Rapid Detection & Diagnosis of Bacterial Pathogens in Clinical Specimens Using Laser-Induced Breakdown Spectroscopy

Presented at 2022 CAP Congress

Emma Blanchette
Department of Physics
University of Windsor

Introduction and Motivation

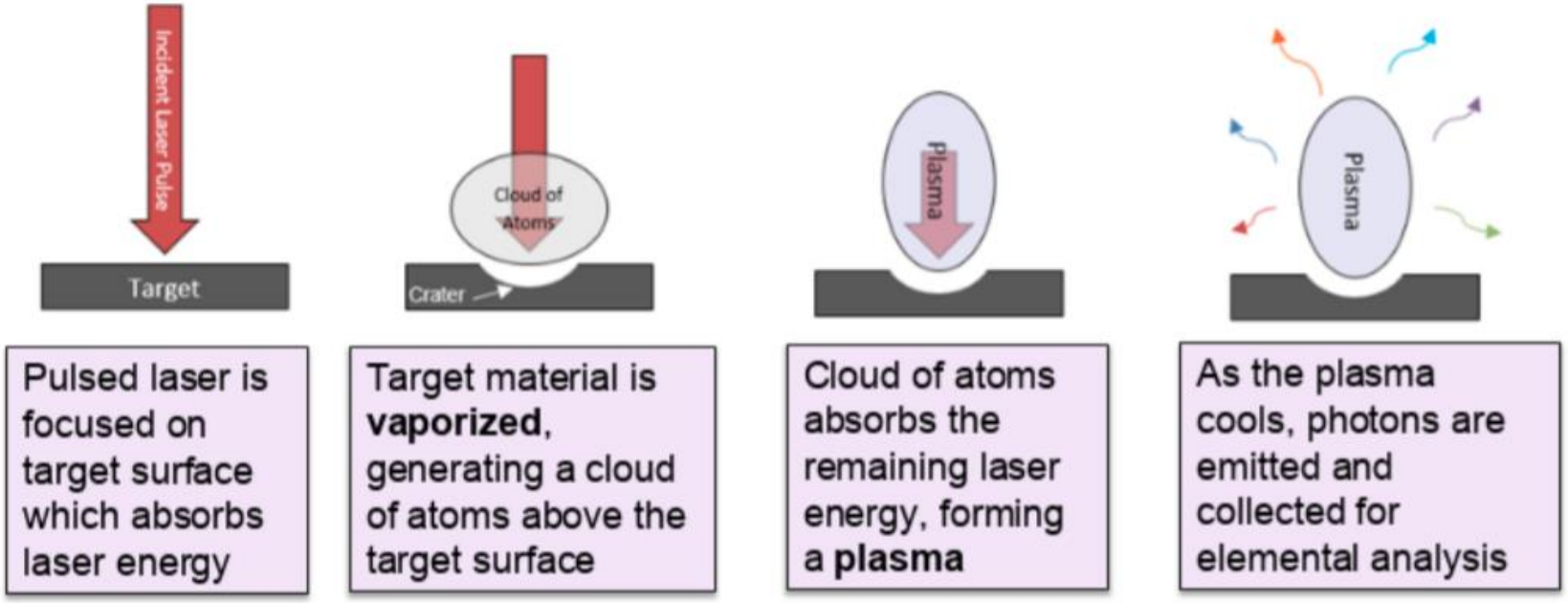
- We are using laser induced breakdown spectroscopy (LIBS) to rapidly diagnose bacterial pathogens

- Current methods of diagnosis takes ~ 1-3 days
  - Lack of technology for fast diagnosis ⟶ use of broad spectrum drugs
  - Sepsis requires fast treatment; preferably within an hour of diagnosis
  - UTI's are the second most common infection people seek treatment for

Goal: Develop rapid technique to diagnose bacterial infection in clinical setting

LIBS has Potential for This Application… it's Fast!

- A laser is focused onto a target to create a high temperature microplasma
- Time-resolved spectra is recorded… all in under 1 minute!

