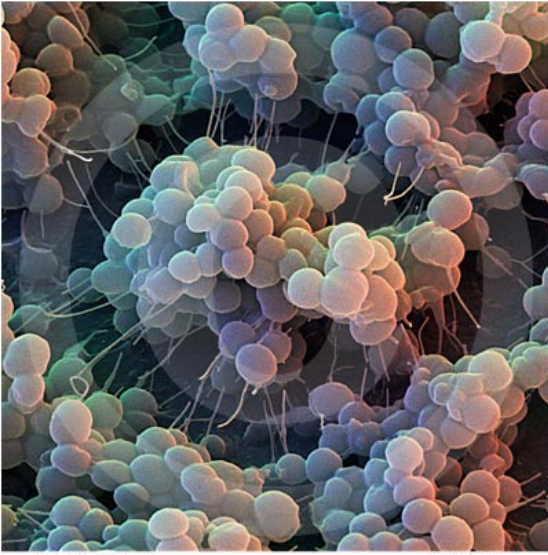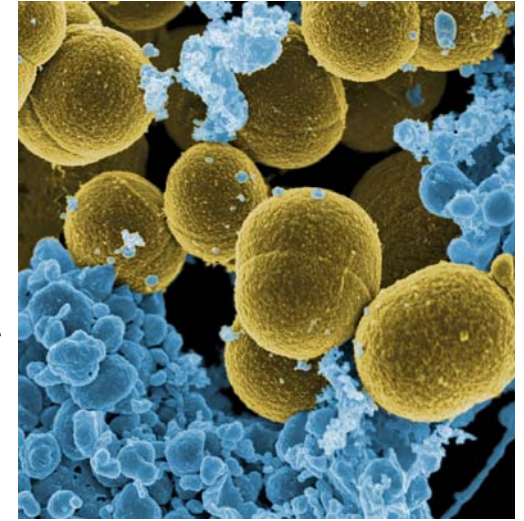# Laser-Induced Breakdown Spectroscopy (LIBS):

