Laser-induced breakdown spectroscopy (LIBS): a new paradigm for rapid pathogen identification

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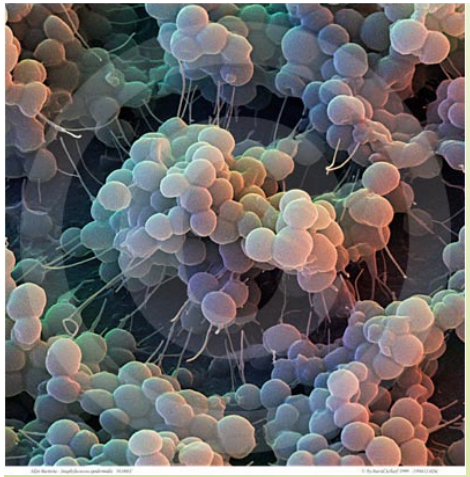
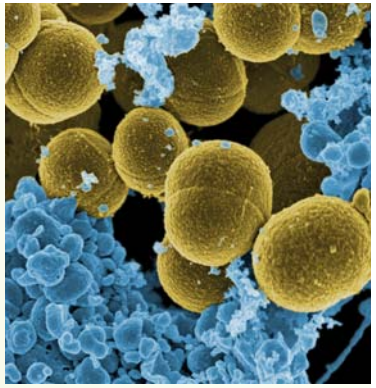


Staph. epidermidis

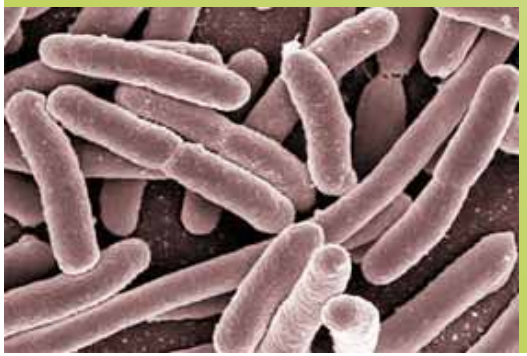
Staph. aureus



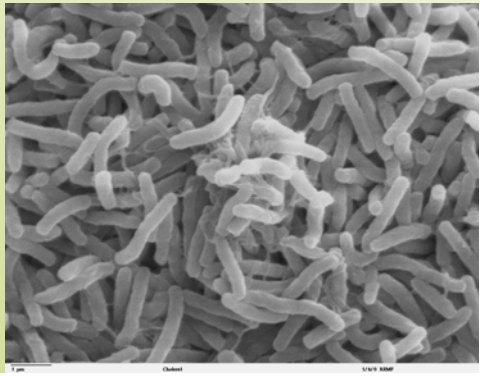
there is an urgent need right now in the military, civilian (hospital, food processing, environmental), and first responder communities for a "…rapid point-of-care (multiplex?) diagnostic for disease-causing pathogens."

NIH claims to be looking for the, "…next-generation of novel or emerging rapid and innovative clinical diagnostic technologies that do not involve nucleic acid amplification."

E. coli



V. cholerae



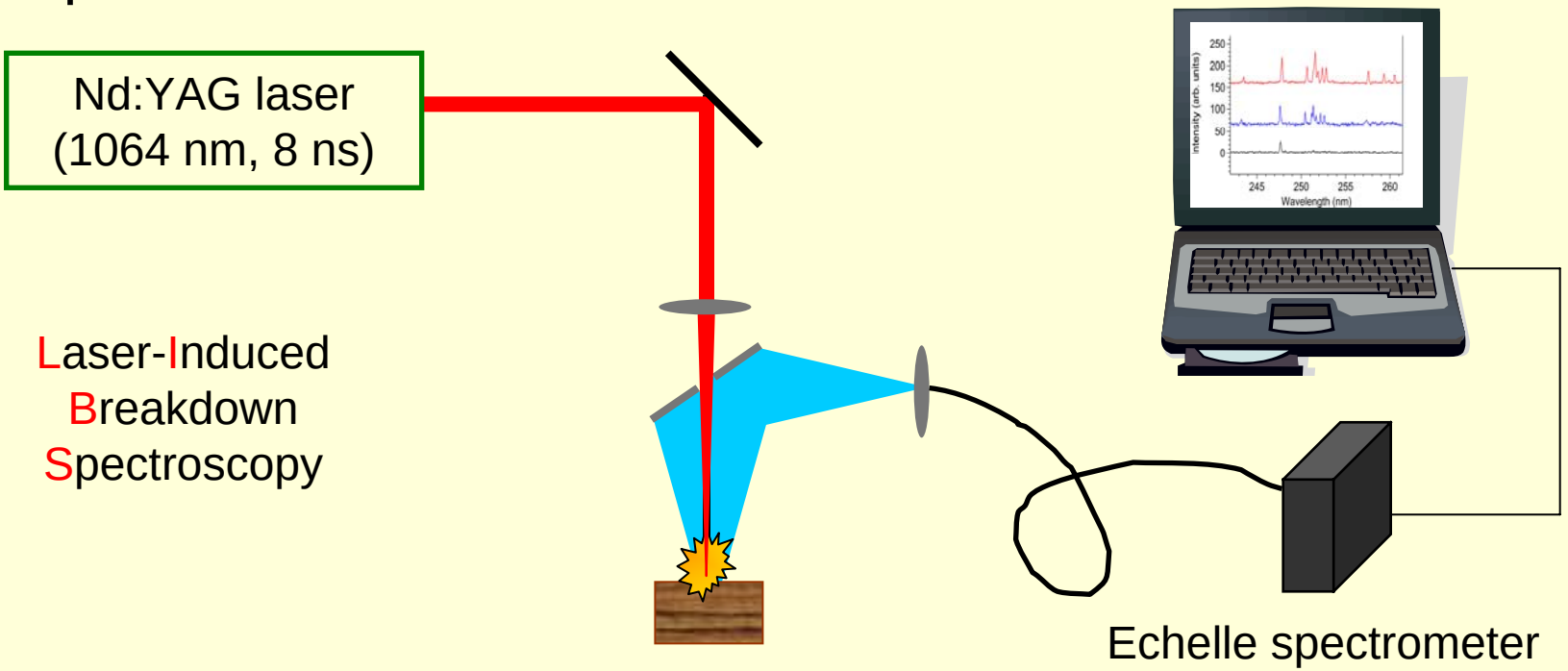
How do we identify bacteria?

4 ways

- genetic
- serological (antigenic)
- microbiological
- compositional
  - LIBS
  - Raman
  - MALDI-TOF-MS

EMMA: Elemental Multivariate Microbiological Analysis

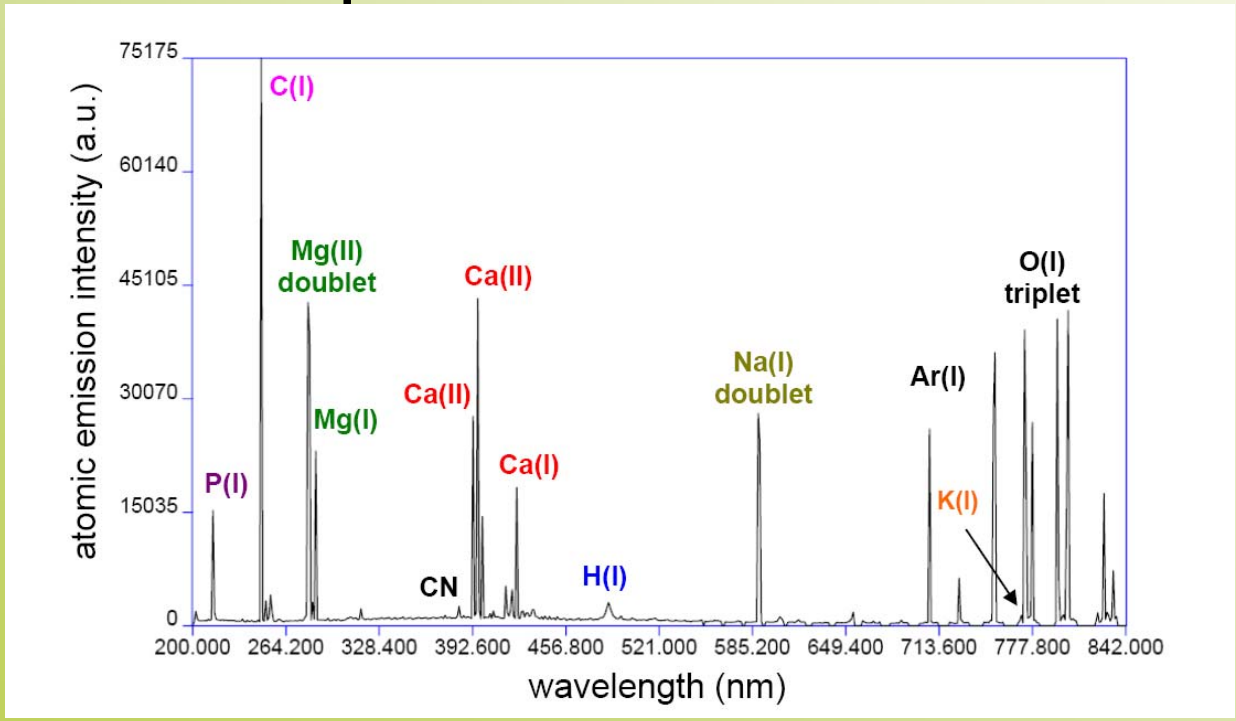
- utilizes laser-induced breakdown spectroscopy (LIBS) to measure the unique atomic or elemental composition of bacteria



