Lenima Field Diagnostics

Molecular Diagnostic Point of Care Testing

Wan Shih

Founder

March30, 2014

Lenima Diagnostics Vision

- Improving healthcare by developing accurate, easy to use, rapid, and cost effective molecular diagnostic tests to the point of care.

MDX Fastest Growing Segment Within IVD Space

- $5.9 Billion (2011) and estimated to grow to $10.9 billion 2015 (Research and Markets, 2/12 Molecular Diagnostics: Market Segmentation and opportunities)
  - Chronic Diseases of Aging Population
  - Increased Availability of Tests
  - Further adoption of pharmacogenomics/personalized medicine
- Major competitors include BioPharma (Abbott, Roche), IVD/MDX pure play companies (Myriad Genetics, Cepheid, Gen-Probe), and research tool based companies (Illumina, Life Technologies)
- M&A deals valued at $4.7 Billion in 2010 with 45 deals
  - Growth will continue with drivers that include companion diagnostics and early detection attracting interest from large diagnostic companies and pharma

First Application

A rapid, low-cost, accurate and point-of-care *Clostridium difficile* infection (CDI) diagnostic tool

Team

Wan Y. Shih, PhD

—Associate professor of Biomedical engineering, Drexel University

Wei-Heng Shih, PhD

—Professor of Materials Engineering, Drexel University

Chris Emery, MD

—Director, Microbiology laboratory, Hahnemann University Hospital

—Associate Professor of Pathology, Drexel University College of Medicine

Suresh Joshi, MD, PhD

—Associate Professor of Microbiology , Drexel University College of Medicine

Richard Hamilton, MD

— Chair & Professor of Emergency Medicine, Drexel University College of Medicine

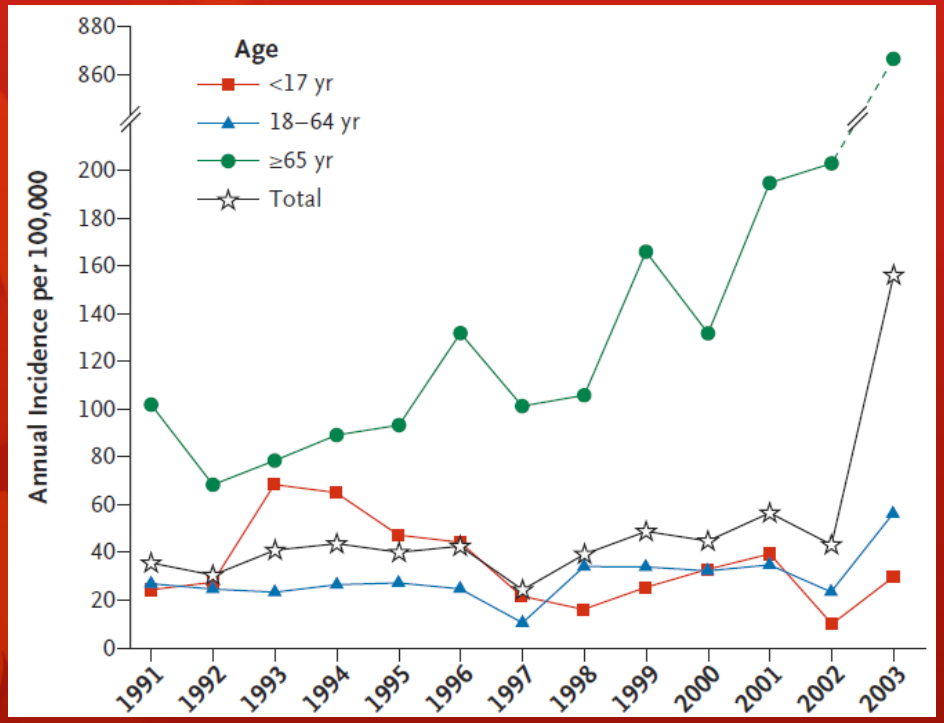
Joe Zack

—Business Advisor

Clostridium difficile Infection (CDI)

- *Clostridium difficile* (CD) is an anaerobic, spore forming, gram-positive rod-like bacterium that produces toxins A and B
- CD spores persist on surfaces for months, can only be destroyed by bleach.
- CD spores are transferred to patients via hands of healthcare personnel who have touched a contaminated surface or item
- CDI is a serious healthcare-associated infection (HAI) for all types of healthcare facilities
  - including hospitals, nursing homes, and outpatient clinics.