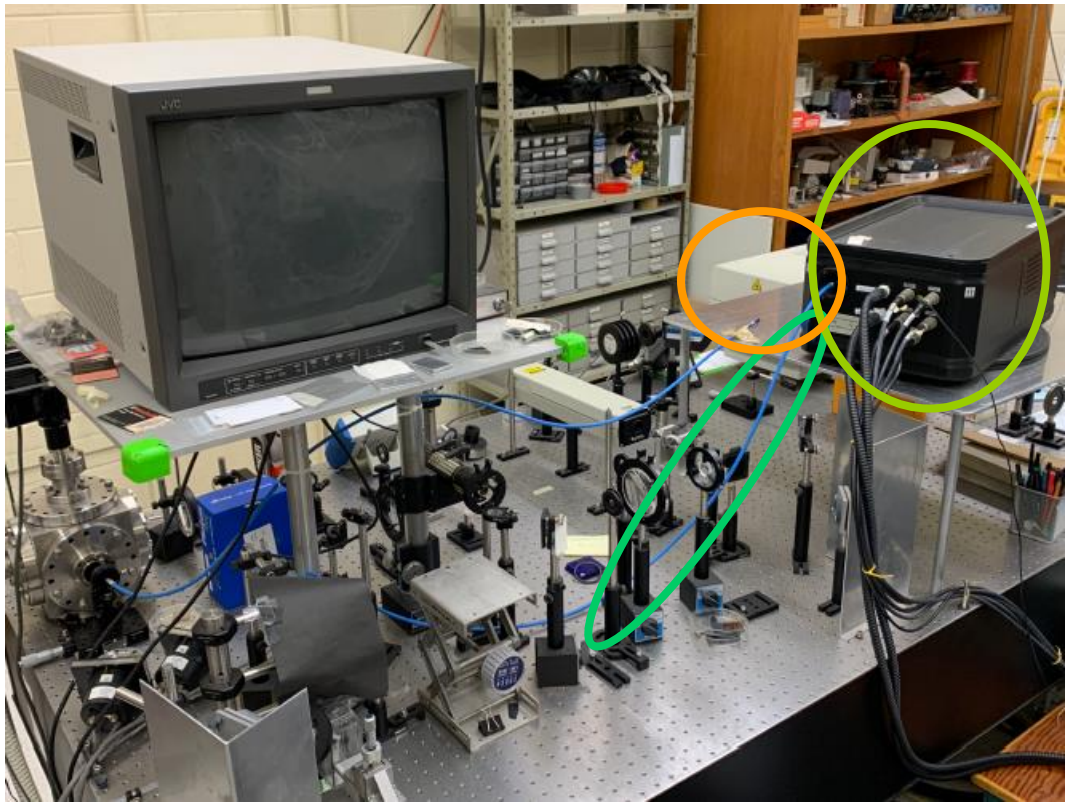


Experimental Setup and Parameters

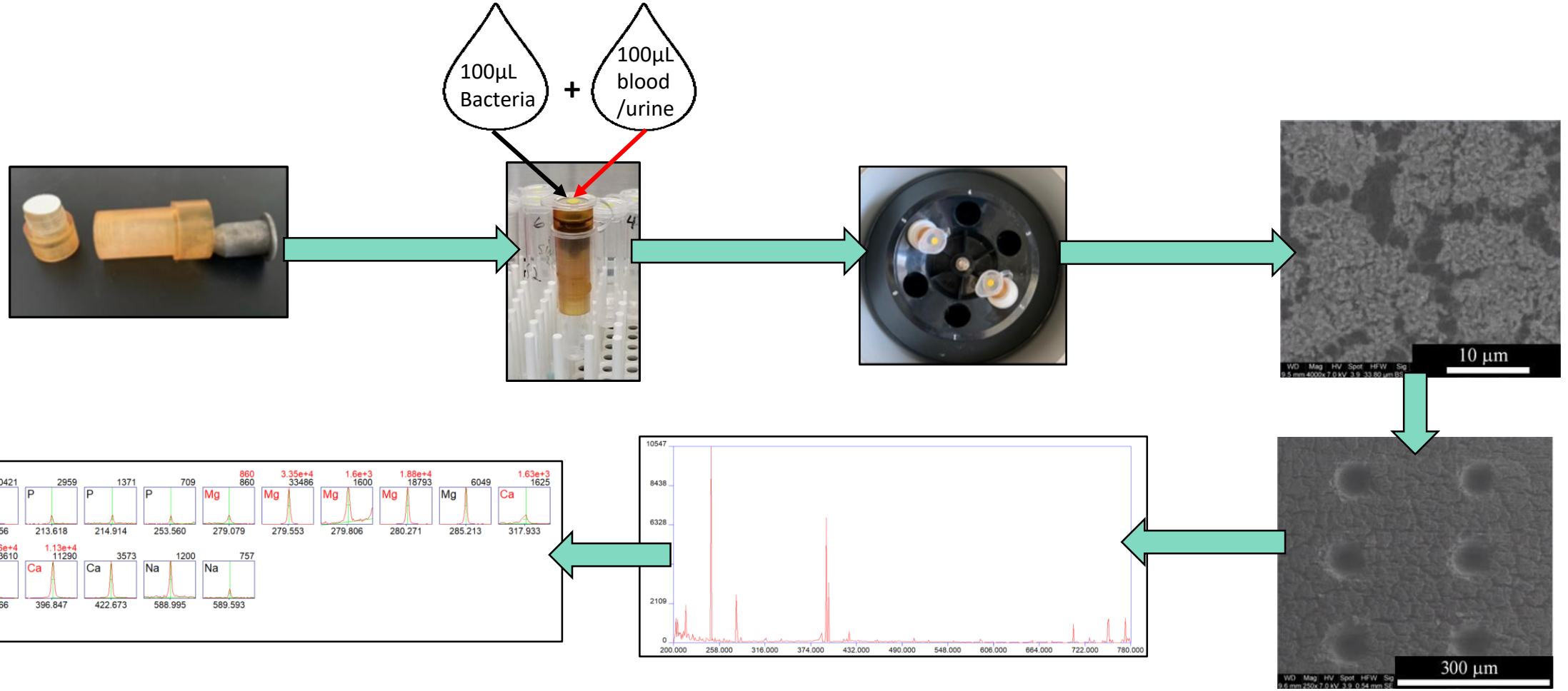
- Nd:YAG laser, with 10 ns pulse duration and 10 Hz pulse frequency

- Light is collected from ablation events and fed into a steel-encased optical fibre
  - NA = 0.22, core ϕ = 600 µm

- Echelle spectrometer detects the light from fibre and generates a spectrum
  - Spectrometer uses an ICCD camera to convert photons to signal

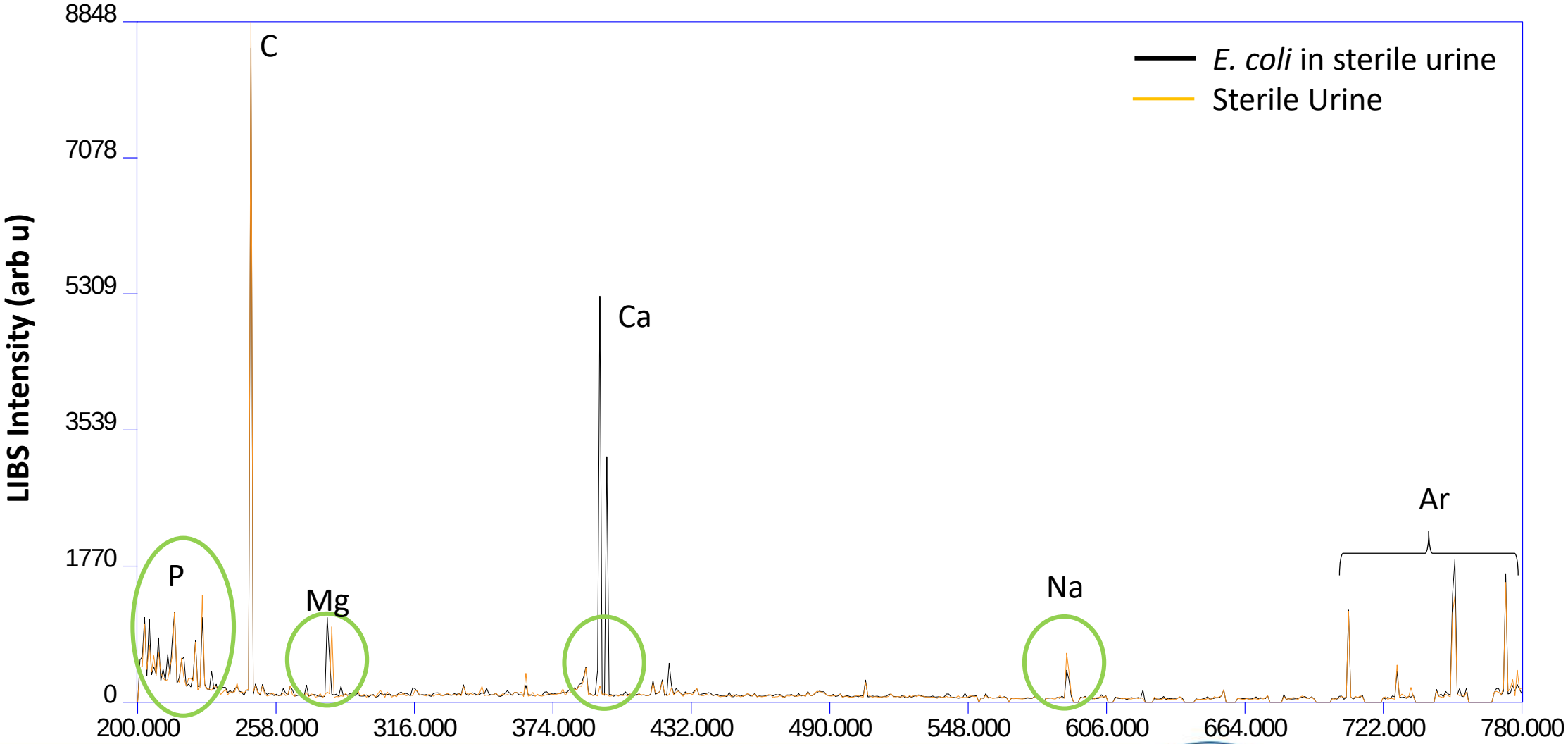


Sample Preparation (Blood & Urine + Bacteria)