LIBS Spectrum is like a Spectral Fingerprint: Unique for Each Sample

Bacterial Composition

LIBS Spectrum



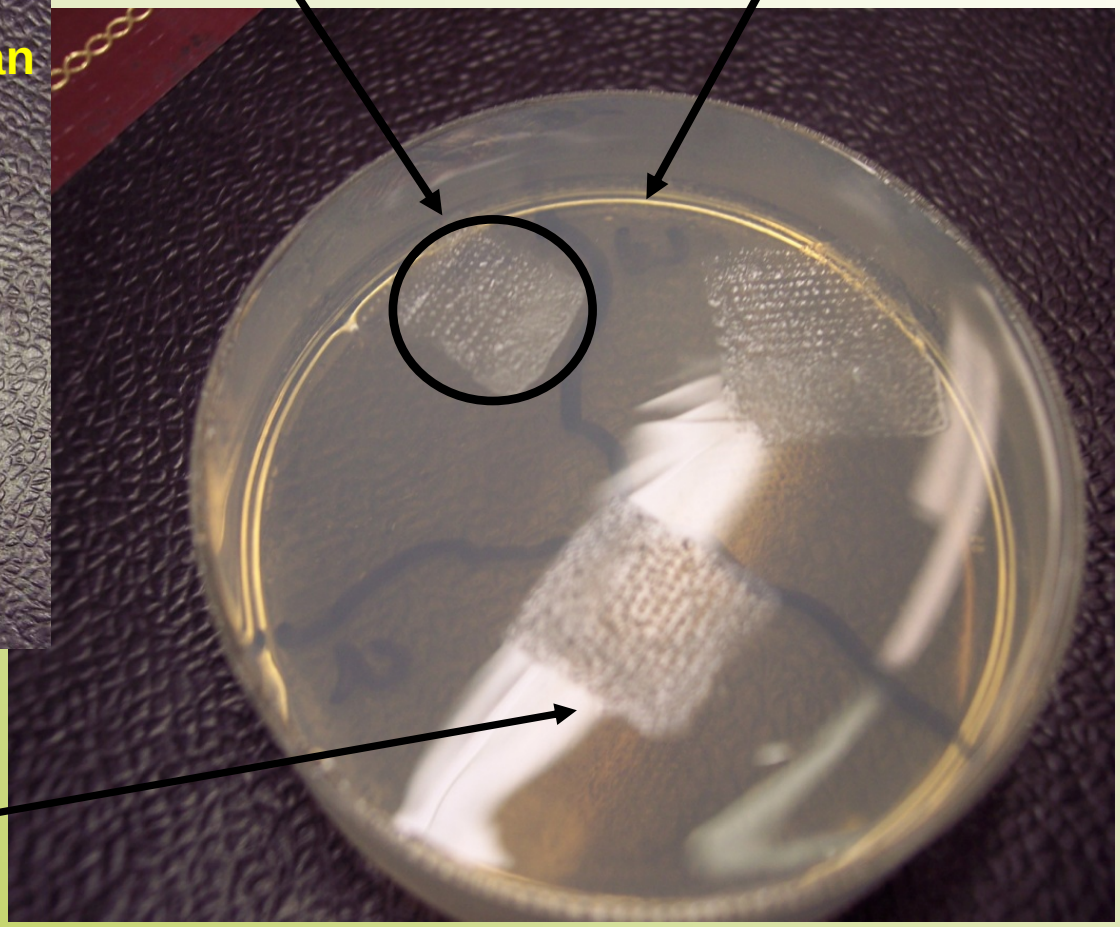
from "*The Bacteria: A Treatise on Structure and Function*" I.C. Gunsalus and R.Y. Stanier, eds

Element	% of fixed salt fraction
Sodium	2.6
Potassium	12.9
Calcium	9.1
Magnesium	5.9
Phosphorus	45.8
Sulfur	1.8
Iron	3.4

How we did it…

10 microliter of bacteria pellet

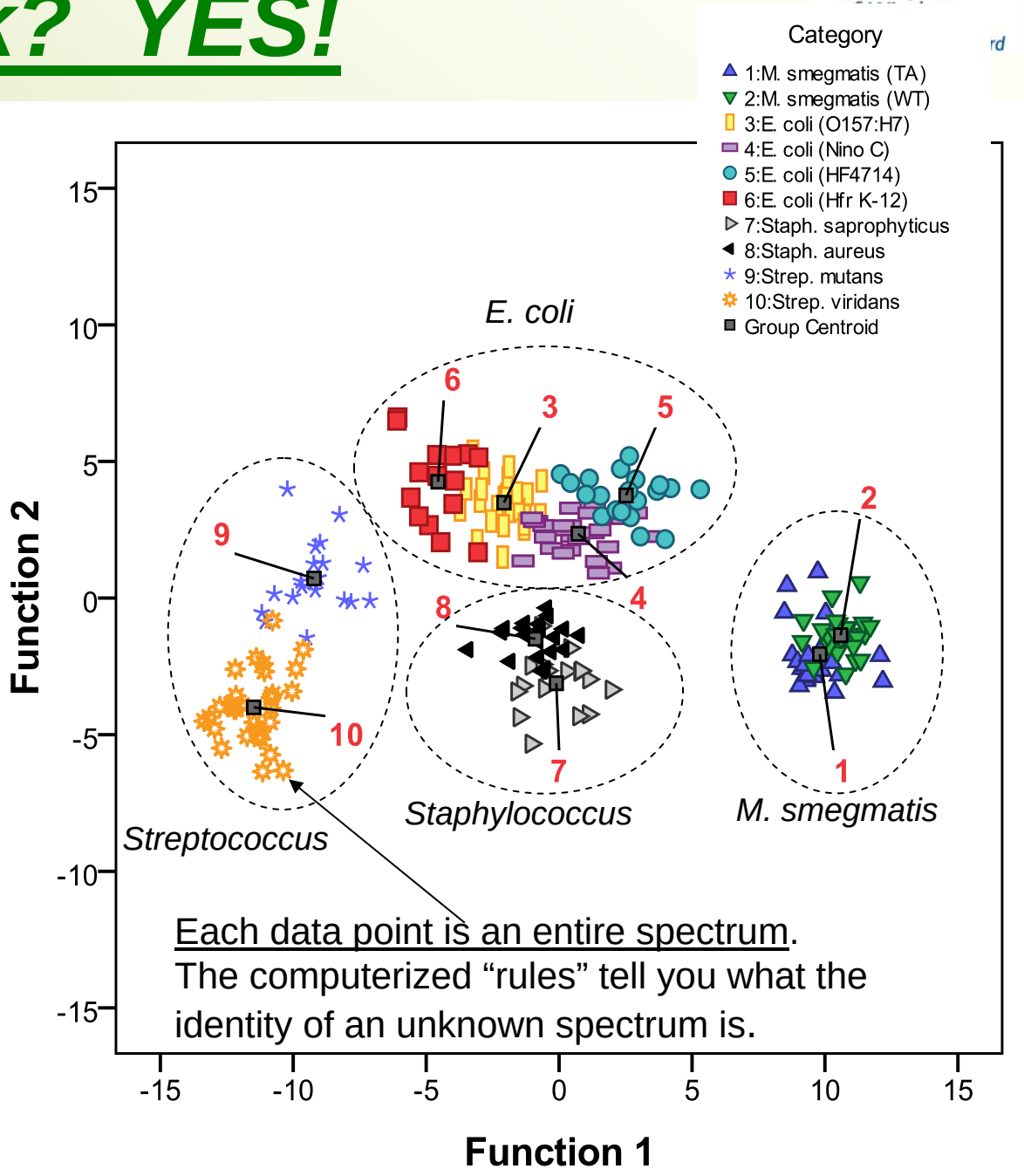
bacto-agar (99% water)



about 500-1500 bacteria per sampling location

Does it work? YES!

