

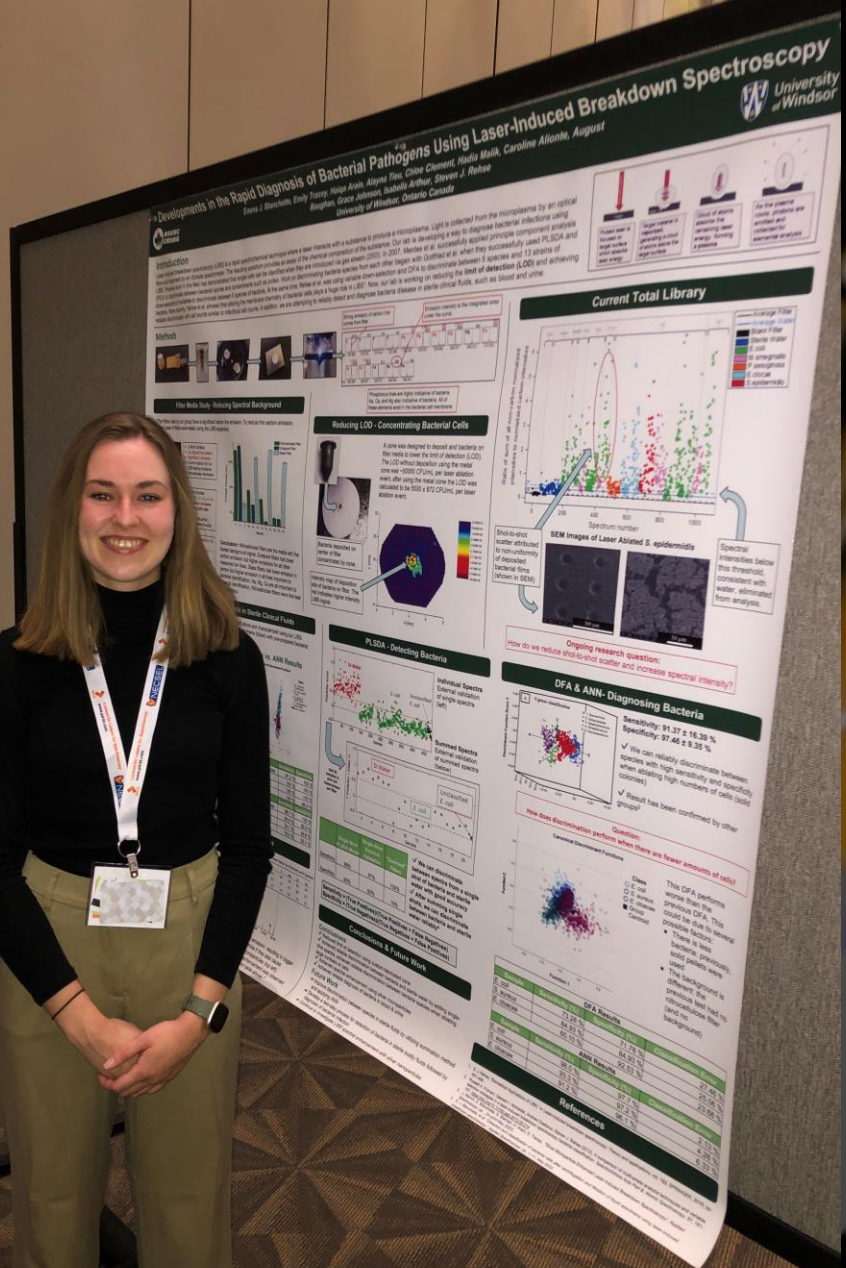
# Laser-Induced Breakdown Spectroscopy for the Identification of Bacterial Pathogens in Blood and Urine

Steven J. Rehse, Emma J. Blanchette, Emily A. Tracey, Caroline N. Alionte, August Baughan, and Hadia Malik

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



**4-7 SEPTEMBER 2023**



This work defended by Master's student **Emma Blanchette**, December 2022.


Emma received a 48TH IEEE ICOPS "Best Student Paper" award, September 2021.

E.J. Blanchette. *Detection and Diagnosis of Bacterial Pathogens in Blood and Urine Using Laser-Induced Breakdown Spectroscopy*. Master's thesis, University of Windsor, 2022.

E.J. Blanchette et al., "Detection and Classification of Bacterial Cells After Centrifugation and Filtration of Liquid Specimens Using Laser-Induced Breakdown Spectroscopy," *Applied Spectroscopy* 76, 2022, pp. 894-904.

# Funding and Acknowledgements

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- An **Ontario Research Fund** Small Infrastructure Funds grant
- **University of Windsor** Outstanding Scholars program
- **University of Windsor** Faculty of Science 



2005-2011

Wayne State University

# Already Shown...

- ✓ We can identify a bacterial species, certainly its genus, with high sensitivity and specificity (confirmed by others).

