

# Direct Detection of Bacterial Contaminants in Platelet Concentrate in Near Real-Time

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**Background.** Currently approved culture-based methods for the bacterial testing of platelet concentrates (PCs) require an extended period of time to obtain results. Proof-of-principle data are presented for a new rapid approach to screen PCs for bacterial contamination at the point-of-issue. The approach relies on monitoring the response from chemically stressed bacteria in PC.

**Methods.** Both apheresis- and whole blood-derived PCs were spiked with bacteria and incubated to ensure adaptation and growth prior to testing. All inoculated PCs were treated with a proprietary cationic membrane-acting chemical stressor at a sub-lethal concentration which caused the development of stress in the bacteria while not affecting human cells. The treated suspensions were immediately tested using the BioSense Z-Sense™ Differential Impedance Platform specifically designed to monitor subtle changes in the dielectric properties of biological suspensions.

**Results.** Profiles characterizing the impedance response of the stressor-treated contaminated PC suspensions will be presented. These include Gram-positive *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Streptococcus pneumoniae*, and Gram-negative *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa* grown to final test concentrations ranging from  $10^3$  CFU/ml to  $10^6$  CFU/ml out of 17 bacterial species tested. For all species studied, viable bacterial cells in the infected PCs were easily detectable within 30 minutes of measurement without the need for cell lysis or additional concentration. Results were confirmed by quantitative culturing of the sample on agar plates. No measurable responses from stressor treated platelets or other human blood cells present in PCs were observed.

**Conclusions.** These proof-of principal experiments demonstrate that monitoring the development of stress in bacteria is a rapid and effective way to detect bacterial contamination in PCs. Detecting the stressed bacteria directly in PC represents a significant improvement to our previously described assay that involved lysis of the platelets. Based on these results, a simple assay can be developed that is based on a one-step protocol and is amenable to point of issue testing in hospitals and blood centers.

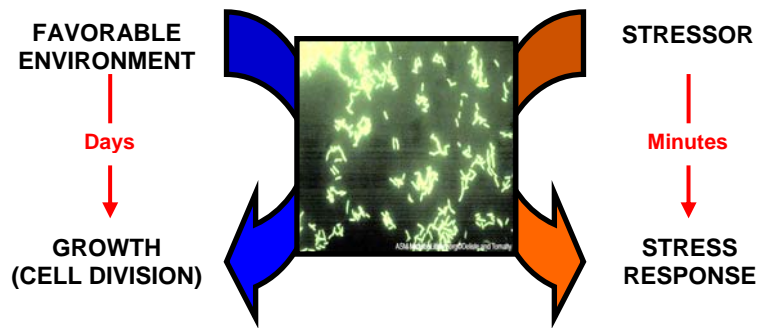
## Introduction

We have previously described a new rapid approach for identifying bacterially contaminated platelet units by monitoring intentionally stressed bacteria using differential impedance sensing.<sup>1</sup> The approach was initially implemented with a protocol that used Triton X-100 to lyse all human blood cells while simultaneously inducing a stress response in any contaminating bacteria.

The present study describes significant improvements to our earlier work from the identification of a chemical stressor that induces a measurable stress response in bacteria but not in human cells. The ability to distinguish between bacteria and human cells enables the direct detection of stressed bacteria in PC using a one-step protocol that does not require lysis, filtration, or centrifugation as preparation steps.

## Physiological Stress Response and Rapid Testing

The ability to respond to adverse environments effectively along with the ability to reproduce are *sine qua non* conditions for all sustainable cellular forms of life. These two conditions are shown graphically in Fig. 1.



**Fig. 1. Two Possible Strategies for Detecting the Presence of Bacteria in Platelet Concentrates.**

Given the availability of an appropriate sensing modality, the ubiquity and immediacy of the stress response forms the basis for a new approach to the routine quality control testing of PC units with a simple and rapid assay. We have found that measuring the dielectric permittivity of a cellular suspension, an easily measurable electronic property, is an effective way to monitor the response of bacterial cells to adverse conditions continuously.