- "Area under the curve" of 13 emission lines from 6 inorganic elements input as independent variables into a DFA.
- This test shows only the first two discriminant function scores for 10 different bacterial types (multiple genera, species, strains)



Group	Predicted Group Membership (%)									
	1	2	3	4	5	6	7	8	9	10
1:*M. smegmatis* (TA)	82.4	17.6	0	0	0	0	0	0	0	0
2:*M. smegmatis* (WT)	28.0	72.0	0	0	0	0	0	0	0	0
3:*E. coli* (O157:H7)	0	0	96.0	4.0	0	0	0	0	0	0
4:*E. coli* (C)	0	0	3.6	96.4	0	0	0	0	0	0
5:*E. coli* (HF4714)	0	0	0	0	100.0	0	0	0	0	0
6:*E. coli* (HfrK-12)	0	0	6.7	0	0	93.3	0	0	0	0
7:*Staph. saprophyticus*	0	0	0	0	0	0	94.1	5.9	0	0
8:*Staph. aureus*	0	0	0	0	0	0	0	100.0	0	0
9:*Strep. mutans*	0	0	0	0	0	0	0	0	95.0	5.0
10:*Strep. viridans*	0	0	0	0	0	0	0	0	0	100.0

The Windsor/Wayne State team has already demonstrated…

EMMA spectral fingerprint is:

- growth-medium independent
- independent of state of growth (how “old” the bacteria are)
- independent of whether the bacteria are live or dead or inactivated by UV light
- obtainable even when other types of bacteria or contaminants are present (mixed samples)
- capable of strain discrimination
- obtainable from about 500 bacteria
- unaffected by the presence of biochemicals in urine

7 publications in Applied Physics Letters, Journal of Applied Physics, Applied Optics, Applied Spectroscopy, and Spectrochimica Acta B

Due to certain well-recognized advantages, laser-induced breakdown spectroscopy (LIBS) is an attractive diagnostic candidate technology

- speed / portability / durability (ruggedness)
- lack of complicated sample preparation
- no expertise required
- no genetic or antigenic precursors (consumables) necessary
- same technology / hardware useful for explosives, chemical, other threats (CBRNE capable)
- capability of sensor fusion

MP-LIBS A full laboratory High-Resolution Broadband LIBS system in a portable backpack

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Backpack contains broadband high-resolution spectrometer, laser power supply, computer, and battery

Head's-up display

Hand-held probe contains laser, joystick for control, and focus optics

Microplasma/ LIBS Event

courtesy of Ocean Optics.

Where I Think We Should Go

(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*.

Long-Term Objectives

(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.

Bruker Daltonics

BRUKER

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MALDI Biotyper: The next generation microbial identification system for the 21st century

The MALDI Biotyper enables an unbiased identification of microorganisms. It can be applied to gram-positive and gram-negative bacteria, yeast and multicellular fungi without any presumptions or pretesting. Starting from culture plates identification results can be generated in a couple of minutes. The MALDI Biotyper covers applications from clinical microbiology, food and feed safety and analysis, as well as industrial quality control.

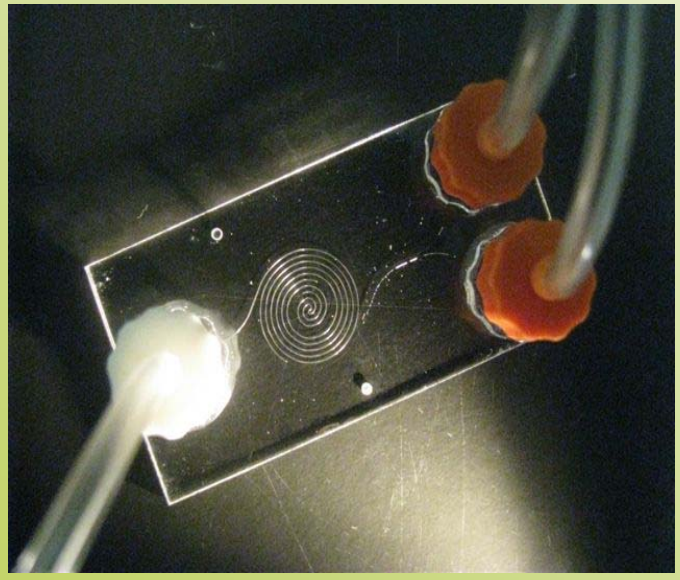
The MALDI Biotyper for identification of microorganisms is a system that meets all the demands defined for a revolutionary new approach - based on advanced, yet well acknowledged technology: mass spectrometry.

Bruker offers the next generation for identifying microorganisms in your lab:

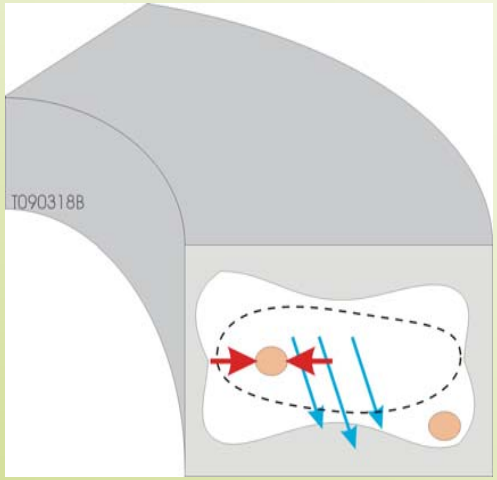
- Easy sample preparation
- Fast
- Robust
- Reliable mass spectrometric instrumentation
- Easy to use software (non MS-expert approved)

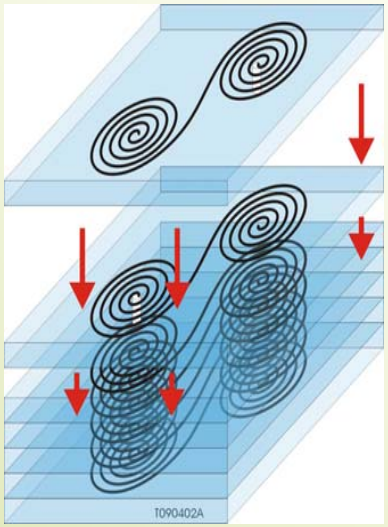
Microfluidic separation/concentration

(Translume, Inc. Ann Arbor, MI)



monolithically fabricated devices in glass

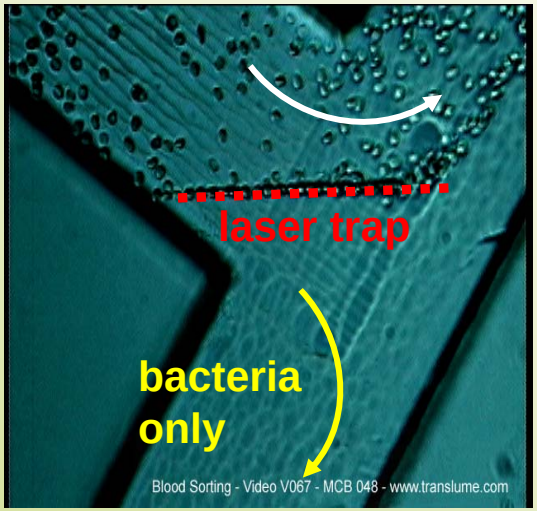




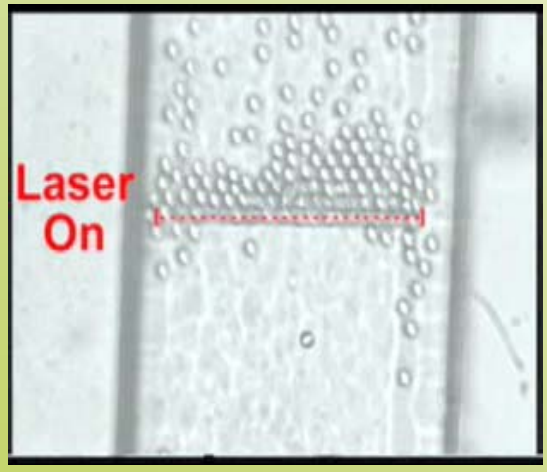
hydrodynamic (microfluidic) separation of heavier cells from lighter cells

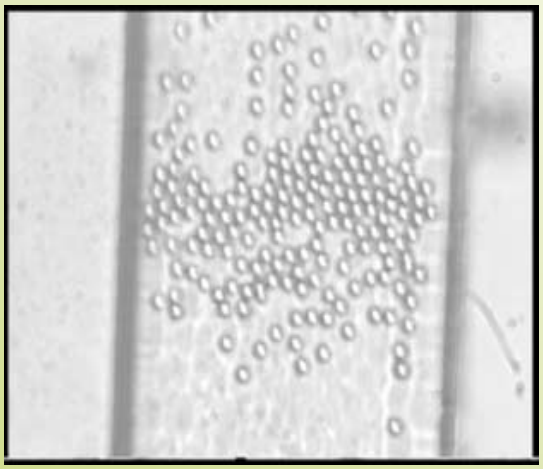
Microfluidic separation/concentration

(Translume, Inc. Ann Arbor, MI)



optical trap-based separation of heavier cells from lighter cells





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Thank you for the invitation!

