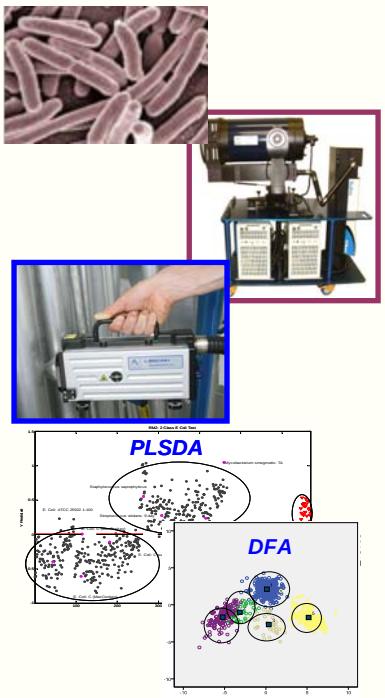
# *Laser-induced breakdown spectroscopy (LIBS) as a rapid bacterial pathogen diagnostic: a novel use of the analytic plasma*

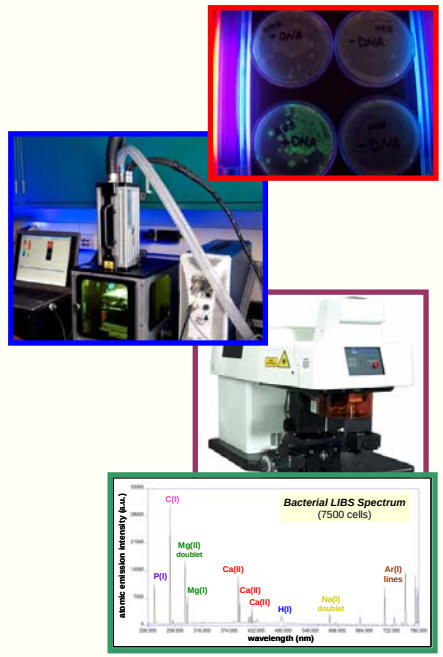
*presented at the 2013 Colloque de Plasma-Québec*
*Montreal, QC  May 2013*



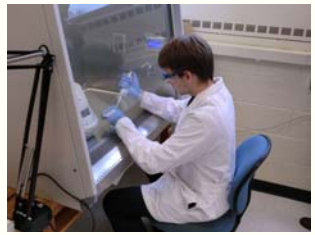

**Steven J. Rehse**

***Department of Physics***
***University of Windsor***

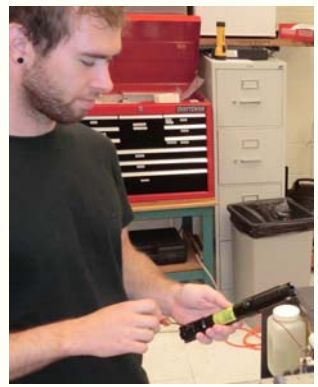
***Windsor, Ontario, Canada***

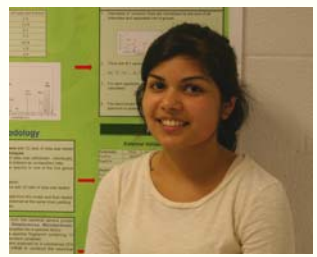


***Qassem Mohaidat and Khozima Hamasha***
*Wayne State University*
*Department of Physics and Astronomy*



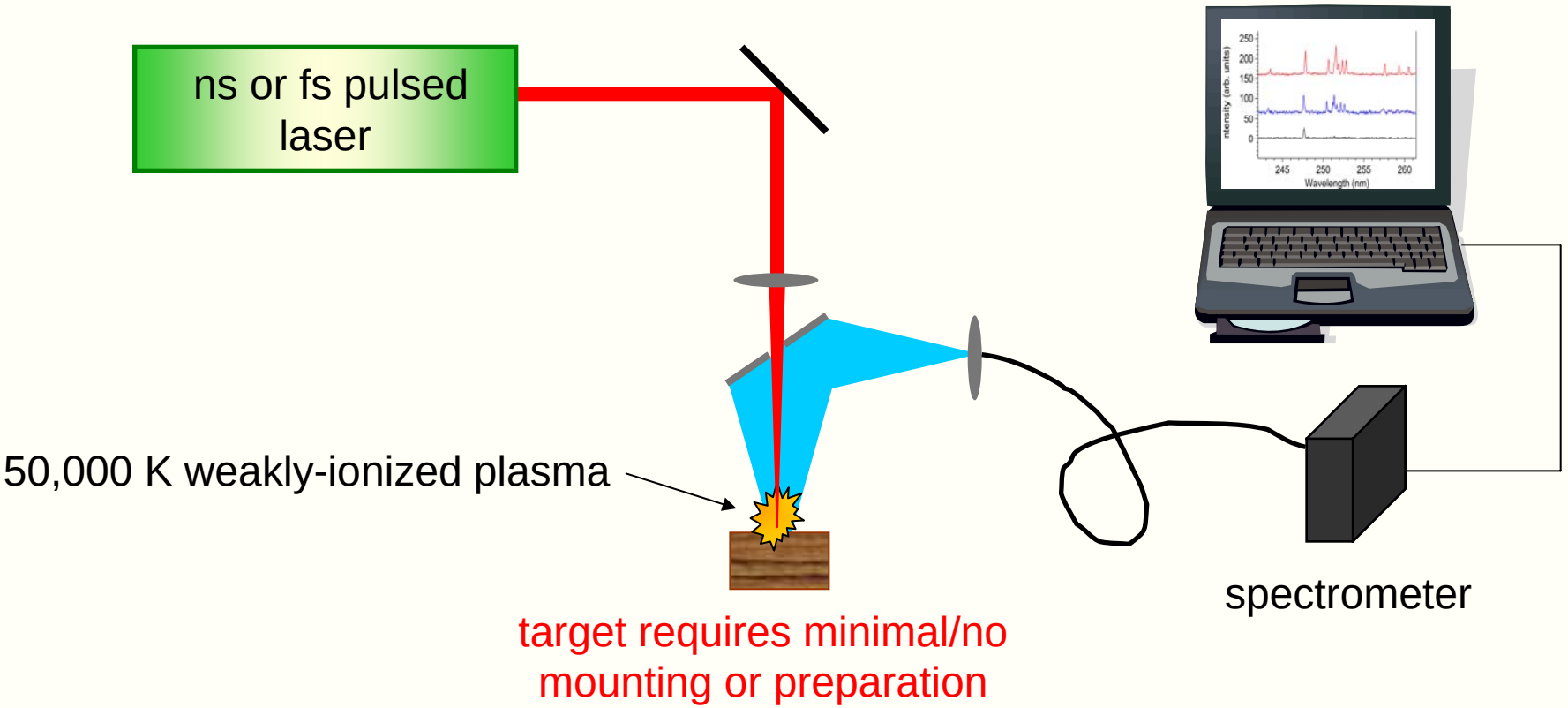
***Khadijia Sheikh, Russell Putnam, Andrew Daabous,***
***Ryan Woodman, Daniel Trojiand, Eric Lessard,***
***Derek Gillies, Hanieh Afkhamiardakani***
*University of Windsor*
*Department of Physics*



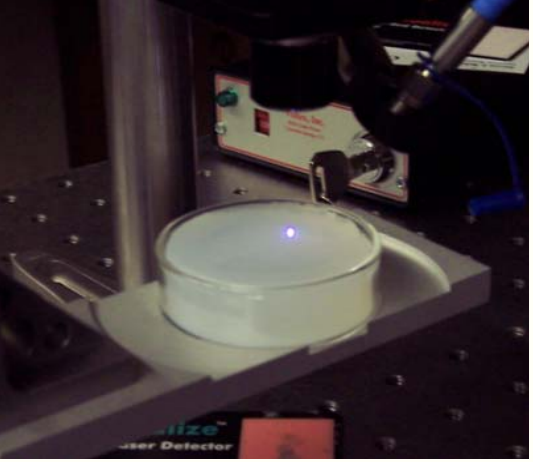


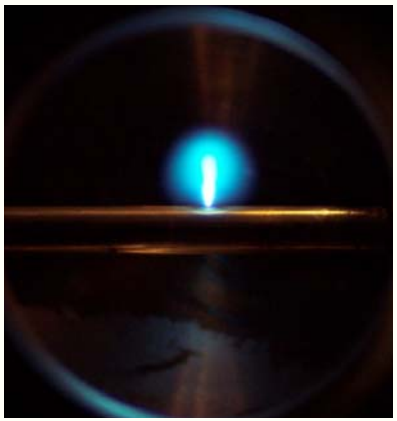
# *Laser-Induced Breakdown Spectroscopy*

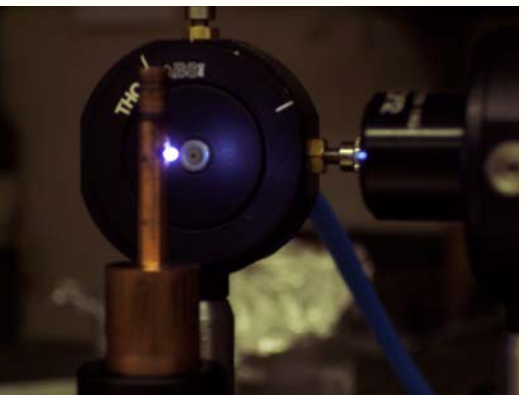
a non-resonant laser-based atomic spectroscopy technique

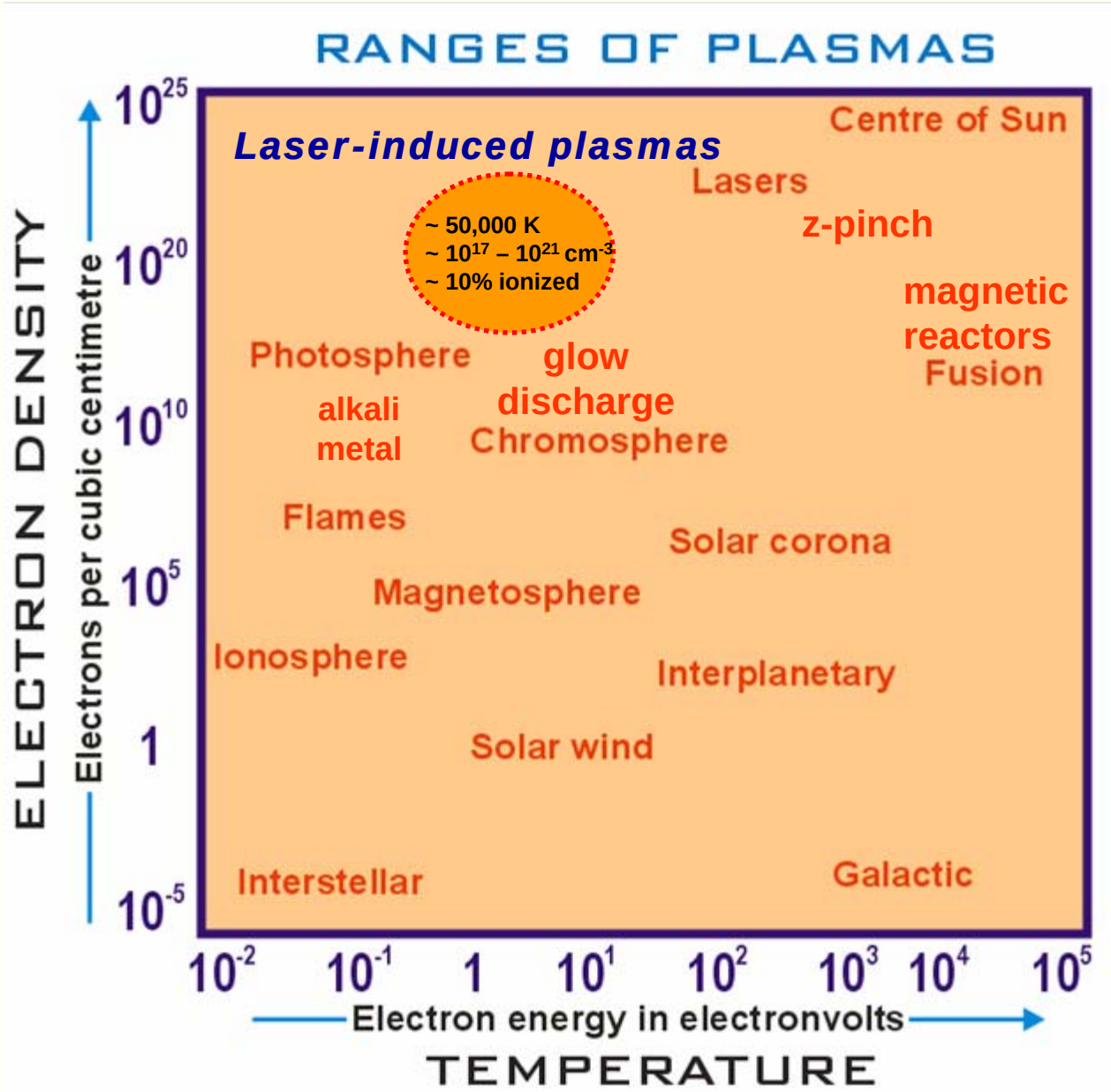



***performs an elemental assay (all elements detected without bias) in under one second!***








RANGES OF PLASMAS
ELECTRON DENSITY
Electrons per cubic centimetre
$10^{25}$
$10^{20}$
$10^{10}$
$10^{5}$
1
$10^{-5}$
Laser-induced plasmas
~ 50,000 K
~ $10^{17}$ – $10^{21}$ cm$^{-3}$
~ 10% ionized
Centre of Sun
Lasers
z-pinch
magnetic reactors
Fusion
Photosphere
glow discharge
alkali metal
Chromosphere
Flames
Solar corona
Magnetosphere
Ionosphere
Interplanetary
Solar wind
Interstellar
Galactic
$10^{-2}$
$10^{-1}$
1
$10^{1}$
$10^{2}$
$10^{3}$
$10^{4}$
$10^{5}$
Electron energy in electronvolts
TEMPERATURE