The use of differential impedance sensing enables the dielectric permittivity of  $10^3$  CFU/mL bacteria mixed among  $10^{10}$  PLT/mL to be measured directly in less than 30 minutes total time including sample preparation.

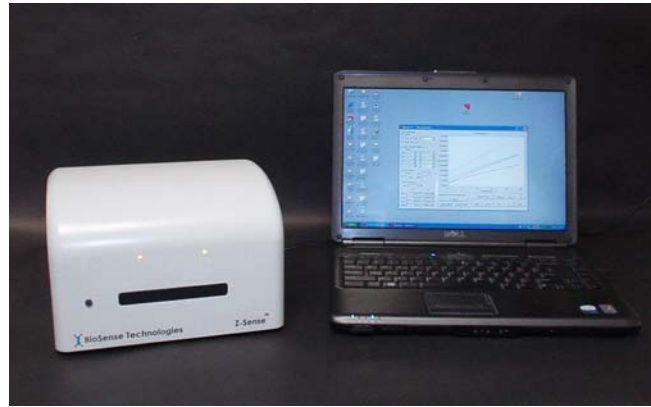
In addition, the approach avails itself of the following major advantages:

- Detects aerobic and/or anaerobic species with the same assay
- Does not require specific culture conditions
- Discerns viable from dead organisms

## Experimental Methods

A total of 141 in- and out-dated apheresis-derived single donor and leukocyte-reduced whole blood-derived platelets were spiked with 17 model bacteria implicated in post-transfusion septic reactions. PC samples were tested at final concentrations from  $10^3$  to  $10^6$  CFU/mL. All bacterial strains used in this study were adapted and growing in human platelet concentrates prior to measurement. The spiked PCs were treated with a proprietary chemical compound designated STR-103 which induced a measurable

stress response in all bacteria but not in the human cells. Subtle changes in the dielectric permittivities of the spiked platelet samples were measured using the BioSense Technologies Z-Sense™ differential impedance sensing platform shown in Fig. 2.

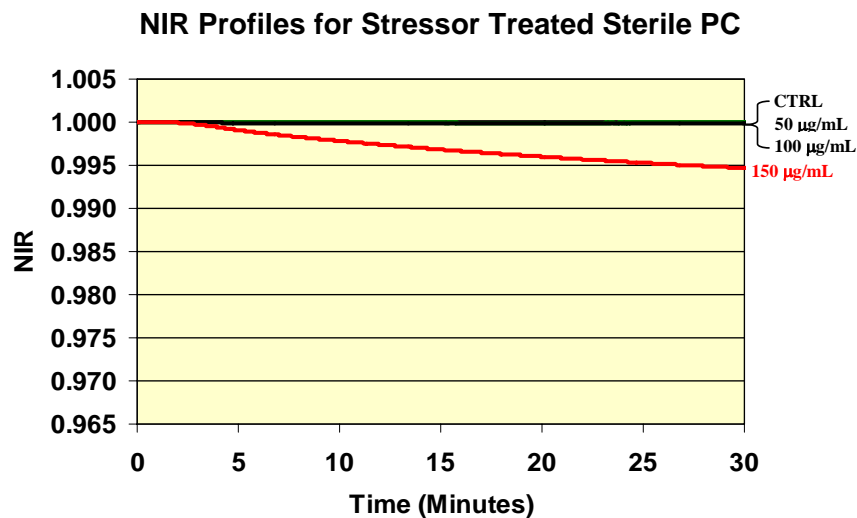


**Fig. 2. Z-Sense™ Differential Impedance Sensing Platform.**

One chamber of the Z-Sense™ test cassette was manually filled with PC containing the stressor while the adjacent reference chamber was filled with untreated PC for direct comparison. The filled cassette was then inserted into the analyzer set at 30°C and the impedance signals from each 100 µL volume test chamber were continuously recorded and analyzed. The capacitance components of the respective impedance signals were analyzed together to minimize unwanted background signals and produce the corresponding Normalized Impedance Response Profiles (NIR). The bacterial concentration was determined at the start of each experiment. All experiments were repeated at least three times.