## An Optical Diagnostic Tool
## for the Rapid Identification and Classification of Pathogenic Bacteria

CAP Congress 2011

**Steven J. Rehse**
*University of Windsor, Department of Physics*

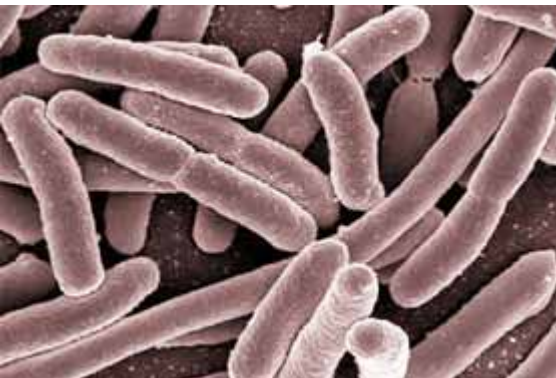University of Windsor
thinking forward

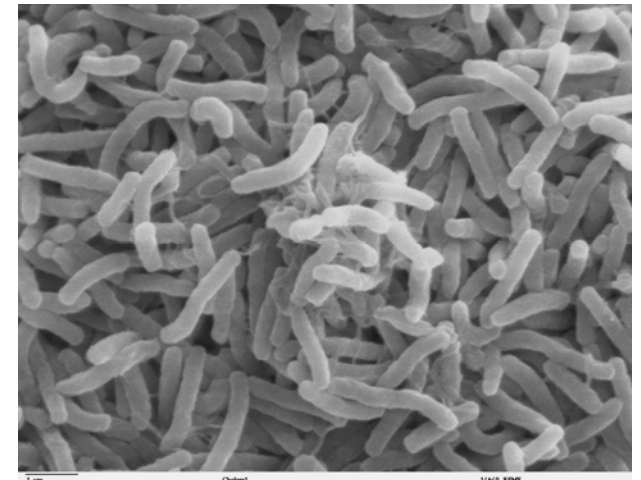*Staph. epidermidis*


*Staph. aureus*

# bacteria are ubiquitous

## 10x more prokaryotic cells in your body than eukaryotic cells

*V. cholerae*

*E. coli*

updated 9:31 a.m. EST, Mon March 2, 2009

# Antibiotic-resistant infections among children on the rise

CNNhealth.com

updated 12:52 p.m. EDT, Sun August 24, 2008

## Canada links Toronto plant to deadly listeriosis outbreak

USA TODAY ■ Home ■ News ■ Travel ■ Money ■ Sports ■ Life ■

Nation  Inside News ▼

# E. coli kills Idaho toddler; spinach pla probed

Updated 10/5/2006 8:57 PM ET

December 8, 2003

## Staph Infection Kills Football Player
By Norm Jones, Newswatch 16, Scranton, PA

E-mail | Save | F

## Denver News

### CU's Nobel Prize Winner Loses Arm To Flesh-Eating Bacteria

*Eric Cornell Remains In Critical Condition*

The New York Times

eanut Product Recall Grows in Salmonella Scare

GARDINER HARRIS
plished: January 28, 2009

USA TODAY ■ Home ■ News ■ Travel ■ Money ■ Sports ■

News » Health & Behavior ■ Medical Resources ■ Health Information

# CDC: 756 ill from salmonella-tainted tomatoes

CBCnews

Home  World  Canada  Politics  Health  Arts & Entertainment  Technology & Science  Money  Cons

REPUBLISH | EMAIL | PRINT | Text Size: S M L XL | REPORT TYPO | SEND YOUR FEEDBACK  SHARE

## New superbugs emerge in U.K., Asia

Canadian cases reported in Vancouver, Alberta

Last Updated: Sunday, August 15, 2010 | 10:18 PM ET  Comments 447
CBC News

Windsor Star Digital

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The Windsor Star | Calendar ▾ 3 Nov 2010 | Table of Contents ▾ A3 WINDSOR & REGION

# MYSTERIOUS POWDER INVESTIGATED

## Source of Germany's deadly E coli outbreak may never be discovered

Reuters

Jun 9, 2011

## Superbug NDM-1 identified in Canada

Global News: Monday, May 30, 2011

NICK BRANCACCIO/The Windsor Star

A hazardous materials team with Windsor Fire Services take readings and samples from the contents of a recycling box at the Transit Windsor garage located beside the Essex-Windsor Solid Waste Authority Central Avenue transfer station on Tuesday. Windsor police, fire and ambulance personnel descended on the garage on North Service Road Tuesday after an employee discovered white powder inside a pencil case that had been left behind on a bus. Police said an employee found the pencil case while cleaning the bus. Just after 4 p.m., two firefighters donned white hazmat suits, rubber boots, oxygen tanks and masks to prepare to handle the material. The workers sifted through the found objects and took samples of the powder to be examined by police. Police have not yet determined the origin or makeup of the powder. It has been taken to a laboratory in Etobicoke for testing.
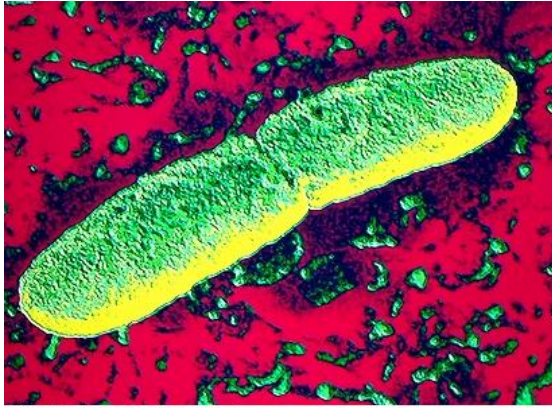
*Yersinia pestis*
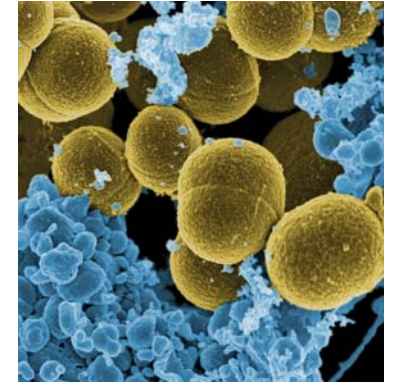
*MRSA*


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."
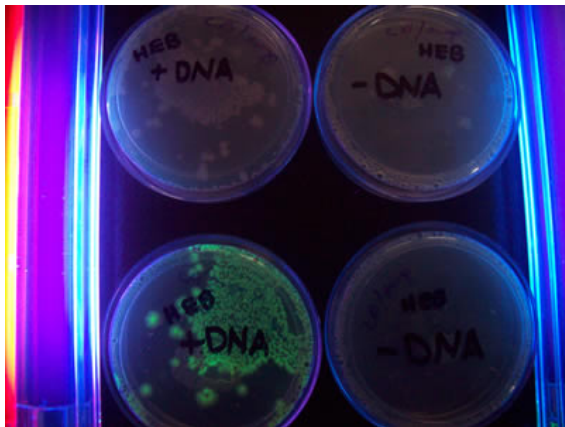
*Acinetobacter baumannii*

*Bacillus anthracis*

# *Why?*

"It is well-accepted that the microbiological *expertise* and *cost* required to perform… identifications preclude their common use as a screening mechanism to prevent human infection."[1]



[1]Tarr, P.I. 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clin. Infect. Dis. **20**, 1-8.