Confirmation by Caceres Group

Talanta 84 (2011) 730–737

Identification and discrimination of bacterial strains by laser induced breakdown spectroscopy and neural networks

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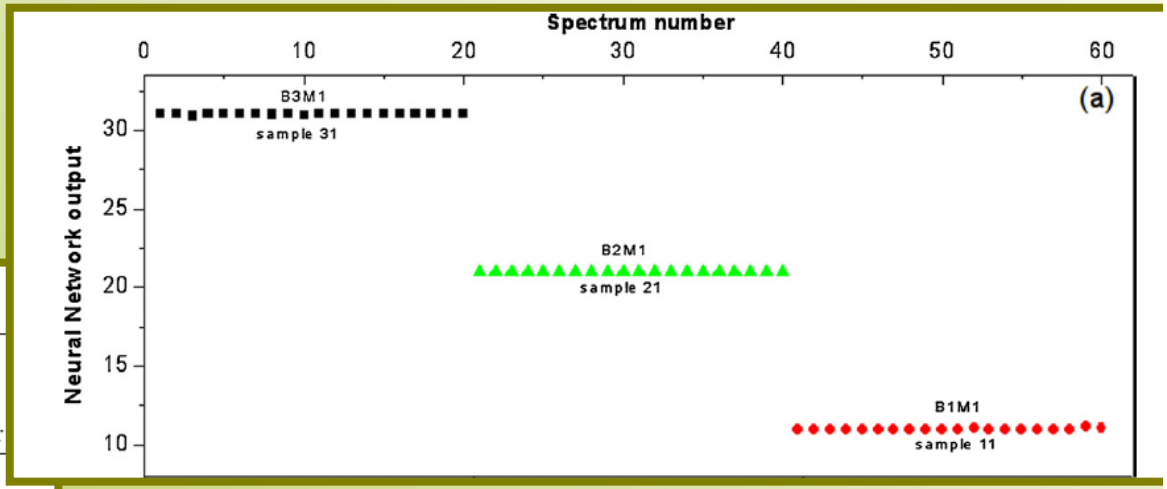

Table 1
Nomenclature used for the samples.

Bacterial strains	Culture media 1 LB agar	Culture media 2 MacConkey agar	Culture media 3 Brucella anaerobic agar
Pseudomonas aeruginosa (B1)	B1M1 (11)	B1M2 (12)	B1M3 (13)
Escherichia coli (B2)	B2M1 (21)	B2M2 (22)	B2M3 (23)
Salmonella typhymurium (B3)	B3M1 (31)	B3M2 (32)	B3M3 (33)

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

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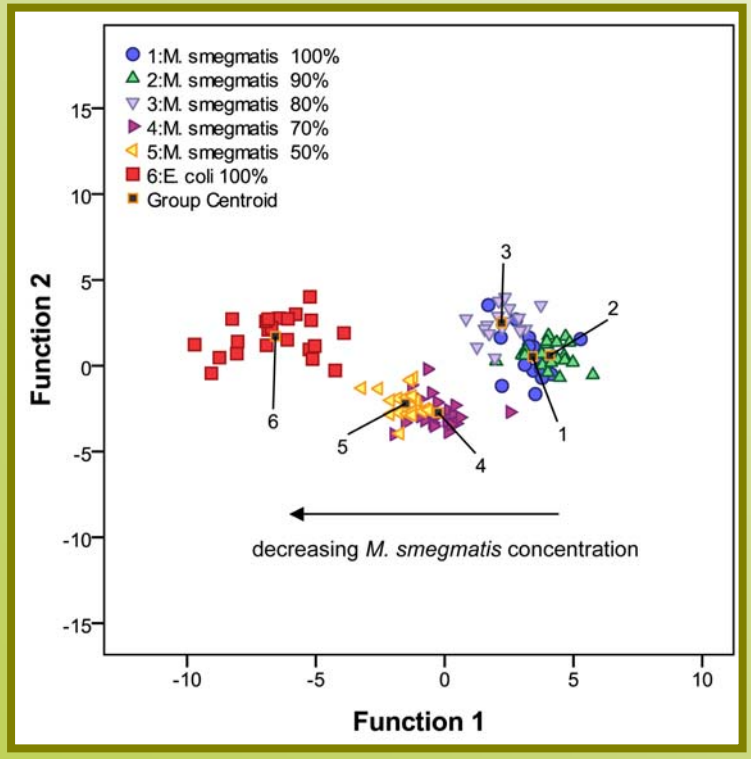
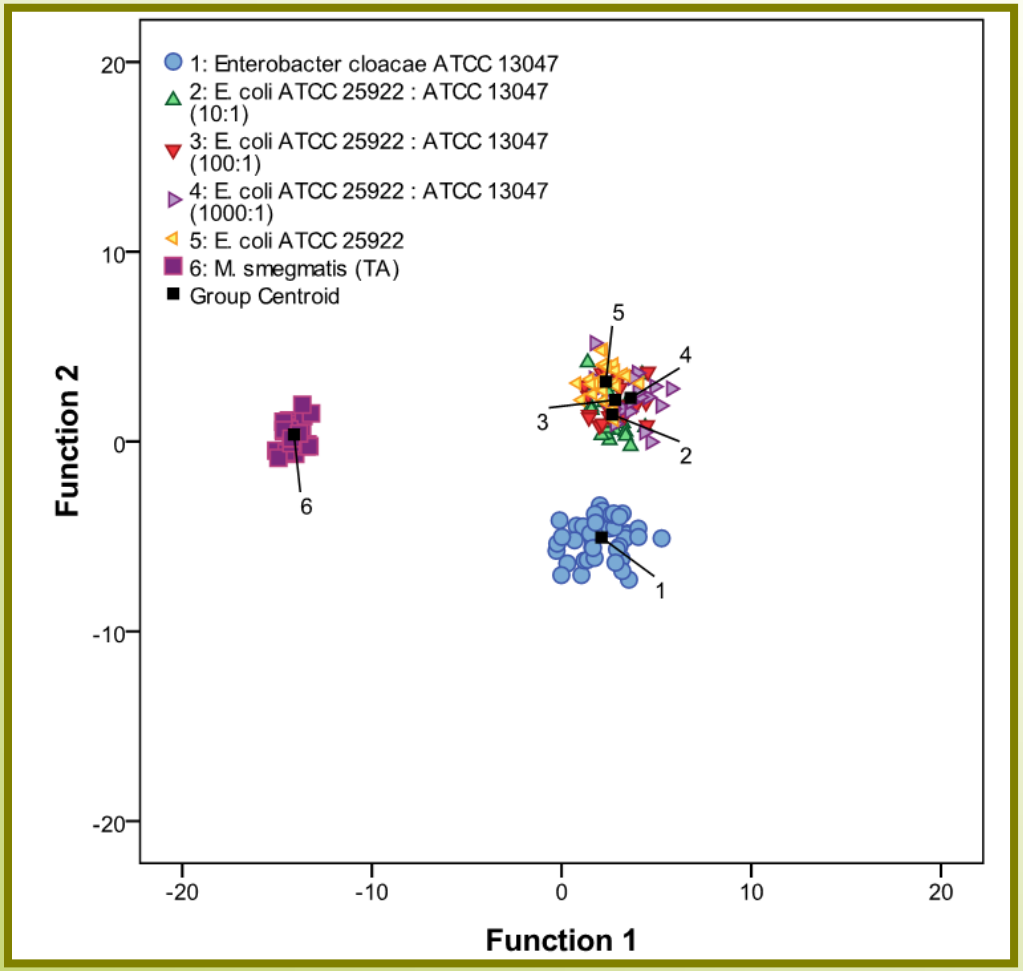
2010