### Results

The Normalized Impedance Response (NIR) profiles for sterile PC treated with different stressor concentrations are plotted together in Fig. 3. No measurable deviation from the NIR profile for untreated PC (CTRL) was found for PC treated with less than 150 µg/mL of the stressor. Thus, a stressor concentration of 100 µg/mL was determined to be optimal and used for all spiked PC experiments.



**Fig. 3. Differential impedance response from sterile platelet concentrate treated with stressor compound STR-103.** Three curves corresponding to the control run (“CTRL”; no stressor), PC treated with 50 µg/mL, and 100 µg/mL are plotted together and overlap having the same value. In contrast, the NIR profile for PC treated with 150 µg/mL deviates from these three curves over the 30 minute measurement period.

In contrast to the response of sterile PC, distinct response profiles were obtained without exception for all 17 bacterial species spiked into PC. NIR profiles for *E. coli*, *P. aeruginosa*, *S. marcescens*, *S. epidermidis*, *P. acnes*, and *L. monocytogenes* are plotted in Fig. 4 as representative of the 17 Gram-negative and Gram-positive species tested. Consistent with our previously reported results, the corresponding NIR profiles are characterized by an immediate and continuous decrease in the NIR values with an intensity that is proportional to the bacterial concentration tested. Bacterial presence was established within 5-10 minutes for high inoculums ( $10^5$  and  $10^6$  CFUs/mL) while low inoculums ( $10^3$  and  $10^4$  CFUs/mL) were usually detectable within 20 minutes.

The statistical significance of the different NIR profiles was analyzed and determined to be significant for all species.

All impedance-based results were confirmed by quantitative culturing of the sample on agar plates. The entire testing process routinely took less than 30 minutes from the point of sampling to the time that the final results were available.

In addition, survival studies of the bacteria in stressor-treated spiked apheresis- and whole blood-derived PC were undertaken to determine if the corresponding NIR profiles were primarily associated with cell death. For all species studied, the numbers of viable bacteria in the stressor-treated PC remained constant over the initial 30 minute measurement period (irrespective of the type of PC) indicating no significant bacterial cell death.

## Summary and Conclusions

These proof-of-principal experiments demonstrate that monitoring the development of stress in bacteria is a rapid and effective way to detect bacterial contamination in PCs. Detecting the stressed bacteria directly in PC represents a significant improvement to our previously described assay that involved lysis of the platelets. Based on these results, a simple assay can be developed that is based on a one-step protocol and is amenable to point of issue testing in hospitals and blood centers.

Future experiments are planned to determine the assay limits of detection and investigate the impedance response at different stages of bacterial growth. The corresponding data will be compared directly with results from other FDA approved methods.

## Acknowledgements

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## References

<sup>1</sup> Rieder, RJ and B. Zavizion. (2008). Monitoring the Physiological Stress Response: A Novel Biophysical Approach for the Rapid Detection of Bacteria in Platelet Concentrate. *Transfusion*, **48**, 2596-605.

**Contact Information.** For more information on our product development or collaboration please contact Dr. Ronald Rieder at BioSense Technologies, Inc. 4 Arrow Drive, Woburn, MA 01801 Tel: (781) 933-3635 or email [info@biosensetech.com](mailto:info@biosensetech.com).