***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
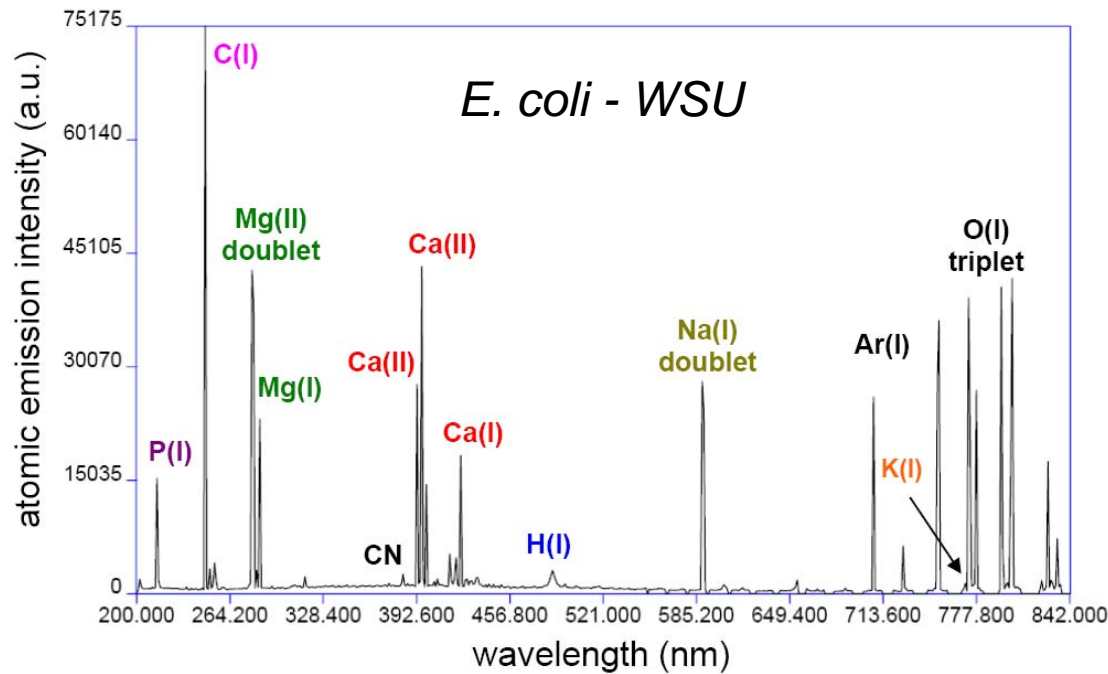- optical technique can be use in "stand-off" mode

# EMMA: Elemental Multivariate Microbiological Analysis

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

Nd:YAG laser
(1064 nm, 8 ns)