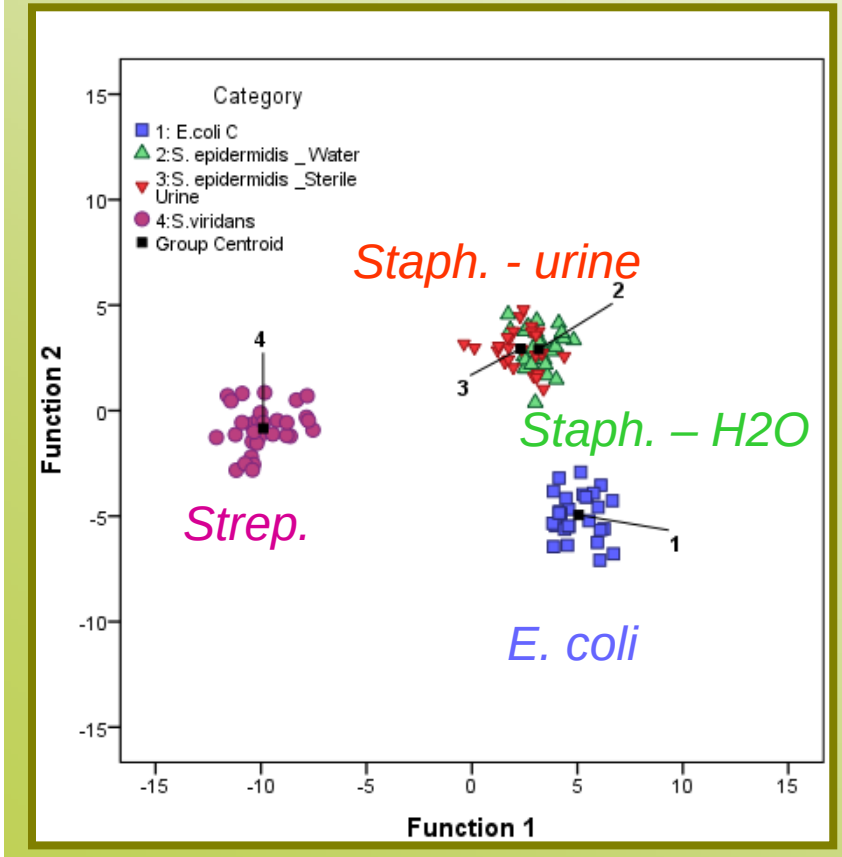


2011

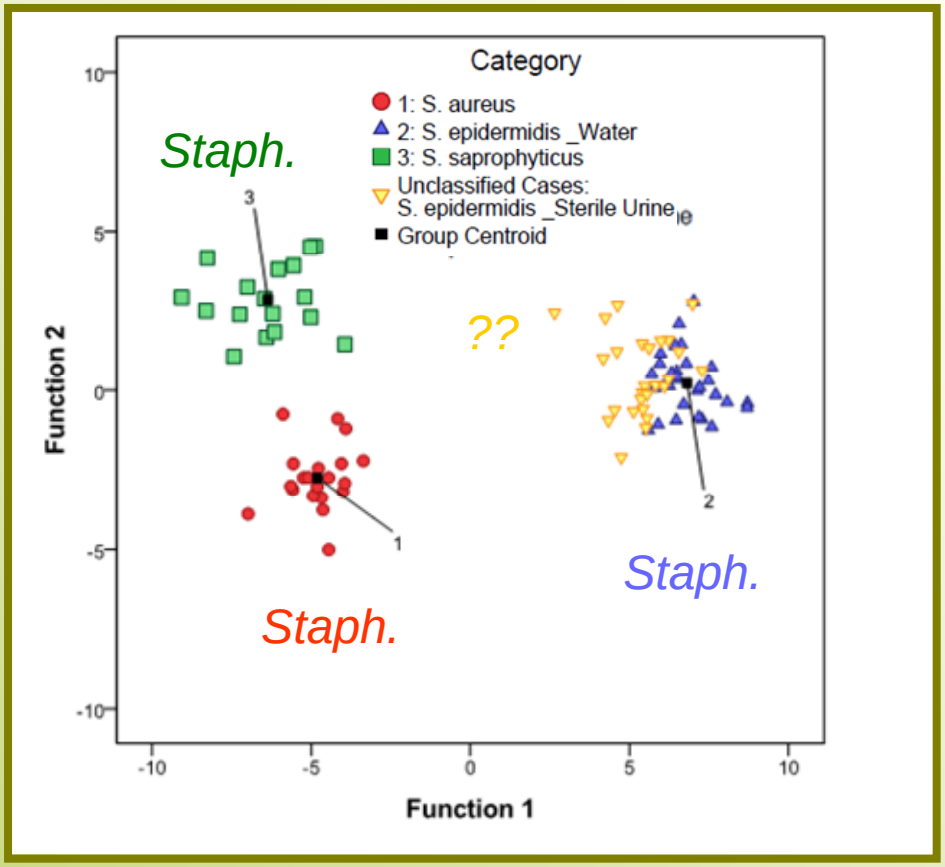


Simulated Clinical Specimens:
sterile urine

2011



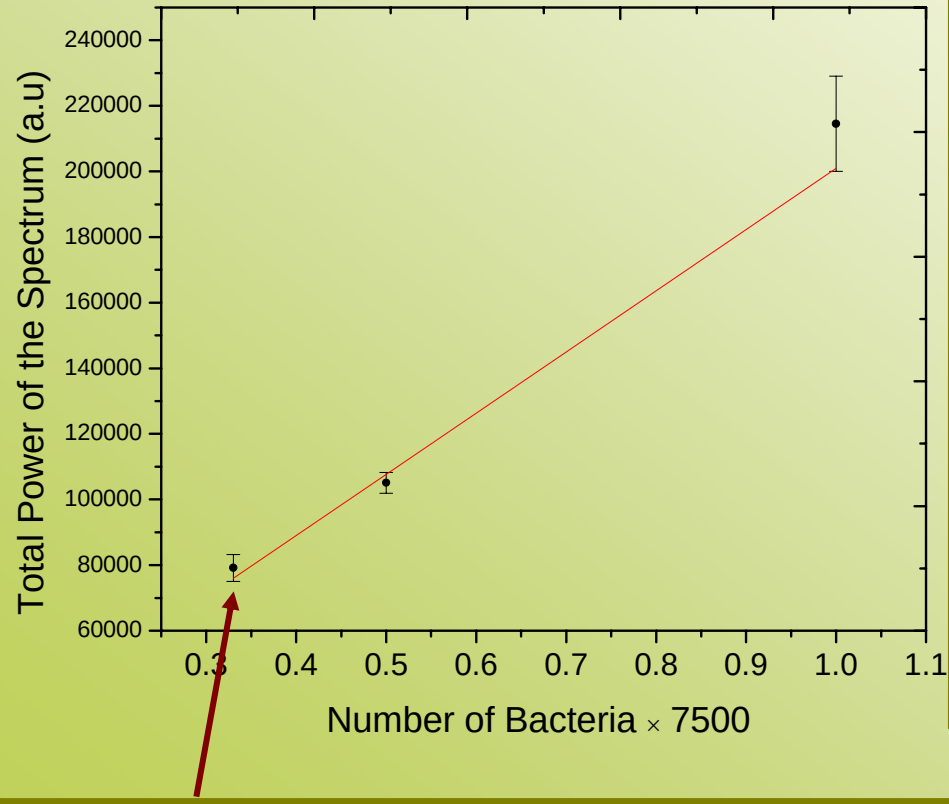
2011

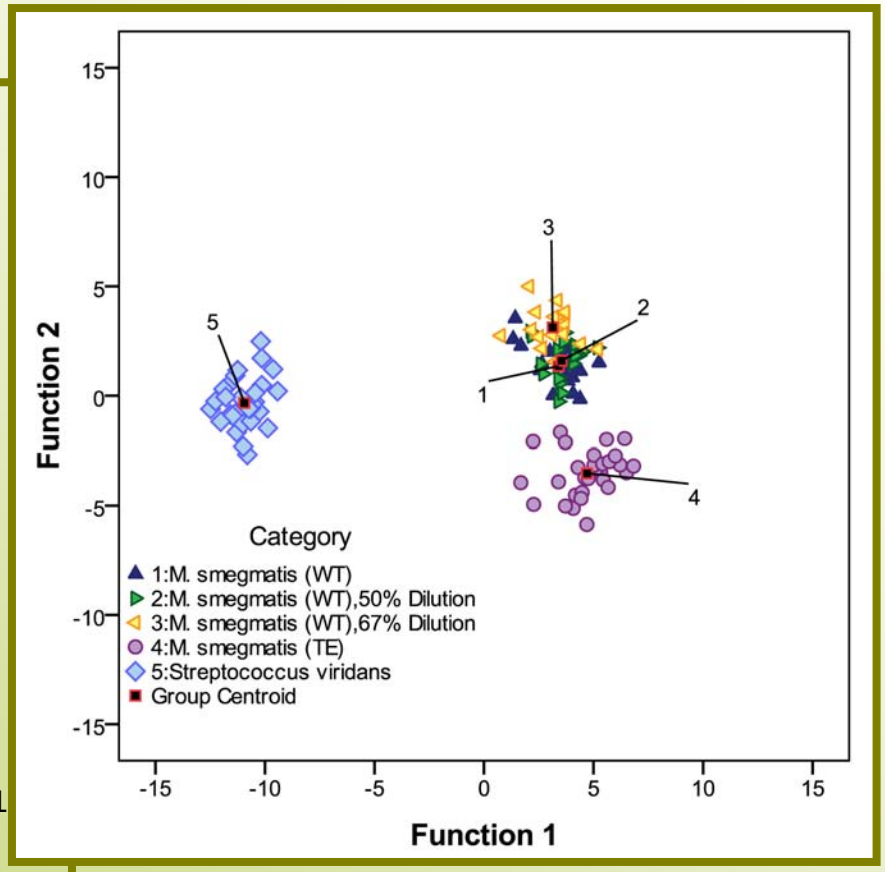


Dilution

specimens of various titer

2010

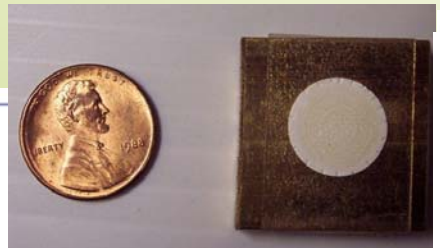




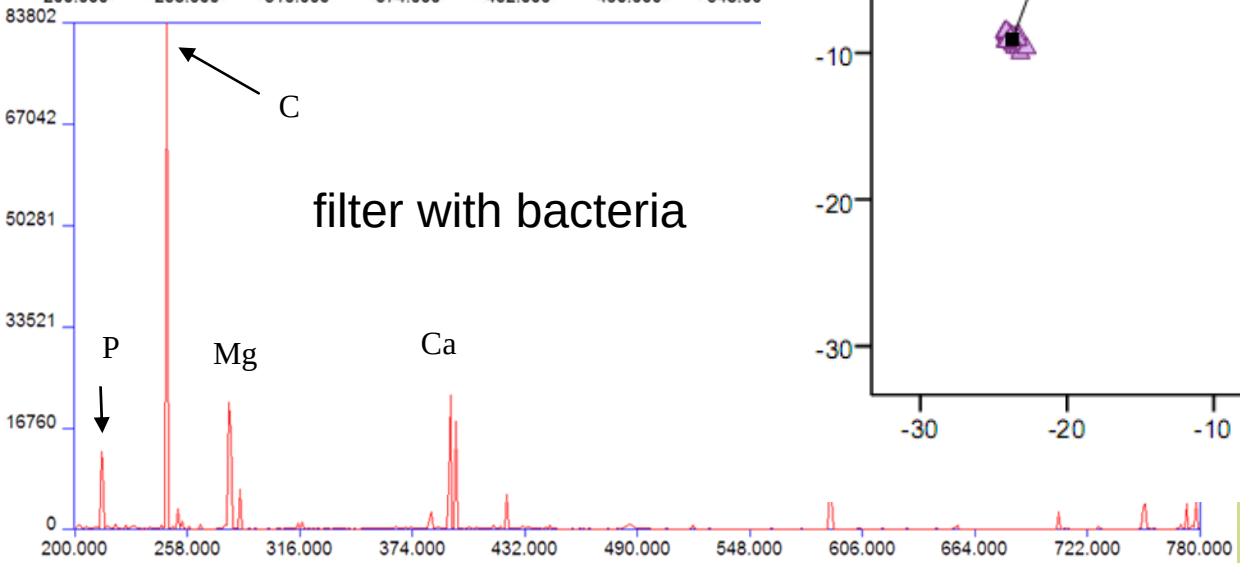
5 laser sampling locations