# *LIBS…as a medical diagnostic?*

- Since 80's LIBS has been known as a *fast*, *sensitive*, and *robust* spectroscopic technique for rapid elemental analysis (inexpensive, on-line, in situ, portable)

- Not enough people outside the LIBS community realize that it is currently being used for a wide variety of intriguing and important *medical and biomedical applications*

**Prospects for laser-induced breakdown spectroscopy for biomedical applications: a review**

Vivek Kumar Singh · Awadhesh Kumar Rai

Lasers Med Sci (2011) 26:673–687

**Assessment of LIBS for Spectrochemical Analysis: A Review**

ASHOK KUMAR PATHAK,[1] ROHIT KUMAR,[1]
VIVEK KUMAR SINGH,[2] RAHUL AGRAWAL,[3]
SHIKHA RAI,[1] AND AWADHESH KUMAR RAI[1]

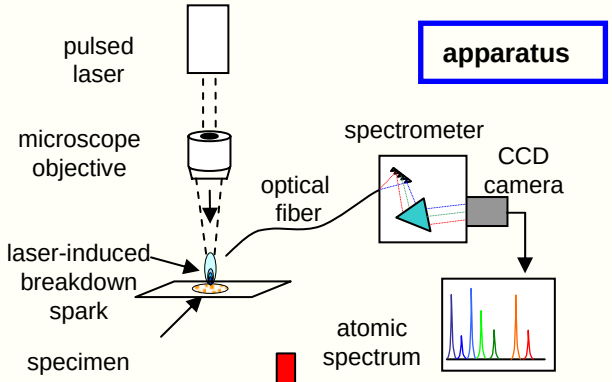
*Applied Spectroscopy Reviews*, 47:14–40, 2012

REVIEW

**Laser-induced breakdown spectroscopy (LIBS): an overview of recent progress and future potential for biomedical applications**

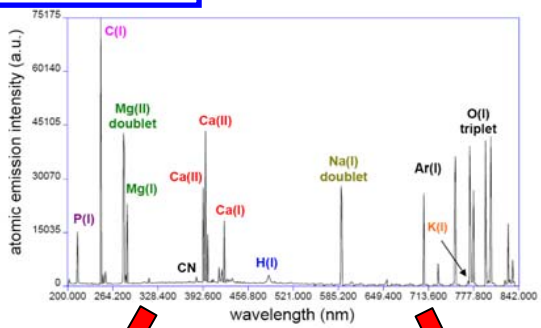
S. J. Rehse*,[1], H. Salimnia[2] and A. W. Miziolek[3]

*Journal of Medical Engineering & Technology*, 2012; 36(2): 77–89

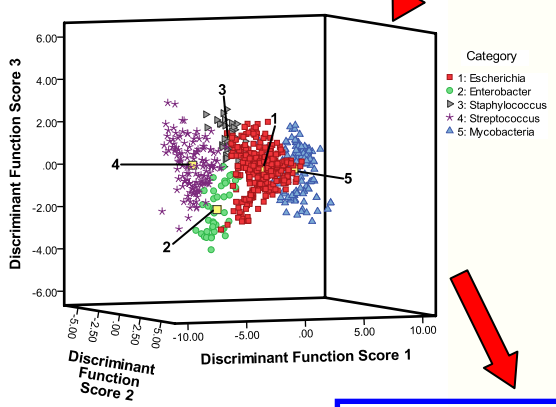



**apparatus**

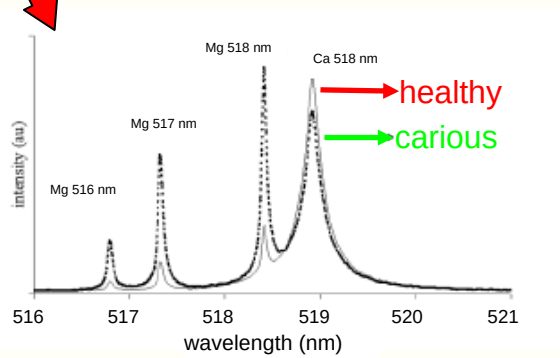
**LIBS spectrum**




**computerized chemometric analysis**




**analysis of element concentration**




**diagnosis of disease state or classification of specimen, either autonomously or by practitioner**

**ANALYSIS OF HARD/CALCIFIED TISSUES**
- *Calcified tissues*
- *Dental studies*
- *Stones and calculi*
- *Fingernails*

**ANALYSIS OF SOFT TISSUES**
- *Organs*
- *Cancerous/malignant tissues*
- *Hair/skin*

**ANALYSIS OF BIOMEDICAL SPECIMENS**
- *Blood*
- *Proteins*

**ANALYSIS OF MICROORGANISMS CAUSING HUMAN DISEASE**
- *Bacterial pathogens*
- *Viral pathogens*
- *Molds, pollens, amoeba*

**LIBS-GUIDED SURGERY**
- *Laser-guided surgery*

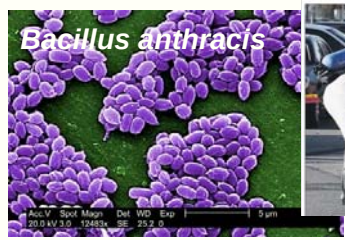
MOTIVATION: there is an urgent need right now in the military, civilian (hospital, food processing, environmental), and first responder communities for a "…rapid point-of-care diagnostic for disease-causing pathogens"

***multiply drug-resistant bacteria (MDRB)***



***food contamination***

***bioterrorism &threats of bioterrorism)***





***emerging "super-bugs"***



- ✓ lower health care costs
- ✓ improve patient outcomes
- ✓ slow the emergence of antibiotic resistance

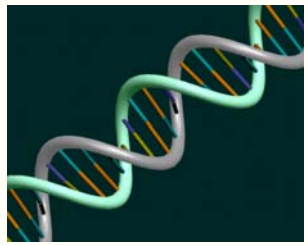
ideally this diagnostic should NOT require:

1. *a priori* knowledge of nucleic acid sequences for genetic testing
2. possession of antibodies against known bacterial antigens

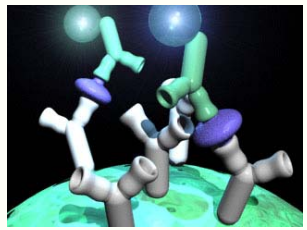
# Infectious Pathogen Diagnosis

***microbiological***


***genetic***



***serological***



## ***compositional***

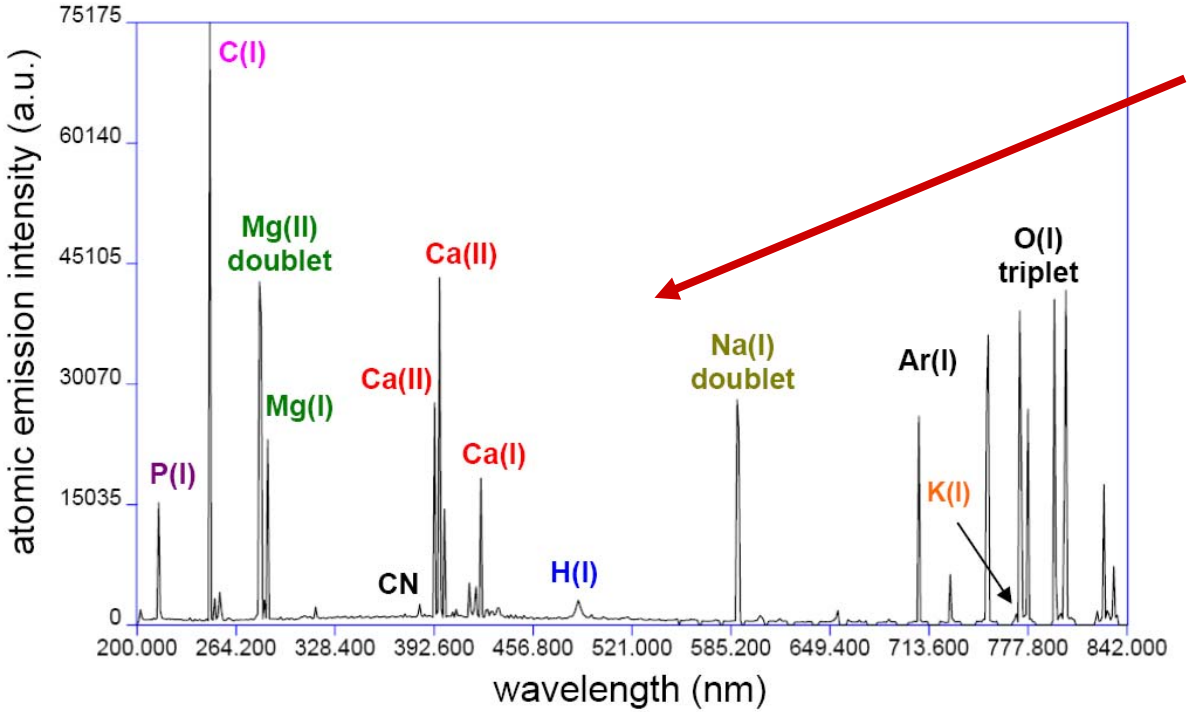
spectroscopic/spectrometric

***Raman spectroscopy***

***Laser-induced breakdown spectroscopy (LIBS)***

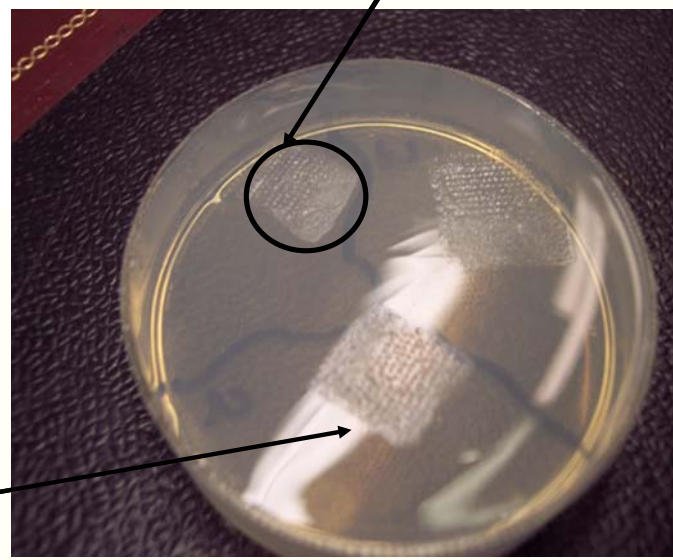
***MALDI-TOF-MS***

# *A LIBS spectrum is a sensitive assay of the bacterial cell's inorganic composition*




7500 cells provide a very high SNR atomic emission spectrum that is unique to each bacterial species/strain

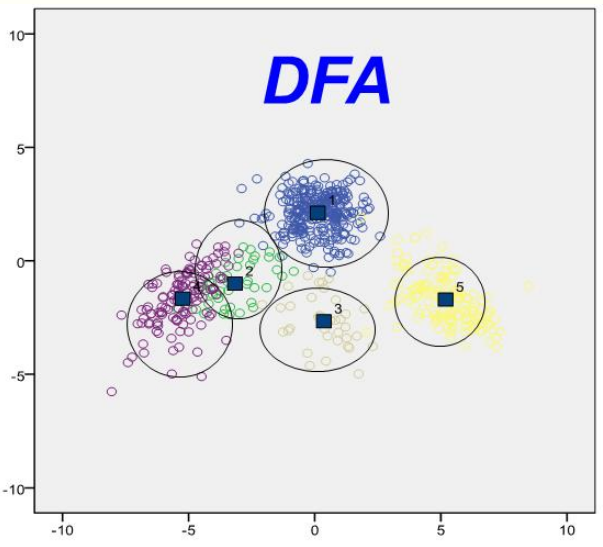

about 500-1500 bacteria per sampling location

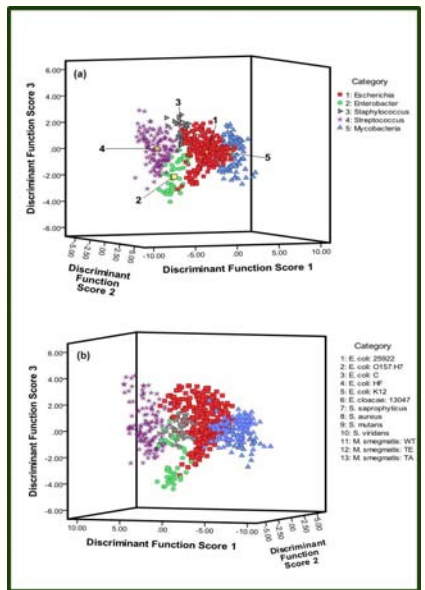


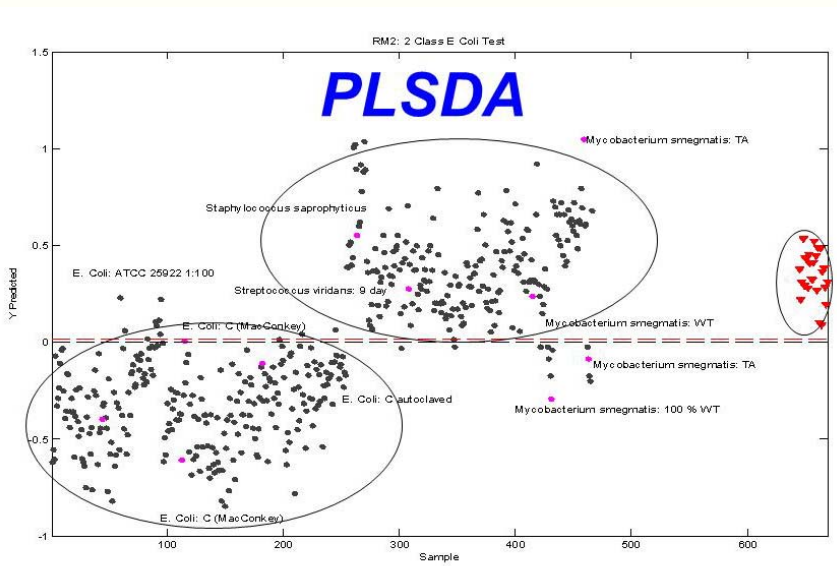
# *To "discriminate" one bacterial spectrum from another, a multivariate analysis ("chemometrics") is required*