**L**aser-**I**nduced
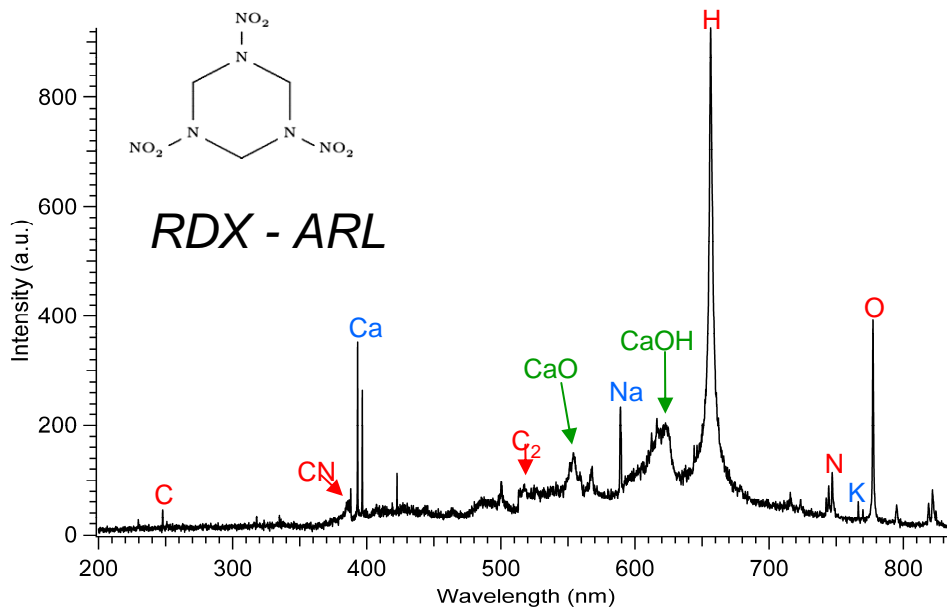**B**reakdown
**S**pectroscopy

spectrometer
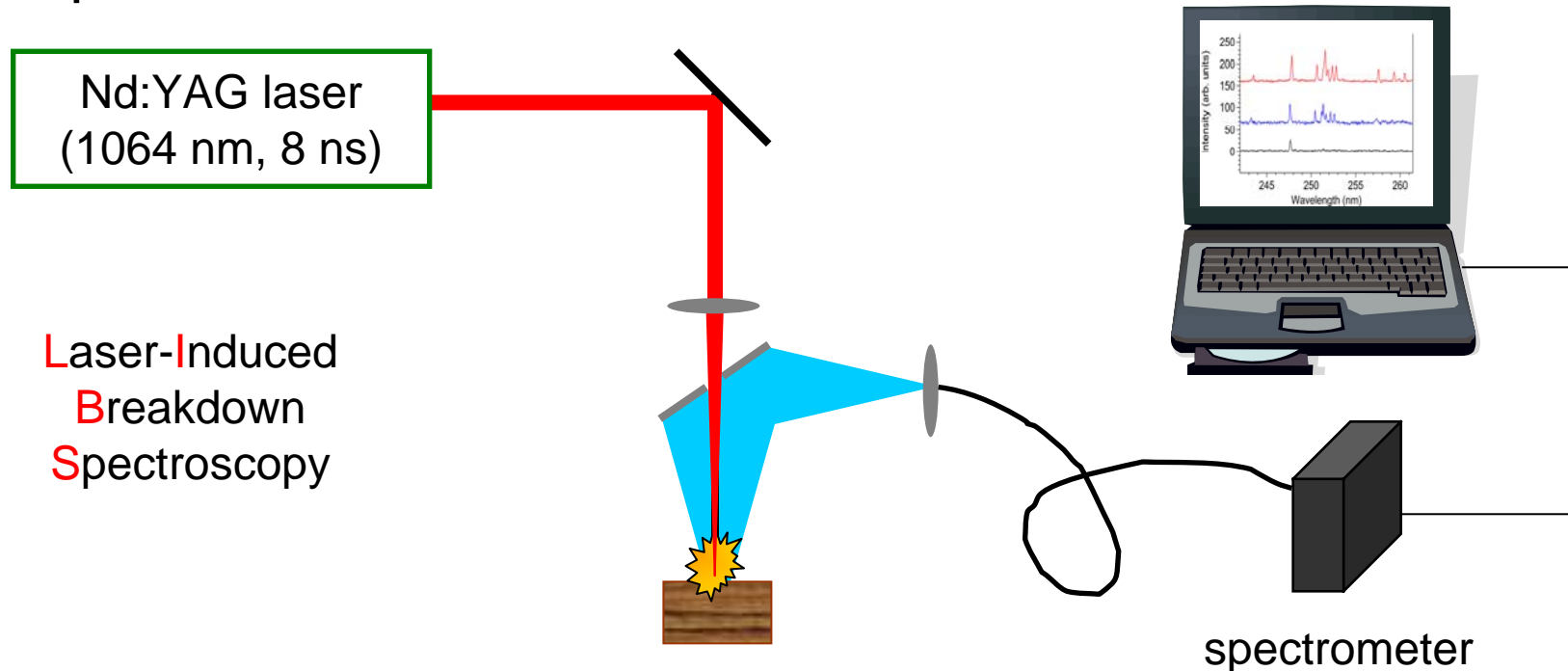
*LIBS Spectrum is like a Bar Code- Unique for Each Sample*

Once a LIBS spectrum is obtained..

