The potential of LIBS to Diagnose Pathogens, Infectious Diseases, and Toxins

NASLIBS2009

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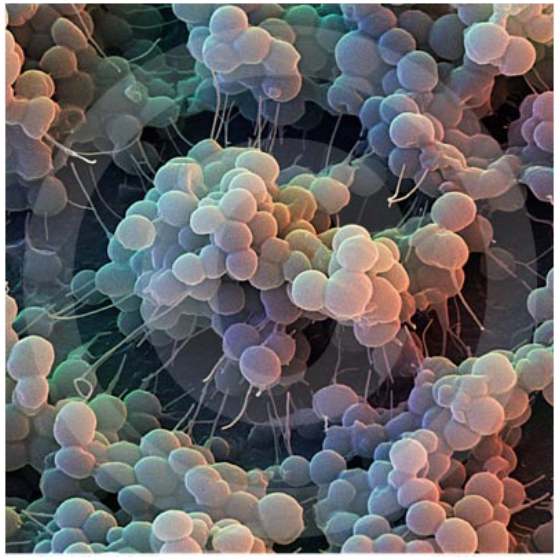
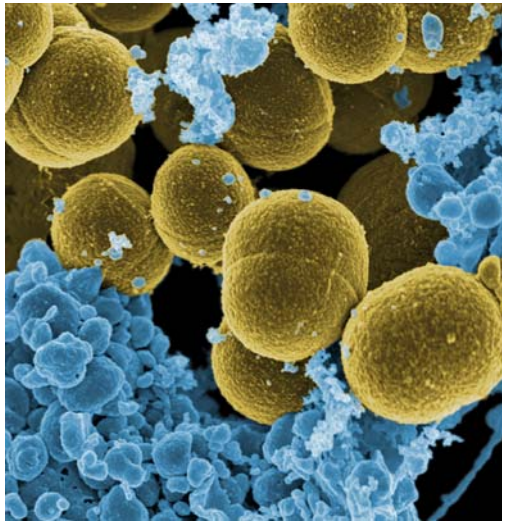


Staph. epidermidis

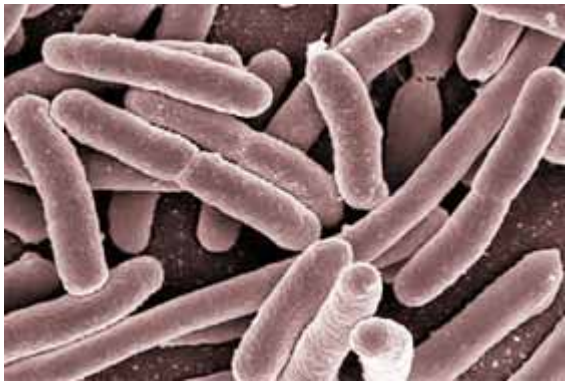
Staph. aureus



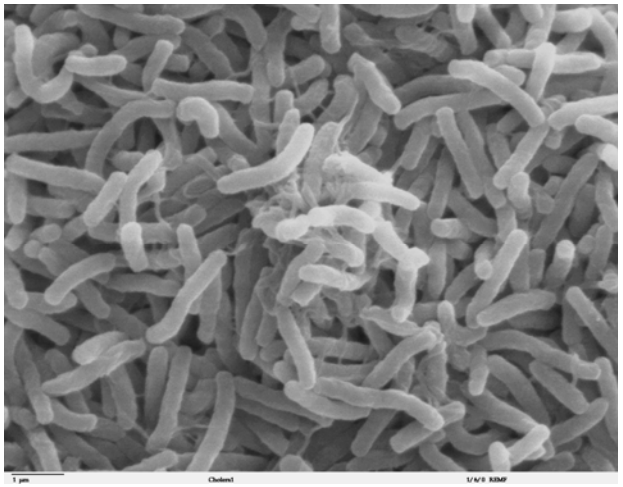
bacteria are ubiquitous

10x more prokaryotic cells in your body than eukaryotic cells

E. coli



V. cholerae



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

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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

olished: January 28, 2009

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News » Health & Behavior ▪ Medical Resources ▪ Health Information

CDC: 756 ill from salmonella-tainted tomatoes

INDEPTH: INSIDE WALKERTON

Canada's worst-ever E. coli contamination

CBC News Online | Updated Dec. 20, 2004

So why?