- Intensities of lines or ratios of intensities used as independent variables in a DFA or PLSDA
  - Express the emission intensity data in a basis set that maximizes differences between data sets
  - Build a "library" of known bacterial spectra

- Identify an unknown specimen according to which class it is assigned with the highest probability

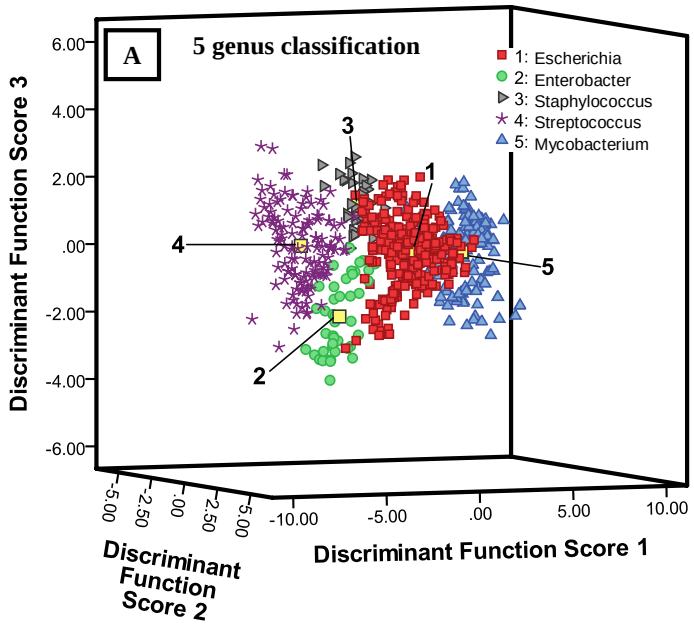


# How unique is “unique”?

- ✓ We can identify a bacterial species, certainly its genus, with high sensitivity and specificity (confirmed by others).
- ✓ We can differentiate strains of *E. coli* (demonstrated by others in MRSA).
- ✓ Multiple multivariate techniques effective at discriminating spectra.




PLSDA

| E. COLI | True | False |
|---|---|---|
| Positive | 95.65% | 9.17% |
| Negative | 90.83% | 4.35% |

| STAPHYLOCOCCUS | True | False |
|---|---|---|
| Positive | 54.05% | 0.51% |
| Negative | 99.49% | 45.95% |

| STREPTOCOCCUS | True | False |
|---|---|---|
| Positive | 95.59% | 1.02% |
| Negative | 98.98% | 4.41% |

| MYCOBACTERIUM | True | False |
|---|---|---|
| Positive | 88.31% | 1.06% |
| Negative | 98.94% | 11.69% |

DFA

| E. COLI | True | False |
|---|---|---|
| Positive | 89.63% | 15.95% |
| Negative | 84.05% | 10.37% |

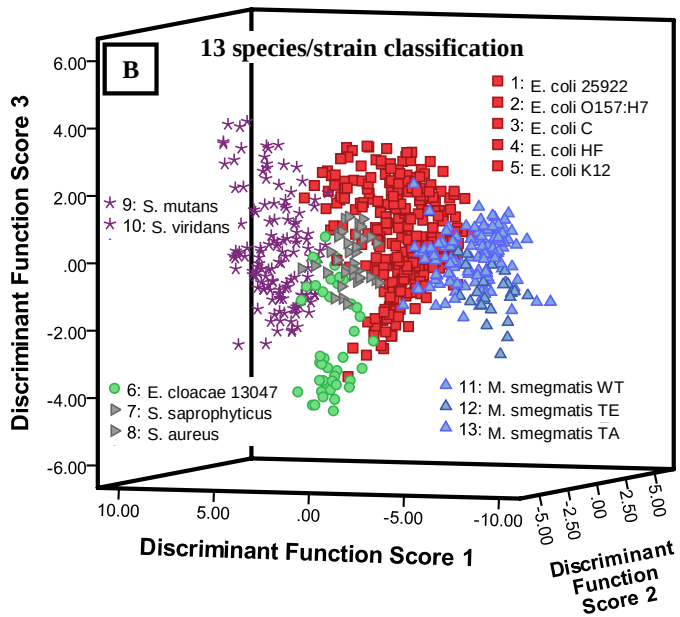
| STAPHYLOCOCCUS | True | False |
|---|---|---|
| Positive | 86.49% | 5.85% |
| Negative | 94.15% | 13.51% |

| STREPTOCOCCUS | True | False |
|---|---|---|
| Positive | 99.26% | 13.32% |
| Negative | 88.68% | 0.74% |

| MYCOBACTERIUM | True | False |
|---|---|---|
| Positive | 96.10% | 4.08% |
| Negative | 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 %



# *Bacterial spectra are unique. Are they robust?*

**2007& 2011** Bacterial identification appears to be independent of the growth condition and culture medium in which the bacteria were grown.

**2011** This result confirmed by Marcos-Martinez et al. on three similar growth media

**2011** *Salmonella enterica* serovar Typhimuriumin identified at various concentrations in various liquids such as milk, chicken broth, and brain heart infusion.

**2007-2012** The bacterial LIBS spectrum for a given species is stable and does not change with time (experiments conducted on the same *E. coli* strain over the course of multiple years).

**2011** Bacterial LIBS spectra do not change with time as the bacteria age on an abiotic surface

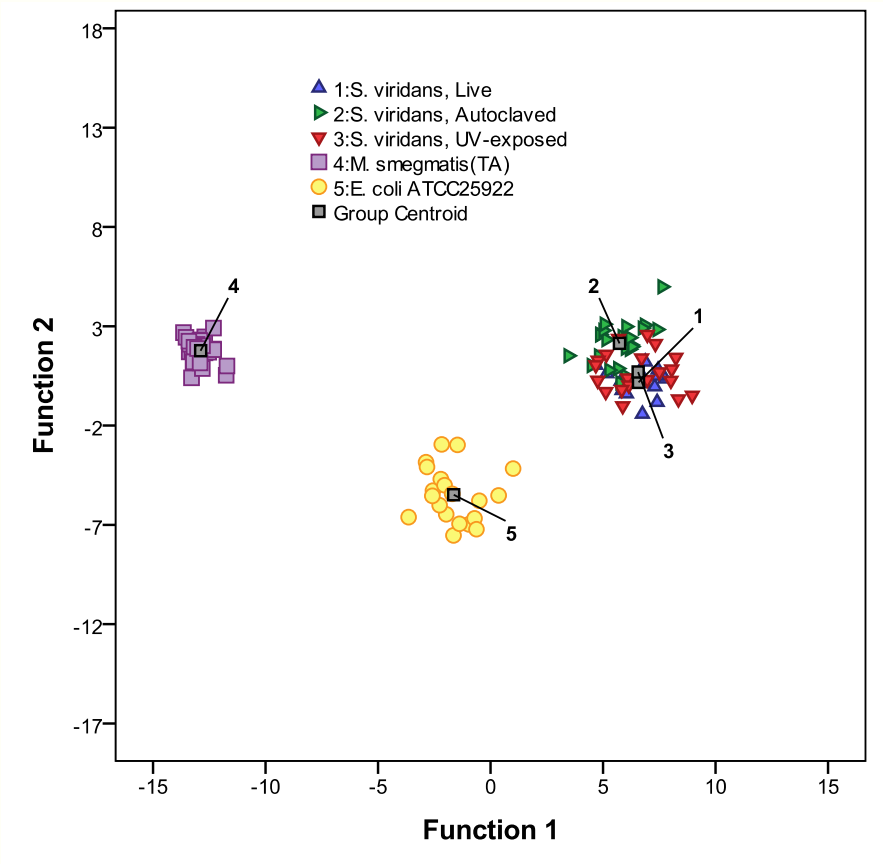
**2011** Bacterial LIBS spectra can be obtained from killed (via autoclaving) or inactivated (via UV light) specimens, and such treatment (which renders the specimen completely safe for handling) does not decrease identification specificity and does not decrease LIBS spectral intensity.

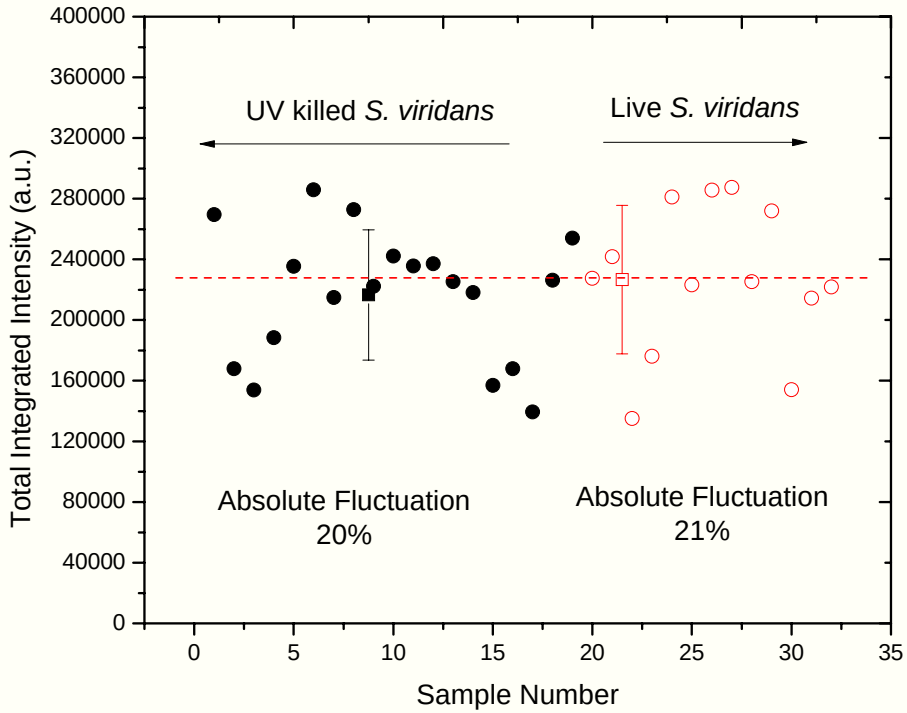
**2012** Bacteria can be identified with high sensitivity and specificity when specimens are obtained from clinical samples (e.g. sterile urine containing organic and inorganic solutes) without the need to remove other compounds present in the sample.

**2012** Live pathogenic *Bacillus anthracis* Sterne strain and *Francisella tularensis* can be differentiated regardless of mounting protocol (as lawn and/or colonies on agar, dilutions on agar, and dilutions on glass slides.)

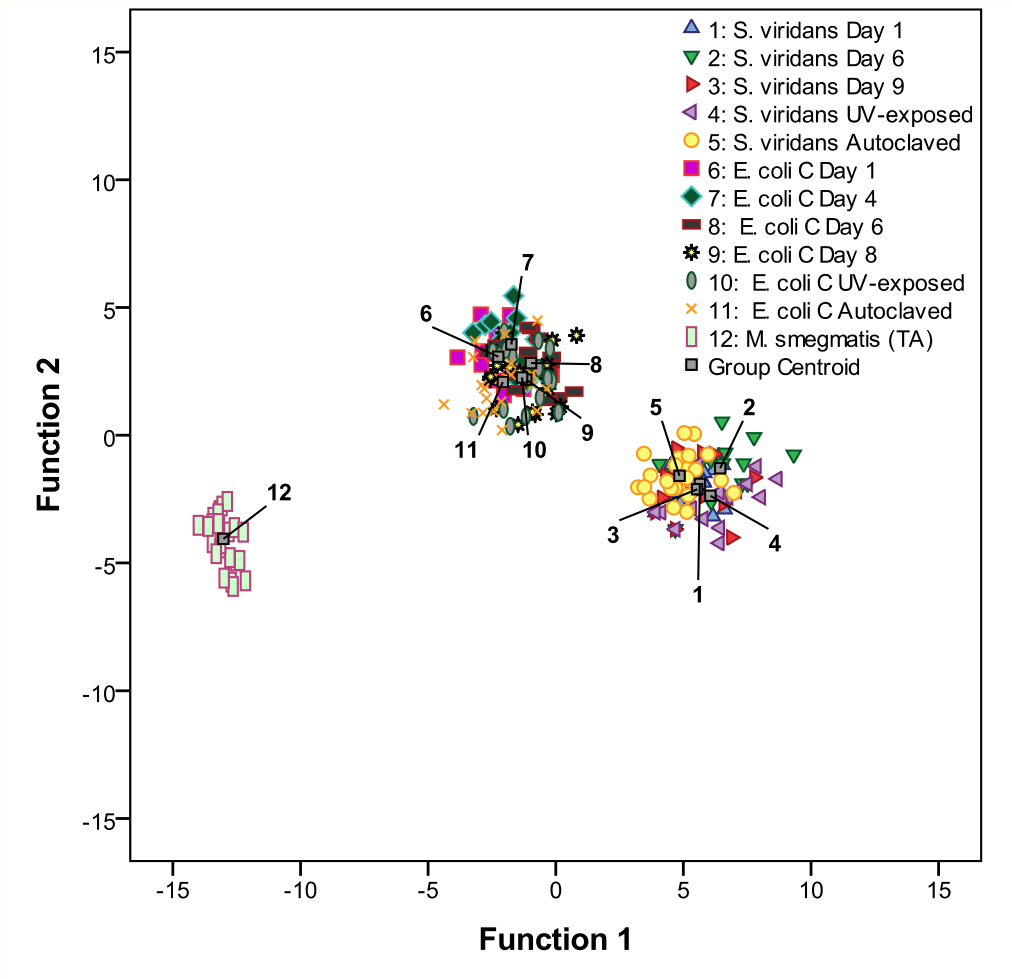
**2011 2012** Bacteria in mixed samples are identifiable. The dominant or majority bacterial component of a two-component bacterial mixture is reliably identified provided it comprises 70% of the mixture or more. Trace mixture or contamination is insignificant.

