Diagnosing Bacterial Urinary Tract Infections Using Laser-Induced Breakdown Spectroscopy

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

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

Motivation

Urinary tract infections (UTIs) are one of the most common infections in adult women, as well as a prominent hospital acquired infection (HAI), creating a burden on the health-care system[1,2]. Currently, the gold standard for diagnosis involves bacteria culturing – a method that is time consuming, costly, and unable to detect all bacteria species[3].

As an alternative diagnostic technique, we investigate laser-induced breakdown spectroscopy (LIBS) for the rapid and accurate identification of pathogenic bacteria in clinical specimens of urine.

Introduction

LIBS is a rapid spectrochemical technique in which a laser pulse is used to ablate a target. During ablation, the laser energy is converted to heat, vaporizing the target and creating a plasma. As the plasma cools, spontaneous emission photons from atoms and ions are collected and dispersed in a spectrometer, producing an optical emission spectrum. This technique allows for the near-instantaneous determination of the elemental composition of the target.

To stimulate clinical UTIs, sterile urine specimens obtained from four patients were spiked with known concentrations of bacterial cells. In this study, three species of bacteria were investigated: *Escherichia coli*, *Staphylococcus aureus*, and *Enterobacter cloacae*.

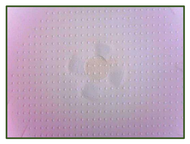
Spectra of sterile urine, as well as spiked urine were obtained using the methodology shown to the right. The spectra were analyzed using:

- **Partial least squares-discriminant analysis** (PLS-DA) for detecting bacteria in urine
- **Artificial neural network analysis with principal component analysis pre-processing** (PCA-ANN) for identifying the type of bacteria

Spectral Analysis

On each filter, there are 30 different sites ablated to obtain 30 single-shot LIBS spectra per filter. These spectra identify the elemental composition of the sample, and the ratios between the intensities of the emission lines are what further allow the differentiation between different bacteria.

Total number of filters: 4 *E. coli*, 4 *S. aureus*, 4 *E. cloacae* and 8 sterile urine.

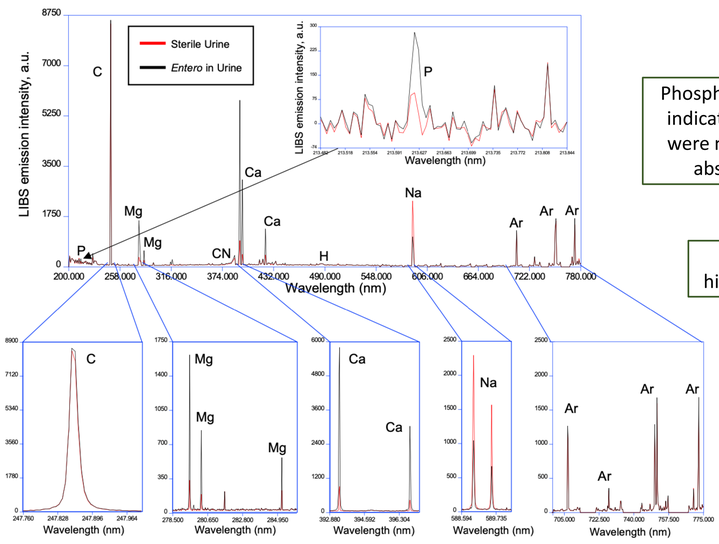


Optical micrograph of laser shots rastered across a filter with bacterial deposition in center.

Strong carbon emission is predominantly from the nitrocellulose filter

Magnesium lines are greater in spiked urine, an indication of bacteria

Calcium lines higher in spiked urine samples, likely due to bacteria



Phosphorus lines are highly indicative of bacteria, and were never present in the absence of bacteria

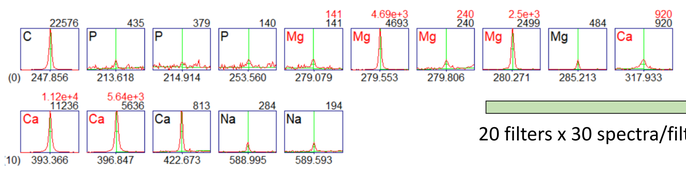
Sodium emission higher in sterile urine

Argon lines are from the chamber environment where the samples are shot in

PLS-DA Results – Detecting Bacteria

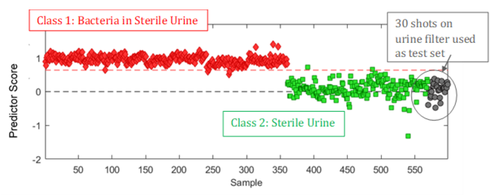
GOAL: Differentiate between sterile urine and spiked urine

Input: 15 emission line intensities and 92 ratios of these lines for a total of 107 variables per spectra.



20 filters x 30 spectra/filter

External Test: One filter withheld and tested against model constructed with 19 remaining filters.