CDI Prevalence & Mortality are Increasing



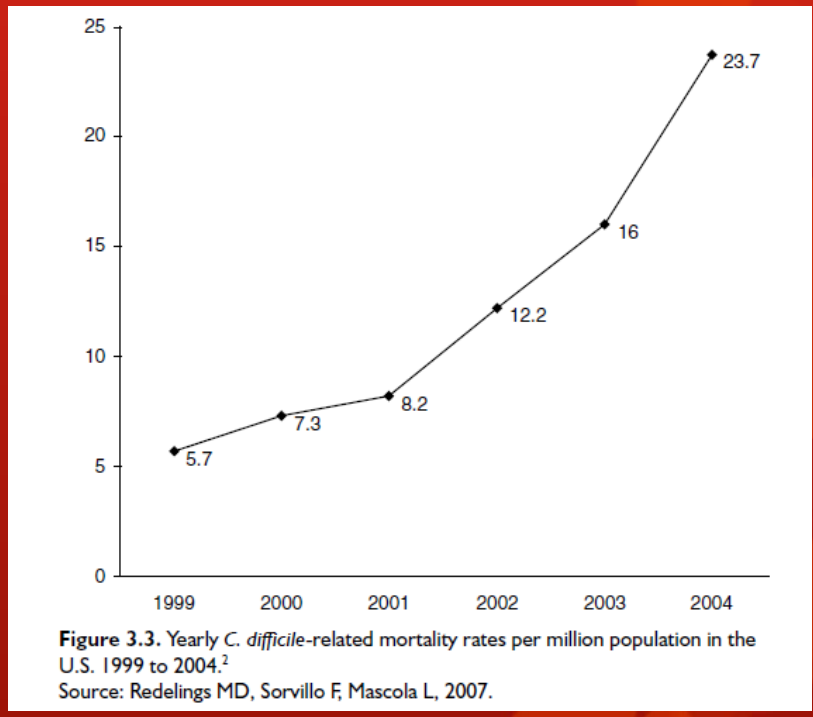


Figure 3.3. Yearly *C. difficile*-related mortality rates per million population in the U.S. 1999 to 2004.[2]
Source: Redelings MD, Sorvillo F, Mascola L, 2007.

N. Engl. J. Med, 2008

- CDI prevalence have more than quadrupled in the past two decades and remain at historically high levels while most types of hospital-associated infections (HAIs) are declining
- Deaths related to CDI increased 400% between 2000 and 2007, due in part to a stronger germ strain

CDI Transmission / Financial Burden

- 3 million CDI cases annually in the US
- Accounts for 20-30% of hospital-associated diarrhea
- Causes 14,000 annual deaths in the US
- Cost > $3B to treat in the US annually
- ~50% CDI occur in people younger than 65, but >90% of deaths occur in people 65 and older
- CDI risk generally increases with age; children are at lower risk
- About 25% of CDI first show symptoms in hospital patients; 75% first show in nursing home patients or in people recently cared for in doctors' offices and clinics

Treatment / Patient management

Treatment

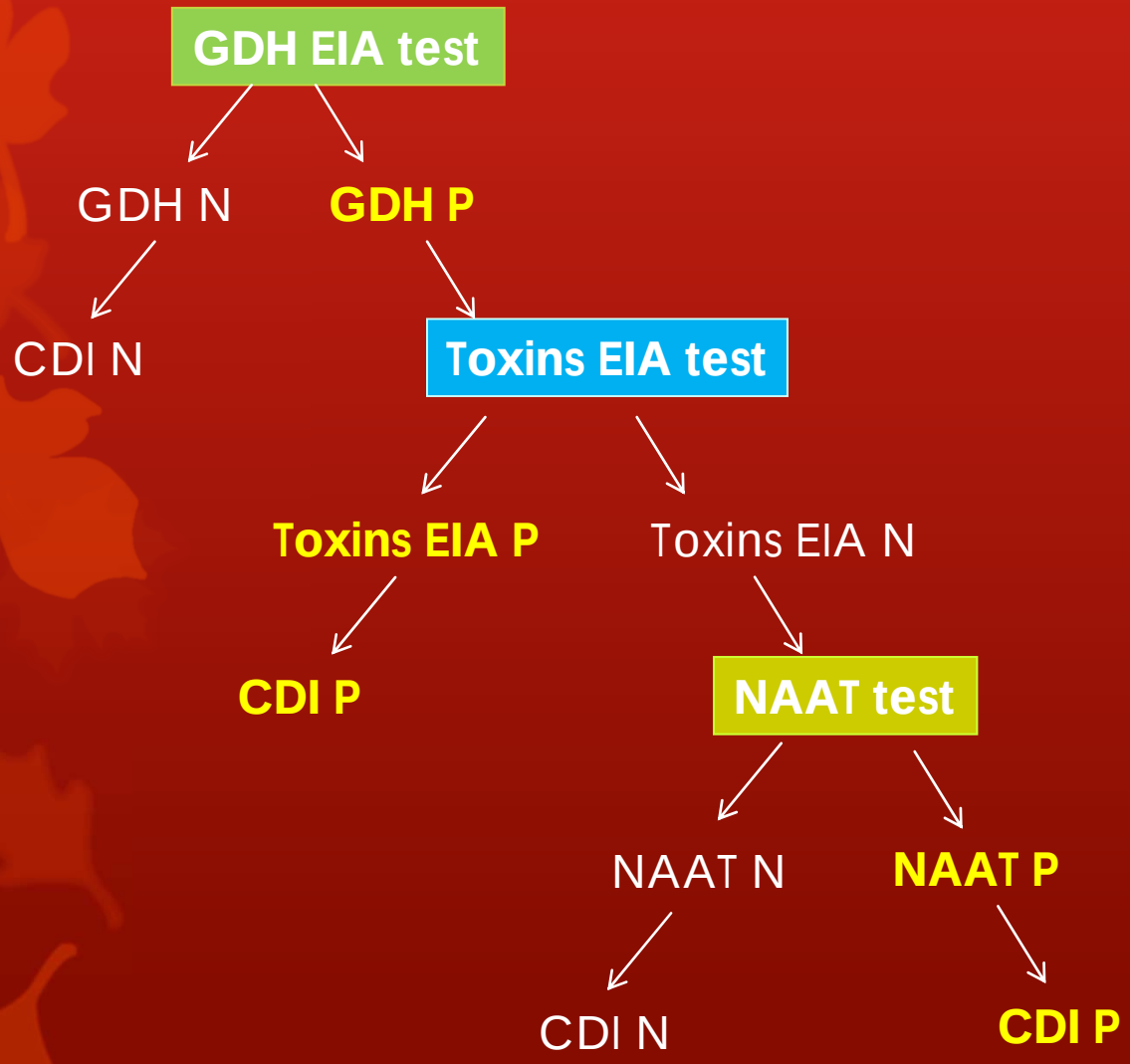
- First step is discontinuation of antibiotic therapy
- Mild diseases are treated with oral Metronidazole
- Severe diseases are treated with Vancomycin
- In rare cases, surgery may be needed
- Relapse or reinfections occurs in 12-24% of patients

