Identification and Elimination of Sources of Extraneous Background Signal in Laser – Induced Breakdown Spectroscopy Spectra of Bacterial Cells Deposited on Filtration Media

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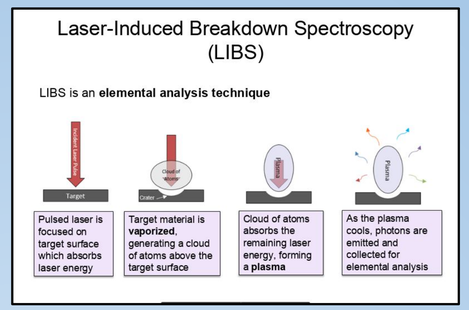
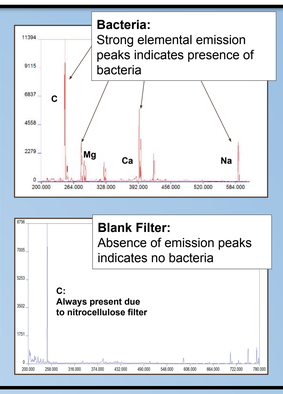
Introduction

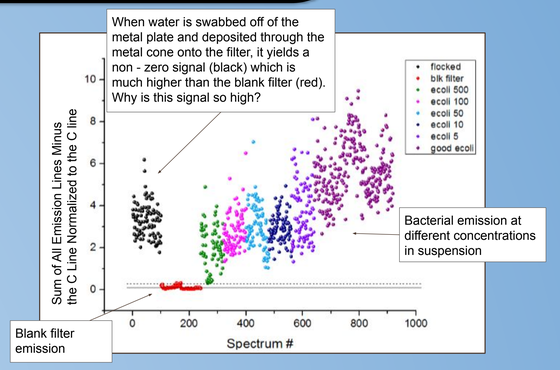
There is an urgent need to develop faster ways to identify pathogenic bacteria. Laser - Induced Breakdown Spectroscopy (LIBS) is a promising technique to achieve this.

Statement of the Problem

Laser ablation of the filter medium and other elemental contaminants yields a non – zero background signal when a control experiment is performed in the absence of any bacterial cells. The purpose of this experiment was to identify the source of this background signal and to introduce new cleaning procedures that reduce this background signal.



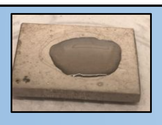




Current Method of Depositing Bacteria On Filtration Media

Metal cone, metal plate, and test tube insert are cleaned in bleach water solution and rinsed with DI water.

Deposit 10 µL of bacteria suspended in DI water onto metal plate and heat for 2 min and 20 s.

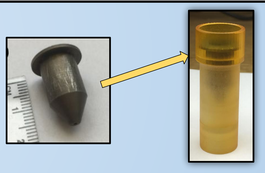


Deposit 1 µL water onto swab tip and swab metal plate.

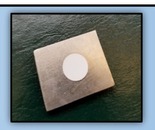
Place swab in centrifuge tube with 1 mL of water and vortex tube for 15 s.

Place filter on the base of the insert. Place the cone in the insert.

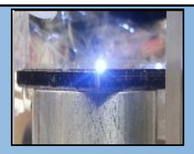
Deposit 1 mL of water from vortexed tube into cone, place insert into clean centrifuge tube. Centrifuge insert at 5000 rpm, 2500 g's for 5 min.



Remove insert from centrifuge, then remove filter from insert. Mount filter on metal plate with double – sided tape.

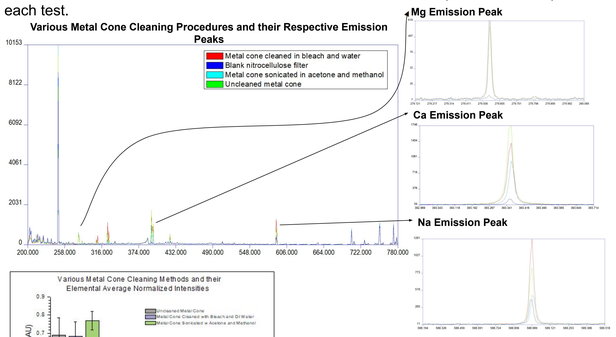


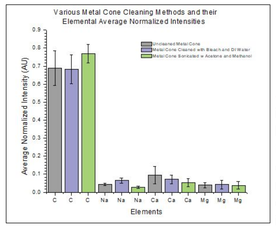
Filter is placed in an argon environment and ablated using a pulsed 1064 nm Nd: YAG laser.



Q: What is the effect of cleaning the metal cone?

The current procedure for cleaning the metal cone consists of rinsing the cone in a 1:10 bleach - water solution for approximately 15 s and letting it air dry. It is then rinsed in DI water for approximately 15s and again letting it air dry. To test whether altering the cleaning procedure had a significant impact on the background signal observed in the spectra, three cleaning procedures to compare the elemental intensities of carbon, sodium, magnesium and calcium were tested. The first test utilized DI water deposited directly into an uncleaned metal cone - with contaminants from fingerprints, dust, etc - and centrifuged at 5000 rpm for 5 min. The second test followed the standard protocol which consisted of pipetting DI water into the metal cone which was rinsed in a 1:10 bleach solution and DI water. The third test repeated this same procedure except the metal cone was sonicated in acetone for 5 min and then sonicated in methanol for 5 min. 20 spectra were acquired in each test.