~500 bacteria per locations

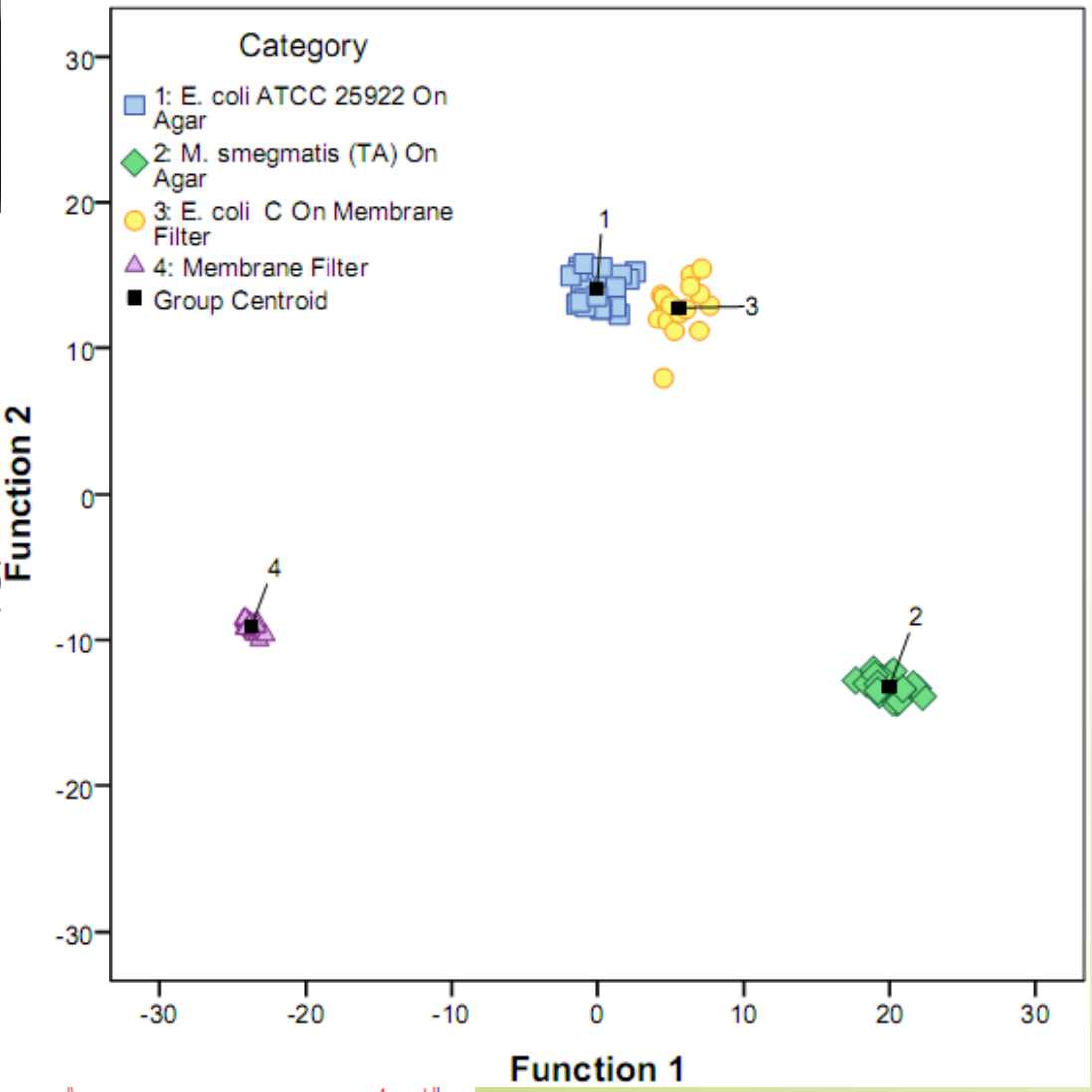
Cellulose Filter



filter

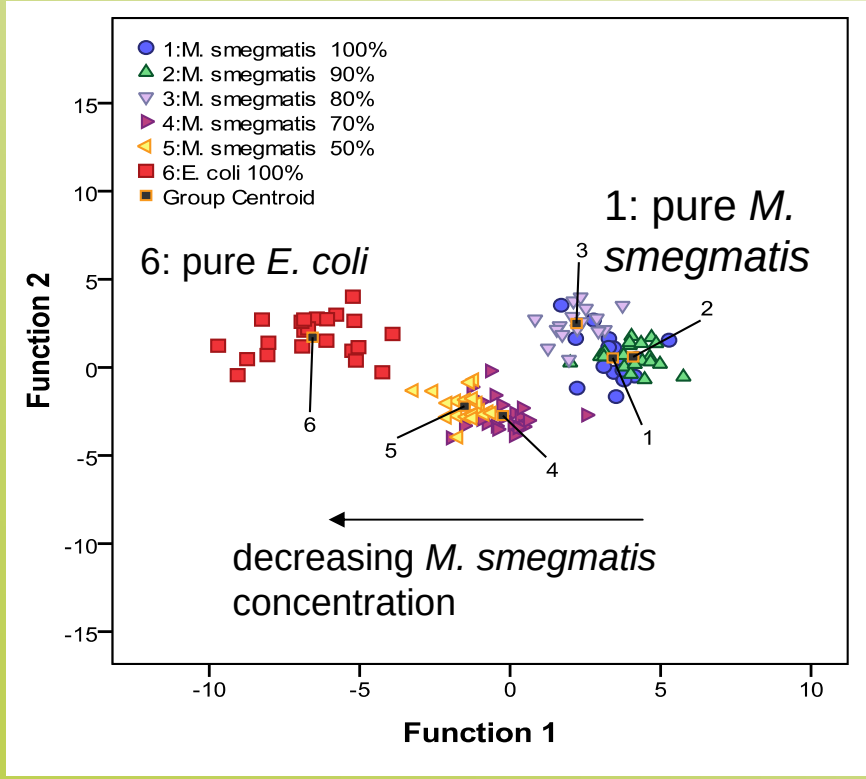


filter with bacteria



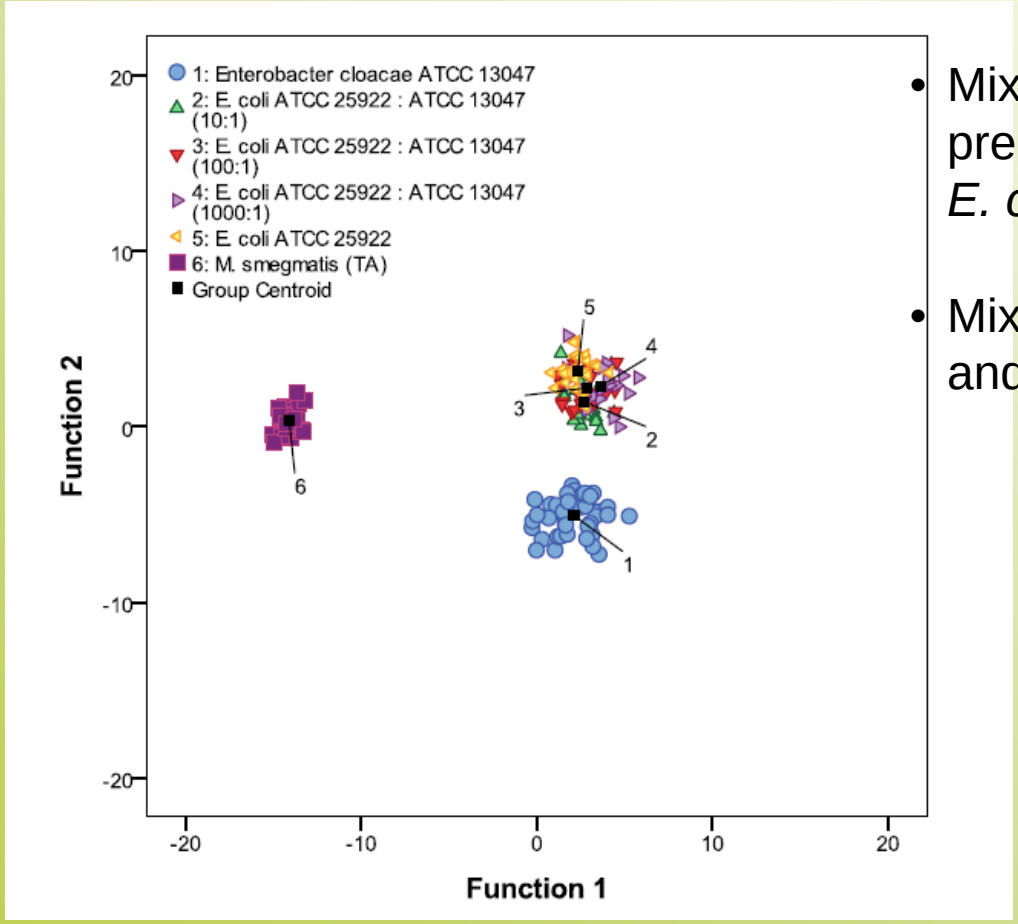
"Mixed" Samples

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%



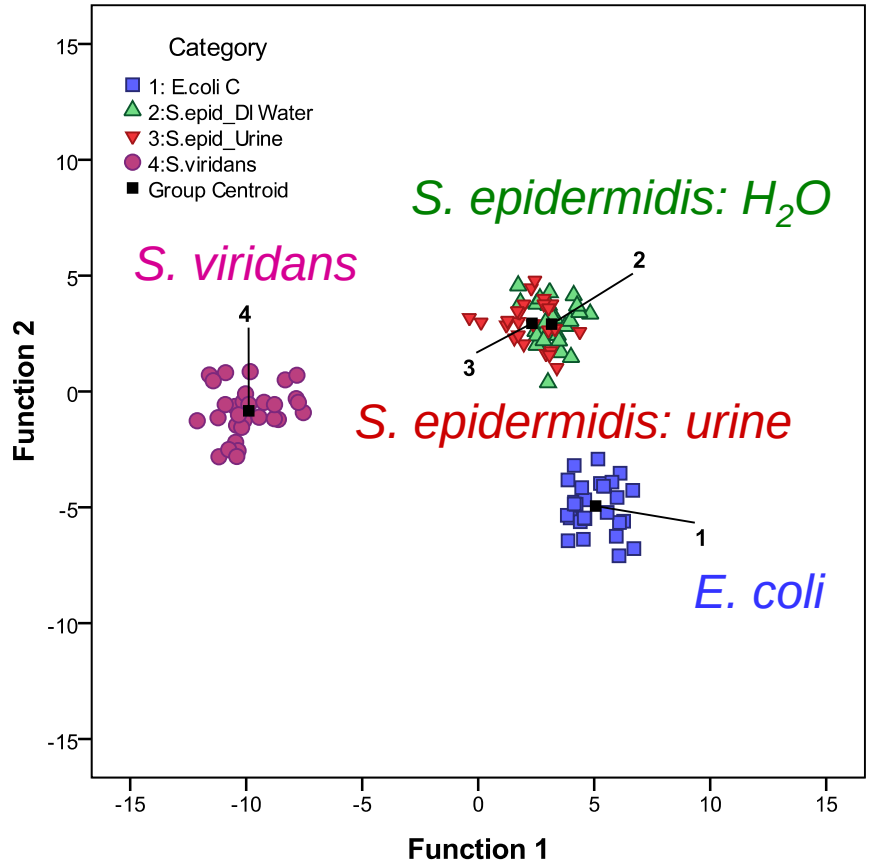
- Six separate mixtures of known mixing fraction were prepared from suspensions *M. smegmatis* and *E. coli* C.