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

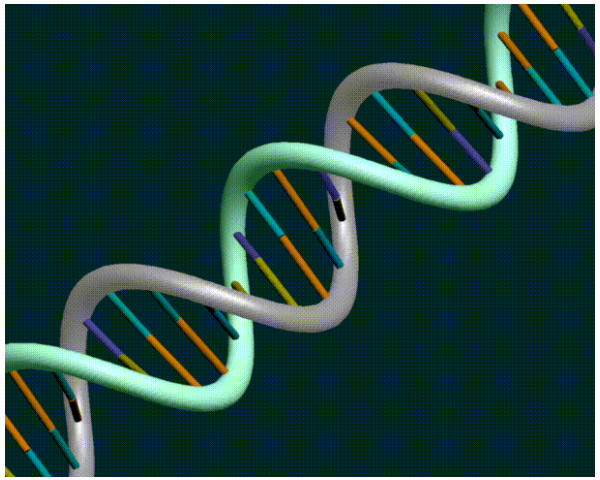
[1]Tarr, P.I. 1995. *Escherichia coli* O157:H7: clinical, diagnostic, and epidemiological aspects of human infection. Clin. Infect. Dis. 20, 1-8.

How do we identify bacteria?

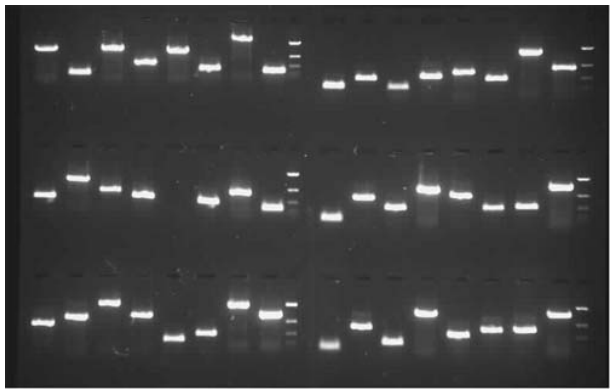
4 ways

- genetic
- serological (antigenic)
- microbiological
- compositional (LIBS)

genetic



- PCR (polymerase chain reaction)
- (random primed) RAPID-PCR
- FISH (fluorescence *in situ* hybridization)



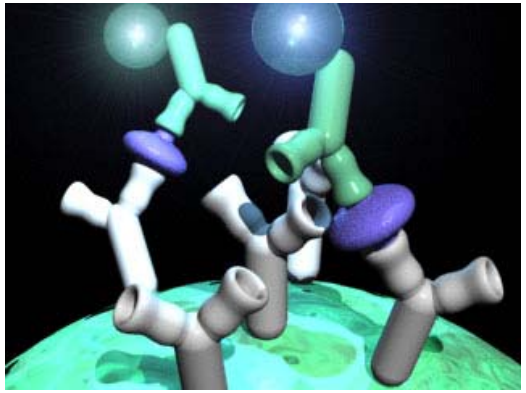
requires

- *a priori* knowledge of genetic sequence (16s RNA gene is conserved in most)

