Differentiation of *Staphylococcus* and *Streptococcus* Species Using Laser-Induced Breakdown Spectroscopy

Jasmine Fric, Rachel Chevalier, Abdullah Mustafa, Lauren Dmytrow, Simona Brezeanu, Will Conlon, Danielle Renard, Katherine Keller, Emma Pesce, Jack Holland, Ayman Younes, Faiza Fric, Steven J. Rehse

NSERC CRSNG

University of Windsor

Introduction

Bacterial meningitis is a dangerous disease caused by infection and inflammation of the meninges. Among the causative infectious agents for meningitis are certain *Streptococcus* and *Staphylococcus* species. These bacterial infections often present with overlapping symptoms and may be easily mistaken. However, as treatments differ, it is clinically essential to distinguish the pathogen responsible.

Problem and Solution

Presently, meningitis is diagnosed after analyzing a sample of cerebrospinal fluid, which can only be obtained through an invasive lumbar puncture. Then, the culture confirmation to determine the specific pathogen can take days, putting a patient more at risk of delay or inappropriate treatment. The risk of delaying treatment is especially harmful, as the odds of an unfavourable outcome rise by 30% every hour [1]. Thus, a rapid method to determine the pathogen responsible is needed.

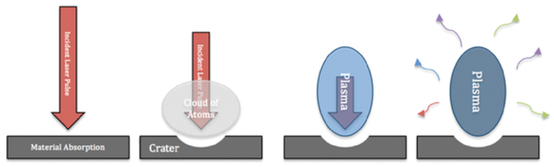
Laser-induced breakdown spectroscopy (LIBS) could provide the necessary solution to these impediments, being a rapid analytical technique capable of differentiating various bacterial species.

LIBS Methodology

In LIBS, a laser ablates the surface of a material, creating a high-temperature plasma. The light emitted by this plasma is then analyzed to determine its constituent atoms and ions, making a rapid elemental assay of the bacterial cells.

Apparatus:

- Nd:YAG 1064 nm laser
- 10 Hz pulse reptition 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
- Spectral emission is collected 2 microseconds after plasma formation
- Roughly 11,000 cells are ablated per laser pulse (producing one spectrum)



Pulsed laser is focused on the target's surface, which absorbs laser energy.

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

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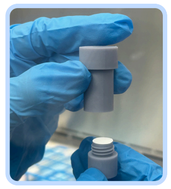
Photons are emitted and collected for elemental analysis.

Bacterial Preparation and Deposition

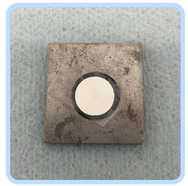
The two species were cultured on blood TSA agar plates, harvested, and suspended in artificial cerebral spinal fluid (aCSF) to replicate the environment of the meninges. Known aliquots of *Staph. aureus* and *Strep. salivarius*, along with sterile aCSF for a control, were then deposited onto a nitrocellulose medium using a custom centrifuge tube insert to concentrate bacterial cells at the centre of the filter.



Filter is placed on centrifuge insert α.

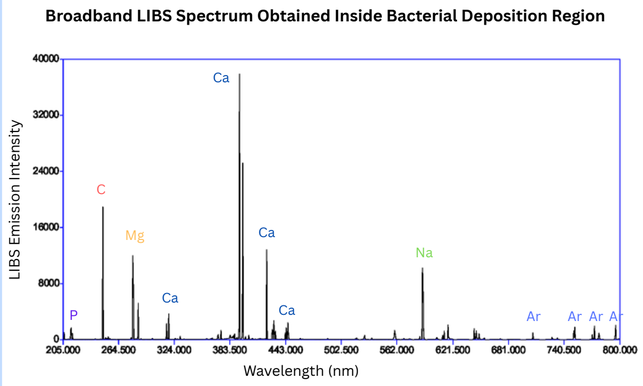
Centrifuge insert β is screwed in until tight and placed in a centrifuge tube. 100 µl of bacterial suspension are pipetted in ten 10 µl aliquots through the aperture in β.

Sample is centrifuged at 2400 rpm for 5 minutes, allowing for aCSF to drain through filter as bacteria are concentrated at centre.



After drying, filter is mounted onto a steel piece with double-sided tape.