# *LIBS specificity and sensitivity are not dependent on bio-activity of the bacteria*



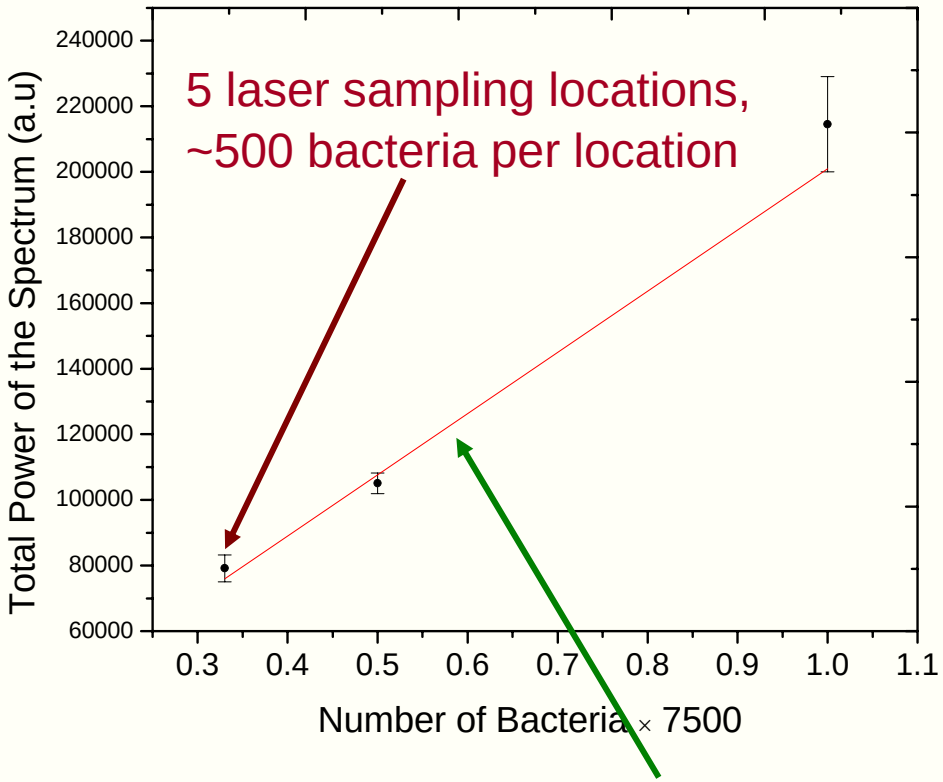
# *LIBS specificity and sensitivity are <u>not</u> dependent on bio-activity of the bacteria*

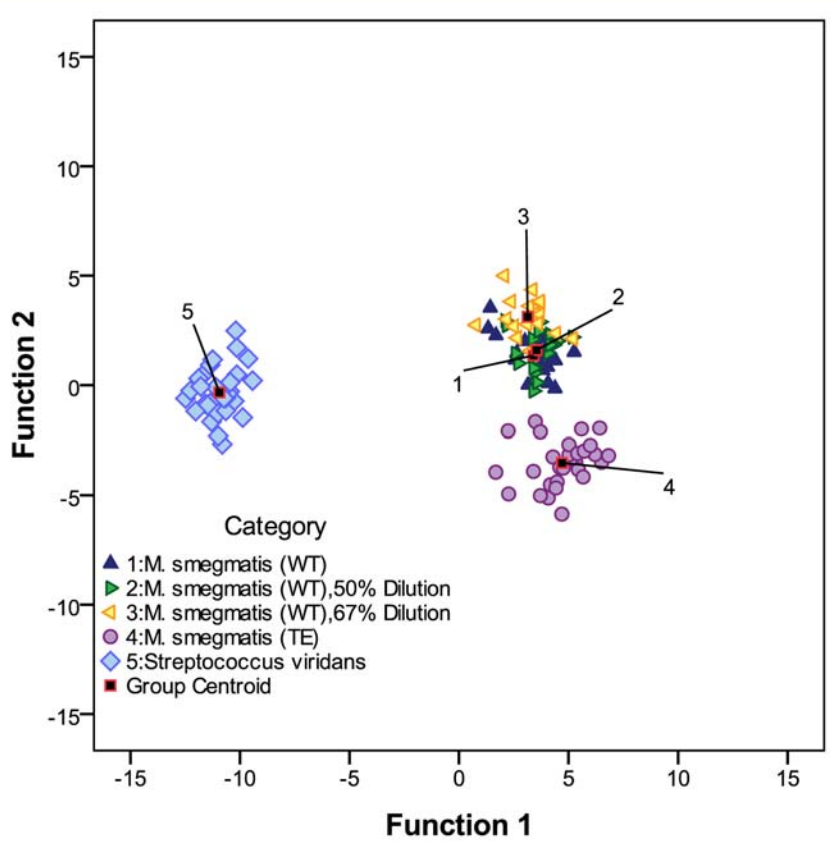



- Two species of bacteria tested
- All specimens prepared separately and left to sit on a nutrient-free medium for up to 9 days at room temperature
- This graph also includes the UV-irradiated and the autoclaved specimens
- All species 100% accurately identified

# Dilution

## *specimens of various titer*

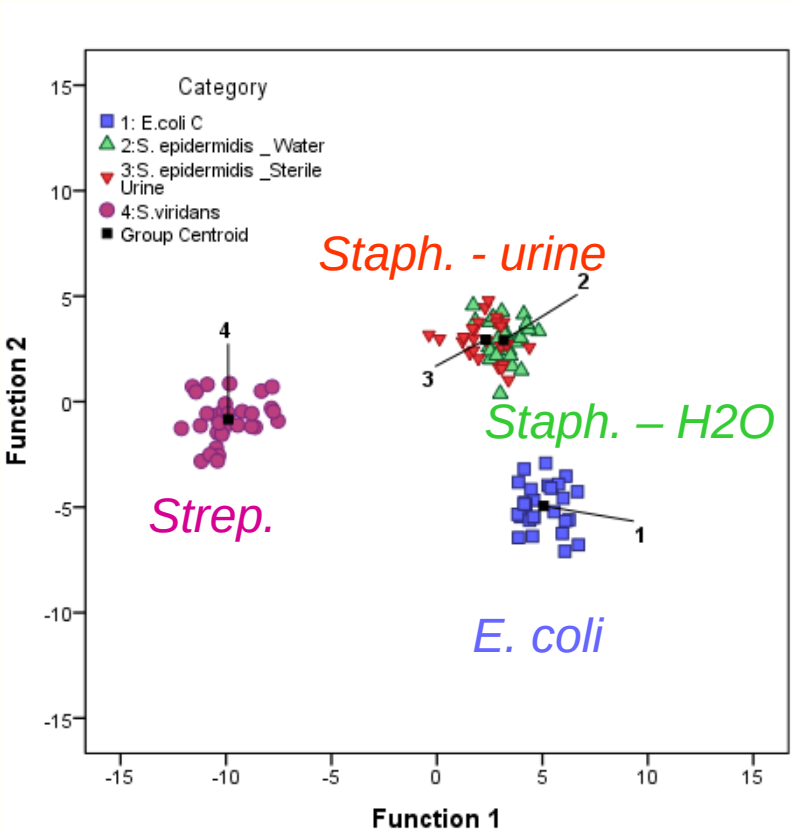


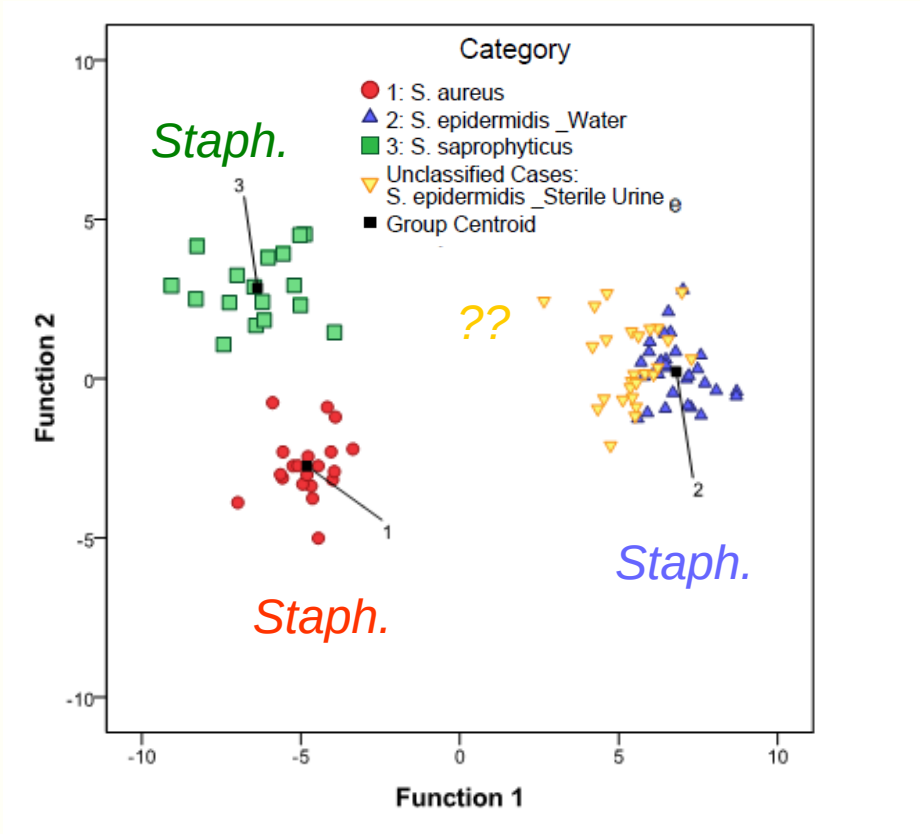




Doubling of cell number easily identifiable:
suggests antibiotic resistance test?

# *Simulated Clinical Specimens*

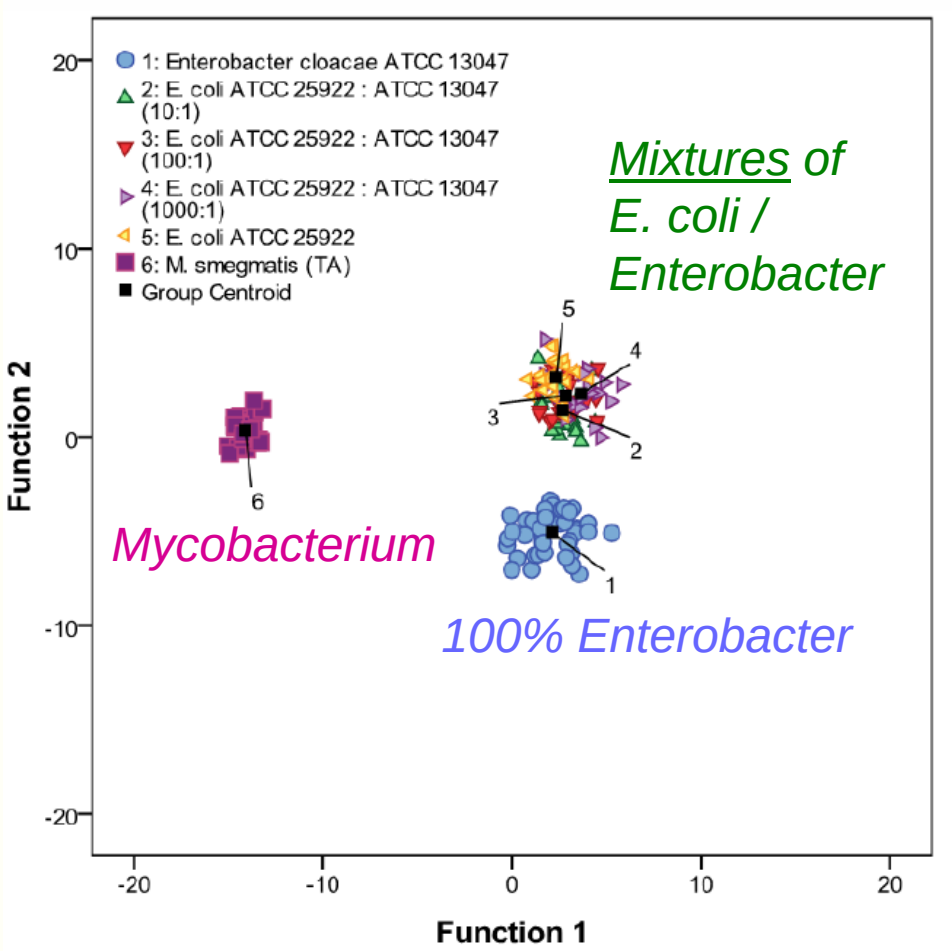
## *sterile urine*



# ***Simulated Clinical Specimens***

## *contaminations / mixtures*



# *Where are we going next?*

**(1)** Clinical specimens that should be normally sterile and contain minimal other cellular components (i.e. urine, cerebral spinal fluid)

- detect the presence of bacteria
- make a rapid classification of that bacteria

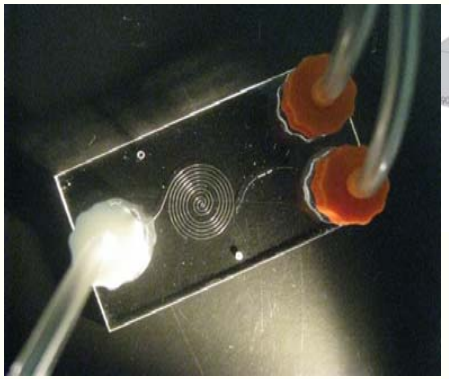
**(2)** Strain classification (particularly antibiotic-resistant pathogen strains such as MRSA)

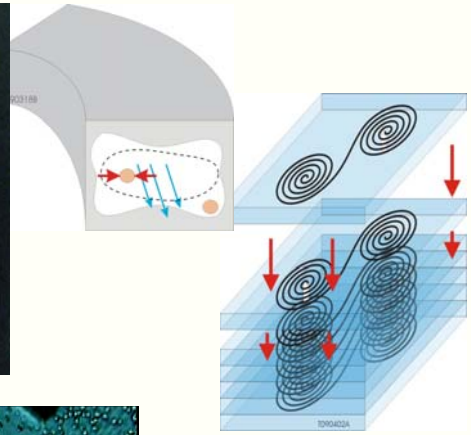
These two applications alone (MRSA infections and UTI's) are responsible for over $2 billion of medical costs worldwide every year.