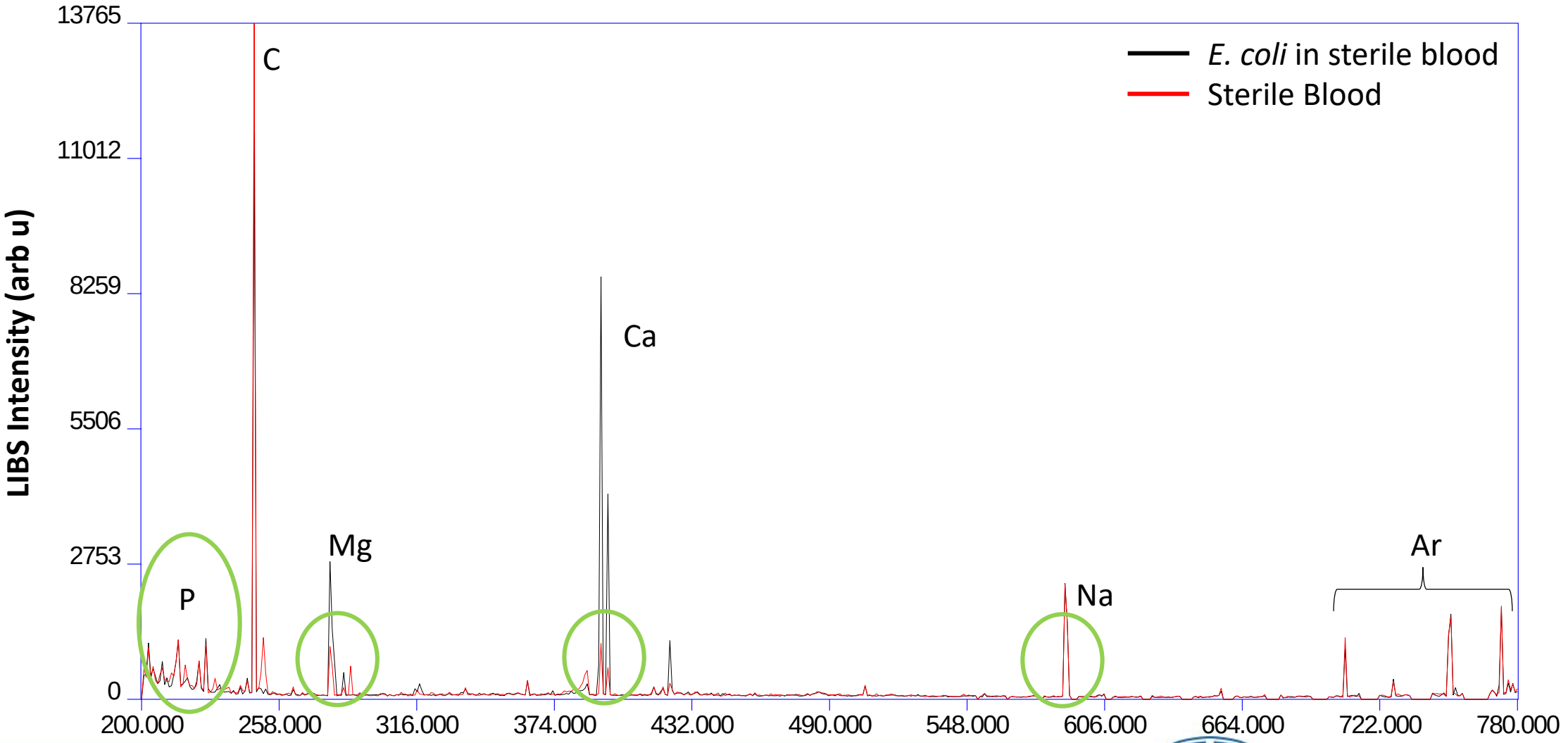
Results: Urine Spectrum

E. coli in sterile urine and Sterile Urine
2 µs delay after plasma initiation
20 SCFH Argon environment
Single laser shot



Results: Blood Spectrum

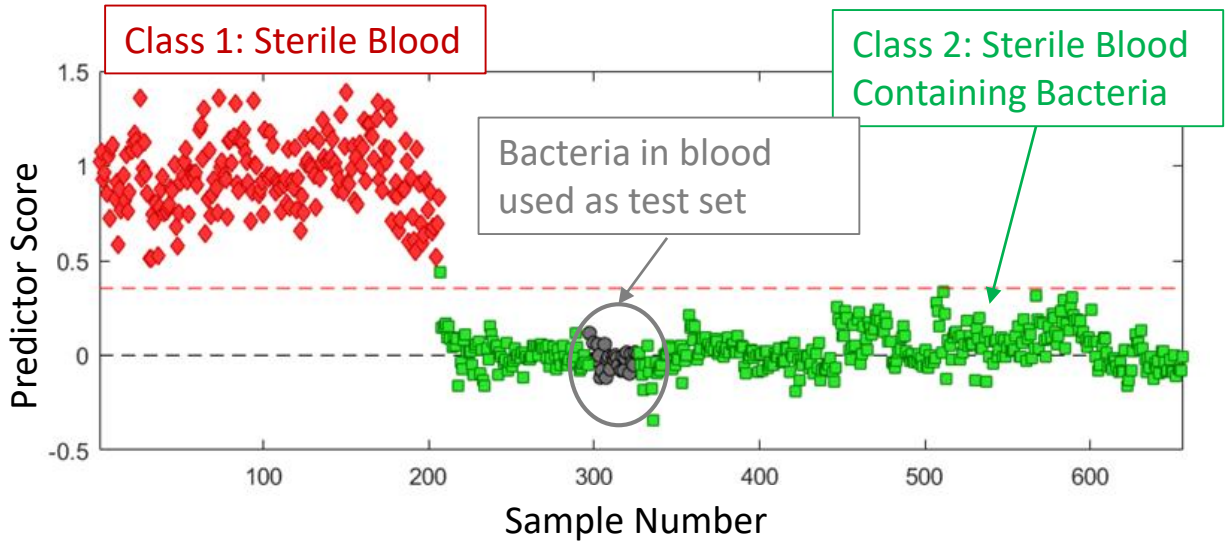
E. Coli in sterile blood and Sterile Blood
2 µs delay after plasma initiation
20 SCFH Argon environment
Single laser pulse



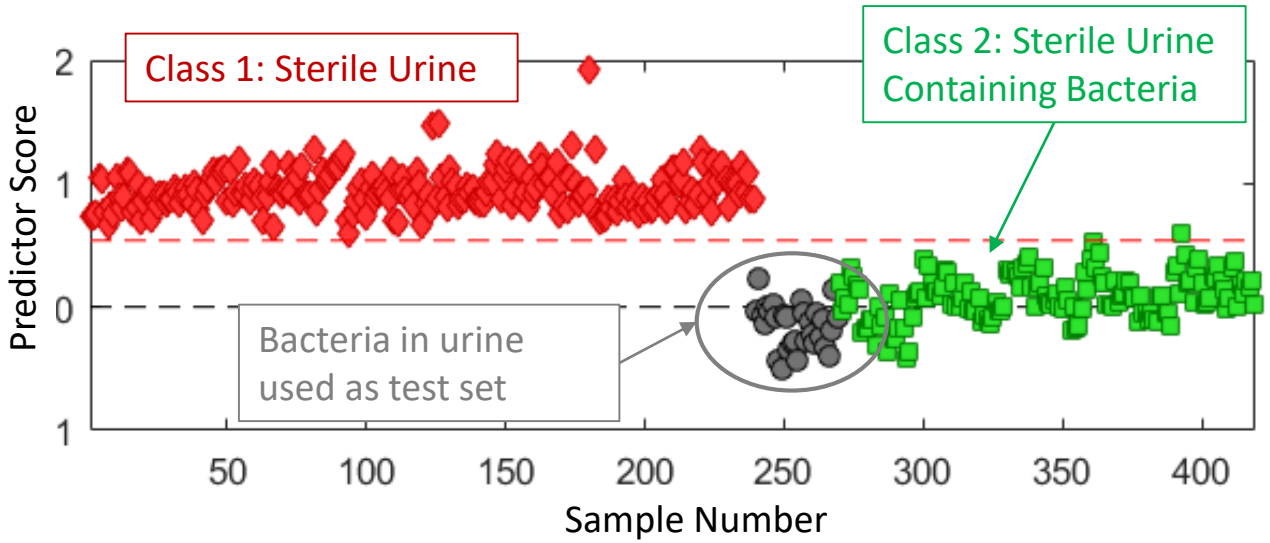
Is the Fingerprint of Blood/Urine Different than Bacteria?

- A **partial least squares discriminant analysis (PLSDA)** test was conducted using external validation to determine if bacteria can be detected in blood and urine

✓ We can detect several types of bacteria in blood and urine reliably



Average Sensitivity: 99.56%
Average Specificity: 100%



Average Sensitivity: 98.90%
Average Specificity: 100%

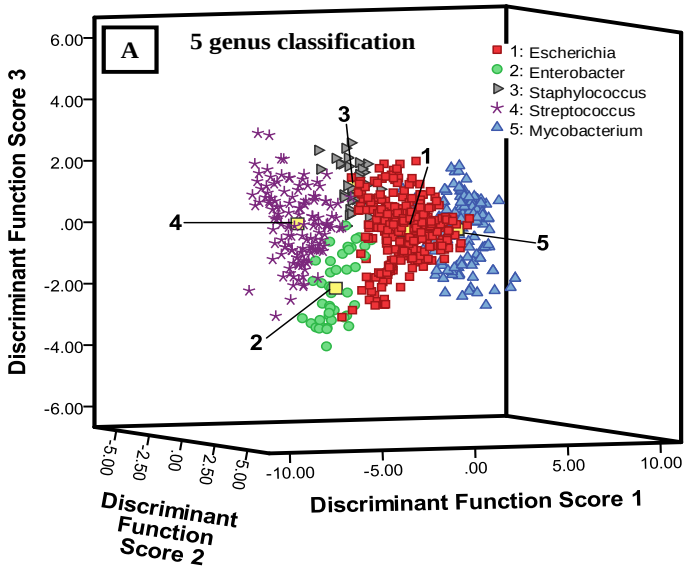
Can we differentiate between species?