1) concentrations of elements (or ratios of concentrations) become independent variables in a *chemometric multivariate analysis*
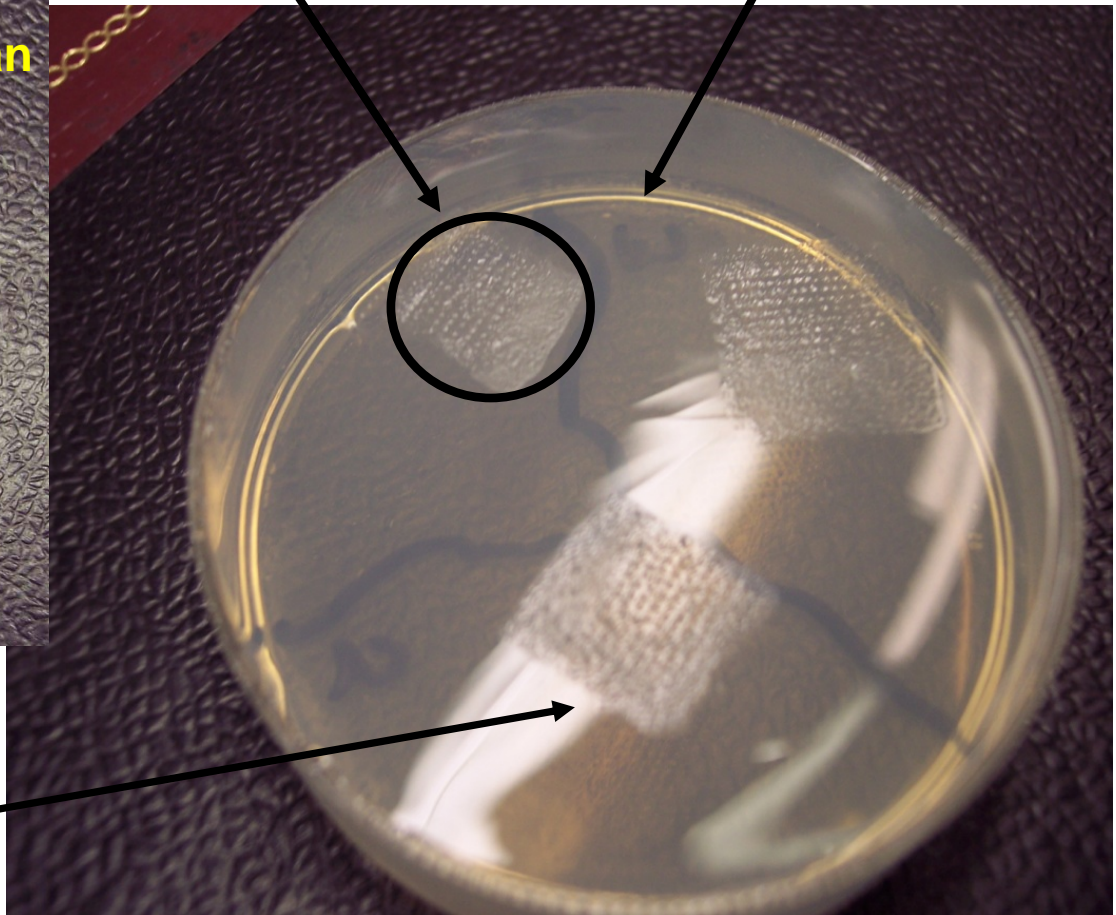
2) the chemometric algorithms classify/ identify the unknown target on the basis of its unique atomic signature