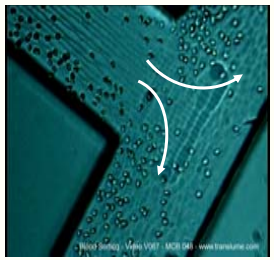
Most deaths from meningitis occur in less than a day from onset of the fever. It is most commonly caused by one of three types of bacteria: *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*.

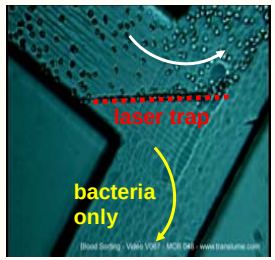
# Where are we going next?

## Microfluidic separation/concentration



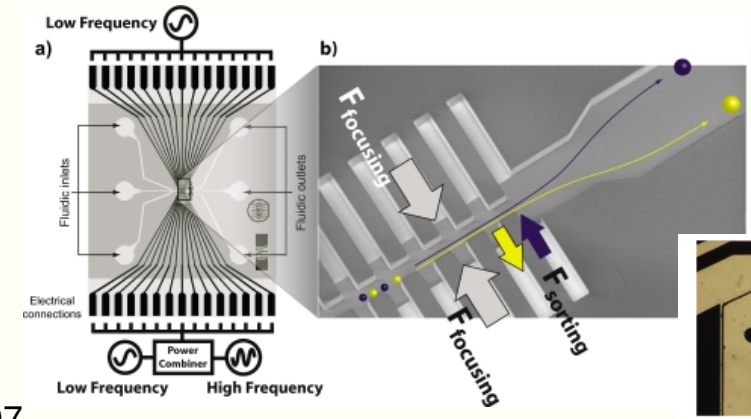


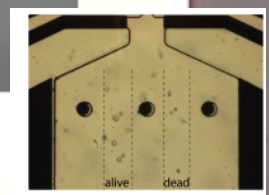


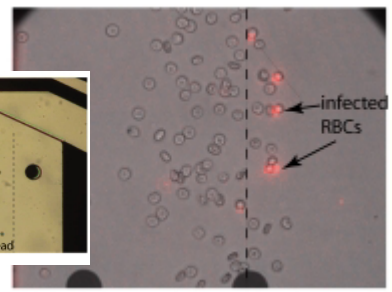



## Centrifugation/filtration

## Dielectrophoresis




***A miniaturized continuous dielectrophoretic cell sorter and its applications***

Ana Valero, Thomas Braschler, Nicolas Demierre, and Philippe Renaud

Biomicrofluidics. 2010 June; 4(2): 022807.

# *Long-term goals to improve the health of Canadians.*

**(1)** LIBS-based pathogen identification must be applicable to blood samples.

- The cellular components of blood?
- More complex sample-preparation steps for bacterial separation and identification needed.
- New sample-handling techniques needed.
- Advances made in the application of LIBS to liquid samples should be integrated to allow the rapid testing of the bacteria in fluid media.

**(2)** In all cases, efforts should now be made to include clinical collaborators.

- Allows the testing of clinical specimens in blind tests.
- All results initially confirmed by more traditional but rigorous microbiological (genetic and molecular microbiology) methods.

**(3)** Results published in medical journals and prototypes developed.

## *Much remains to be done…*

…but all tests to date have proven the possibility of using LIBS for a rapid pathogen diagnostic, as well as numerous other biomedical applications.

## *Work continues, with generous help from:*


- University of Windsor

University of Windsor
thinking forward

- NSERC Discovery Grant

Natural Sciences and Engineering Research Council of Canada
Conseil de recherches en sciences naturelles et en génie du Canada

- CFI-LOF grant

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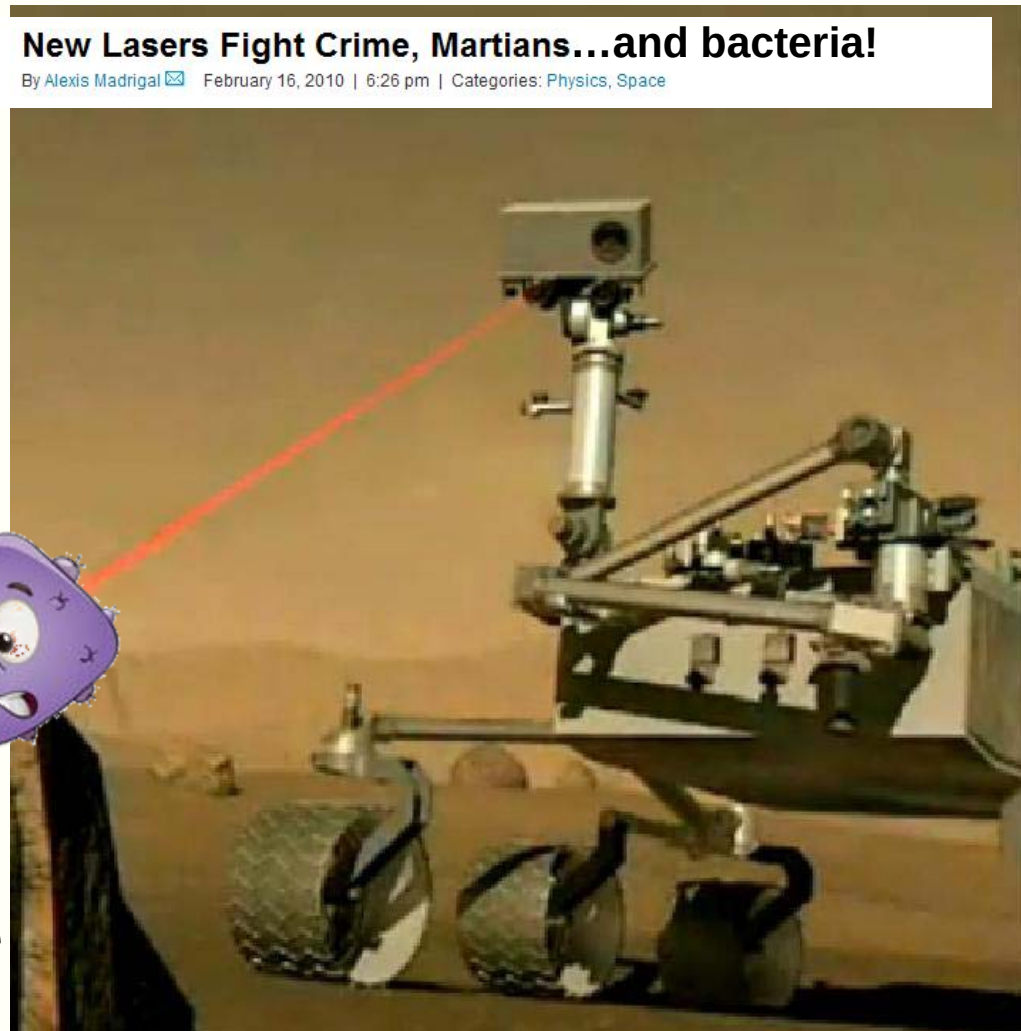
# *Thank you for your attention!*

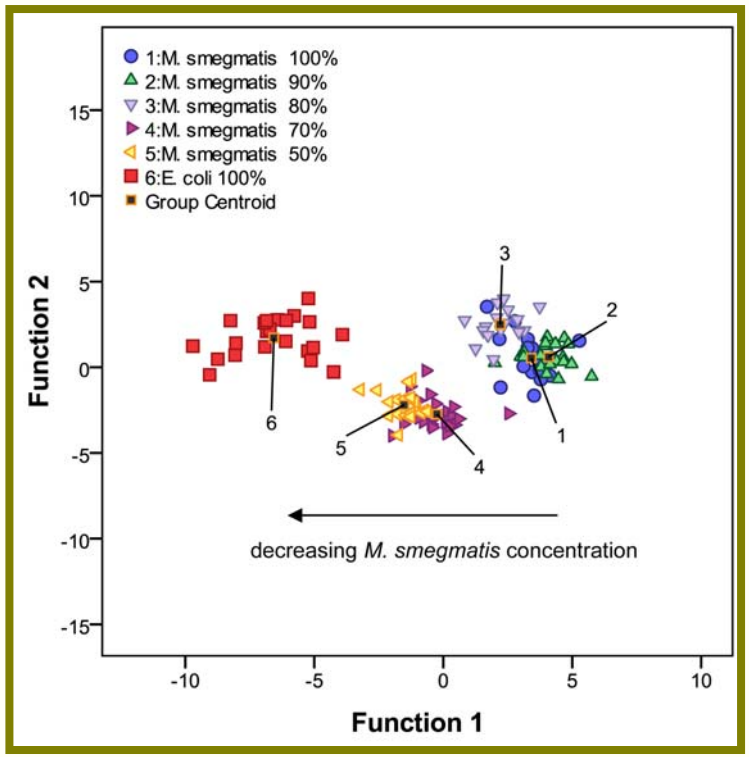
**http://www.uwindsor.ca/rehse/**



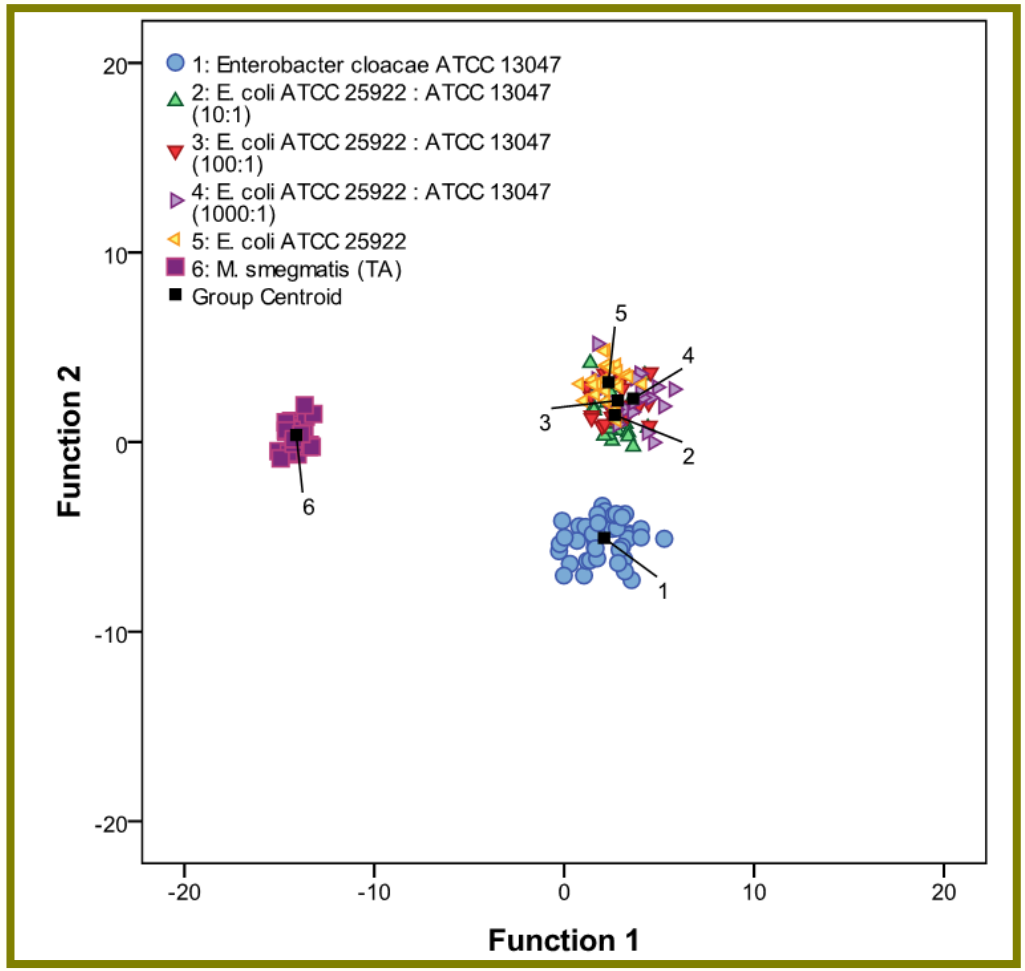

The Mars Science Laboratory “Curiosity” uses the ChemCam LIBS package to ablate rocks looking for signs of habitable environments.