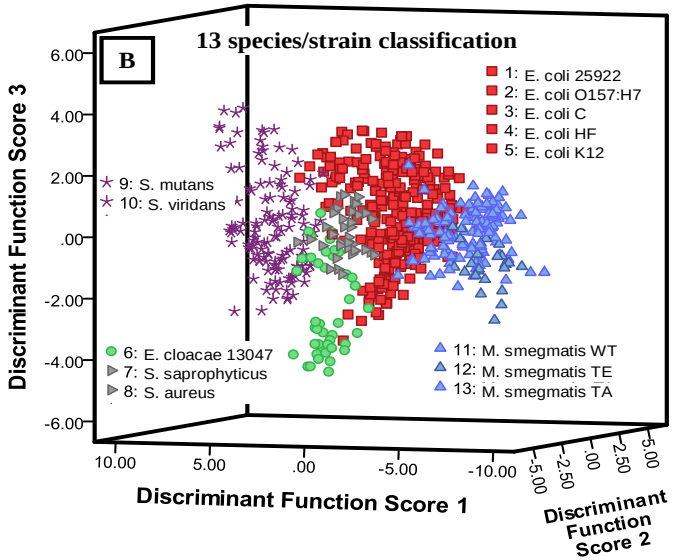
- ✓ We can discriminate between species with high specificity and sensitivity (confirmed by others) using **discriminant function analysis (DFA)**
- ✓ We can differentiate between strains of *E. coli*
- ✓ Many multivariate techniques work[2]

	DFA		PLSDA	
E. COLI	True	False	True	False
Positive	95.65%	9.17%	89.63%	15.95%
Negative	90.83%	4.35%	84.05%	10.37%
STAPHYLOCOCCUS	True	False	True	False
Positive	54.05%	0.51%	86.49%	5.85%
Negative	99.49%	45.95%	94.15%	13.51%
STREPTOCOCCUS	True	False	True	False
Positive	95.59%	1.02%	99.26%	13.32%
Negative	98.98%	4.41%	88.68%	0.74%
MYCOBACTERIUM	True	False	True	False
Positive	88.31%	1.06%	96.10%	4.08%
Negative	98.94%	11.69%	95.92%	3.90%

DFA: *Sensitivity*: 91.37 ± 16.39 % *Specificity*: 97.46 ± 9.35 %

PLSDA: *Sensitivity*: 93.13 ± 10.25 % *Specificity*: 90.60 ± 21.33 %





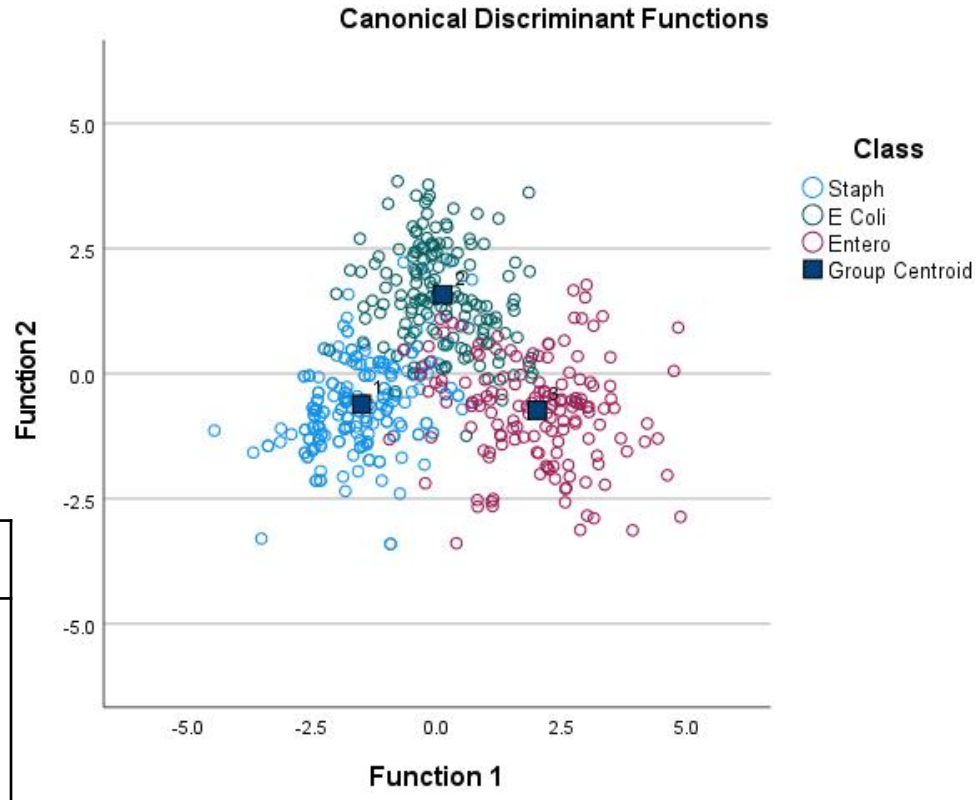
2. Russell A. Putnam, Qassem I. Mohaidat, Andrew Daabous, Steven J. Rehse (2013). A comparison of multivariate analysis techniques and variable selection strategies in a laser-induced breakdown spectroscopy bacterial classification. *Spectrochimica Acta Part B: Atomic Spectroscopy, 87*, 161-167. https://doi.org/10.1016/j.sab.2013.05.014

DFA – Diagnosing a Bacterial Infection in Blood

- Attempting to replicate previous results with:
  - Fewer cells
  - Non-zero background (coming from filter and blood)

✓ Average Sensitivity = 80.97 %

✓ Average Specificity = 90.8 %*



Bacteria	Sensitivity	Specificity	Classification Error
E. coli in sterile blood	78.8 %	90.4 %	15.40 %
S. aureus in sterile blood	86.1 %	89.4 %	12.25 %
E. cloacae in sterile blood	78.0 %	92.5 %	14.75 %

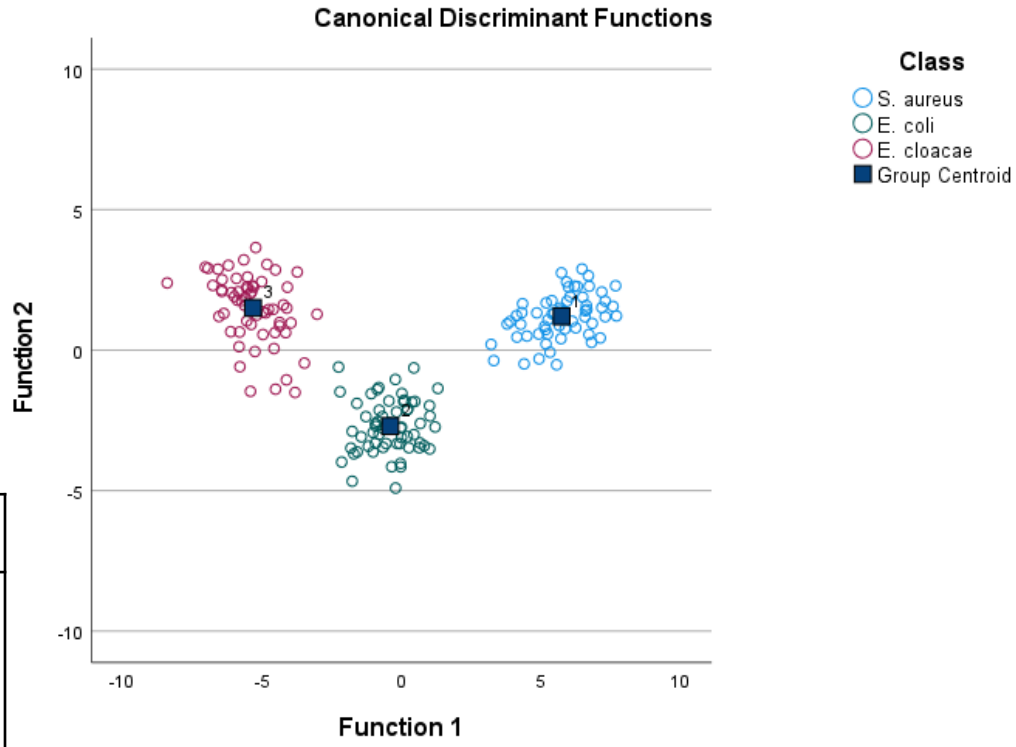
*Calculated by averaging sensitivity and specificity for each species