# how we did it…

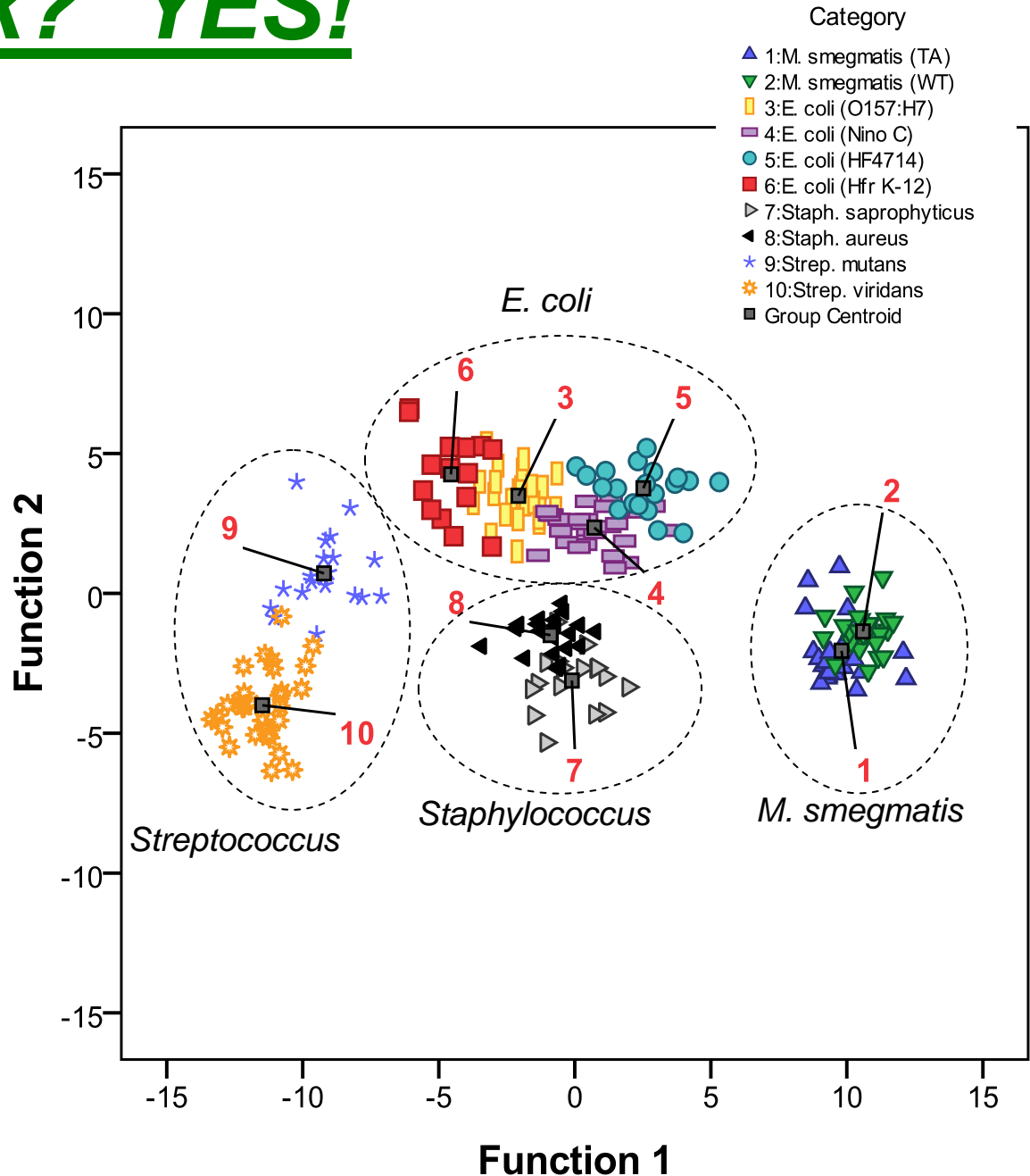10 microliter of bacteria pellet

bacto-agar (99% water)

*E. coli* from liquid specimen. Centrifuged than supernatant removed

about 500-1500 bacteria per sampling location

# *Does it work?  YES!*

- Intensity of lines, ratios of intensities used in a statistical multi-variate analysis

- Discriminant function analysis (DFA)
  - principal component analysis (PCA)
  - partial least squares – discriminant analysis (PLS-DA)
  - linear discriminant analysis (LDA)

| 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 |

# *We have 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
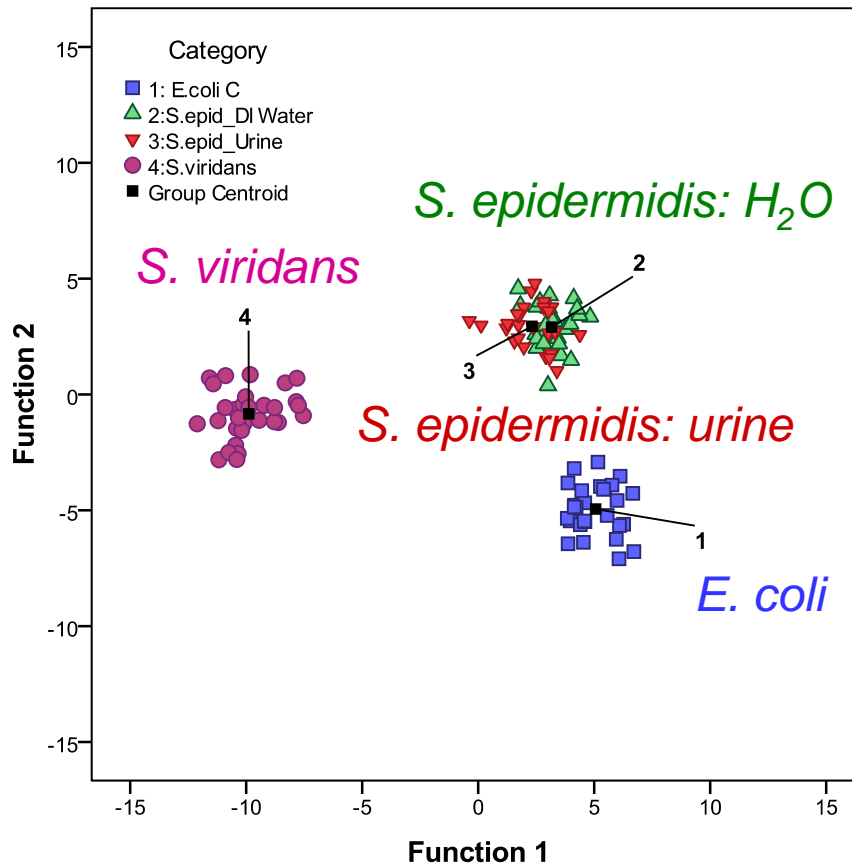- obtainable from about 500 bacteria