Patient management

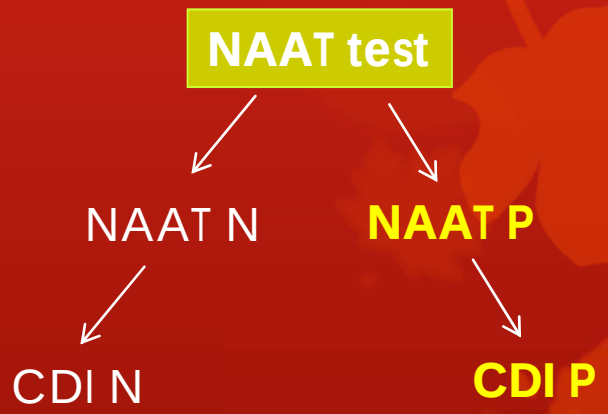
- CDI patients are isolated in a single room or cohorted with other CDI patients
- All healthcare workers and visitors must wear gloves and gowns when entering the room of CDI patients

Current CDI Diagnosis

(A) Combination of EIA and NAAT



(B) NAAT stand alone test



NAAT: Nuceic Acid Amplification Test (of toxin genes)

EIA: Enzyme enhanced ImmunAassay

GDH: surface antigen

Emerging Epidemic Hyper-virulent Strains

- Since 2005, hyper-virulent strains such as BI/NAP1/027 are emerging
- Hyper-virulent strains possess a third toxin, **binary toxin gene**

- CDI 30-day mortality rate
  - 17% without binary toxin gene
  - **28% with binary toxin gene**

- CDI recurrence rate
  - 17% without binary toxin gene
  - **28% with binary toxin gene**

- **Early detection and correct treatment is critical to reduce severe outcomes**
- **Detection of the binary toxin gene in addition to the toxins genes is important to combat CDI**

Unmet Need

Accurate, Affordable, multiplexed, Rapid and Point-of-Care test

NAAT

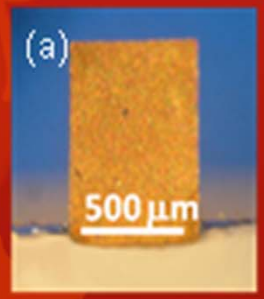
Meridian, Cepheid, Nanosphere, BD, Prodesse

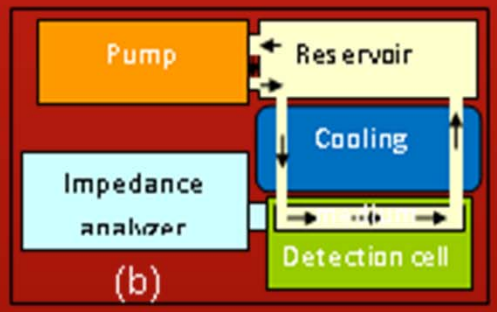
$35-$58/gene

Sensitive (>90%) but expensive
Does not multiplex, adding binary toxin gene would further increase the cost