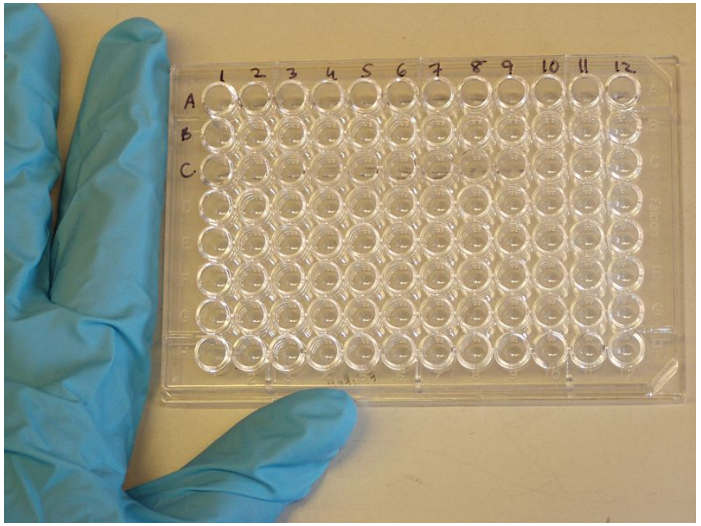
drawbacks

- amplification time (multiple generations needed)
- nonspecific reactivity
- still need to do gel electrophoresis
- very contamination sensitive

serological



- immunoassays
- microwell devices
- ELISA (enzyme-linked immunosorbent assay)
- fluorescently labeled antibody techniques
- MEMS



requires

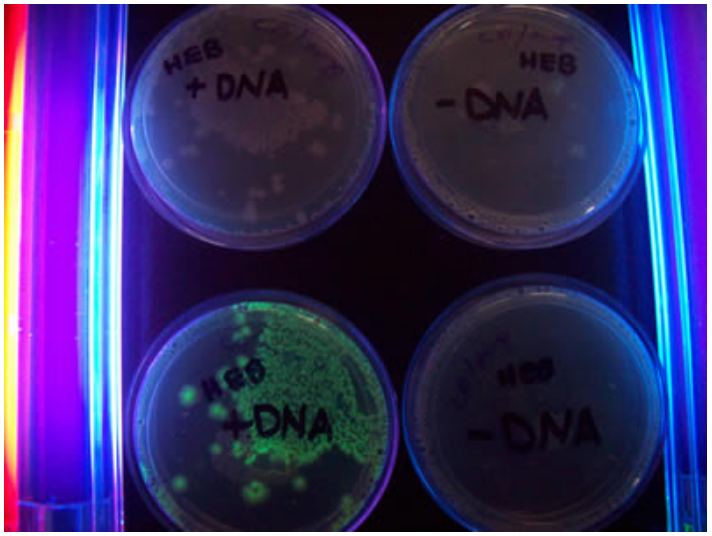
- *a priori* knowledge of serology (surface antigens)

drawbacks

- any mutation (common) undetectable
- antibodies are not stable (shelf-life)
- consumables
- binding affinities may be low

microbiological

- culturing and colony counting
- phenotyping
- sensitivity to immunochemicals
- Gram staining



requires

- time
- expertise
- LOTS of supplies
- *a priori* clinical knowledge (case-history)