DFA – Diagnosing a Bacterial Infection in Urine

- Attempting to replicate previous results with:
  - Fewer cells
  - Non-zero background (coming from filter and urine)

✓ Average Sensitivity = 91.70 %

✓ Average Specificity = 95.8 %*



Bacteria	Sensitivity	Specificity	Classification Error
E. coli in sterile urine	96.7 %	98.3 %	2.5 %
S. aureus in sterile urine	91.7 %	91.7 %	8.3 %
E. cloacae in sterile urine	86.7 %	97.5 %	7.9 %

*Calculated by averaging sensitivity and specificity for each species

ANN Results – Diagnosing a Bacterial Infection in Blood & Urine

ANN on Bacteria in Blood

- Can discriminate between species with good specificity and variable sensitivity
- Slightly better than DFA
- **Avg Sensitivity: 82.5 %**
- **Avg Specificity: 91.3 %***

Sample Type	Sensitivity	Specificity
Sterile blood containing *S. aureus*	87.5 %	89.2 %
Sterile blood containing *E. coli*	79.2 %	91.3 %
Sterile blood containing *E. cloacae*	80.8 %	93.3 %

ANN on Bacteria in Urine

- Can discriminate between species with high specificity and high sensitivity
- Better than DFA
- **Avg Sensitivity: 95.8 %**
- **Avg Specificity: 98.9 %***

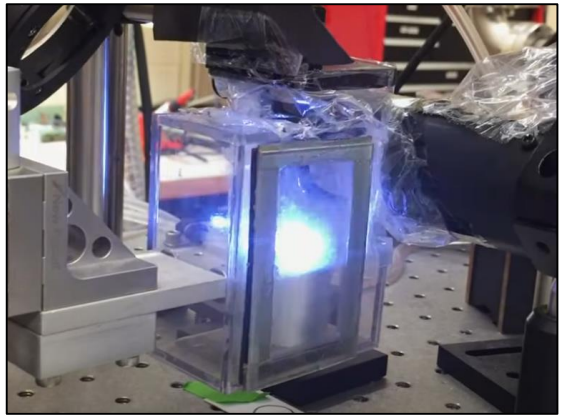
Sample Type	Sensitivity	Specificity
Sterile Urine containing *S. aureus*	98.9 %	100 %
Sterile Urine containing *E. coli*	89.5 %	99.6 %
Sterile Urine containing *E. cloacae*	99.1 %	97.1 %

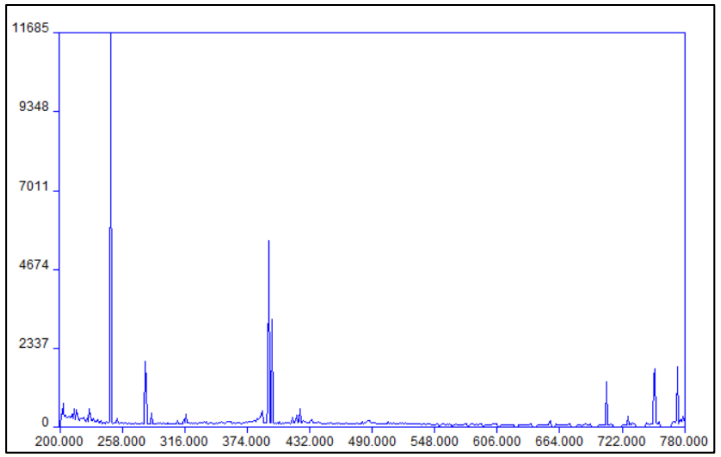
*Calculated by averaging sensitivity and specificity for each species

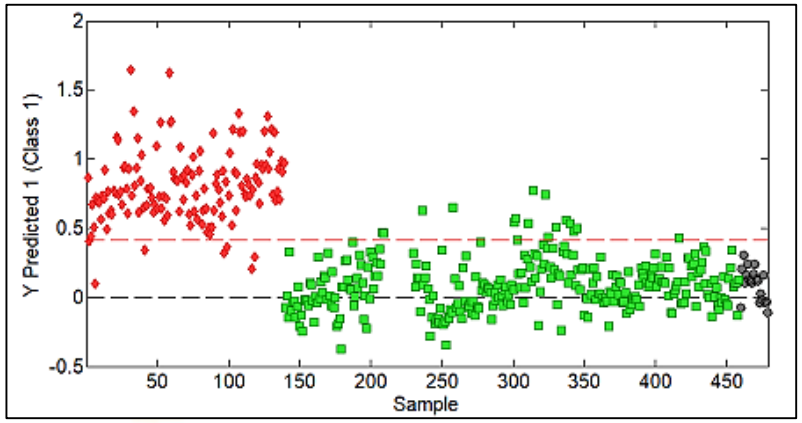
Parameters:
Test size = 20% of data (80% is used for the model)
Hidden layers = 1
Batch size = 32
Epochs: determined by algorithm (based on loss curve)
Optimizing: hidden nodes & patience for each data set

Conclusions

- ✓ We have determined that spectra of blood/urine and bacteria are different
- ✓ We can reliably detect bacteria in sterile blood and urine
- ✓ DFA and ANN shows promising results for discrimination between species present in blood







Special Issue

Applied Spectroscopy
www.s-a-s.org

Detection and Classification of Bacterial Cells After Centrifugation and Filtration of Liquid Specimens Using Laser-Induced Breakdown Spectroscopy

Applied Spectroscopy
2022, Vol. 0(0) 1–11


Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/00037028221092789
journals.sagepub.com/home/asp
SAGE

Emma J. Blanchette[1], Sydney C. Sleiman[1], Haiqa Arain[1], Alayna Tieu[1], Chloe L. Clement[1], Griffin C. Howson[1], Emily A. Tracey[1], Hadia Malik[1], Jeremy C. Marvin[1] and Steven J. Rehse[1]

Special Issue

Applied Spectroscopy
www.s-a-s.org

Silver Microparticle-Enhanced Laser-Induced Breakdown Spectroscopy

Applied Spectroscopy
2022, Vol. 0(0) 1–12


Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/00037028221096483
journals.sagepub.com/home/asp
SAGE

Jeremy C. Marvin[1], Emma J. Blanchette[1], Sydney C. Sleiman[1], Haiqa Arain[1], Emily A. Tracey[1] and Steven J. Rehse[1]

Acknowledgements

- Advisor: Dr. Steven Rehse

- Colleagues:
  - Haiqa Arain
  - Emily Tracey
  - Griffin Howson
  - Alayna Tieu
  - Chloe Clement
  - Hadia Malik
  - Caroline Aliontе
  - Grace Johnson
  - August Baughan
- Sponsors:
  - Natural Sciences and Engineering Research Council of Canada (NSERC)
  - University of Windsor Outstanding Scholars

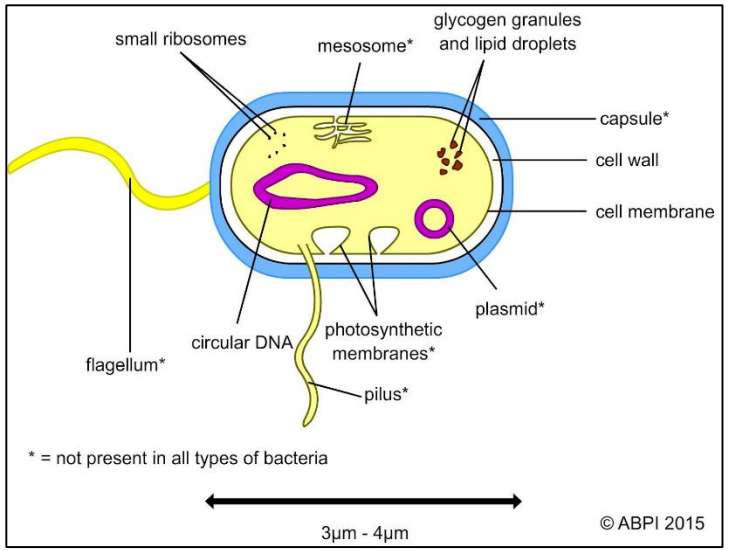
Commercial benchtop systems have been built…