	Single-Shot Filters	Averaged Filters
Sensitivity	98.3%	100%
Specificity	97.9%	100%

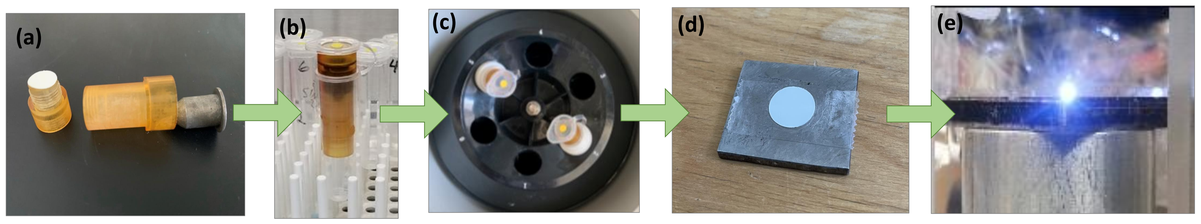
30 spectra (one filter) withheld and tested individually against a model constructed from the remaining 570 spectra

30 spectra of each filter averaged, and then tested against a model constructed from the 19 other averaged spectra

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}} \times 100\% \quad \rightarrow \text{ tests if all infected samples correctly diagnosed}$$

$$\text{Specificity} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}} \times 100\% \quad \rightarrow \text{ tests if any uninfected samples are diagnosed as infected}$$

Methodology

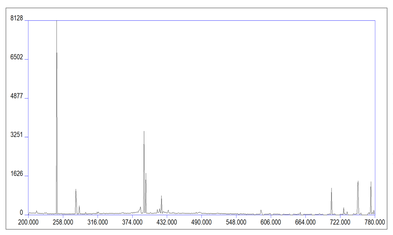


(a) A 3D-printed centrifuge insert which has a metal cone attachment is assembled and placed in a test tube **(b)** where bacteria is concentrated onto a nitrocellulose filter. **(c)** The sample is centrifuged and then the filter is mounted onto a piece of steel **(d)** which is then placed in an argon environment and ablated by a 1064 nm ND:YAG laser **(e)**. Finally, a broadband atomic spectrum is obtained.

PCA-ANN Results – Identifying Bacteria

GOAL: Classify the species of bacteria in urine

Input: Entire LIBS spectrum from 200 nm to 590 nm (42,000 variables)



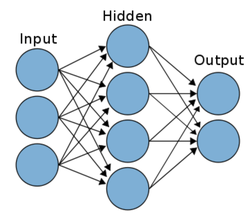
12 spiked urine filters x 30 spectra/filter

PCA: Spectrum is reduced to 10 principal components (PC) scores, through the use of a Python code developed from several libraries

1	2	3	4	5	6	7	8	9	10

Python libraries used: *sklearn.decomposition*, *Pandas*, *Numpy*, and *mpl_toolkits*

ANN: Neural network developed on Python code relates 10 PC scores to bacterial species



Python libraries used: *Pandas*, *Numpy*, *Tensorflow*, and *Scikit-Learn*

80:20 Cross Validation				
	E. coli	*S. aureus*	*E. cloacae*	Overall Test
Sensitivity	100%	100%	91.67%	97.22%
Specificity	95.83%	100%	100%	98.61%
Classification Accuracy	97.91%	100%	95.83	97.91%

80% of the data is randomly selected to train the model on, while the other 20% is tested against this model.

External Validation				
	E. coli	*S. aureus*	*E. cloacae*	Overall Test
Sensitivity	63.6%	86.9%	62.3%	70.9%
Specificity	87.7%	85.9%	82.8%	85.5%
Classification Accuracy	79.7%	86.3%	76.0%	80.6%

Spectra of a single filter tested against a model constructed from the remaining 11 filters. This test was repeated 10 times, and the average result was used to determine the test's performance.

$$\text{Classification Accuracy} = \frac{\text{True Positive} + \text{True Negative}}{\text{True Positive} + \text{True Negative} + \text{False Positive} + \text{False Negative}} \times 100\% \quad \rightarrow \text{ number of predictions correct out of the total predictions}$$

Conclusions and Future Work

Conclusions:

- ✓ LIBS was used to **detect and identify bacteria in urine specimens** to investigate its efficacy as a UTI diagnostic technique
- ✓ Successful use of PLS-DA to detect bacteria, as well as concluding that averaging 30 shot of one filter provides 100% sensitivity and specificity
- ✓ Implementation of PCA-ANN models to identify the type of bacteria in the urine, including an 80:20 cross-validation and an external validation method, with a test **sensitivity** of 97.22% and 70.9% and a **specificity** 98.61 and 85.5%, respectively.

Future Work:

- Exploration of new deposition apparatus to improve reproducibility of bacterial concentration
- Investigate enhancements in PCA-ANN classification algorithms, including improving the quality of LIBS data by obtaining more consistent spectra shot-to-shot and optimizing the signal to noise ratio
- Test analysis method on a wider variety of bacterial species, obtained from a greater number of patients

References

[1] T.K. Price, E.E. Hilt, T.J. Dune, E.R. Mueller, A.J. Wolfe, L. Brubaker, Urine trouble: should we think differently about UTI?, Int. Urogynecol. J. 29 (2018) 205-210, https://doi.org/10.1007/s00192-017-3528-8

[2] World Health Organization, Prevention of hospital-acquired infections: a practical guide, 2nd ed., eds: G. Ducel, J. Fabry, L. Nicolle, World Health Organization, Geneva, 2002, pp. 1-64.

[3] M. Barza, Urinary Tract, 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.