Inexpensive, Rapid, Multiplexed, and Accurate CDI Test Solution…

Piezoelectric Plate Sensor (PEPS) Array



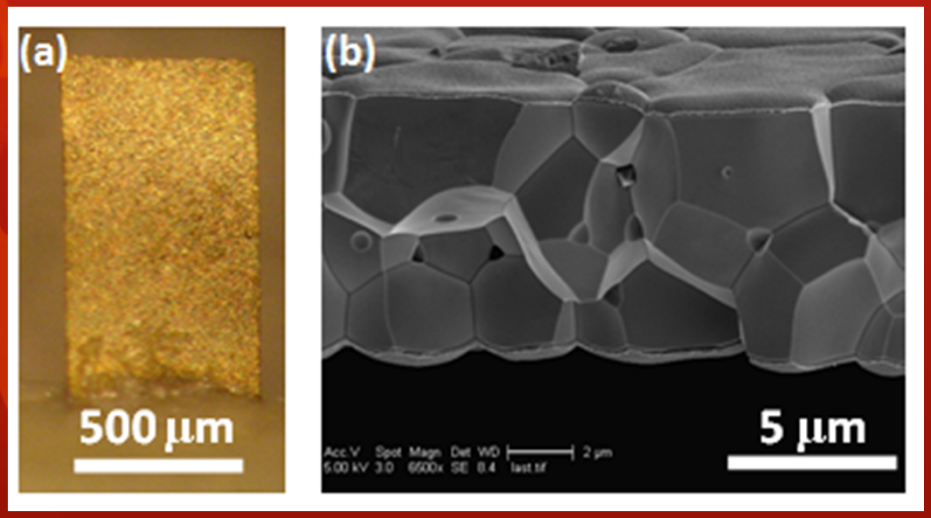


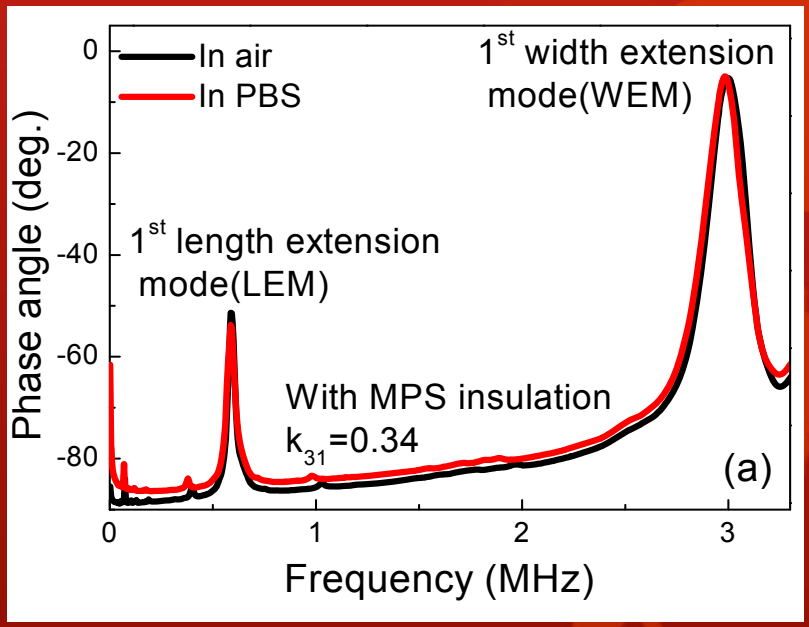
- Rapid, sensitive, and yet low-cost detection using PEPS with
  - *in situ* bacteria lysing,
  - *in situ* DNA release,
  - *in situ* DNA denaturing,
  - *in situ* DNA detection All in 40 min

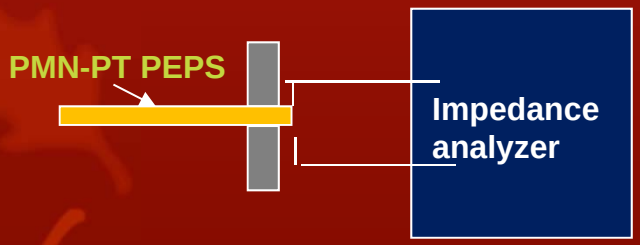
- With PCR-like sensitivity but no DNA extraction, concentration, and amplification
- Real-time genetic detection using array piezoelectric plate sensors (PEPS) with a $500 impedance analyzer

