data presented here establish that the susceptibilities of the six organisms tested with stressor STR-103 are similar enough to define a single statistically significant threshold cNIR value that corresponds to low clinically relevant limits of detection for each organism. A conservative threshold value for cNIR equal to -2.12 was calculated that would encompass all statistically significant data obtained in this investigation irrespective of the species tested. When applied to the entire data set, estimated CFU/mL levels for each organism at the threshold value were calculated and compared with the minimum detectable levels measured. Little significant difference was found for five of the six of the organisms tested.

According to the calculation, the threshold level for *E. coli* was extrapolated to be $3.1 \pm 0.6 \times 10^2$, half of its already low measured detectable level of 560 CFU/mL in PC. While the cNIR value reflects the metabolic activity of stressed live bacteria, an increase in the number of cells or an increase in stress cannot be distinguished. Overall colony growth for *E. coli* is at least one order of magnitude lower than all other colony producing experiments. Consequently, measured cNIR values could be influenced by increasingly stressed organisms and the resulting extrapolated value for *E. coli* may be artificially low.

Further comparison of the NIR profiles obtained in these experiments with those from our earlier publication[5] also suggest similar susceptibilities for other relevant organisms and a like common cNIR value.

False Positive or True Positive? Of particular interest are the two *E. coli* experiments where statistically significant cNIR values were measured despite the absence of any colony growth (Figure 5B). In both experiments the *E. coli* bacteria were only partially adapted to

the pooled PC. Consequently, less than robust growth and poor survival rates are not unexpected.

The assay presented here monitors induced changes in cellular metabolic activity of living organisms directly and non-invasively. A statistically significant cNIR value determines only that susceptible bacteria were alive and able to respond to stressor STR-103 at the time of measurement compared to bacteria not exposed to the compound. The assay cannot determine the overall health of the cells and whether the bacteria will form colonies if transferred to solid growth medium.

Although uncommon, viable but nonculturable growth is well documented in the microbiological literature for *E. coli*. [11-13]

These *E. coli* data suggest that the underlying assumption that bacteria are not viable and will not proliferate when transfused if and only if colony growth cannot be produced is incomplete. These data show that it is possible for some partially adapted cells not robust enough to form plate counts to survive in PC. What cannot be easily tested is if these living bacterial cells will replicate if transfused into a human being.

Monitoring the metabolic activity of intentionally stressed bacteria using differential impedance provides additional and important information about the physiological status of the cells that would be lost by relying on plating results alone.

Testing in a clinical environment

Currently, bacterial testing of PC is performed at the blood center where the PC is collected. POI testing would relocate the physical testing to a hospital setting where the transfusion will occur. An effective POI test must be rapid, sensitive, produce easy to interpret results, and be operationally simple to implement for successful adoption in a clinical setting.

While sterile PC can be set aside for control measurements in laboratory experiments, clinical samples are either infected or all sterile and obtaining a negative control in parallel with the test sample is not possible. Consequently, baseline information must come from extensive *a priori* laboratory experiments characterizing the cassette and different PC units. The reproducibility of the negative control for this investigation is based on 21 sterile WBD PC units and multiple cassettes. This result is characterized by the histogram in Figure 3 and the average cNIR value of the distribution is 0.30 ± 0.50 based on a Gaussian curve fit to the data.

Applying a cNIR threshold value equal to -2.12 would include all statistically significant measurements for the six species tested in this study. Once the cNIR value reaches this threshold value, the operator would be alerted that an infected unit has been identified.

It is anticipated that a commercial manufactured version of the platform would include a disposable cassette and an automated fill station. For these experiments using manually filled, hand-assembled reusable cassettes, sample preparation for each time point typically required between two and four minutes followed by 30 minutes for data collection. For a

large number of measurements detection thresholds were reached in as few as 15 minutes resulting in a significantly shorter time to results.