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## Spectrochimica Acta Part B

journal homepage: [www.elsevier.com/locate/sab](http://www.elsevier.com/locate/sab)



Invited Review

A review of the use of laser-induced breakdown spectroscopy for bacterial classification, quantification, and identification

Steven J. Rehse\*

University of Windsor, Department of Physics, Windsor, Ontario N9B 3P4, Canada



strated by

ive at

3,712 CFU per filters using a

2011-2023

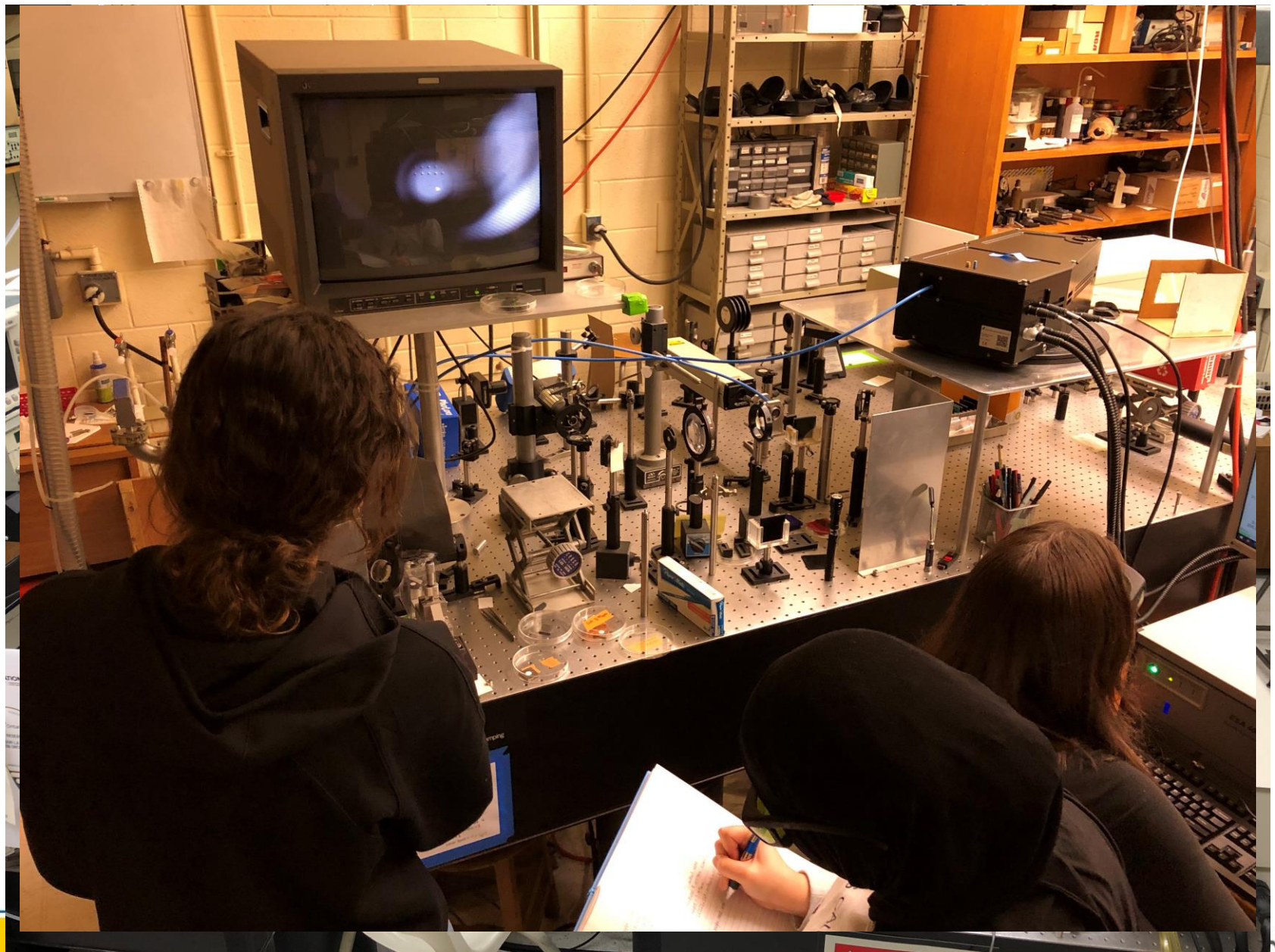
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Can we develop this LIBS test to be:

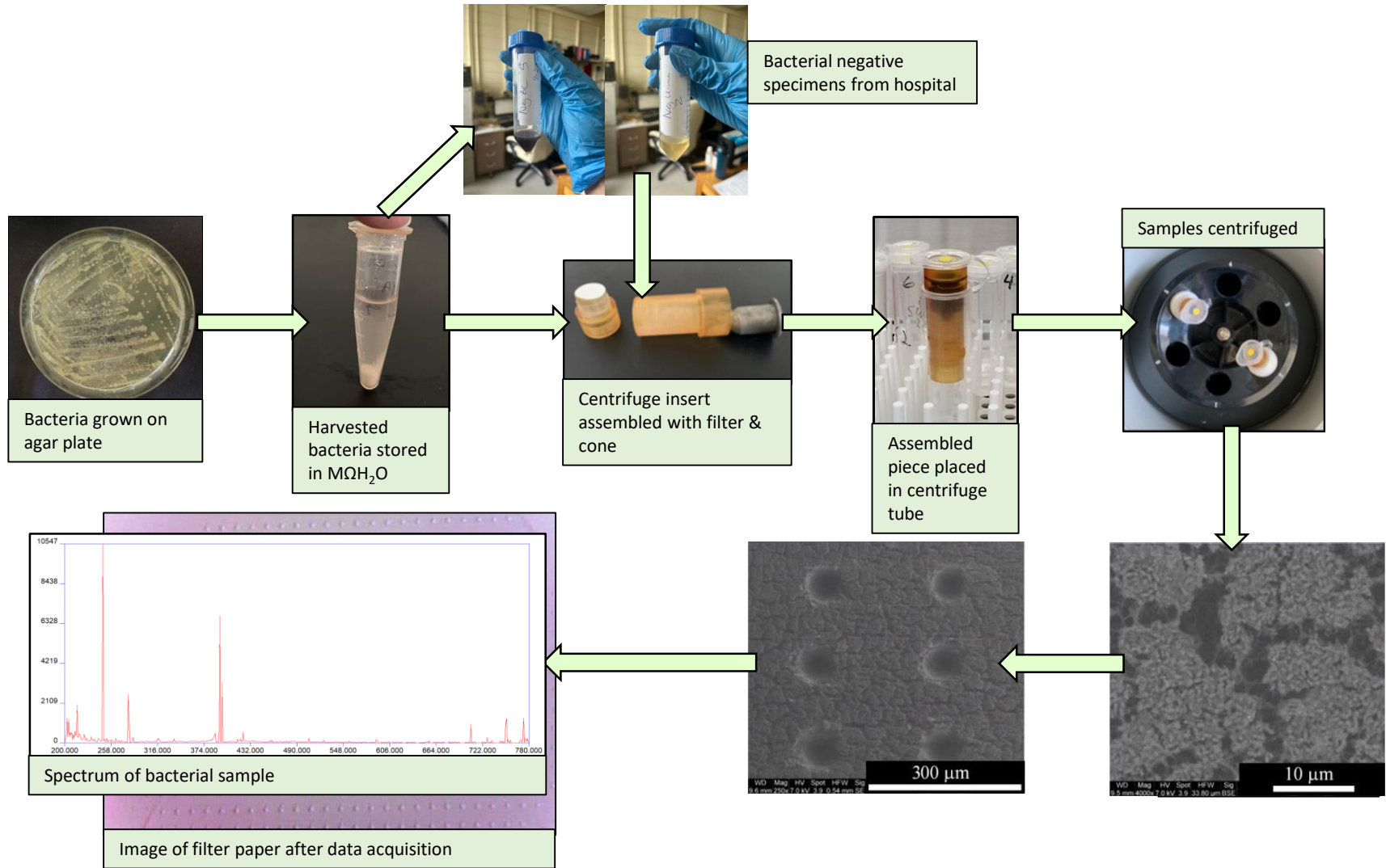
1. convenient (for clinicians) to administer?
2. autonomous (using ML / chemometrics to make an objective diagnosis)?