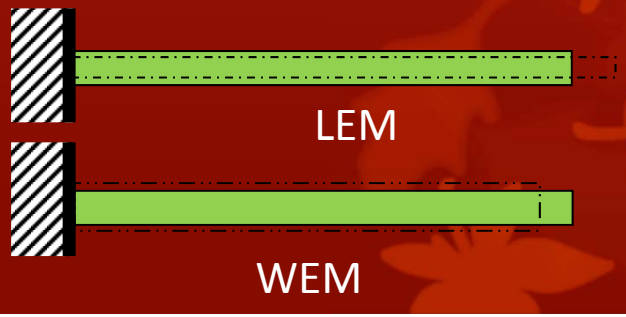
PMN-PT piezoelectric plate sensor (PEPS)

PMN-PT PEPS: (1) 1 mm x 0.5 mm made
(2) made of PMN-PT freestanding film 8 μm thick
(3) operated at length extension mode (LEM)
or width extension mode (WEM)



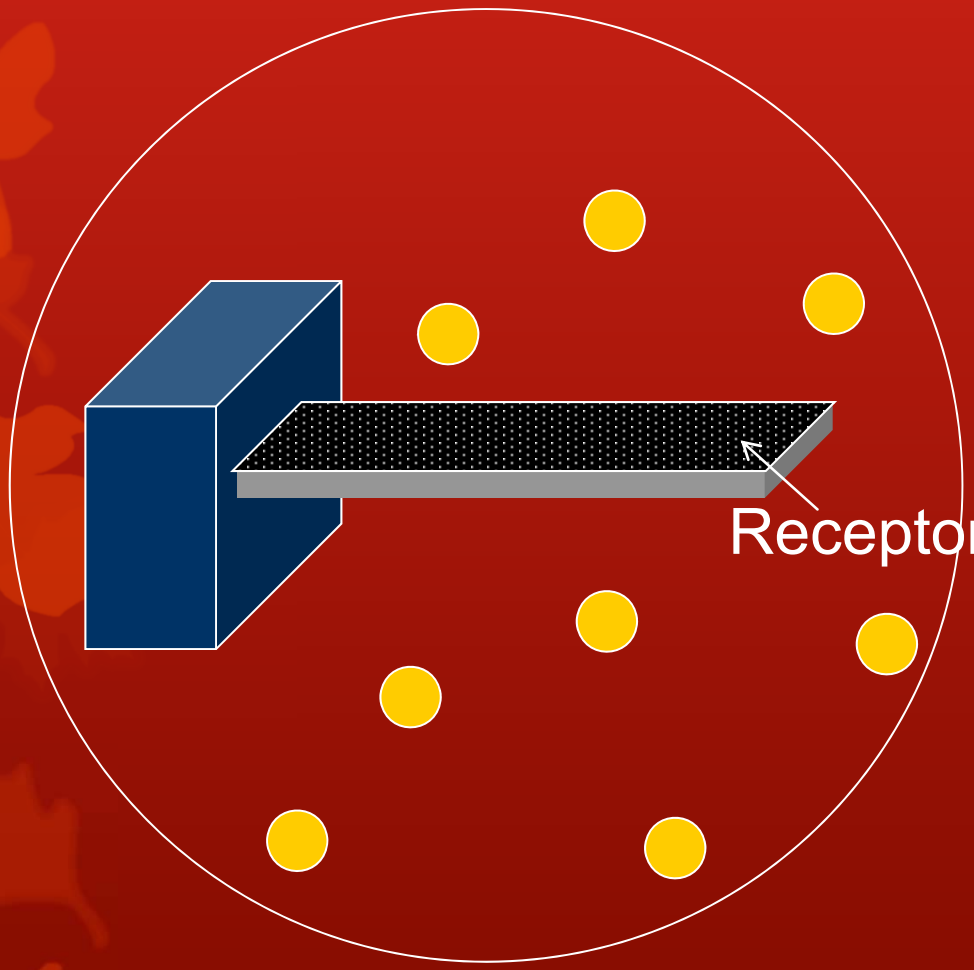


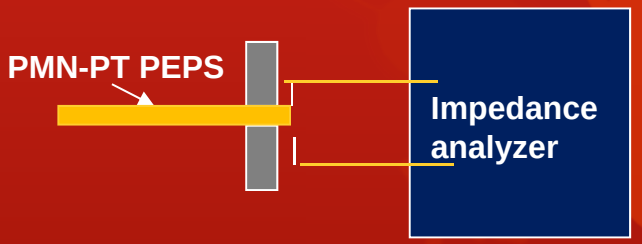


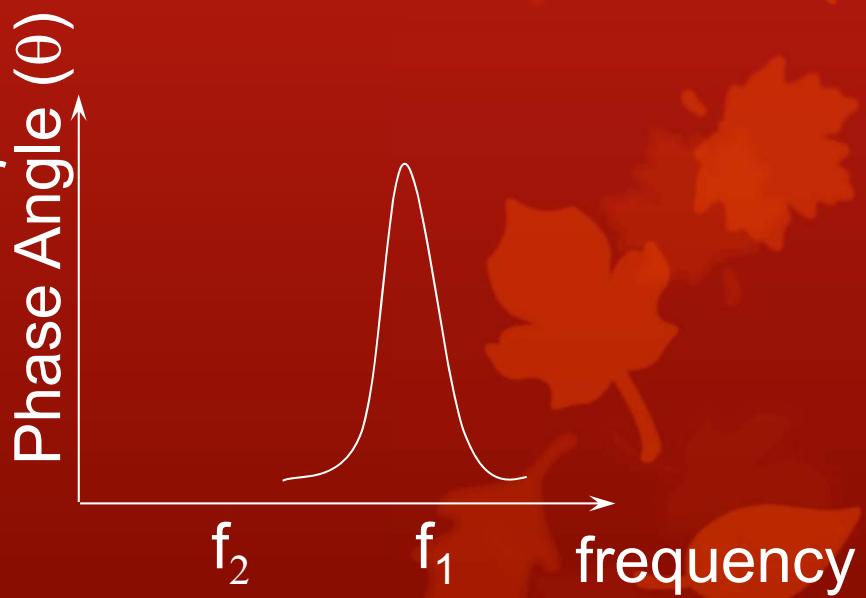


PMN-PT Piezoelectric Plate Sensor (PEPS)