drawbacks

- slow/labor intensive
- requires experts

compositional

- Raman
- Mass-spectrometry
- **LIBS**

requires

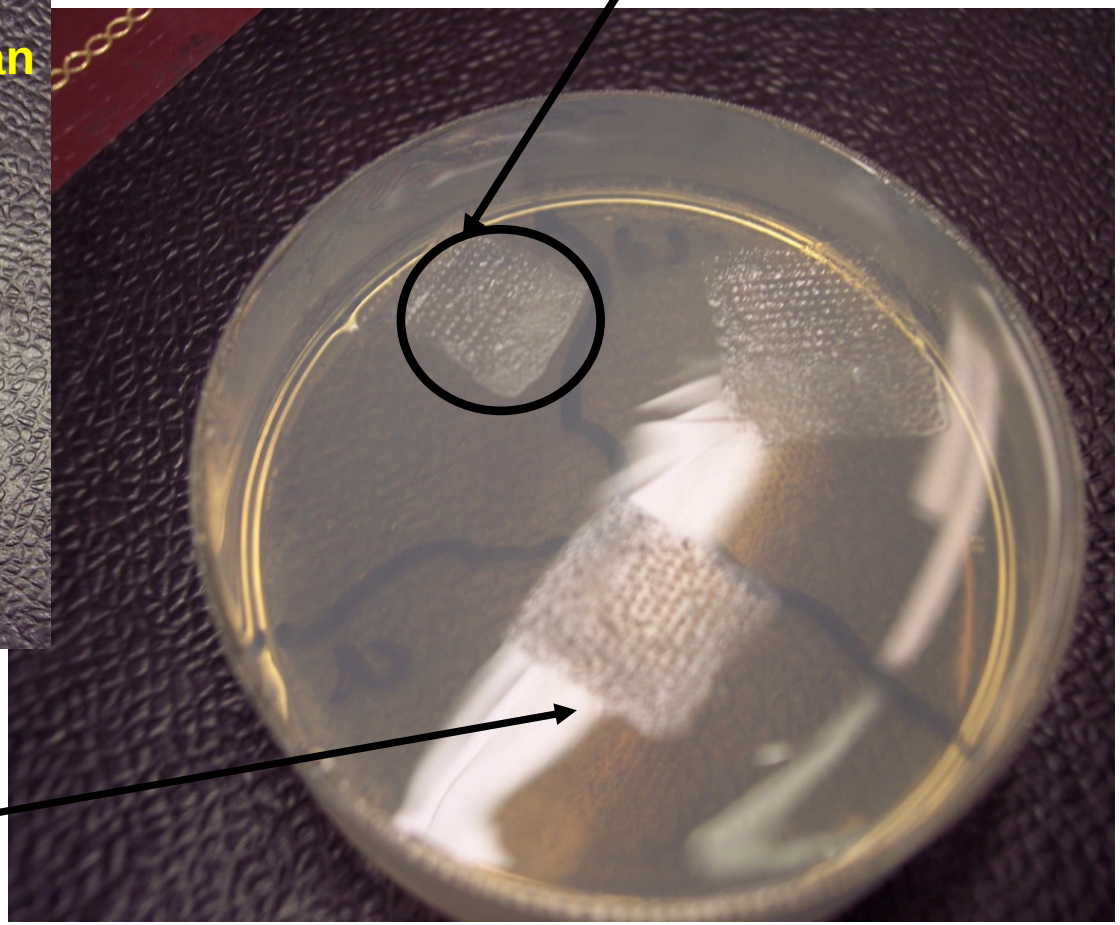
- no *a priori* knowledge of serology (surface antigens)
- no *a priori* knowledge of genetic sequence
- no consumables (hopefully)
- no expertise

drawbacks

- sensitivity (no amplification)
- hardware probably expensive (relative)
- specificity?