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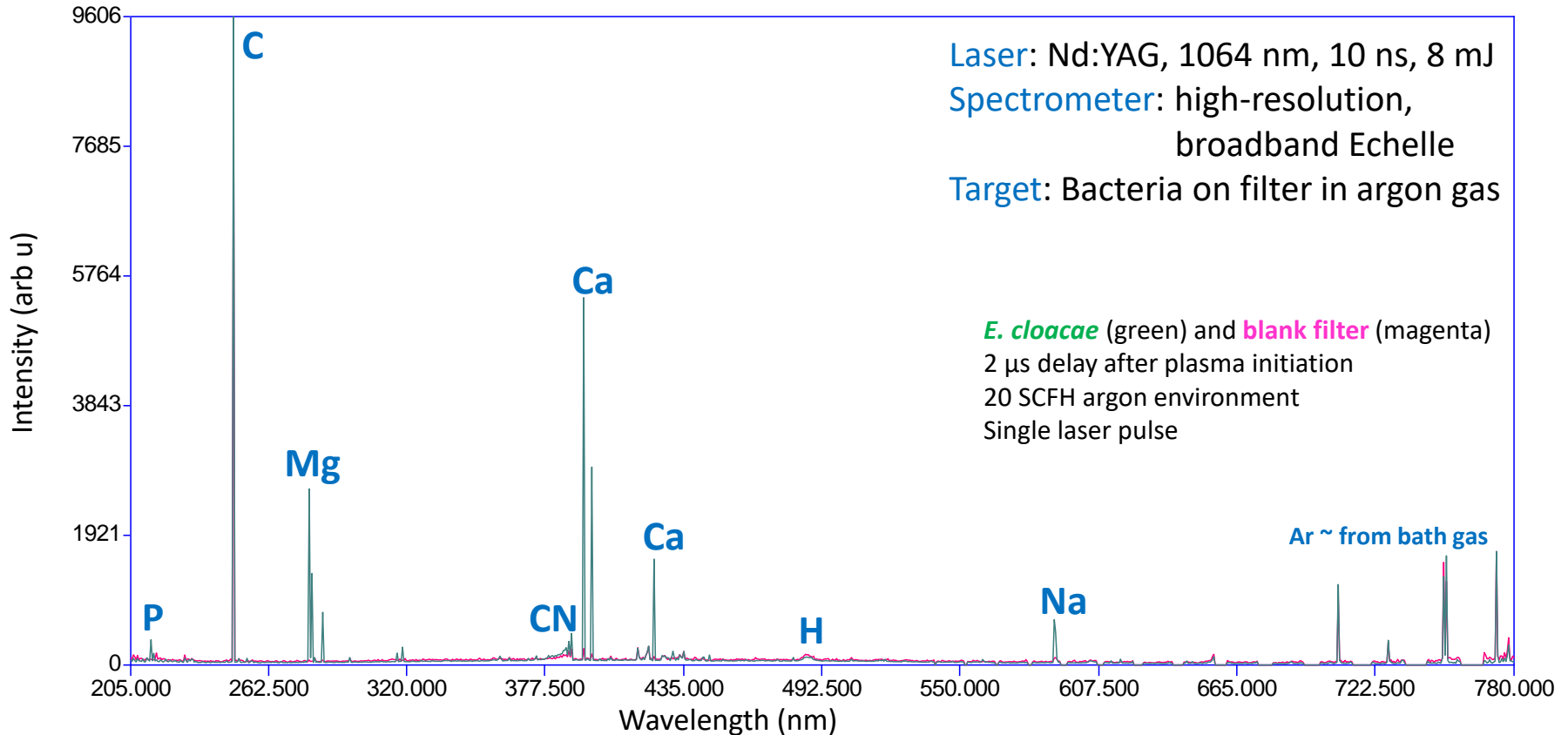


# Methodology – Bacterial Growth & Sample Prep



# Spectrum From Bacteria

An elemental assay of the bacterial cell composition!



Suggests a real-time method for pathogenic bacterial diagnosis.



# Diagnosing Species in Water with Machine Learning

“Ratio Model” consists of:

- 15 emission line intensities and 92 simple ratios
- 107 independent variables

DFA on  
Ratio Model

ANN on Ratio  
Model

PCA-ANN on Ratio  
Model

PCA-ANN on Full  
Spectrum Data\*

- Started using the “whole spectrum” from 200 nm - 590 nm. 42,000 variables.
- Perform unsupervised PCA first (implemented in Python), reduce to 10 PC's.
- Models are trained on 80% of single shot data, 20% reserved for testing. (~15 seconds).

PCA-ANN With Full Spectrum Data			
	E. coli	S. aureus	E. cloacae
<b>Sensitivity</b>	98.04 %	93.27 %	91.23 %
<b>Specificity</b>	97.71 %	97.22 %	96.12 %
<b>Classification Error</b>	2.13 %	4.28 %	6.33 %

\*The full spectrum spans 200 nm – 760 nm, but no lines of interest > 590 nm







# Detecting Infection in Urine with a PLS-DA



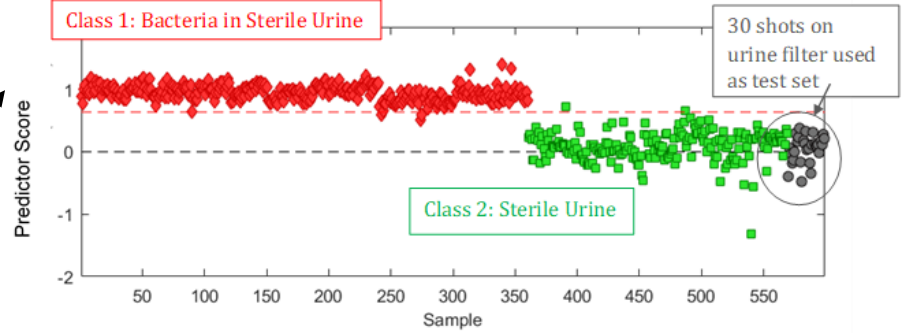
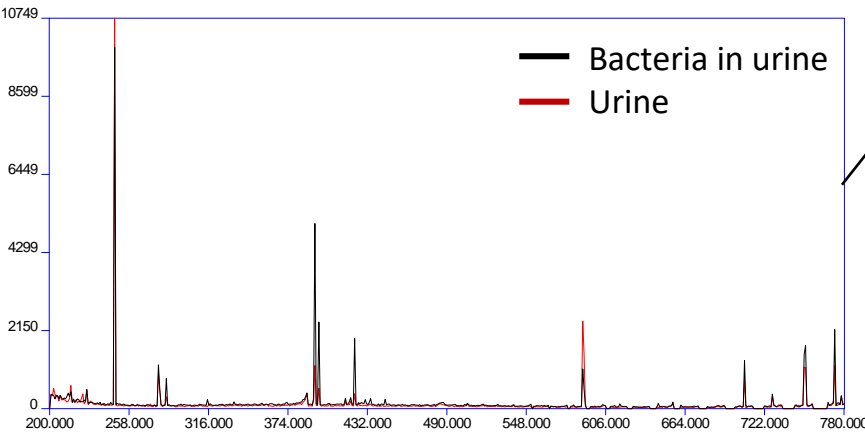
Bacterial-negative specimens obtained from Windsor Regional Hospital