### NIR Profiles for Stressor Treated Spiked PC

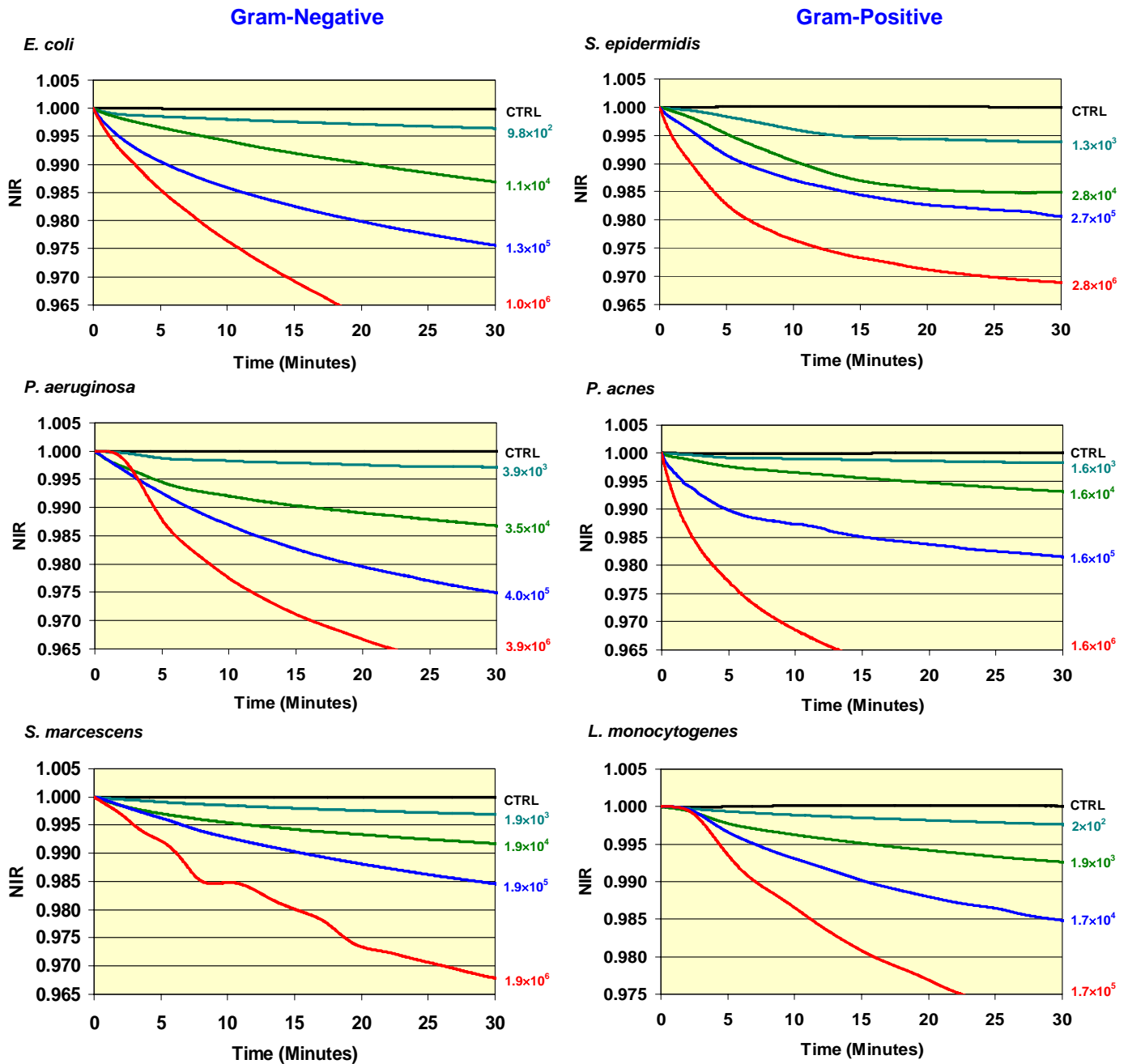


Figure 4. Normalized Impedance Response (NIR) profiles from PC infected with six representative bacterial species out of 17 tested treated with 100 mg/mL of STR-103. NIR profiles for PC infected with between  $10^3$  CFUs/mL to  $10^6$  CFUs/mL of the Gram-negative species *E. coli*, *P. aeruginosa*, *S. marcescens*, and Gram-positive species *S. epidermidis*, *P. acnes*, and *L. monocytogenes*. The actual bacterial concentrations tested are displayed next to their corresponding NIR profiles. Sterile but treated PC is plotted as a negative control (CTRL) in all plots.