# *"Mixed" Samples*
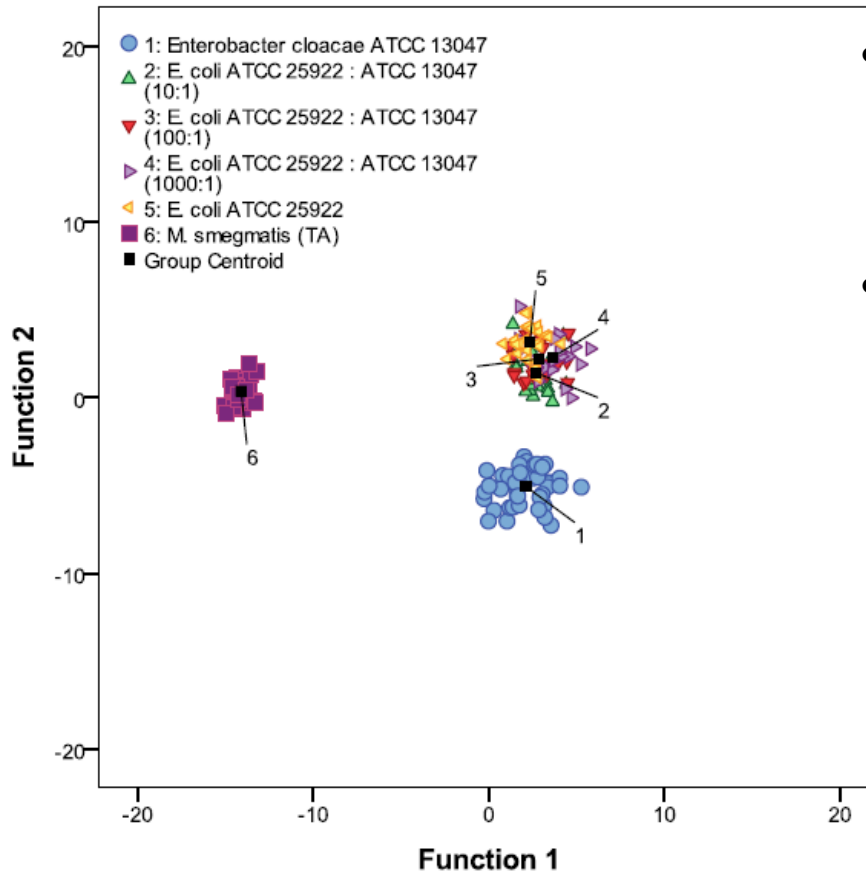


- Mixtures of known mixing fraction were prepared from suspensions *E. coli* C and *E. cloacae*.

- 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*

# *Strain discrimination confirmed by others…*

- 100% accuracy exhibited in blind trials of 4 MRSA strains and one *E. coli* strain
- lyophilized ("freeze-dried") specimens used

# We Must Proceed, and Faster…

LIBS research must proceed along two equally important avenues:

- fundamental research to explore the microbiological diversity that can occur in specimens