# Contamination of samples will <u>not</u> degrade specificity

**2010**

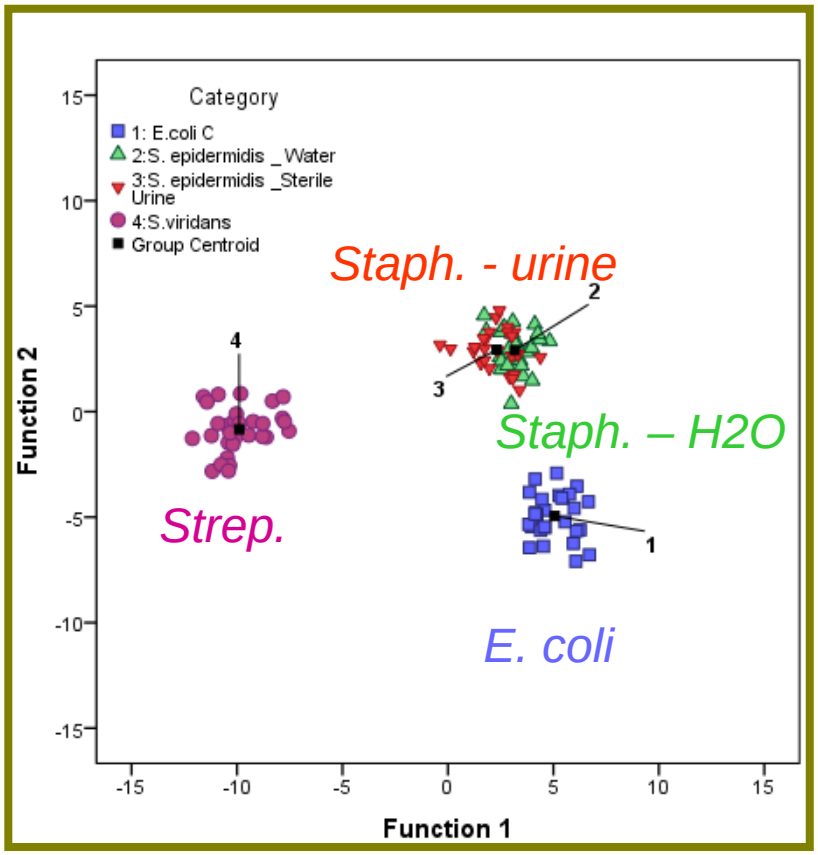



**2011**



# Simulated Clinical Specimens:
## *sterile urine*

**2011**

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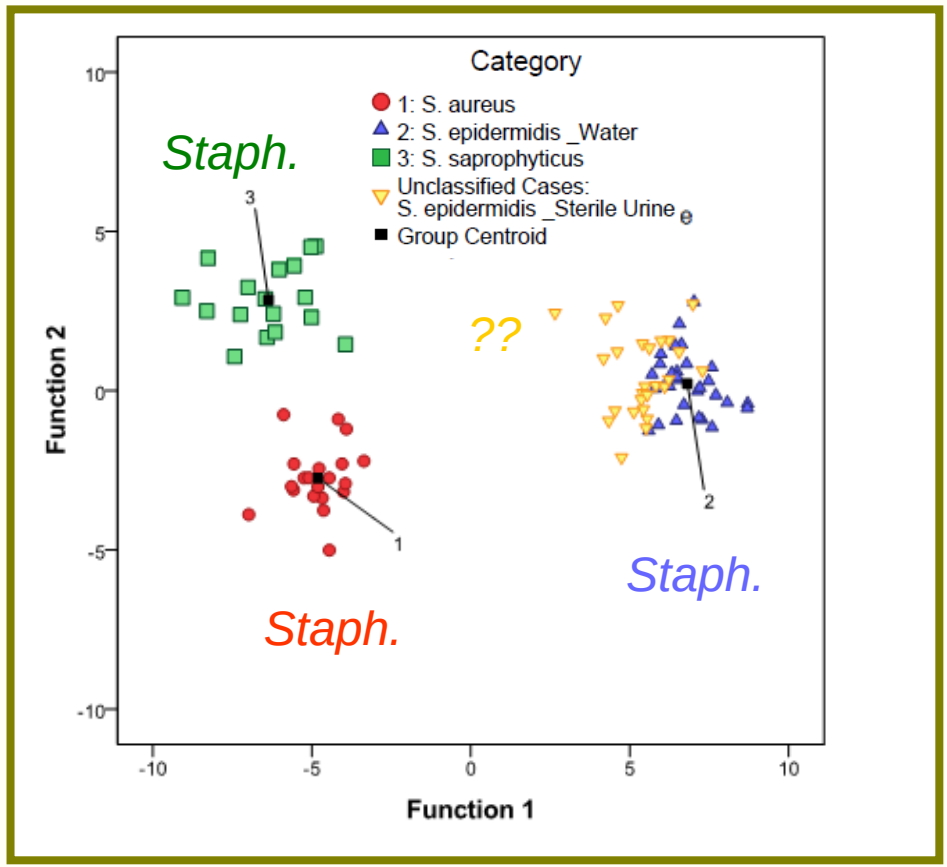
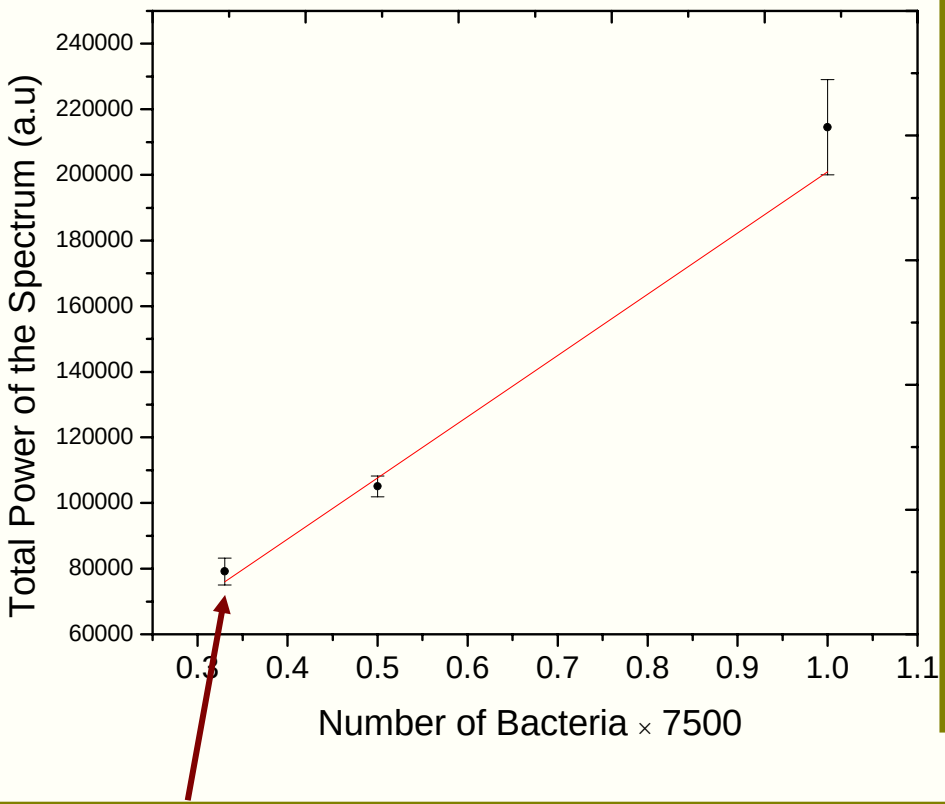
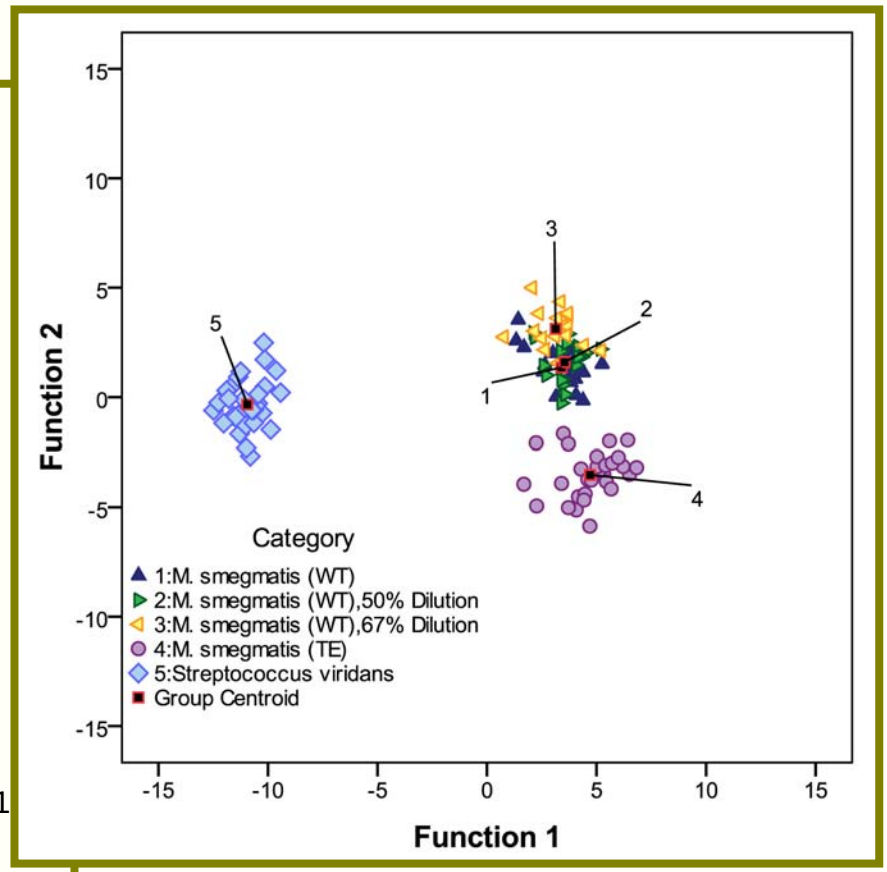