Rapid, Label-Free Sensing





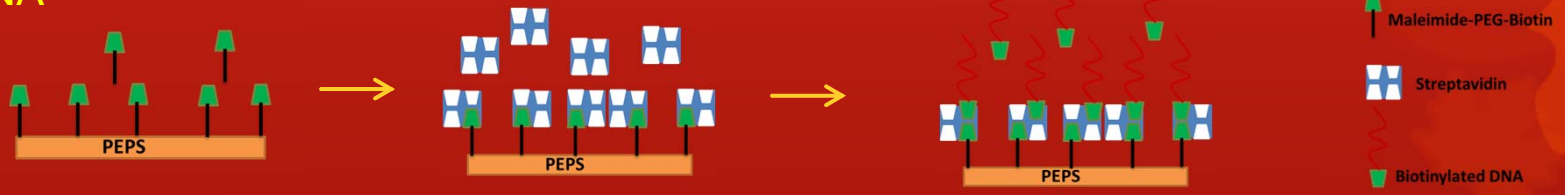


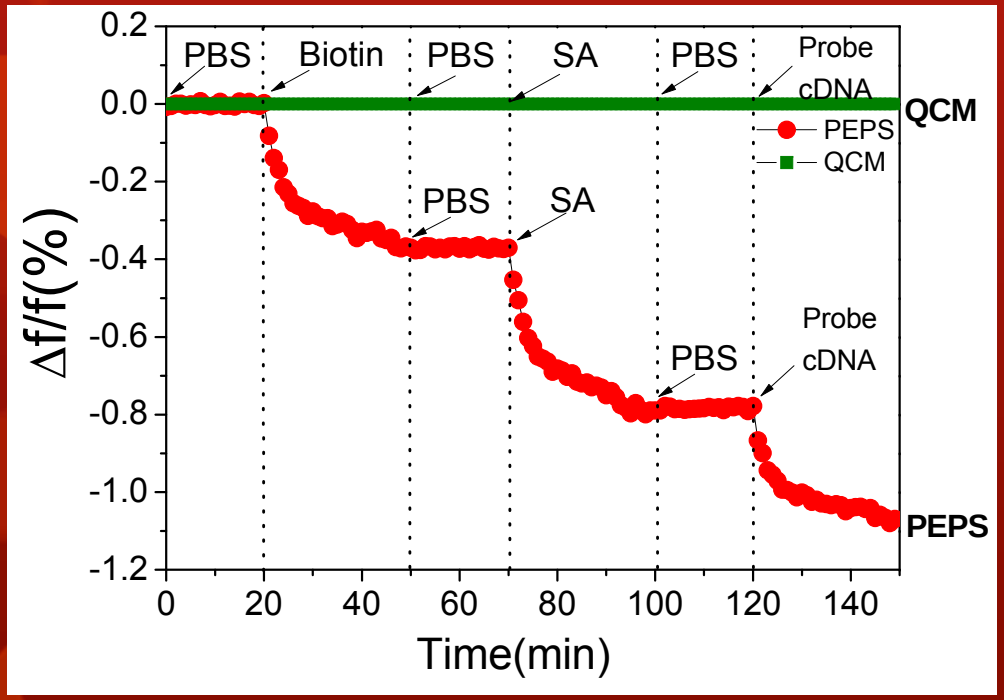
Target antigen/analyte

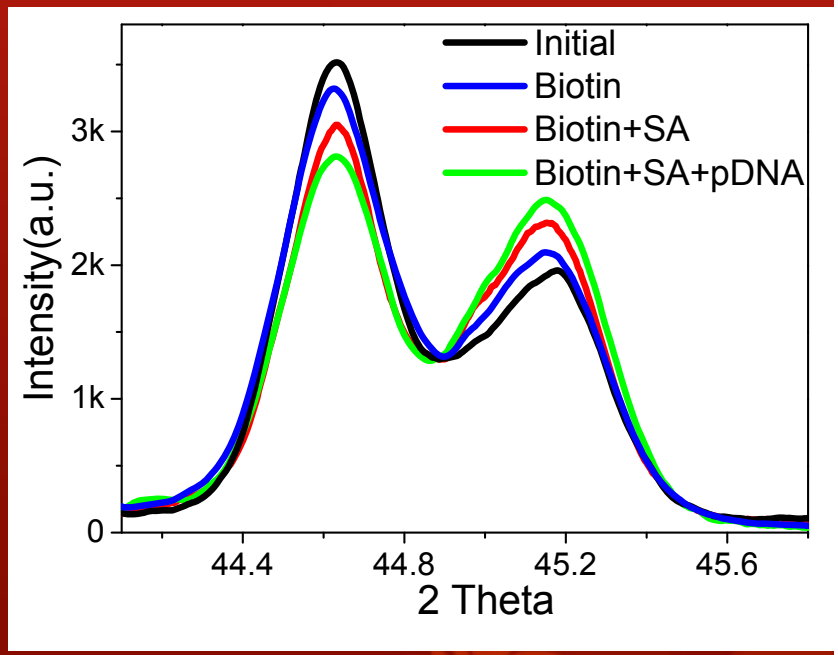
- WYS and WHS have worked on PEPS and its predecessor, PEMS
  - For more than 15 years
  - with more than $4M federal/state funding
  - more than 10 PhD theses
  - 10 patents/patent applications
  - more than 40 published journal papers
- The piezoelectric-material and sensor development is ripe

1000 times Self Enhancement of Detection Δf/f