### DETECTION IN URINE (individual spectra)

98.9% sensitivity  
100% specificity

### DETECTION IN URINE (spectra added)

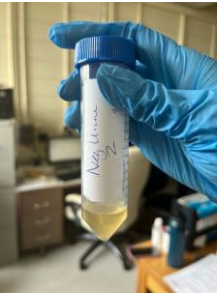
12 of 12 filters positive, 100% sensitivity  
8 of 8 filters negative, 100% specificity



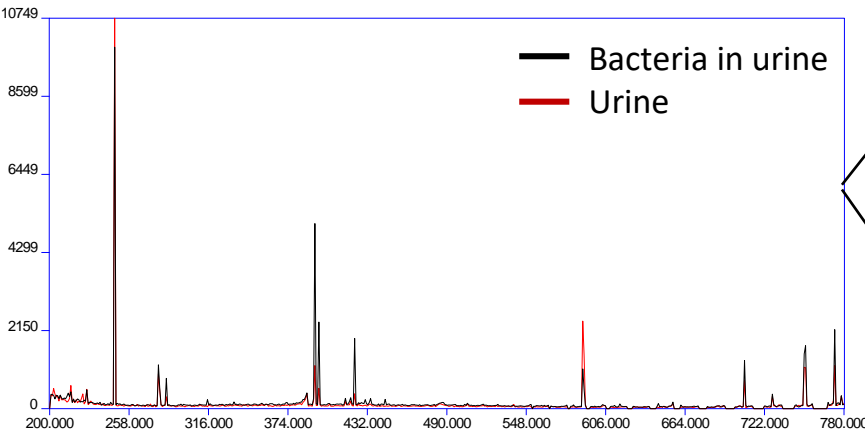
- 360 spectra of bacteria in urine
  - 3 species, four filters of each
- 240 spectra of sterile urine
  - 8 filters



# Diagnosing Infection in Urine with an ANN



Bacterial-negative specimens obtained from Windsor Regional Hospital

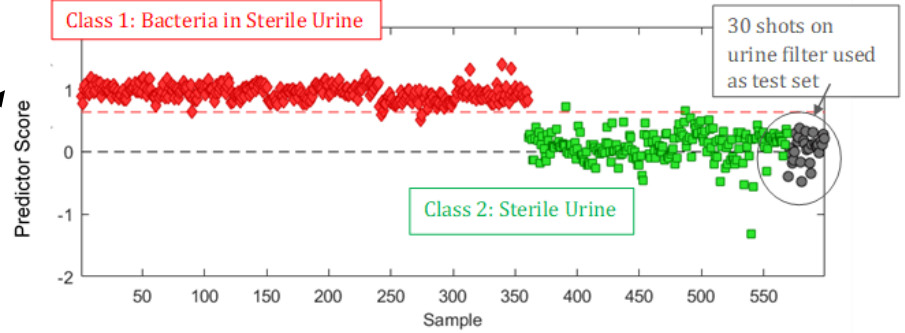


DETECTION IN URINE (individual spectra)

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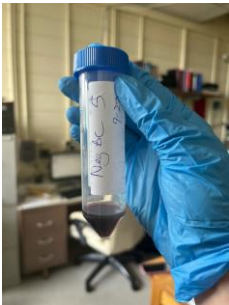
- 360 spectra of bacteria in urine
  - 3 species, four filters of each
- 240 spectra of sterile urine
  - 8 filters

PCA-ANN With Full Spectrum Data*			
	<i>S. aureus</i>	<i>E. coli</i>	<i>E. cloacae</i>
<b>Sensitivity</b>	100 %	100 %	91.67 %
<b>Specificity</b>	100 %	95.83 %	100 %
<b>Classification Error</b>	0.00 %	2.09 %	4.17 %