- As long as the majority bacterium comprised 80% of the mixture, we saw 100% identification.

"Mixed" Samples



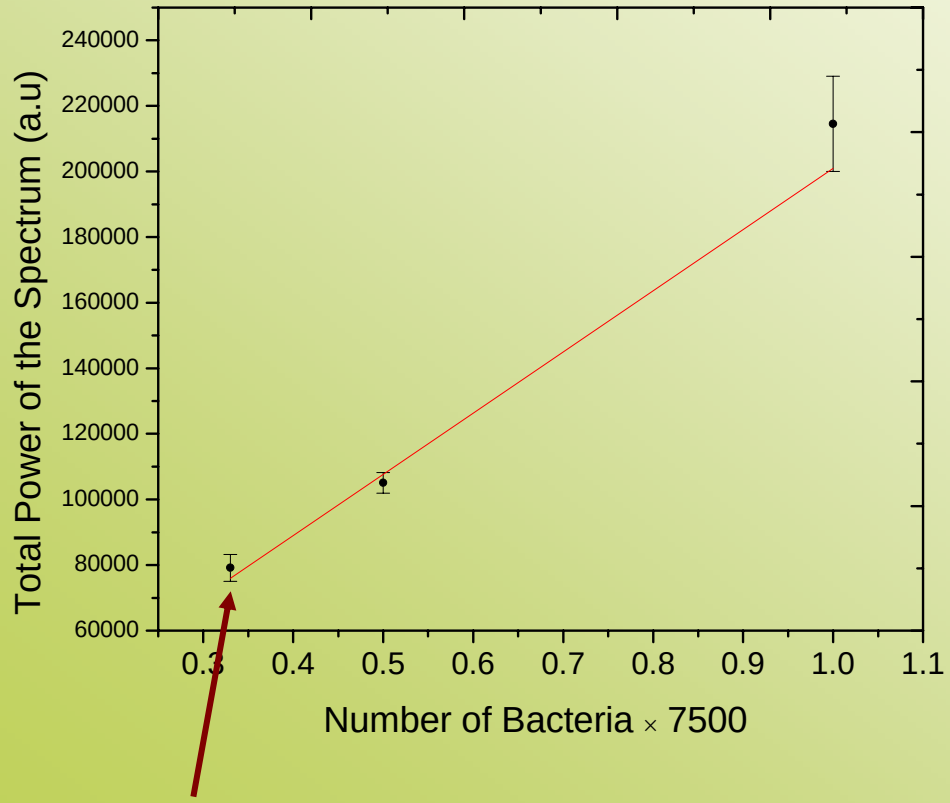
- Mixtures of known mixing fraction were prepared from suspensions *E. coli* C and *E. cloacae*.
- Mixing represent "clinical" contaminations and/or mixtures (i.e. 10:1, 100:1, 1000:1).