CONCLUSIONS

In conclusion, the data presented here confirm the capability of differential impedance sensing to identify PC contaminated with bacteria rapidly with sensitivity below current clinical thresholds for PC transfusion-induced septic reactions. Monitoring induced changes in metabolic activity provides additional biological information having potential clinical relevance that is not easily obtained by other methods. When combined with its operational simplicity, the assay is an ideal candidate for POI testing.

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Fig. 1. Differential Impedance Instrument

Photograph of the differential impedance sensing analyzer with calibration, four-chamber test, and cleaning cassettes. The cassette is loaded into a drawer located on the face of the analyzer designed to hold the cassette during measurement.

Fig. 2. Normalized Impedance Response (NIR) profiles from PC infected with *S. aureus* treated with STR-103 (60 $\mu\text{g/mL}$)
 NIR profiles are plotted for: sterile PC with STR-103 as a negative control (CTRL), infected PC at the start of the experiment (t=0h), and the infected PC obtained after 12 hours of incubation (t=12h).

Fig. 3. Histogram of the cNIR values for Sterile Stressor-Treated PC

The histogram data include 21 measurements from 7 different PC units fitted with a Gaussian curve.

Fig. 4. Detection of Gram-Negative and Gram-Positive Bacteria Growing in Whole-Blood Derived Platelet Concentrate using Differential Impedance Sensing

Pooled WBD PCs infected with Gram-positive or Gram-negative bacteria were sampled at two-hour intervals and tested using a differential impedance analyzer. The average cNIR values (bar) and associated standard deviations are plotted as a function of time along with the corresponding plate counts (line) for each PC sample tested. A negative value for cNIR corresponds to a population of stressed bacteria. Statistically significant responses ($p < 0.05$) are indicated by (*).

Fig. 5. cNIR values of Representative *S. marcescens* and *E. coli* Time-Course Experiments Having No Colony Growth

cNIR values for representative *S. marcescens* and *E. coli* experiments are plotted in Fig. 5A and Fig. 5B, respectively, along with corresponding plate counts for each time point. Four experiments total (two for each organism) were conducted using PC infected with partially adapted bacteria. No colony growth was measured for all four experiments. No statistically significant cNIR values were measured for *S. marcescens*. In contrast, significant metabolic activity was measured for *E. coli* after 6 hours of incubation.

Table 1
Average of Lowest Statistically Significant Detectable Bacterial Levels

Bacterial Species	Gram Characterization	N	CFU/mL	cNIR	P-value (Range)	CFU/mL THRESHOLD (cNIR = -2.12)
<i>Staphylococcus aureus</i>	Gram-positive	2	$2.0 \pm 1.2 \times 10^3$	-2.50 ± 0.83	0.011 – 0.012	$1.7 \pm 1.0 \times 10^3$
<i>Listeria monocytogenes</i>	Gram-positive	2	$1.8 \pm 0.4 \times 10^3$	-2.31 ± 0.47	0.005 – 0.045	$1.7 \pm 0.3 \times 10^3$
<i>Staphylococcus epidermidis</i>	Gram-positive	3	$2.3 \pm 0.1 \times 10^3$	-2.52 ± 0.51	0.007 – 0.012	$2.0 \pm 0.1 \times 10^3$
<i>Klebsiella pneumoniae</i>	Gram-negative	2	$9.3 \pm 0.2 \times 10^2$	-1.74 ± 0.71	0.003 – 0.009	$1.1 \pm 0.3 \times 10^3$
<i>Serratia marcescens</i> *	Gram-negative	2	$6.2 \pm 0.4 \times 10^3$	-1.50 ± 0.40	0.007 – 0.017	$8.4 \pm 0.5 \times 10^3$
<i>Escherichia coli</i> *	Gram-negative	1	$5.6 \pm 1.1 \times 10^2$	-4.20 ± 0.13	0.001	$3.1 \pm 0.6 \times 10^2$

* Measurements without corresponding plate counts results are not included in these final averages. N is the number of time-course experiments using different PC units; all measurements within each time-course experiment were performed in triplicate.