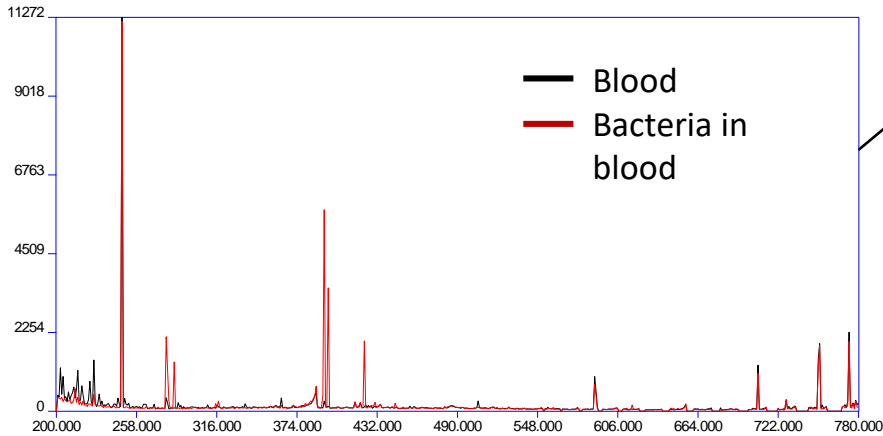
\*classification using 80:20 split



# Detecting Infection in Blood with a PLS-DA



Bacterial-negative specimens obtained from Windsor Regional Hospital



- 600 spectra of bacteria in blood
  - 4 species, five filters of each
- 206 spectra of sterile urine
  - 7 filters

## DETECTION IN BLOOD (individual spectra)

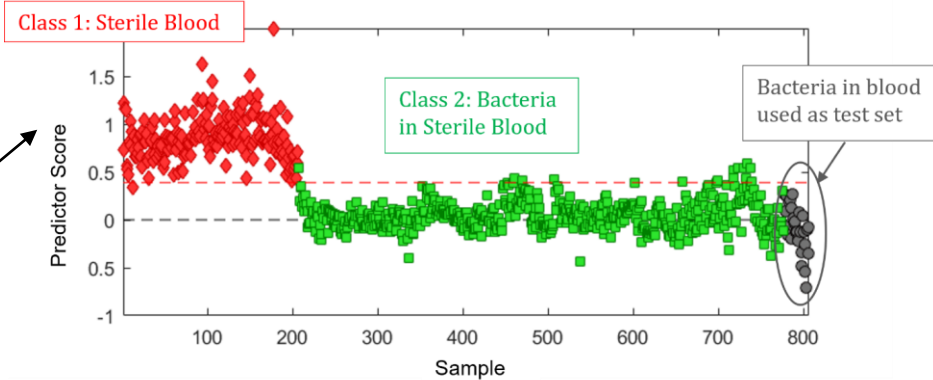
96.3% sensitivity

98.6% specificity

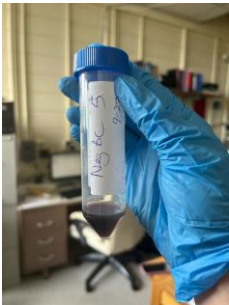
## DETECTION IN BLOOD (spectra added)

19 of 19 filters positive, 100% sensitivity

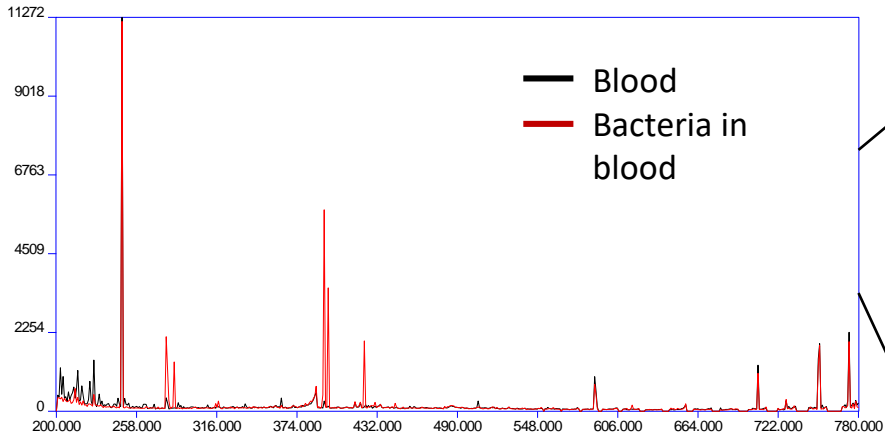
7 of 7 filters negative, 100% specificity



# Diagnosing Infection in Blood with an ANN



Bacterial-negative specimens obtained from Windsor Regional Hospital



- 600 spectra of bacteria in blood
  - 4 species, five filters of each
- 206 spectra of sterile urine
  - 7 filters