Bacterial LIBS Spectra



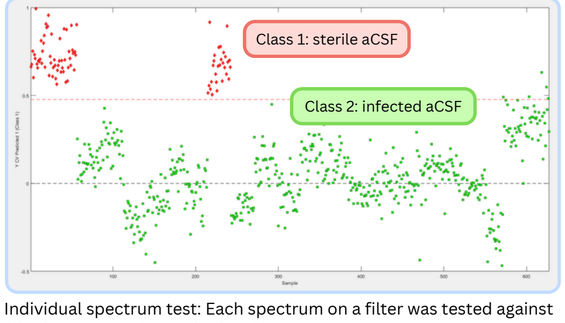
- **P** emission lines are highly indicative of the presence of bacteria
- **Ar** emission lines are from the chamber environment in which samples are ablated in
- **C** emission line comes from the nitrocellulose filter
- **Mg** and **Na** emission lines are indicative of bacterial cells, though they are also present in the sterile aCSF solution
- **Ca** emission lines are very strong when bacteria are added to aCSF

Light emitted from the ablated sample is collected by the spectrometer, which produces an emission spectrum (above) in which the intensity of each spectral line is proportional to its concentration in the sample.

Results: PLS-DA

The spectra from the sterile aCSF and both bacterial species were classified using partial least squares discrimination analysis (PLS-DA). The chemometric algorithm PLS-DA predicts the test data class identity given a set of known independent variables. This test was done to determine whether the system was capable of detecting bacterial presence.

3 filters of sterile aCSF were classified against 20 filters of infected aCSF (10 with *Staph*., 10 with *Strep*.). The model was constructed from 84 spectra of sterile aCSF (red, class 1) and 543 spectra of infected aCSF (green, class 2).



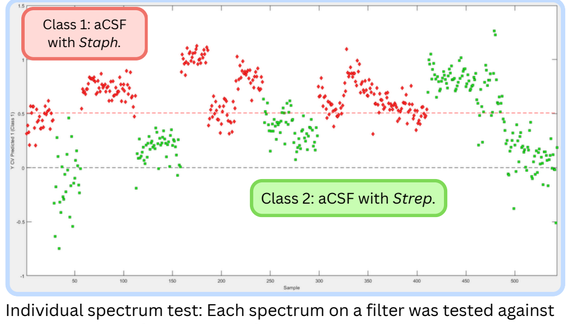
Individual spectrum test: Each spectrum on a filter was tested against all other spectra from all other filters

The sensitivity of the test was determined to be 99.1%, and the specificity of the test was determined to be 100%. The classification accuracy was determined to be 99.2%.

Results: PLS-DA

The spectra from the aCSF infected with *Staph.* and aCSF infected with *Strep.* were then classified using PLS-DA, to determine whether the system was capable of diagnosing bacterial identity.

10 filters of aCSF infected with *Staph*. were classified against 10 filters of aCSF infected with *Strep*. The model was constructed from 280 spectra of aCSF with *Staph*. (red, class 1) and 263 spectra of aCSF with *Strep.* (green, class 2).



Individual spectrum test: Each spectrum on a filter was tested against all other spectra from all other filters

The sensitivity of the test was determined to be 79.6%, and the specificity of the test was determined to be 68.8%. The classification accuracy was determined to be 74.4%.

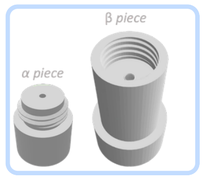
TP = True Positives FP = False Positives
TN = True Negatives FN = False Negatives

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100\%$$

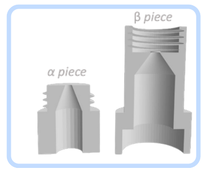
$$\text{Specificity} = \frac{TN}{TN + FP} \times 100\%$$

$$\text{Classification Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \times 100\%$$

Custom 3D-Printed Centrifuge Insert



Top view



Cross section

3D-printed cone with two components: α (small insert) and β (large insert). The two pieces screw together with the filter in between. The aperture in the large insert concentrates the bacteria in a 1.8 mm diameter circle, while the ridge prevents bacterial cells from escaping.

The aperture on the small insert allows water and ions to drain through while larger bacterial cells remain on the filter surface, forming an easily-identifiable indent.

Conclusions

LIBS was used to obtain elemental emission spectra from aCSF solutions with *Staphylococcus* and aCSF solutions with *Streptococcus*.

The PLS-DA test was successful in detecting the presence of pathogenic bacteria in aCSF, with a classification accuracy of 99.2%. The system was moderately successful in diagnosing bacterial identity, with a classification accuracy of 74.4%.

Future Work

- Improve chemometric and machine-learning algorithms to enhance efficiency and optimize accuracy
- Improve reproducibility in bacterial deposition
- Incorporate ultrasonic dismembration to homogenize bacterial solutions
- Train the data on an artificial neural network (ANN) to better predict bacterial identities.

References

(1) M. Glimåker, B. Johansson, M. Bell, M. Ericsson, J. Bläckberg, M. Brink, J. Sjölin, Early lumbar puncture in adult bacterial meningitis—rationale for revised guidelines. Scandinavian Journal of Infectious Diseases, (2013). 45(9), 657–663. https://doi.org/10.3109/00365548.2013.799289