Coriosity Laser Imager - Elemission

J200 – Applied Spectra

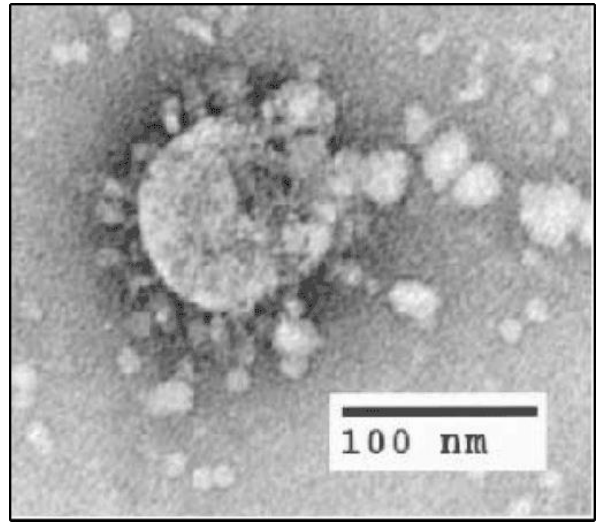
ChemReveal LIBS Desktop Elemental Analyzer – TSO

LIBS on Viruses? Size matters!



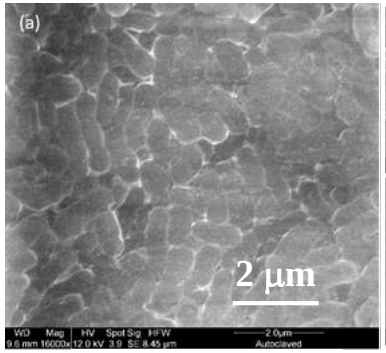
https://www.abpischools.org.uk/topic/pathogens/2/1

- Bacteria are ~1-3 µm
- Corona viruses are ~100-300 nm
- Volume is roughly 1,000 – 10,000 lower!
- Also, viruses are not rich / don't contain trace metals, as bacteria do.



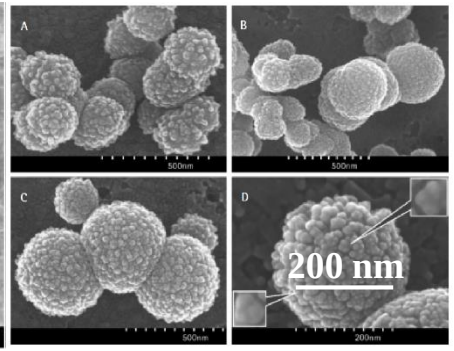
C.S. Goldsmith, CDC,

https://www.cdc.gov/sars/lab/images.html



SEM of *E. coli* specimen from our lab

vs.



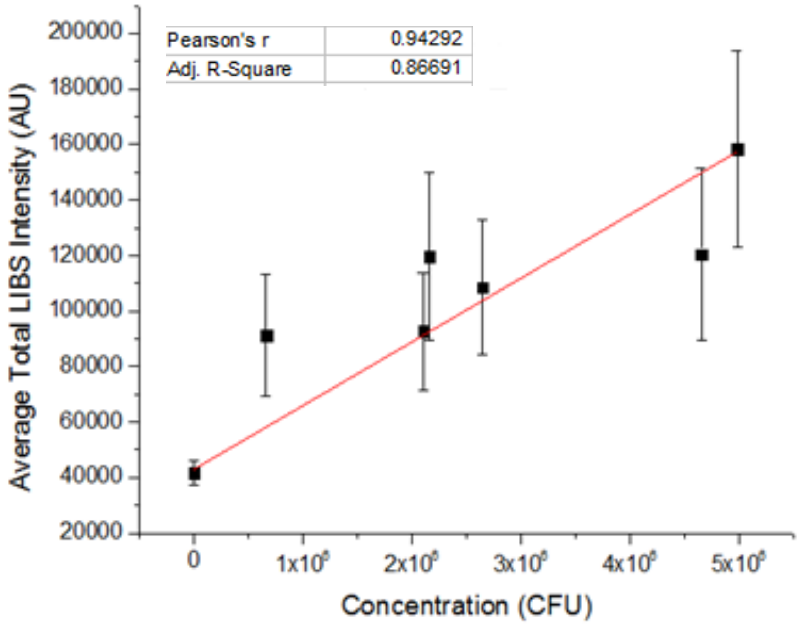
SEM of SARS corona-virus, Antiviral Therapy 9:287-289, 2004

Two known papers on the use of LIBS to identify viruses:
(full details in S.J. Rehse, Spectrochimica Acta Part B 154 (2019) 50–69)

detect the presences of an MS-2 bacteriophage (smallpox surrogate)
J.L. Gottfried, Anal. Bioanal. Chem. 400 (2011) 3289–3301,

differentiation with LIBS of four strains of live *hantavirus*
R.A. Multari et al., Appl. Opt. 51 (2012) B57–B64,

Metal Cone: Limit of Detection



Spectrochimica Acta Part B 157 (2019) 68–75

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Concentration of bacterial specimens during centrifugation prior to laser-induced breakdown spectroscopy analysis☆

Alexandra E. Paulick, Dylan J. Malenfant, Steven J. Rehse*

Department of Physics, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B 3P4, Canada

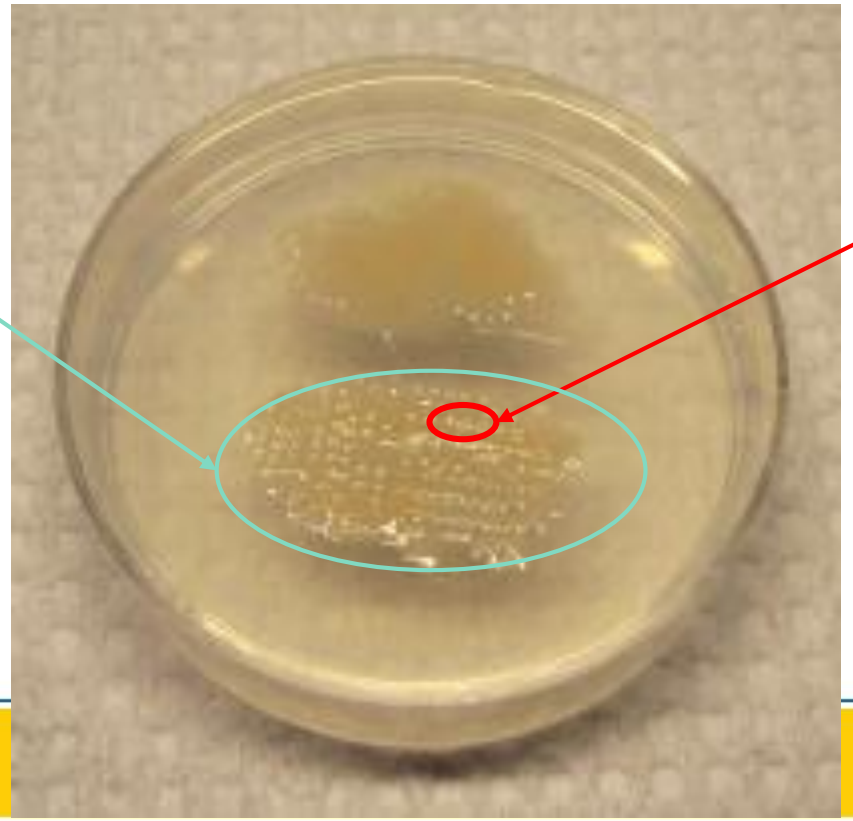
LOD ~ **11 000 CFU** per laser ablation event

metal cone **well-plate** **insert**

Recall:
Well-plate → LOD ~ 50 000 CFU per laser ablation event
Insert → LOD ~ 90 000 CFU per laser ablation event

Bacteria on Agar – Initial DFA Results

- Bacteria was ablated on nutrient-free agar surface providing essentially zero background signal



Confirming the Different Spectral Fingerprints With ANN

- ANN was used to confirm the previous result
- 3 species of bacteria and several samples of sterile blood were input into our ANN