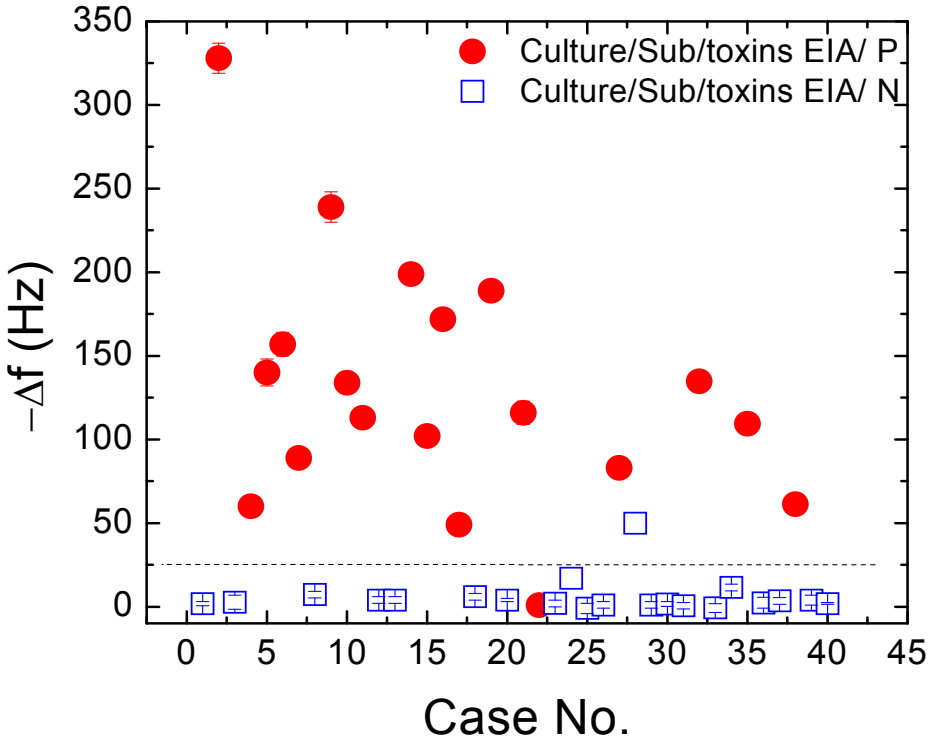
- Due to crystalline orientation switching in “thin” PMN-PT layer induced by binding stress---No such enhancement in other piezoelectric sensor (QCM, SAW…)
- The enhancement increases inversely with a decreasing thickness
- Enhancement is further amplified in DNA detection due to the highly negatively charged nature of DNA







Testing on 40 Blinded Patient Stools



40 stool samples:
20 CDI positive
20 CDI negative
According to stool culture/sub/toxins EIA