# Dilution

*specimens of various titer*

**2010**

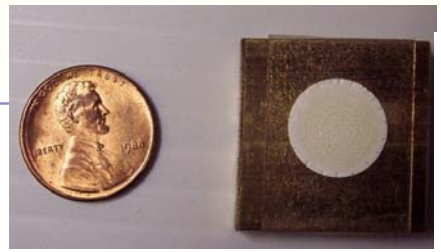



5 laser sampling locations

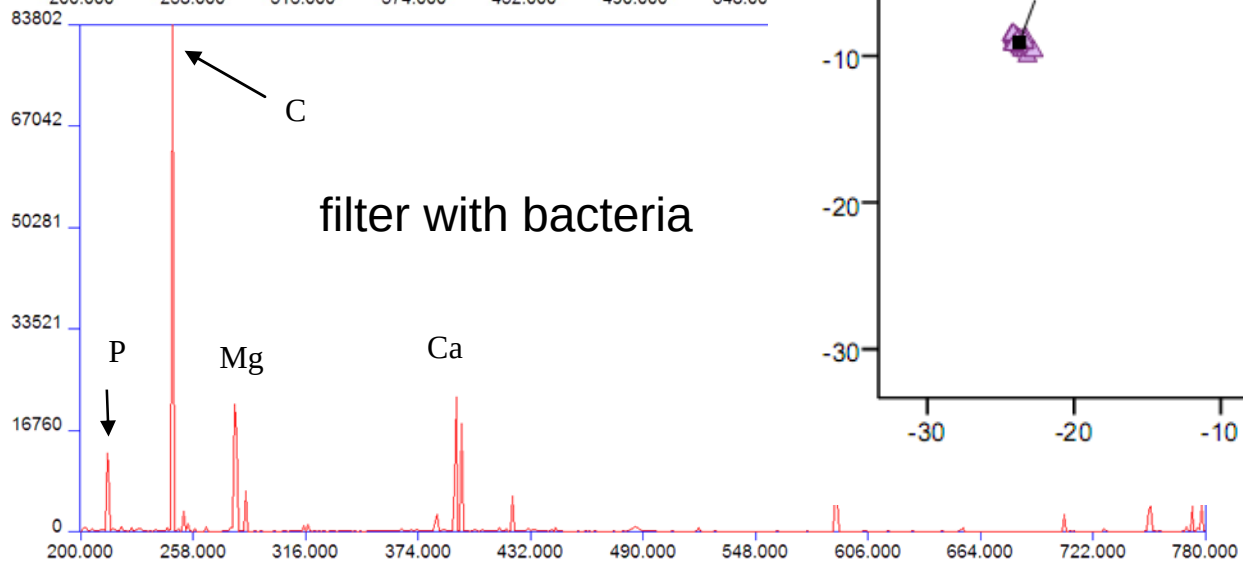
~500 bacteria per locations

# Cellulose Filter

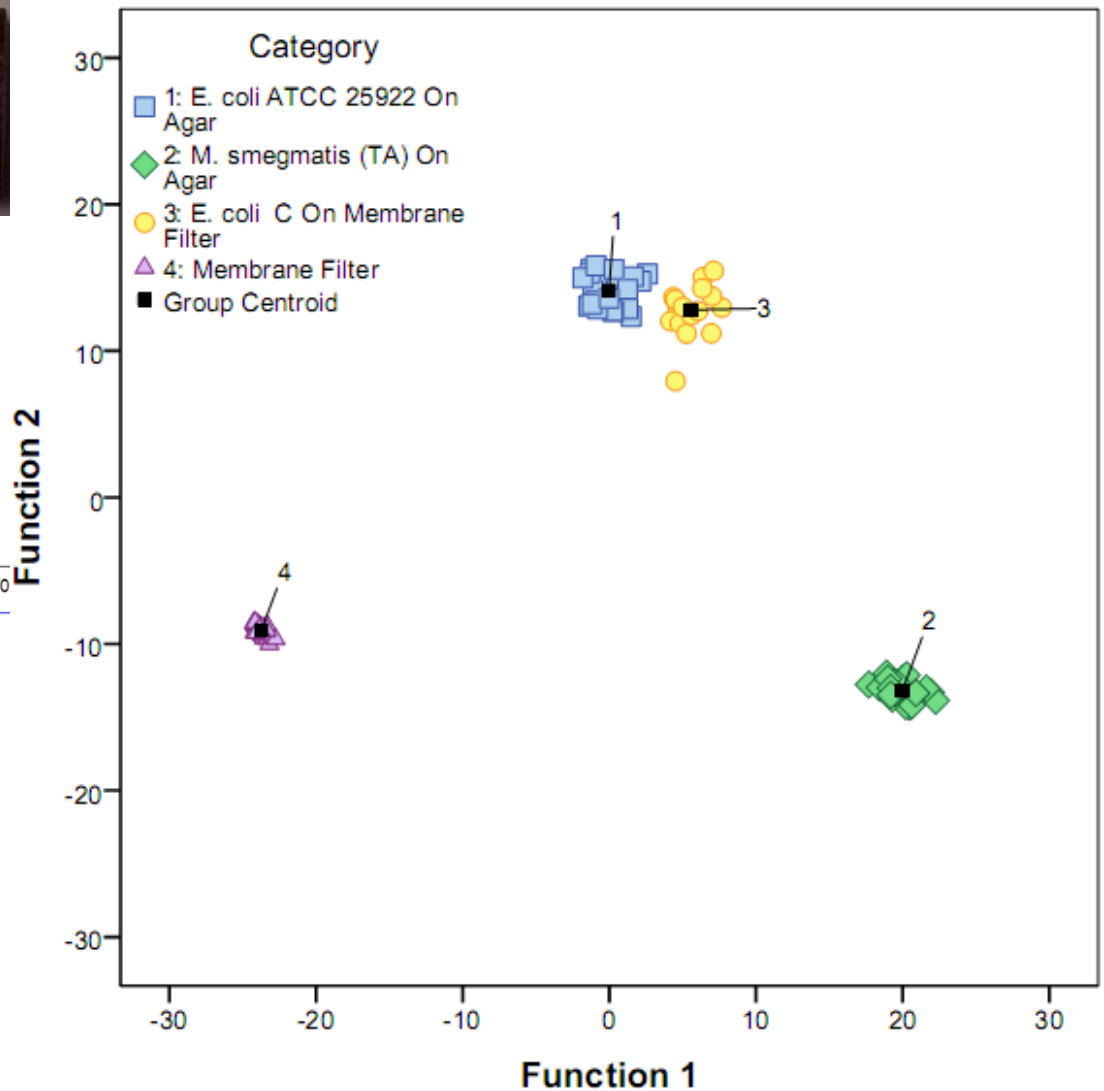





filter

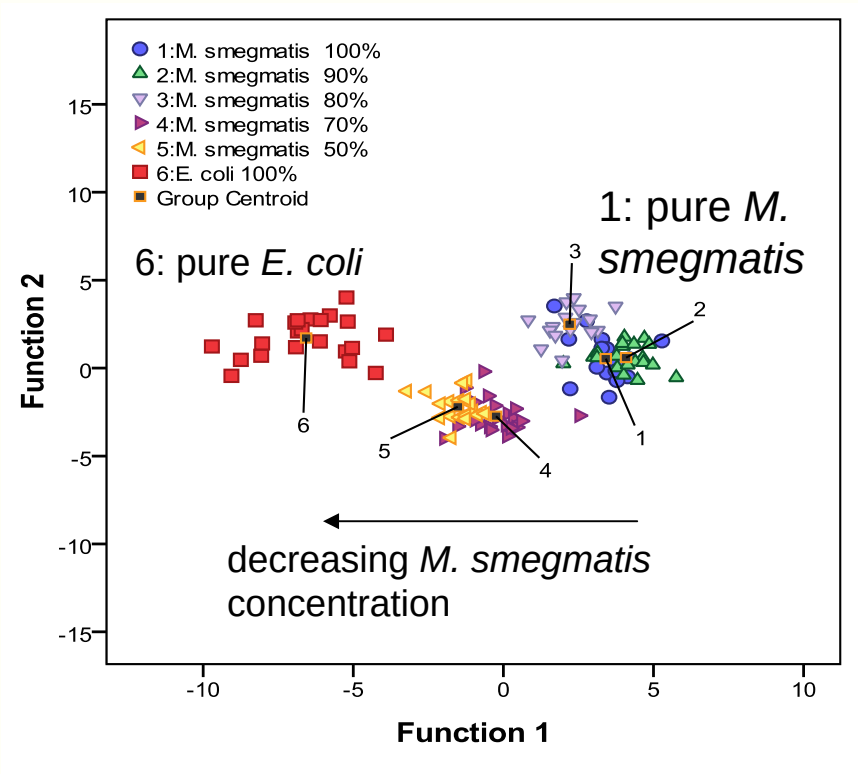



filter with bacteria



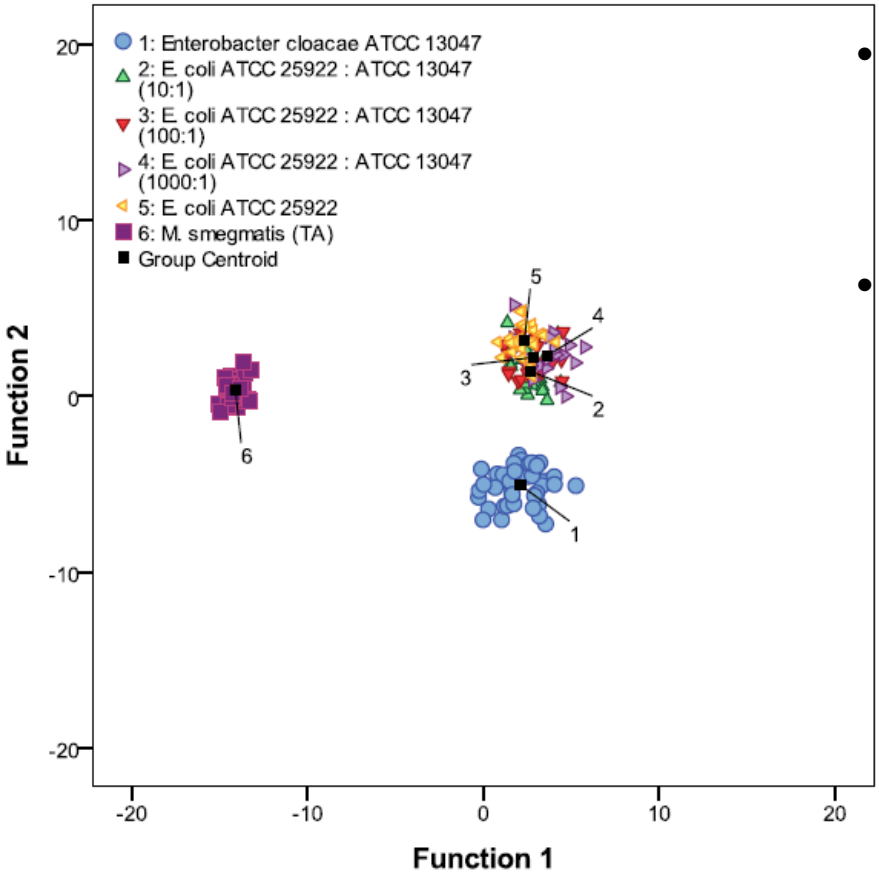
# "Mixed" Samples

| Category | # of Spectra | Classification Results | | |
|---|---|---|---|---|
| | | *M. smegmatis* | *E. coli* | *S. viridans* |
| 100% *M. smegmatis*, 0% *E. coli* | 21 | 100% | 0% | 0% |
| 90% *M. smegmatis*, 10% *E. coli* | 20 | 100% | 0% | 0% |
| 80% *M. smegmatis*, 20% *E. coli* | 16 | 100% | 0% | 0% |
| 70% *M. smegmatis*, 40% *E. coli* | 21 | 76% | 24% | 0% |
| 50% *M. smegmatis*, 50% *E. coli* | 19 | 47% | 53% | 0% |
| 0% *M. smegmatis*, 100% *E. coli* | 25 | 0% | 100% | 0% |



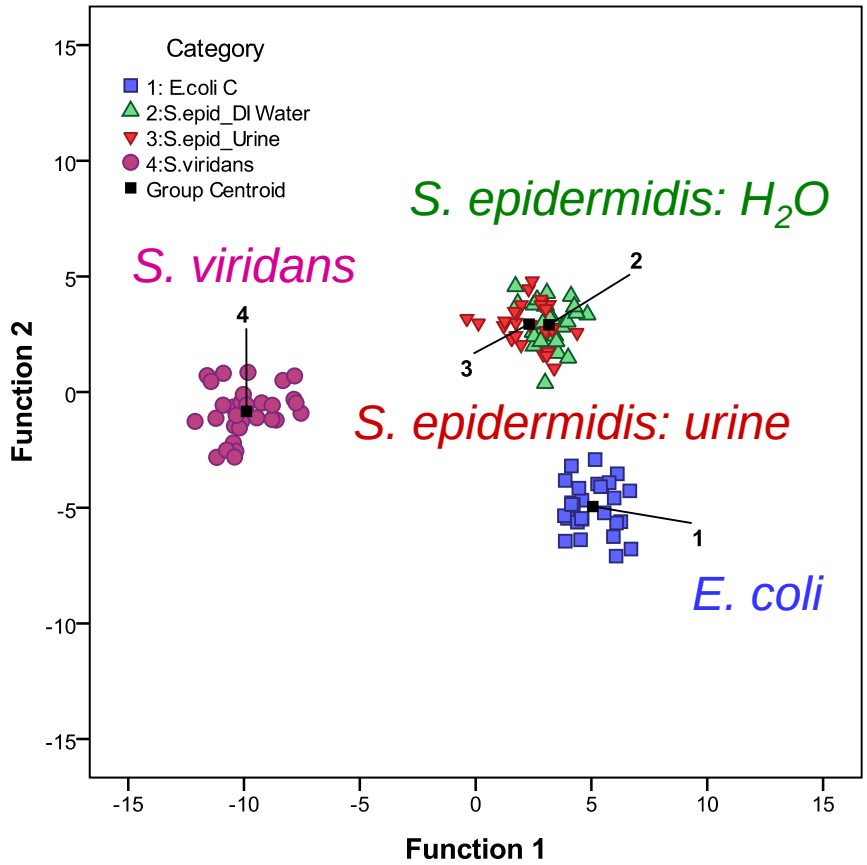

- Six separate mixtures of known mixing fraction were prepared from suspensions *M. smegmatis* and *E. coli* C.
- As long as the majority bacterium comprised 80% of the mixture, we saw 100% identification.

# *“Mixed” Samples*



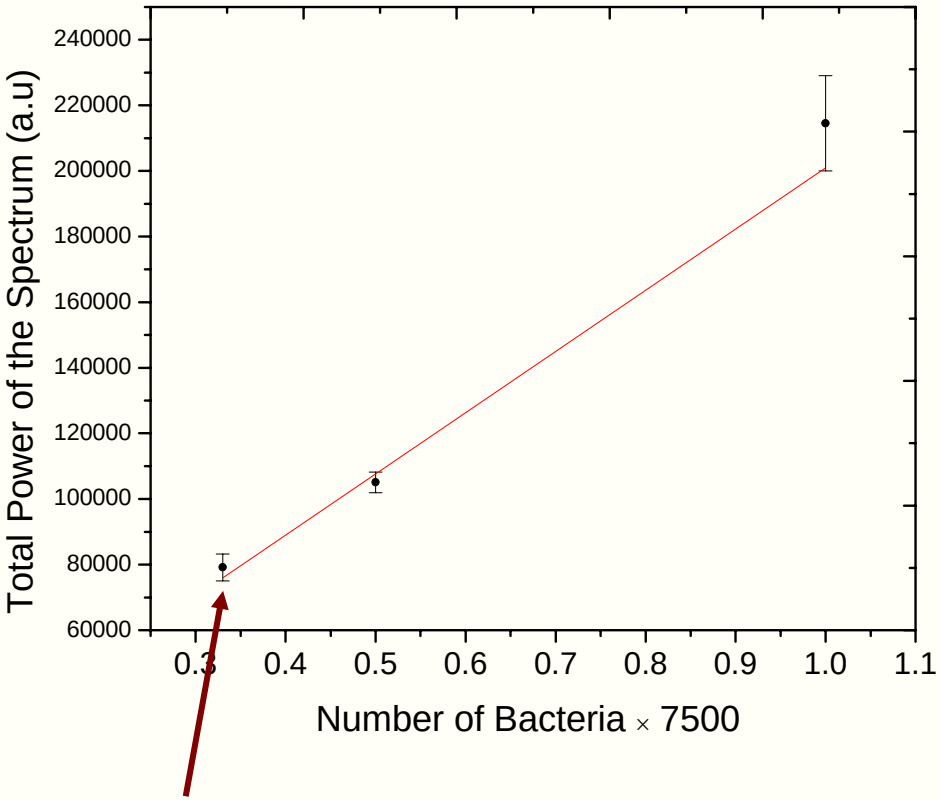

- Mixtures of known mixing fraction were prepared from suspensions *E. coli* C and *E. cloacae*.
- Mixing represent “clinical” contaminations and/or mixtures (i.e. 10:1, 100:1, 1000:1).

# *“Dirty” clinical samples*




- Samples of *Staph. epidermidis* were prepared in DI water and sterile urine.
- Samples were collected and tested via LIBS with NO WASHING.
- LIBS spectral fingerprint from urine-exposed bacteria were identical to water-exposed bacteria.
- EMMA correctly classified 100% of the urine-exposed bacteria as being consistent with *S. epidermidis*