- specimen preparation and handling protocols and techniques to isolate pathogens from contaminants of biological origin
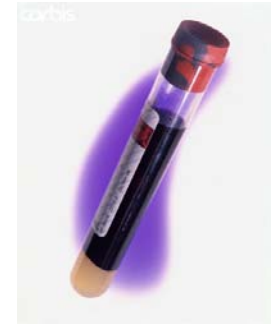
NOTE: we do NOT need to fingerprint hundreds and hundreds of "new" bacteria
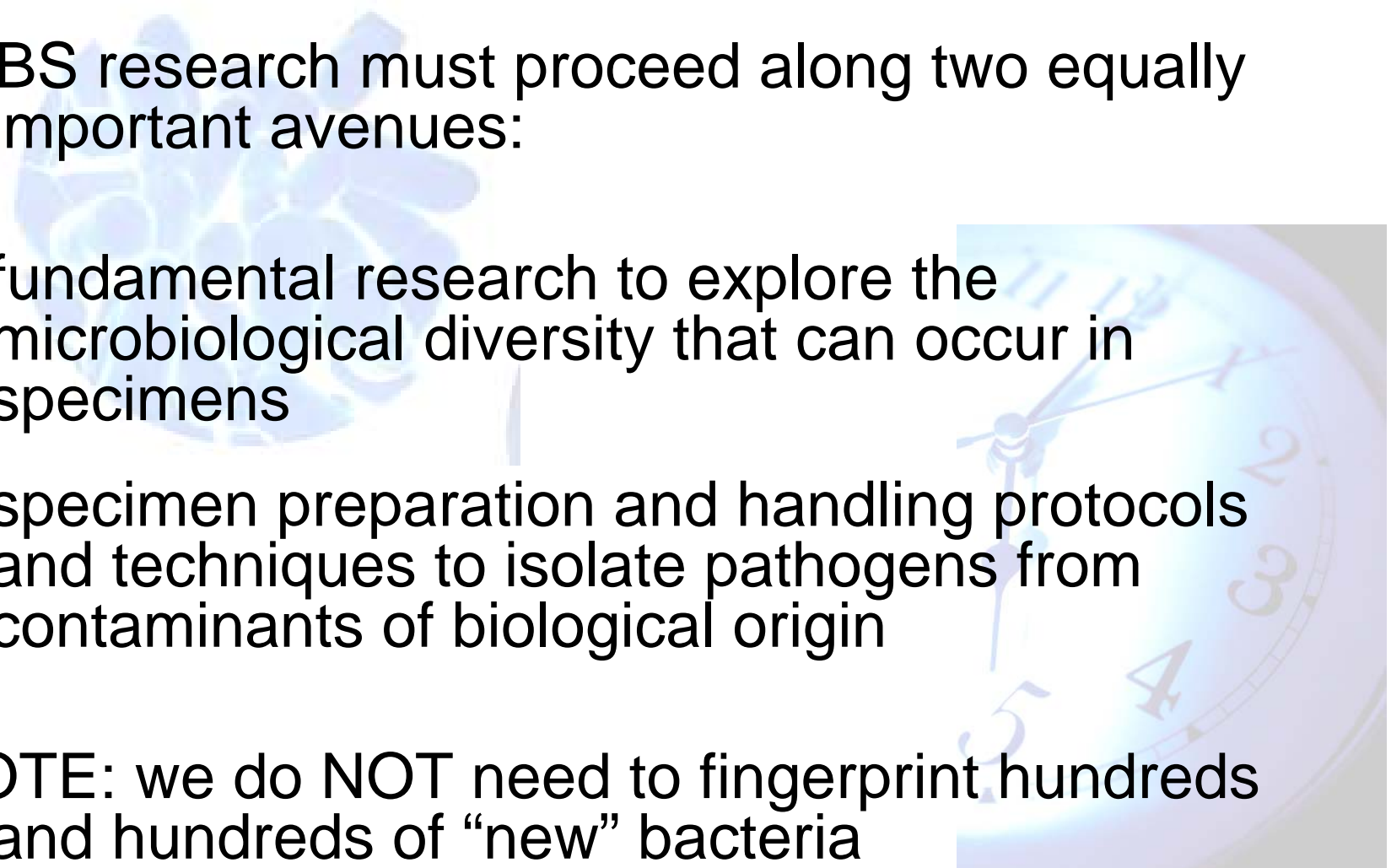
# *what must we do to make LIBS a clinical tool?*

Develop hardware and protocols for clinical sample testing (blood, urine, sputum)

- isolation
- concentration under the laser focus



## *solutions*

1. differential centrifugation
2. filtration (sequential?)
3. optical trapping / separation
4. microfluidic separation
5. antibody isolation/phage display technology (consumables!)

# *Thanks to my students…*

## Graduate Students
- Jon Diedrich, M.S.
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- Arathi Padhmanabhan
- Caleb Ryder
- Qassem Mohaidat, Ph.D.
- Khozima Hamasha, Ph.D.

## Undergraduate Students
- Marian Adamson
- Emmett Brown
- Garrett Godfrey
- Heather Ziola

# *Thanks to my collaborators and sponsors…*

# *Thanks to you for listening...*

Questions?