## DETECTION IN BLOOD (individual spectra)

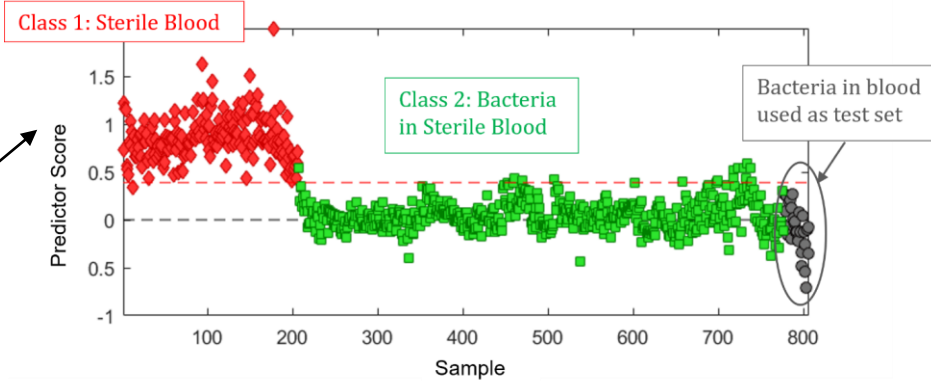
96.3% sensitivity

98.6% specificity

## DETECTION IN BLOOD (spectra added)

19 of 19 filters positive, 100% sensitivity

7 of 7 filters negative, 100% specificity



PCA-ANN With Full Spectrum Data*				
	<i>S. aureus</i>	<i>E. coli</i>	<i>E. cloacae</i>	<i>P. aeruginosa</i>
<b>Sensitivity</b>	100 %	100 %	100 %	100 %
<b>Specificity</b>	100 %	100 %	100 %	100 %
<b>Classification Error</b>	0.00 %	0.00 %	0.00 %	0.00 %

\*classification using 80:20 split



# External Validation of PCA-ANN in Blood and Urine

- External validation done in both urine and blood (whole filters withheld)
- ANN model run 10 times per excluded filter
- Improvements need to be made on quality of spectra, not ML

average sensitivity of 74.6%

Urine	<i>E. coli</i>	<i>S. aureus</i>	<i>E. cloacae</i>	
Average Sensitivity	63.58 %	86.92 %	62.33 %	
Blood	<i>E. coli</i>	<i>S. aureus</i>	<i>E. cloacae</i>	<i>P. aeruginosa</i>
Average Sensitivity	93.73 %	61.73 %	91.60 %	95.0 %

average sensitivity of 85.5%

Urine

*Sensitivity of each E. coli filter removed from the model to be externally validated. ANN run 10 times per filter.*

<i>E. coli</i>	Predicted			Sensitivity
	<i>S. aureus</i>	<i>E. coli</i>	<i>E. cloacae</i>	
Sample #				
1	30	250	20	0.833
2	1	138	161	0.460
3	11	153	136	0.510
4	72	222	6	0.74
Sum	114	763	323	0.636

Blood

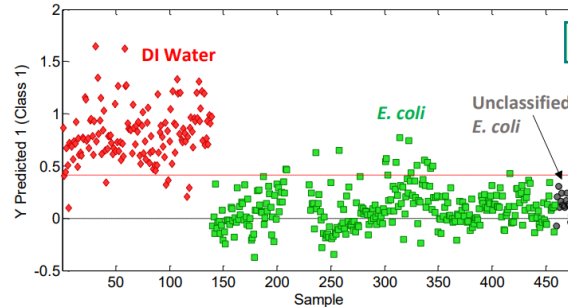
*Sensitivity results for S. aureus filters removed from the model to be externally validated. ANN run 10 times per filter.*

<i>S. aureus</i>	Predicted				Sensitivity
	<i>S. aureus</i>	<i>E. coli</i>	<i>E. cloacae</i>	<i>P. aeruginosa</i>	
Sample #					
1	50	54	86	110	0.167
2	300	0	0	0	1.000
3	1	27	266	6	0.033
4	290	10	0	0	0.967
5	285	13	0	2	0.950
Sum	926	104	352	118	0.617

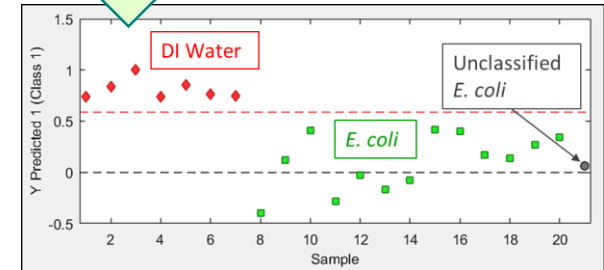


# Conclusions

- Adding spectra improves discrimination in PLSDA
  - Detection of bacteria in water, blood and urine improved; **sensitivity = 100%, specificity = 100%**
- Rigorous cleaning of cone & usage of ultrapure water reduces background signal



Adding 30 single-shot spectra to create one measurement per filter improves classification in PLSDA