“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

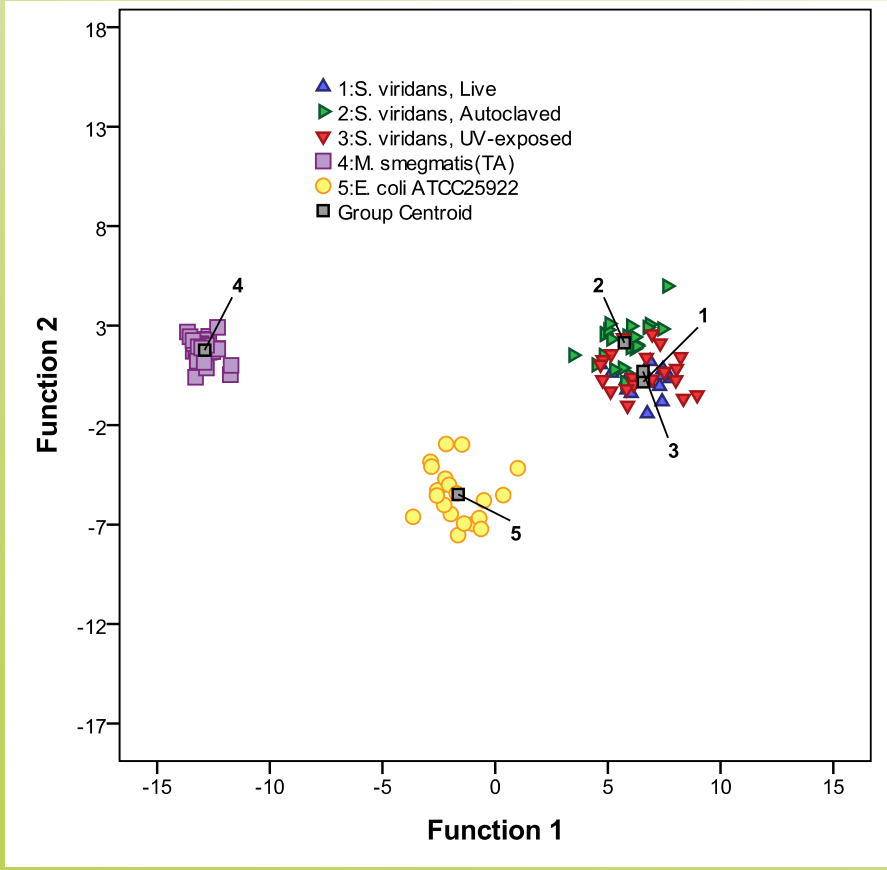


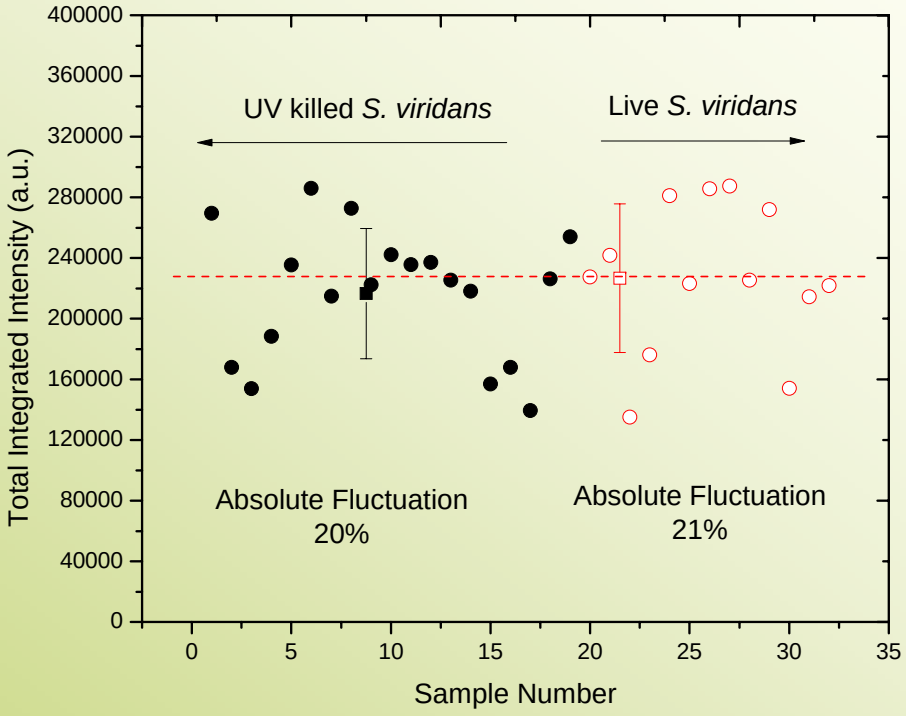
5 laser sampling locations

~500 bacteria per locations

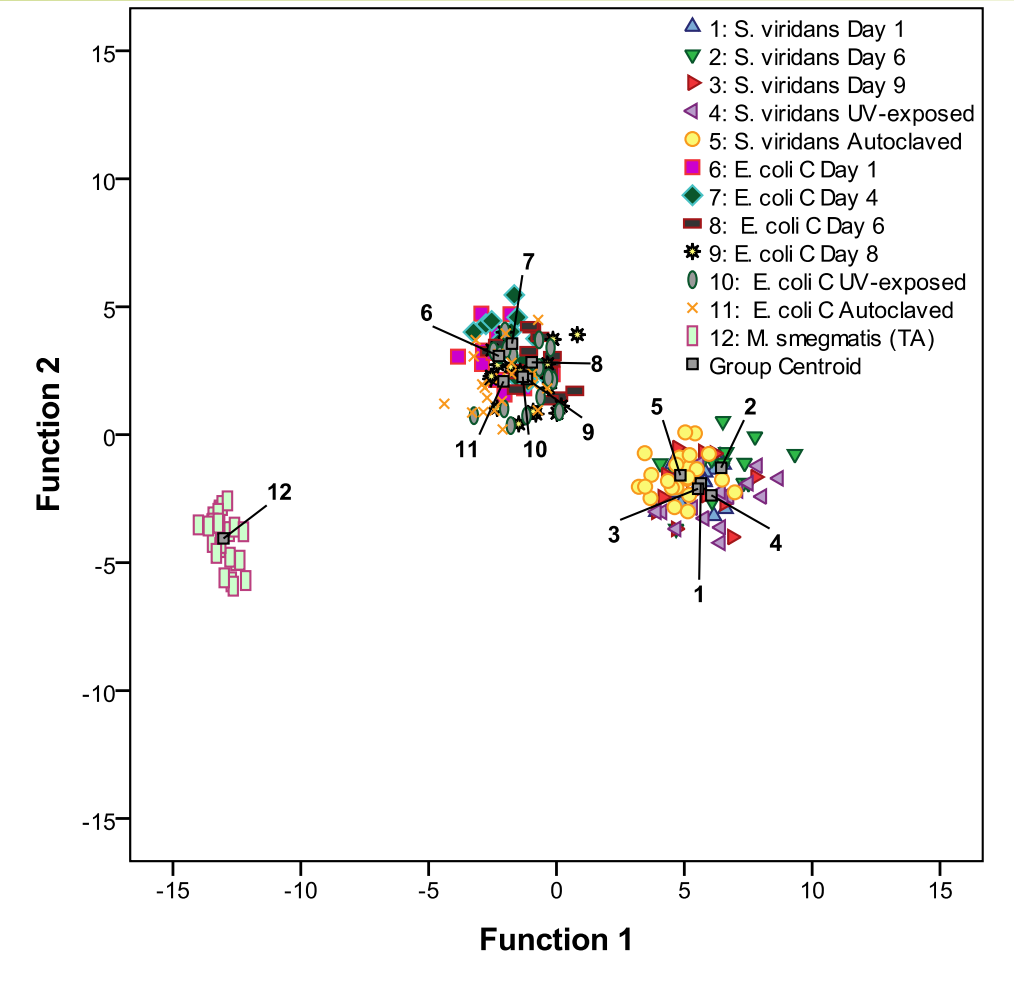
- Samples of *E. coli* with different titer tested on agar.
- Each data point is the average of 5 sampling locations.
- As expected, spectra demonstrate a linear dependence with cell number.
- All spectra were 100% correctly identified (specificity not dependent on number of cells).
- Suggests an antibiotic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

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





LIBS specificity and sensitivity not 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