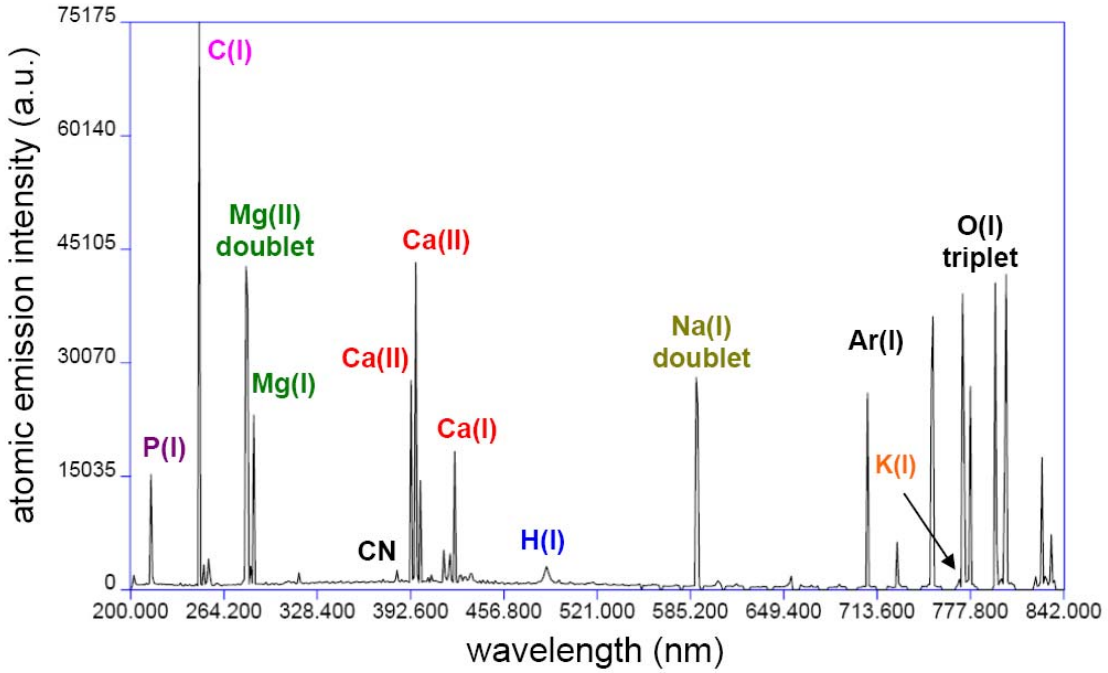
how we do it…


E. coli from liquid specimen. Centrifuged than supernatant removed


10 microliter
about 500-1500 bacteria per sampling location

bacterial composition

LIBS-based pathogen identification is inorganic element based (at this point)

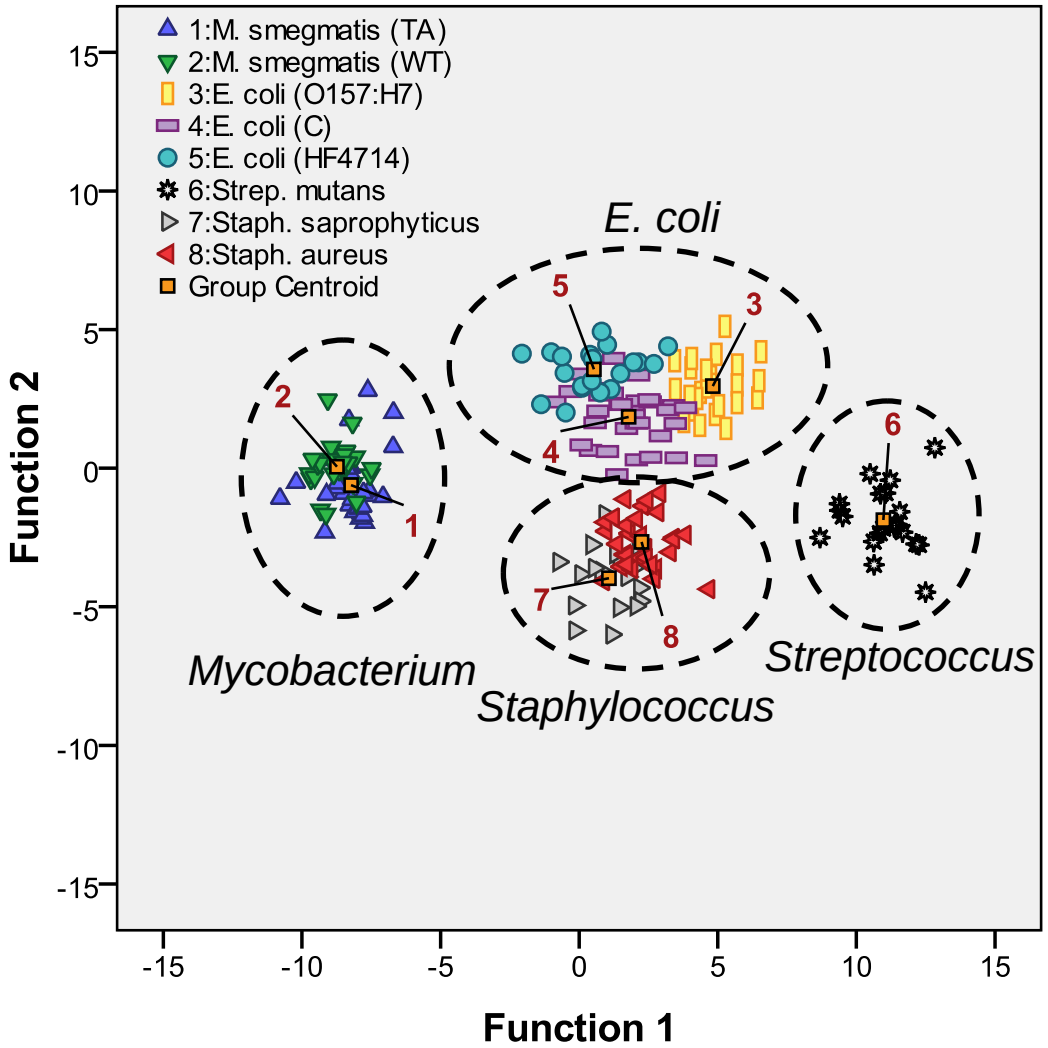


from "*The Bacteria: A Treatise on Structure and Function*" I.C. Gunsalus and R.Y. Stanier, eds