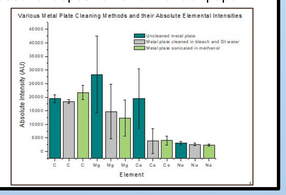




A: Cleaning the metal cone by ultrasonication in acetone and methanol yielded a lower background signal than the uncleaned cone and the bleach water cleaning method for the elements measured. The method of cleaning the metal cone was therefore an important contributor to the background signal produced in the emission spectra.

Q: What is the effect of cleaning the metal plate?

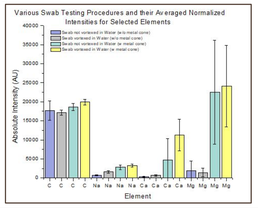
The current procedure for cleaning the metal plate consists of rinsing it in a 1:10 bleach - water solution for approximately 15 s, then allowing it to air dry. It is then rinsed in DI water for approximately 15s, then allowing it to air dry. To investigate whether altering the cleaning procedure had a significant impact on background signal, three variations of the cleaning procedure were tested and the elemental intensities of carbon, sodium, magnesium, and calcium were compared. The variations in cleaning include an uncleaned plate, a plate cleaned in bleach - water, and a plate cleaned by ultrasonication for 5 minutes in acetone followed by 5 minutes in methanol. The procedure used for deposition onto the filter paper was the current method of depositing bacteria onto filtration media. No bacteria were used in this experiment.



A: Both cleaning procedures yielded a lower background signal than the uncleaned plate as expected. The ultrasonic cleaning of the metal plate made little difference in the reduction of the background signal in comparison to cleaning with bleach - water. Cleaning had a significant impact, but the method of cleaning yielded similar background signals.

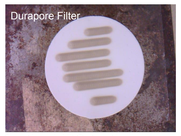
Q: Does vortexing the swab before use have an effect?

The current procedure for testing bacteria involves vortexing the swab after bacteria have been collected, possibly shaking contaminants off of the swab. To investigate the contribution of the swab, a pre - cleaning step before collecting bacteria was introduced. The swab was vortexed in DI water in order to shake contaminants off. Then these swabs and uncleaned swabs were used in the current procedure for swabbing the cleaned metal plate. No bacteria were used in this experiment.

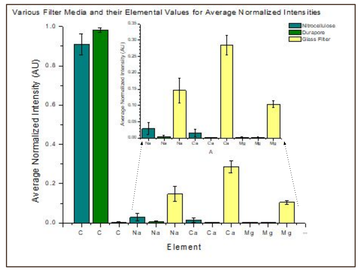


A: The pre - cleaned swab yielded similar results to the non - cleaned swab, indicating that the swab was sterile and not a significant contributor to the observed background emission.

Q: Does the filter medium used have an effect?



The current filter medium used for the LIBS testing procedure is a 22 micron pore size nitrocellulose filter (Millipore). To test whether the choice of filter medium impacted the background signal, two new filters were tested and the normalized elemental emission intensities of four elements of interest were measured: carbon, sodium, calcium, and magnesium. These elements were used for comparison because they are instrumental in the discrimination of different bacteria and their presence in the filter is problematic. The durapore filter (Millipore) and glass microfiber (Whatman) filter were tested against the standard nitrocellulose filter and their varying elemental intensities were compared. The durapore filter has lesser amounts of sodium, magnesium, and calcium, and would be an ideal medium to test bacteria samples on due to this reduced intensity. However, when performing laser ablation, the intensity of the laser was too high for this medium and the excess carbon in the filter produced a significant burn pattern (see picture above) which may not be ideal when testing bacterial samples. The glass microfiber filter showed significant emission from sodium, magnesium, and calcium and is not suitable for testing because it is a non-uniform surface with many ridges and fibres that when shot, created large ablations and disrupted the surrounding media for further testing (see picture above).



A: Nitrocellulose: Ca, Mg, and Na, significant C emission observed.
Durapore: reduced Ca, Mg, and Na observed; C emission increased. Significant scorching.
Glass Microfiber: significant increase in Ca, Mg, and Na observed; significant reduction in C emission. Destruction of testing surface.
Conclusion: Nitrocellulose is the optimal testing surface.

Conclusions and Future Work

The protocols developed in this experiment (cleaning of cone, cleaning of plate) will be introduced as a part of the current testing protocol which will reduce the limits of detection of bacteria with LIBS.

The limits of detection will be determined when bacteria are swabbed off of surfaces and deposited in fluid suspensions in the cone (both simulating clinical tests).

The discrimination of different bacteria when swabbed off of surfaces and deposited in fluid suspensions in the cone will be investigated.

Testing of actual clinical specimens obtained from pathology labs will be initiated including bacteria suspended in complex mixtures (i.e. blood, cerebral spinal fluid). Other areas of interest include automation techniques to make the technique more usable by non - experts and more safe, without loss of sensitivity or specificity.

Acknowledgements

This work was supported by the Outstanding Scholars program, NSERC through the USRA and discovery grant programs, and the Faculty of Science. We would like to thank Dr. Rehse, our supervisor, for his continued guidance on these projects as well as Allie Paulick for her teaching and support.

References

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