✓ Sterile blood is classified correctly 100% of the time

✓ We can reliably detect bacteria in blood

Sample Type	Sensitivity	Specificity
Sterile Blood	100 %	100 %
Sterile blood containing *S. aureus*	73.33 %	91.23 %
Sterile blood containing *E. coli*	53.33 %	98.86 %
Sterile blood containing *E. cloacae*	93.33 %	93.86 %

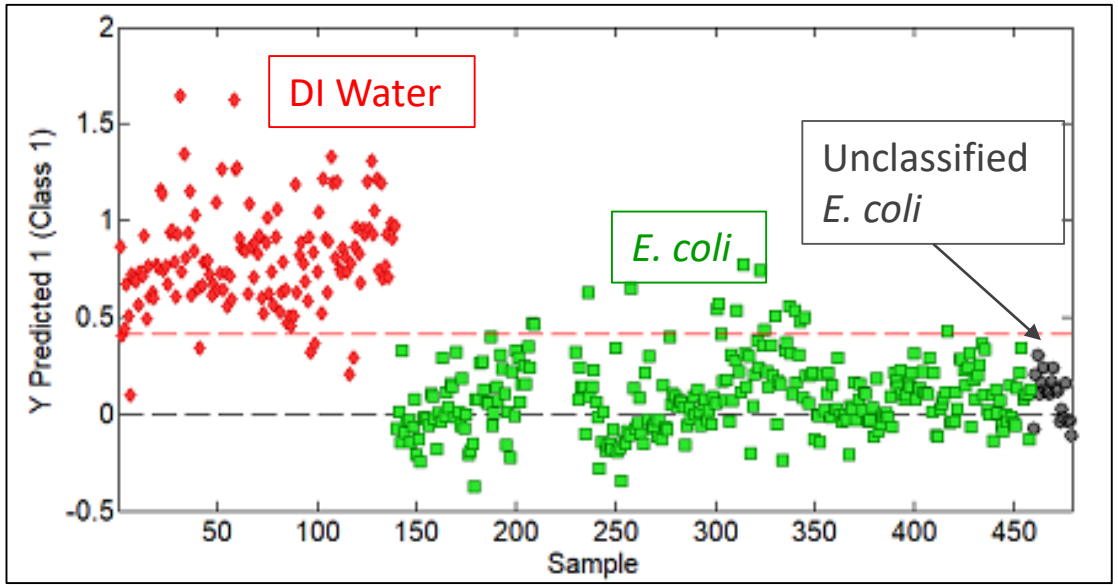
How do we use the bacterial signal?

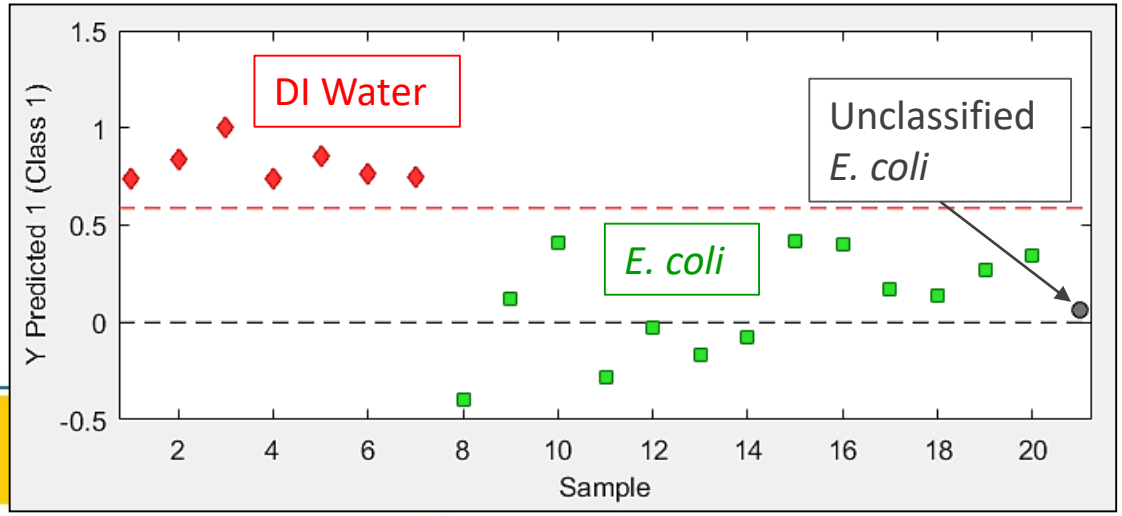
- DETECTION: need to be able to detect the presence of bacteria from sterile sources

✓We can discriminate between single-shot data of bacteria and sterile water with good accuracy[1]

✓After summing all single shots on a filter, we can discriminate between bacteria and sterile water reliably[1]

	Single-Shot Spectra	Added Spectra
Sensitivity	87%	100%
Specificity	93%	100%

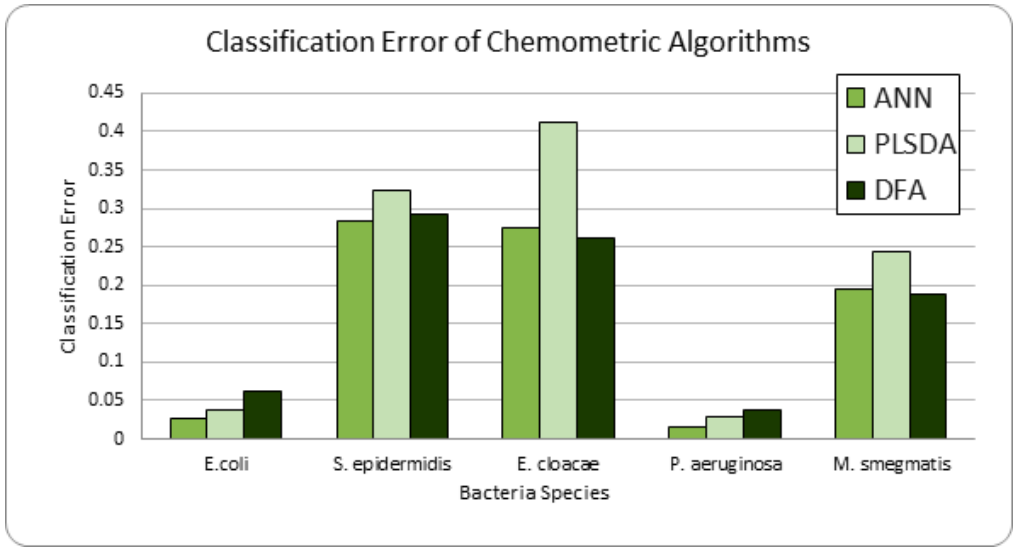




1. Blanchette et al. (2021) Detection and classification of bacterial cells after centrifugation and filtration of liquid specimens using laser-induced breakdown spectroscopy. *Spectrochimica Acta Part B:* In submission.

Sensitivity, Specificity, and Classification Error

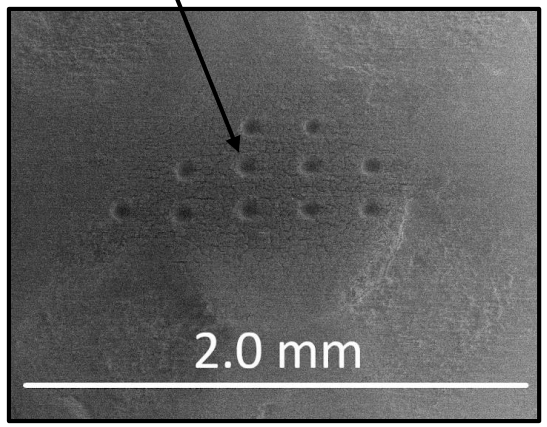
Sensitivity = (True Positives)/(True Positives + False Negatives)
Specificity = (True Negatives)/(True Negatives + False Positives)