- Using PCA-ANN on full spectrum data provides the best results for discrimination between bacterial species (using 80:20 split)
- Average sensitivity = **94 %**
- Average specificity = **96 %**

PCA-ANN With Full Spectrum Data			
	E. coli	S. aureus	E. cloacae
<b>Sensitivity</b>	98.04 %	93.27 %	91.23 %
<b>Specificity</b>	97.71 %	97.22 %	96.12 %
<b>Classification Error</b>	2.13 %	4.28 %	6.33 %

Approximate increase from DFA  $\approx$  30 % (sensitivity), 16% (specificity)



# Conclusions

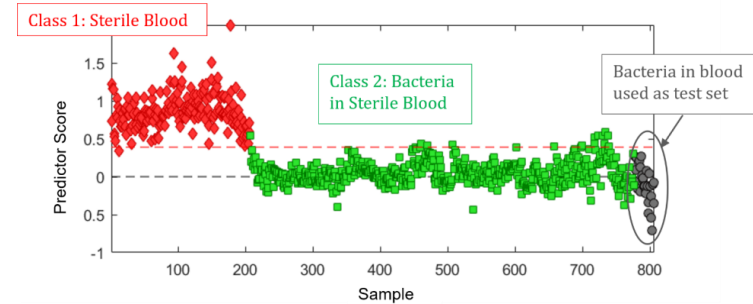
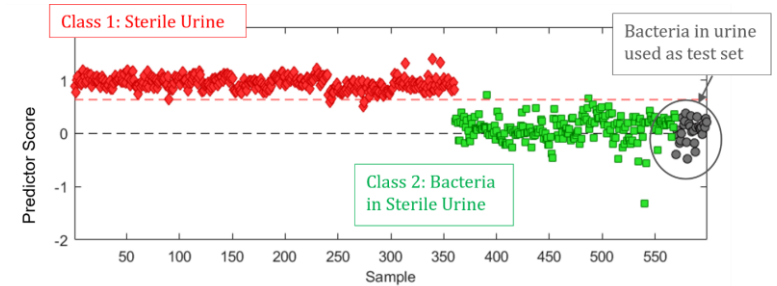
- Bacteria can be **detected** (PLSDA) and **diagnosed** (PCA-ANN) in blood and urine
- Future work:
  - Determine LOD for bacteria in blood and urine
  - Study how bacteria behaves in blood to ensure we are reproducing clinical conditions
  - Apply new deposition method to blood & urine

PCA-ANN With Full Spectrum Data (Urine)

	S. aureus	E. coli	E. cloacae
<b>Sensitivity</b>	100 %	100 %	91.67 %
<b>Specificity</b>	100 %	95.83 %	100 %
<b>Classification Error</b>	0.00 %	2.09 %	4.17 %

PCA-ANN With Full Spectrum Data (Blood)

	S. aureus	E. coli	E. cloacae	P. aeruginosa
<b>Sensitivity</b>	100 %	100 %	100 %	100 %
<b>Specificity</b>	100 %	100 %	100 %	100 %
<b>Classification Error</b>	0.00 %	0.00 %	0.00 %	0.00 %



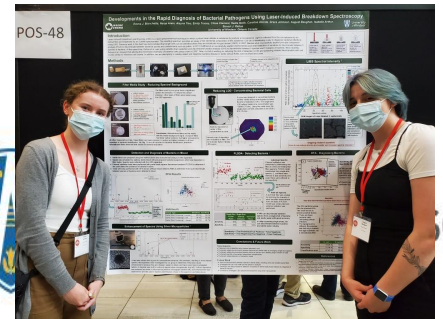
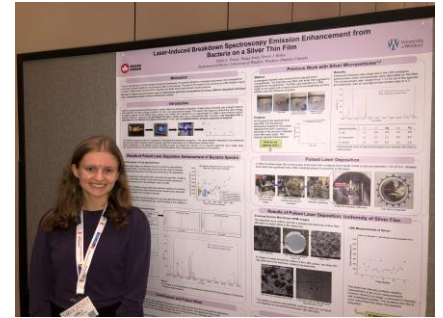
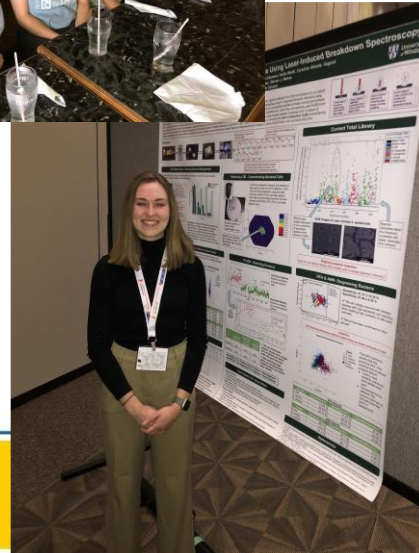


# Future Work



# Acknowledgements for the people who did the work...

2022-2023



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# Thank you!



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