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

chemometrics used

- 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)



what must we do to make LIBS a clinical tool? *(4 things)*

1. Increase sensitivity
   - currently ID'ing 7,500 – 2,500 bacteria
   - Need to do 100

solutions

1. Dual-pulse
2. Better emission collection optics

what must we do to make LIBS a clinical tool? *(4 things)*

2. ID bacteria in “mixed” or “dirty” samples
   - other bacteria may not be a problem
   - other biological objects (cells, etc.)
   - all genetic techniques face same problem

solutions

1. better chemometrics

2. better sample preparation (see next point)

what must we do to make LIBS a clinical tool? *(4 things)*

3. Develop protocols for clinical sample preparation (blood, urine, sputum)
   - isolation
   - concentration under the laser focus

solutions

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

what must we do to make LIBS a clinical tool? *(4 things)*

4. Construct a reference library
   - maybe 30 most important species
   - can add as many strains/species as desired
   - in some cases, a single bacterium would justify the technology

solutions

1. hard work
2. WSU has access to a large clinical repository of stored clinically identified organisms (specimens must be confirmed via other method first)

things that make LIBS-based technology unique

Why do I think this is relevant for clinical, military, first responder, and screening applications?

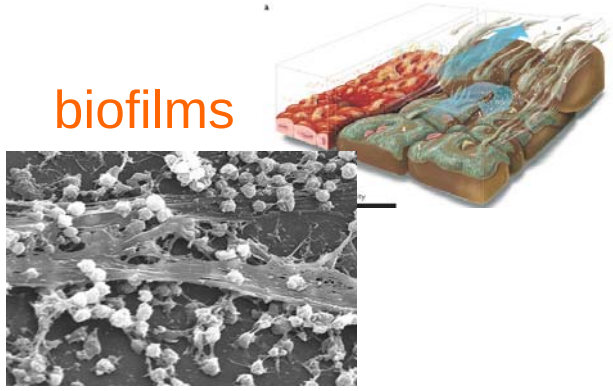
- speed / portability / durability (ruggedness)
  - "rapid point-of-care diagnostic…"
- lack of complicated sample preparation
- no expertise required
- no genetic or antigenic precursors (consumables) necessary
- same technology / hardware useful for explosives, chemical, other threats (CBRNE capable)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

please come see our poster!
P_65

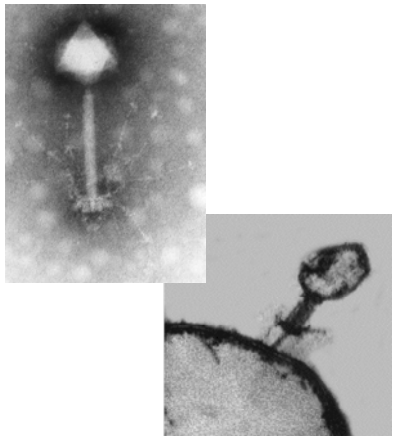
LIBS for Rapid Discrimination / Identification of Gram-negative and Gram-positive Bacteria

novelties

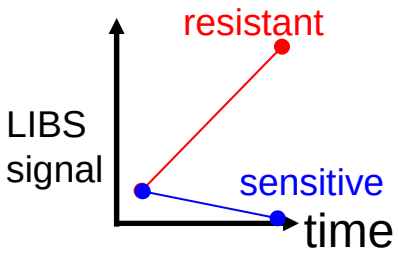
biofilms



phage induction



antibiotic resistance
(quick test based on LIBS signal vs. bacterial number)



Thank you for your attention!

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