# *LIBS intensity linearly dependent on number of bacteria*

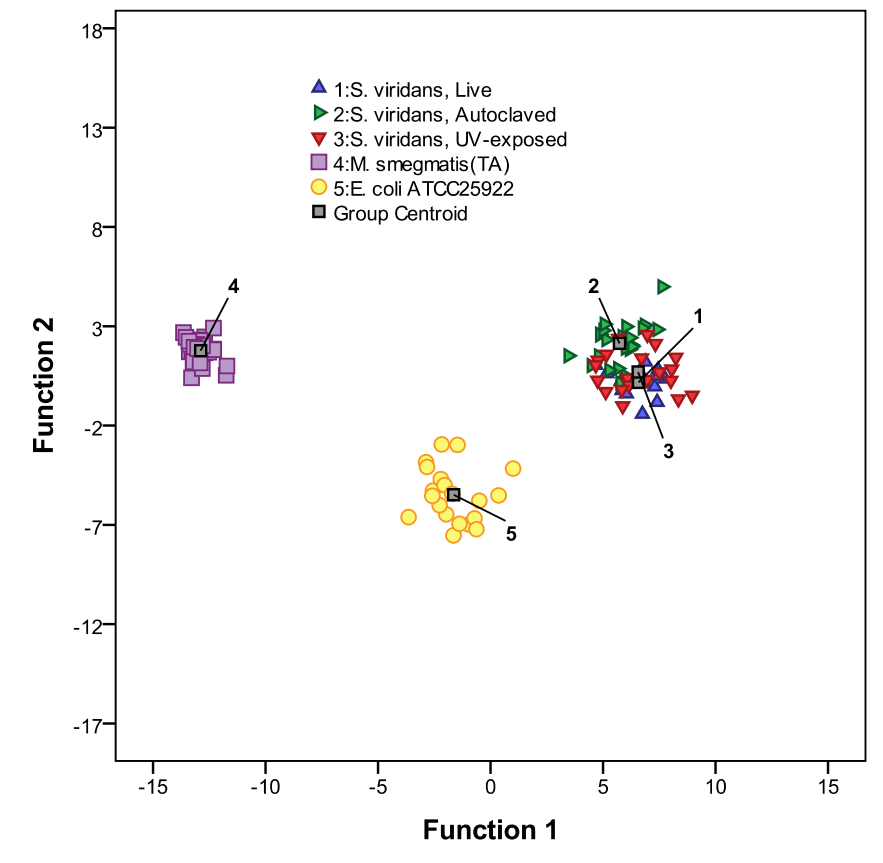


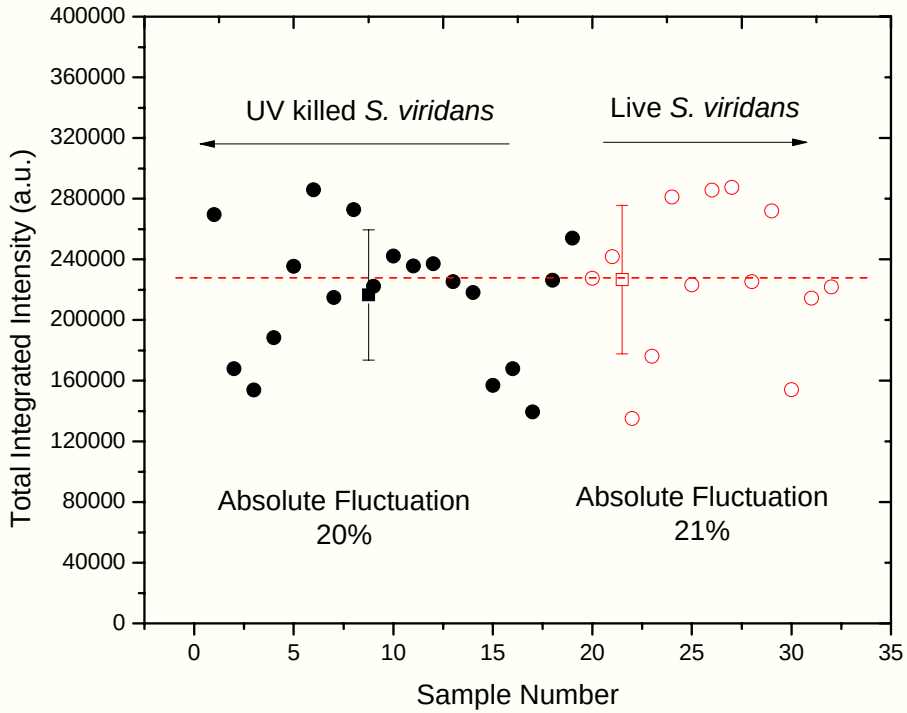

5 laser sampling locations

~500 bacteria per locations

- Samples of *E. coli* with different titer tested on agar.
- Each data point is the average of 5 sampling locations.
- As expected, spectra demonstrate a linear dependence with cell number.
- All spectra were 100% correctly identified (specificity not dependent on number of cells).
- Suggests an antibiotic resistance test?

# *LIBS specificity and sensitivity not dependent on bio-activity of the bacteria*



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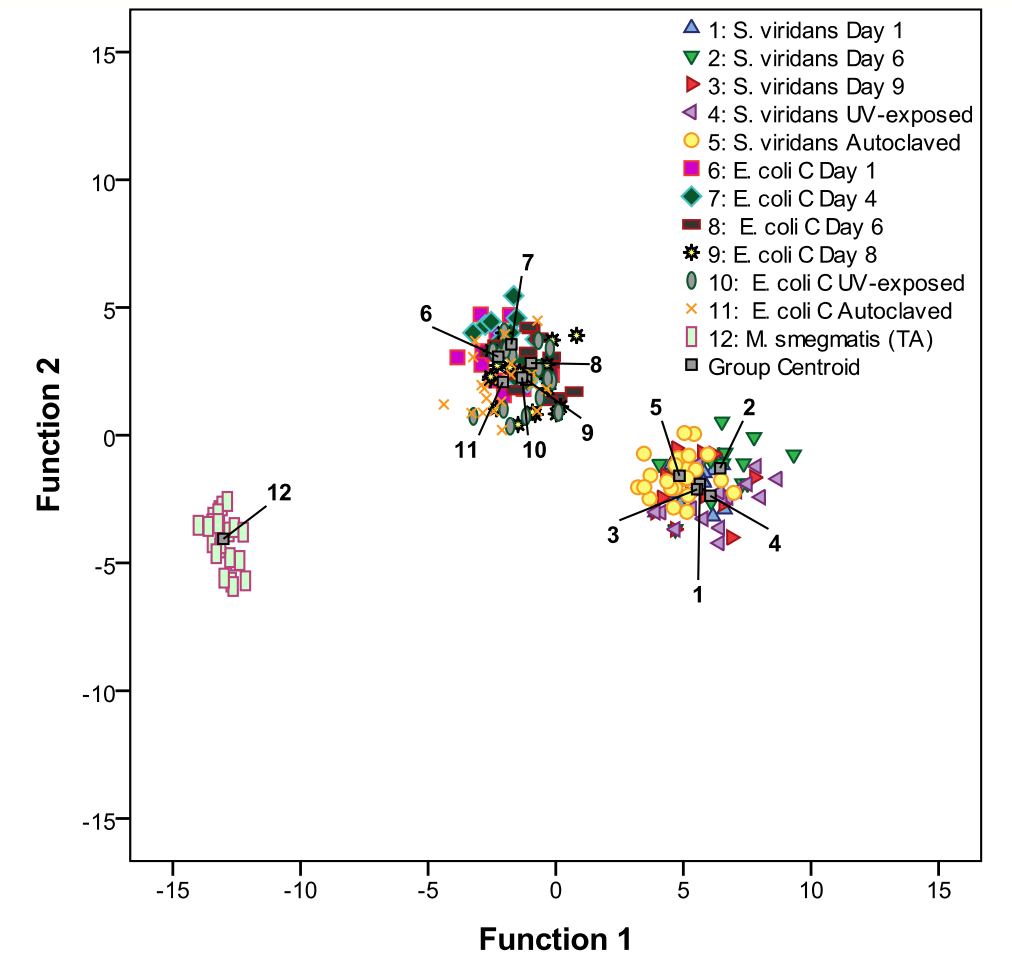


- Two species of bacteria tested
- All specimens prepared separately and left to sit on a nutrient-free medium for up to 9 days at room temperature
- This graph also includes the UV-irradiated and the autoclaved specimens
- All species 100% accurately identified

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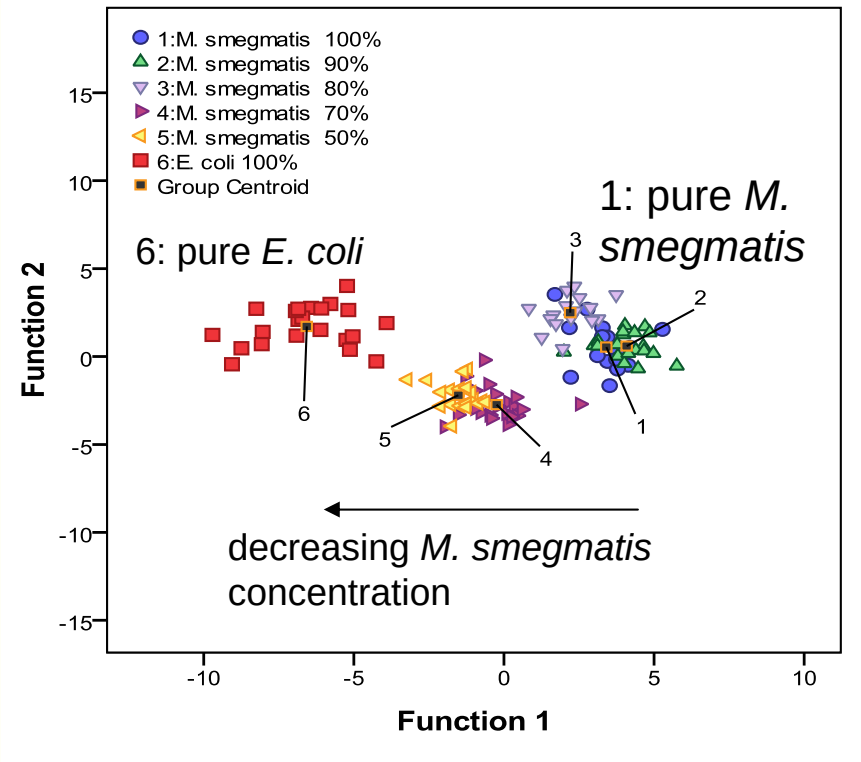


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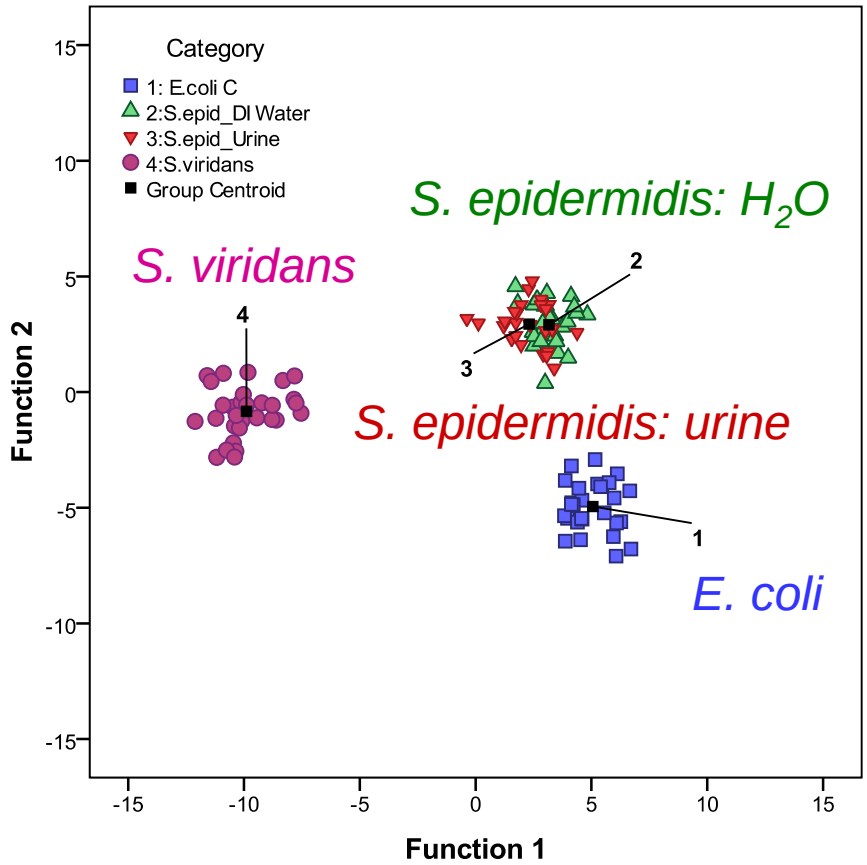
- Six separate mixtures were prepared with a ratio *M. smegmatis* to *E. coli* C given by $M_{1-x}:C_x$ with $x$ = 0.0, 0.1, 0.2, 0.3, 0.5, 1.0.
- Multiple 1.5 mL tubes of these mixtures were prepared, thoroughly agitated via vortex mixing, then centrifuged for 3 minutes at 5000 rev/min.

# *“Dirty” samples*

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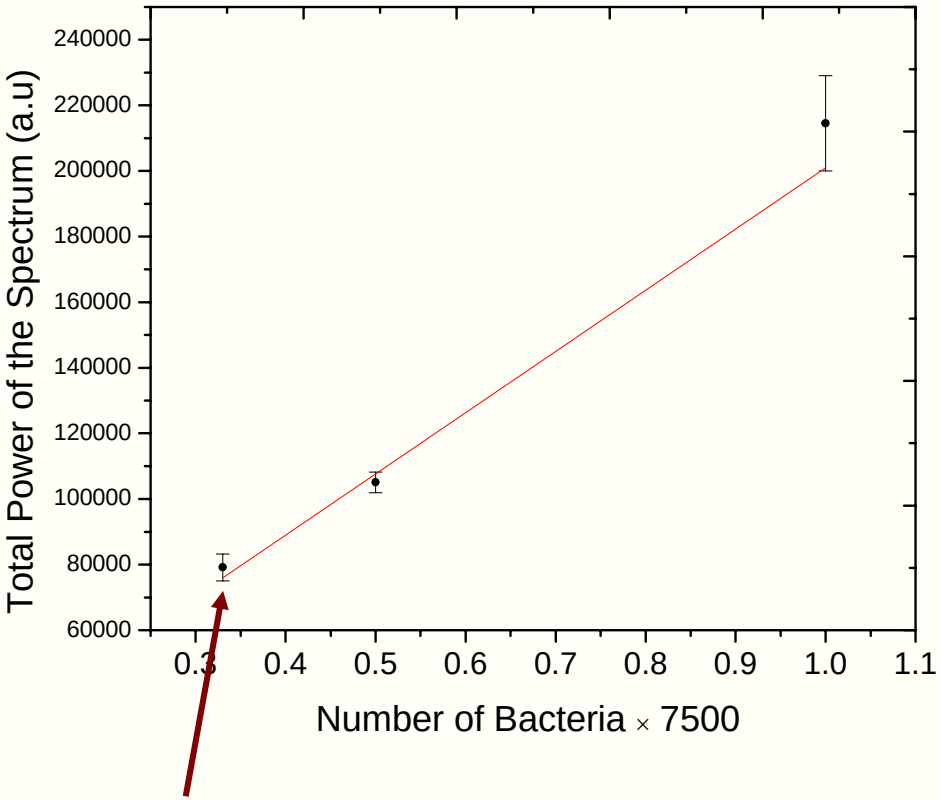


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