Detection of Bacteria in Blood using Laser-Induced Breakdown Spectroscopy

Emma Blanchette, Jasmine Saad, Emily Tracey, August Baughan, Grace Johnson, Hadia Malik, Caroline Alionte, Isabella Arthur, Rachel Chevalier, Nicholas Bolton, Matteo Pontoni, Mila Vasquez, Abdullah Mustafa, Lauren Dmytrow, Steven J. Rehse

Department of Physics, University of Windsor, Windsor, Ontario, Canada

Introduction

Sepsis is a serious, often fatal, blood infection which may lead to septic shock in severe cases. This possesses a 50% mortality rate, even with preferred treatment.[1] Every hour of delay in diagnosing sepsis increases patient mortality.[2]

New **antibiotic resistant strains** of bacteria are emerging, making infections more difficult to treat. This leads to:

- Increased patient mortality
- Significant economic strain on healthcare systems worldwide

Statement of Problem

Currently, the **gold standard diagnostic blood test** consists of a slow and labour-intensive two-step blood culture: an initial culture (up to 120 hours) to determine the presence of bacteria, followed by a secondary subculture (24 – 48 hours) to diagnose the pathogen.

Clearly, a much more rapid method to determine the specific pathogen responsible for bacterial sepsis is desperately needed.

Our Solution

❖ Investigate the use of **laser-induced breakdown spectroscopy (LIBS)** to rapidly detect and identify bacterial cells present in in unadulterated and unprocessed specimens of whole human blood.

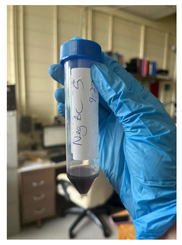
❖ Implement autonomous **machine learning techniques** to reduce the time required to diagnose bacterial infections.

Bacterial Preparation

The Bacteria

Four species of pathogenic bacteria **cultured** in our lab: *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*.

Harvested bacteria cells were suspended in ultra-pure megohmic water (10^7 CFU/mL) to reduce background elemental signal.



The Blood Samples

Five specimens from different patients obtained from local hospital, all tested negative for bacteria.

Specimens were mixed with anticoagulant sodium polyanetholesulfonate (SPS) to prevent blood from clotting.

Blood was several weeks old at the time of testing and had already undergone some hemolysis (red blood cell destruction).

Known aliquots of bacterial suspension "spiked" into sterile blood to simulate sepsis for LIBS testing

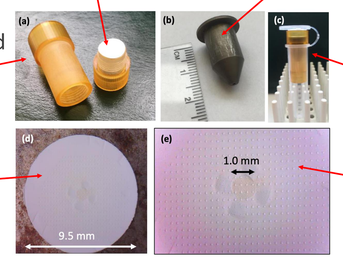
Bacterial Deposition

A custom centrifuge tube insert was used to deposit and concentrate bacterial cells in the center of a disposable nitrocellulose filter.

9.5mm diameter disposable filter

Aluminum cone with 1 mm diameter aperture at apex

Disassembled 3D printed centrifuge insert



100 µL blood + 100 µL bacterial suspension pipetted into reassembled apparatus and sealed in centrifuge tube

Filter after centrifugation at 5000 RPM for five minutes (bacteria concentrated at the center)

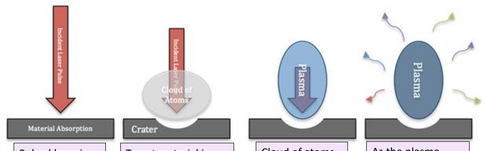
Magnification of the filter with a rastered array of laser ablation sites

Prepared Samples:

- 5 filters each of *S. aureus*, *E. coli*, *E. cloacae*
- 4 filters of *P. aeruginosa*
- 7 filters of sterile blood
- 30 LIBS laser shots from each deposition

LIBS Methodology

LIBS is a **spectrochemical process** in which a laser ablates the surface of targeted material, resulting in a high-temperature plasma. The subsequent light emitted is used to analyze the constituent atoms and ions.



Pulsed laser is focused on target surface which absorbs laser energy

Target material is **vaporized**, generating a cloud of atoms above the target surface

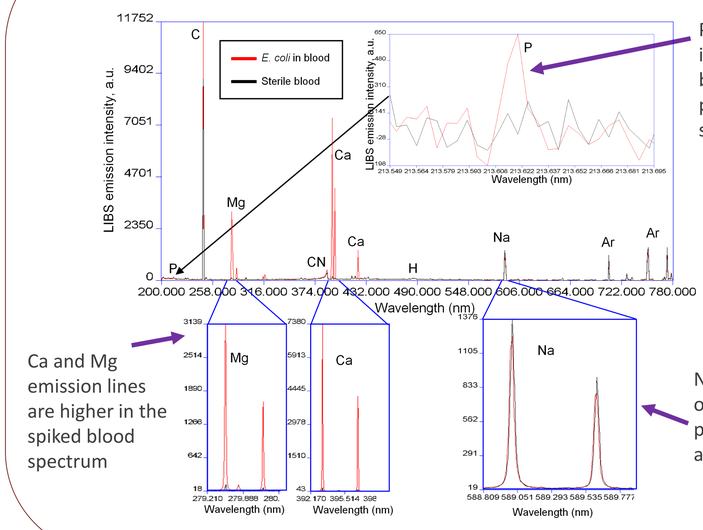
Cloud of atoms absorbs the remaining laser energy, forming a **plasma**

As the plasma cools, photons are emitted and collected for elemental analysis

Apparatus:

- Nd:YAG 1064nm laser
- 10 Hz pulse repetition rate, 10 ns pulse duration
- Argon flow at 20 standard cubic feet per hour (SCFH)
- Echelle spectrometer disperses plasma emission, produces a spectrum from 200 – 840 nm with 12 pm resolution
- Emission collected 2 microseconds after plasma formation
- ~11,000 cells ablated per spectrum (laser pulse)

Bacterial LIBS Spectra



P line is small in the spiked blood, but not present in the sterile blood

Ca and Mg emission lines are higher in the spiked blood spectrum

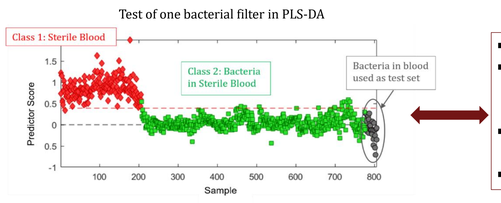
Na emission observed is partially due to anticoagulant

Results: PLS-DA

Chemometric algorithm **partial least squares-discriminant analysis (PLS-DA)** was used to classify unknown specimens into either a sterile blood or spiked blood class.

PLS-DA predicts the dependent variable (test data class identity) given a set of known independent variables (15 emission line intensities + 92 ratios of intensities).

- **Individual spectrum test**: Each spectrum on a filter was tested against all other spectra from all other filters.
- **Averaged spectrum test:** All 30 spectra on every filter were averaged then tested individually against the remaining 25 filters



- Implemented in PLS_Toolbox (Eigenvector Research, Inc.).
- Model constructed from 570 spectra of spiked blood (green, class 2) and 210 spectra of sterile blood (red, class 1).
- Bayesian cut line (red dashed line) differentiates class 1 from class 2.
- Model tested with 30 spectra of spiked blood (grey).

	Individual Spectra	Averaged Spectra
Sensitivity	96.3%	100%
Specificity	98.6%	100%

Results: PCA-ANN

570 spectra from 200 – 590 nm (42,000 independent variables) were preprocessed with an unsupervised **principal component analysis (PCA)**, resulting in 10 PC scores that captured 99.58% of variance. An **artificial neural network (ANN)** was trained on these data to predict the bacterial identities.

External validation test: Model was trained on 18 filters. Each individual filter (30 spectra) was withheld and tested ten times against the model.

External Validation Test Results					
	S. aureus	*E. coli*	*E. cloacae*	*P. aeruginosa*	Four Class Average
Sensitivity	61.7 %	93.7 %	91.6 %	95.0 %	85.5 %
Specificity	96.2 %	97.4 %	90.2 %	96.1 %	95.0 %
Classification Accuracy	87.1 %	96.4 %	90.6 %	95.9 %	92.5 %

80:20 cross-validation: Model was trained on 80% of all data (randomly selected). Remaining 20% of data was tested against this model.

80:20 Test Results					
	S. aureus	*E. coli*	*E. cloacae*	*P. aeruginosa*	Four Class Average
Sensitivity	100 %	100 %	100 %	100 %	100 %
Specificity	100 %	100 %	100 %	100 %	100 %
Classification Accuracy	100 %	100 %	100 %	100 %	100 %

Sensitivity: true positive rate of the test
Specificity: true negative rate of the test
Classification Accuracy: correct prediction rate of the test

ANN Implementation

A PCA script was constructed using Python, along with several libraries: *sklearn.decomposition*, *Pandas*, *Numpy*, and *mpl_toolkits*. The algorithm reduced 42,000 independent variables to 10 PC scores (~20 minutes) for each spectrum—greatly reducing ANN training time.

ANN was developed with Python's *Pandas*, *Numpy*, *Tensorflow*, and *Scikit-Learn* libraries.

Parameters utilized were:

- 170 hidden nodes
- 1 hidden layer
- Patience = 35

➢ **Run time of two seconds.**

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

$$\text{Specificity} = \frac{TN}{TN + FP}$$

$$\text{Classification Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

Conclusions

LIBS was used to **obtain elemental emission spectra** from **sterile blood** and **blood spiked with bacteria**.

The PLS-DA test was **successful in detecting the presence of pathogenic bacteria in blood.**

- 96.3% sensitivity and 98.6% specificity were achieved when single shot LIBS spectra (11,000 cells) were used to detect the bacteria.
- 100% specificity and sensitivity were achieved when the spectra were averaged together (330,000 cells).

PCA-ANN models were used to **diagnose the bacterial species present in infected blood.**

- 85.5% sensitivity and 95.0% specificity in a four species external diagnostic test.
- 100% sensitivity and 100% specificity in a four species 80:20 cross-validation test.

Two filters of *S. aureus* performed very poorly ⇒ decreased sensitivity and classification accuracy.

Future Work

❑ Determine the bacterial limit of detection.
❑ Develop new techniques to maximize the LIBS emission from a reduced number of cells.
❑ Expand the study to include more species of bacteria and a greater number of blood donors.
❑ Improve reproducibility in bacterial deposition.
❑ Improve chemometric and machine-learning algorithms to enhance efficiency and optimize accuracy.

References

(1) E. Whitnack, Sepsis, in: N.C Engleberg, V.J. DiRita, T. Dermody, M. Schaechter (Eds.), Mechanisms of Microbial Disease, 3rd ed, Lippincott Williams & Wilkins, 2007, pp. 564-572.
(2) A. Kumar, D. Roberts, K.E. Wood, B. Light, J.E. Parrillo, et al., Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock, Crit. Care Med. 34 (2006) 1589-1596, https://doi.org/10.1097/01.CCM.0000217961.75225.E9.