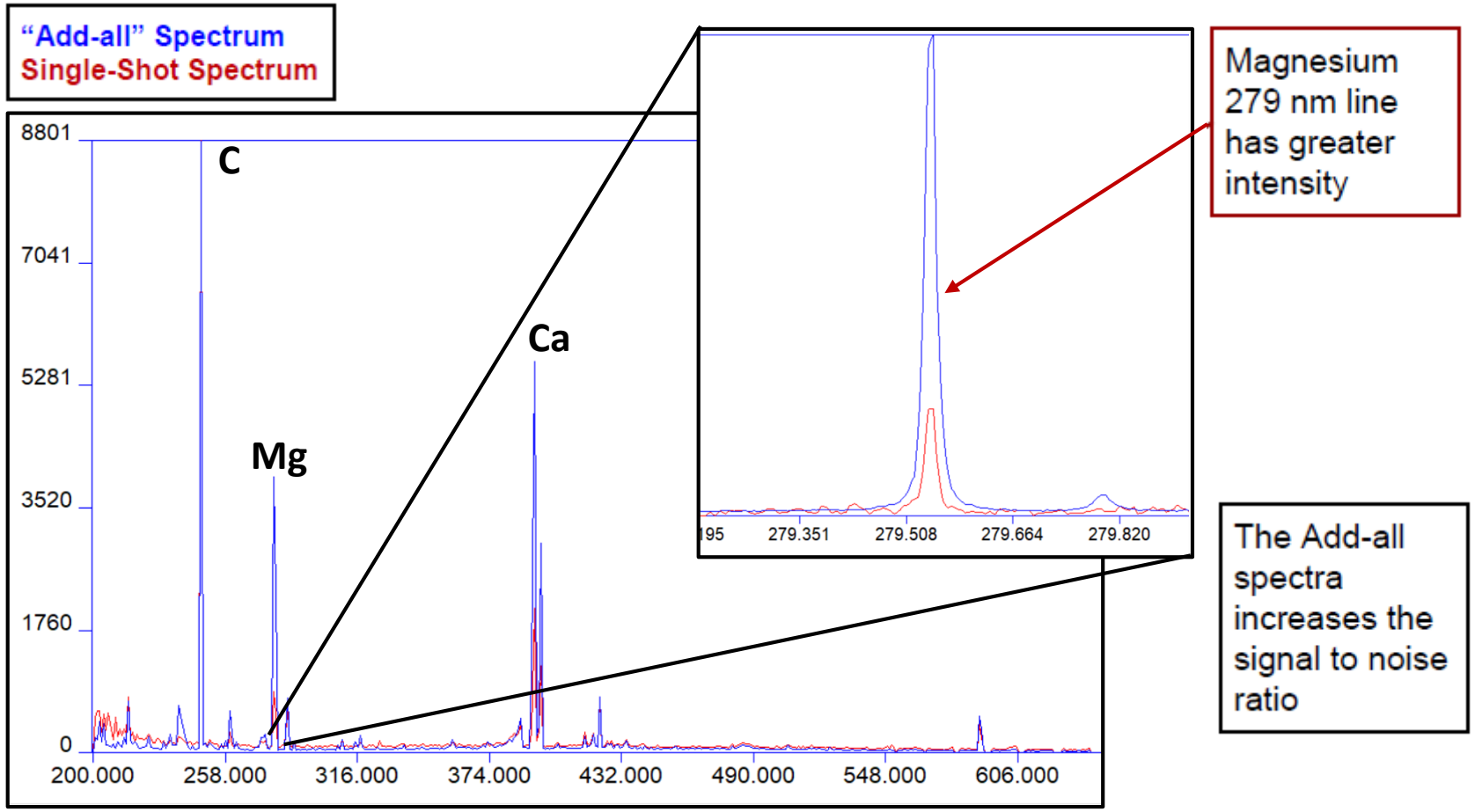


The classification error combines the sensitivity and specificity
Classification error = 1-(sensitivity + specificity)/2

Effect of Adding Spectra

Circular area of filter containing 10 single-shot spectra.

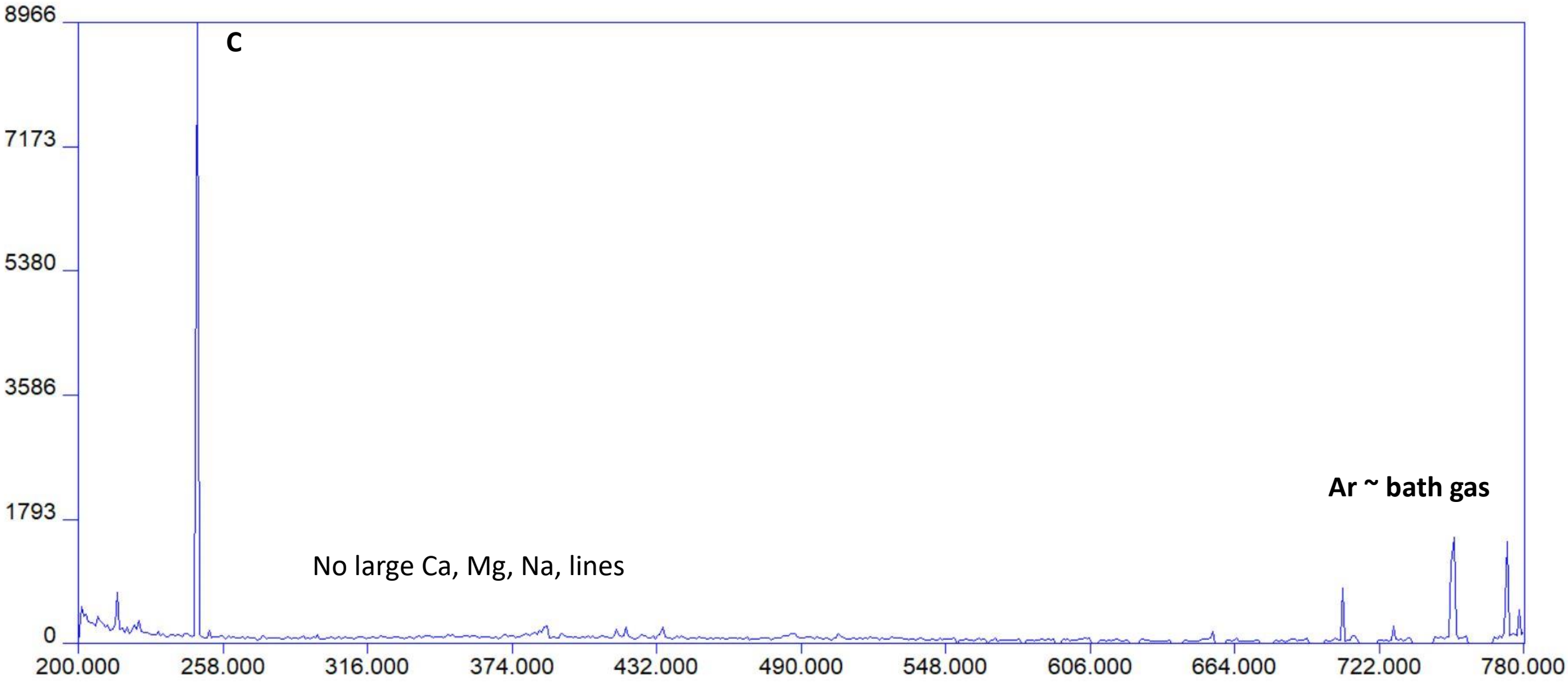




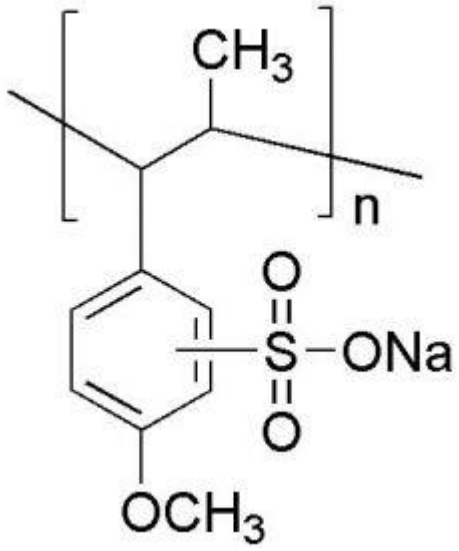
Magnesium 279 nm line has greater intensity

The Add-all spectra increases the signal to noise ratio

Blank Filter Spectrum



Sodium Polyanetholesulfonate (SPS)



- Structure of the blood anti-coagulant; the only thing we see in our spectrum is sodium
- Doesn’t appear to affect detection of bacteria

Image of Plasma