PEPS exhibited

- **95% sensitivity--** positive 19/20 CDI positive stools
- **95% specificity--** negative 19/20 CDI negative stools

—The same as Cepheid Xpert (the best genetic test)

Comparison with Current Technologies

Table 2.1 Competitive Comparison between PEPS and commercially-available CD diagnosis alternatives							
	Equipment	Detection time	CDI diagnosis	Sensitivity	Specificity	severity test	Cost/test
GDH+toxins EIA	$20 – 50k	Hours	No	50-60%	95%	No	$17.5
Genetic test	Free to $150 – 180K	1 hour	Yes	95%	95%	No	$30-$58
GDH/toxin/ Genetic test	$150 – 180K	Hours	Yes	87%	>90%	No	$40
PEPS	**Free to $3K**	**40 min**	**Yes**	**95%**	**95%**	**Yes**	**$20**

Reimbursement from the Centers for Medicare and Medicaid Services

$17.5 for GDH test

$50.27 for bacterial detection using amplification

Hospital Revenue Potential

# of hospitals		Bed size	Avg Estimated CDI tests*	Total Estimated CDI tests*	Revenue Potential	% of Revenue potential
XL-size	75	>800	3,000	225,000	$4,500,000	4%
L-size	430	400-799	2,250	967,500	$19,350,000	17%
M-size	1500	150-399	1,032	1,547932	$30,958,640	27%
S-size	3400	<149	891	3,029,323	$60,586,460	53%
Total	5405		6,282	5,769,756	$115,395,120	100%

*Estimate is based on Hahnemann, a 400-bed hospital that performed 1,500 tests last year.

- $20/test makes it a +$100 million opportunity
- Cepheid Xpert penetrates only 30% and 10% of mid-size and small hospitals, respectively due to its costs.
- Even large hospitals like Temple University Hospital moved away from using Cepheid Xpert and is trying to develop their own PCR method
- Small and mid-size hospitals accounts for 53% and 27% (together 80%) of the market, or *$92MM a year based on $20/test*

IP*

- **Wan Y. Shih**, Qing Zhu, and Wei-Heng Shih, “Enhanced Detection Sensitivity with Piezoelectric Sensors,” US Patent No. 871,663, June 3, 2014.
- **Wan Y. Shih**, W.-H. Shih, W. Wu, M. Soylu, C. Kirimli, H. Guo, S. Joshi, Y.-H. Su, “Piezoelectric Plate Sensor and Uses Thereof,” US provisional patent. December, 2013.

*Optioned from Drexel University

Validation and Regulatory

We are targeting fecal CDI detection as the first application

FDA Approval: 510K Pre-Market Notification

- Predicate Devices:
  - K091109: Cepheid Xpert *C. difficile*
  - K100818: Meridian *Illumigene C. difficile*
  - K123197: Nanosphere Verigene *C. difficile*

Reimbursement

Reimbursement from the Centers for Medicare and Medicaid Services

$17.5 for GDH test

$50.27 for bacterial detection using amplification

Development Plan

- Development of the core technology-24 month process in total
  - Manufacturing of PMN-PT films and PEPS (In House)
    - PMN-PT films and PEPS are the heart of the technology
    - PMN-PT films fabrication has been perfected on the lab scale
    - Will work on mass production of PMN-PT films and mass production of PEPS cutting

    Development cost / duration: **$0.7M / 24 months**

  - Fabrication of PEPS Array (NextFab)
    - PEPS reproducibility issues was solved on the lab scale.
    - Presently working with Nextfab to get an estimate for assemble array PEPS by 3D printing

    Development cost / duration: **$0.1M / 24 months**

  - Automated total system w/ flow & electronic circuitry (Imet, ION Design, NextFab w/ Lenima)
    - a sieve to strain the stool
    - a reservoir at 95°C
    - a cooling module
    - a detection chamber at around 50-60°C
    - AIM 4170 impedance analyzer ($500)

    Development cost / duration: **$1.2M / 24 months**

    Permanent unit price: **$3K**

    Disposal unit: **$0.5-10**

Total Development Cost: ~$2M

Business Strategy

- Build Awareness and Positive Disposition toward Technology Pre-Launch
  - Work with Key Opinion Leaders (KOL's)
  - Publications
  - Build relationships with all the key infectious disease organizations, and get incorporated into appropriate testing guidelines.
- Develop payer strategy to optimize reimbursement
- Assess U.S. Commercialization Requirements
  - Hospital Market is Accessible
- Alternatively, partner with BioPharma or Molecular Diagnositic Companies (MDX) following validation or FDA approval
  - U.S.
  - Europe
  - Developing Countries
- Work closely with WHO and other key international organizations/non-profits to leverage their relationships and infrastructure

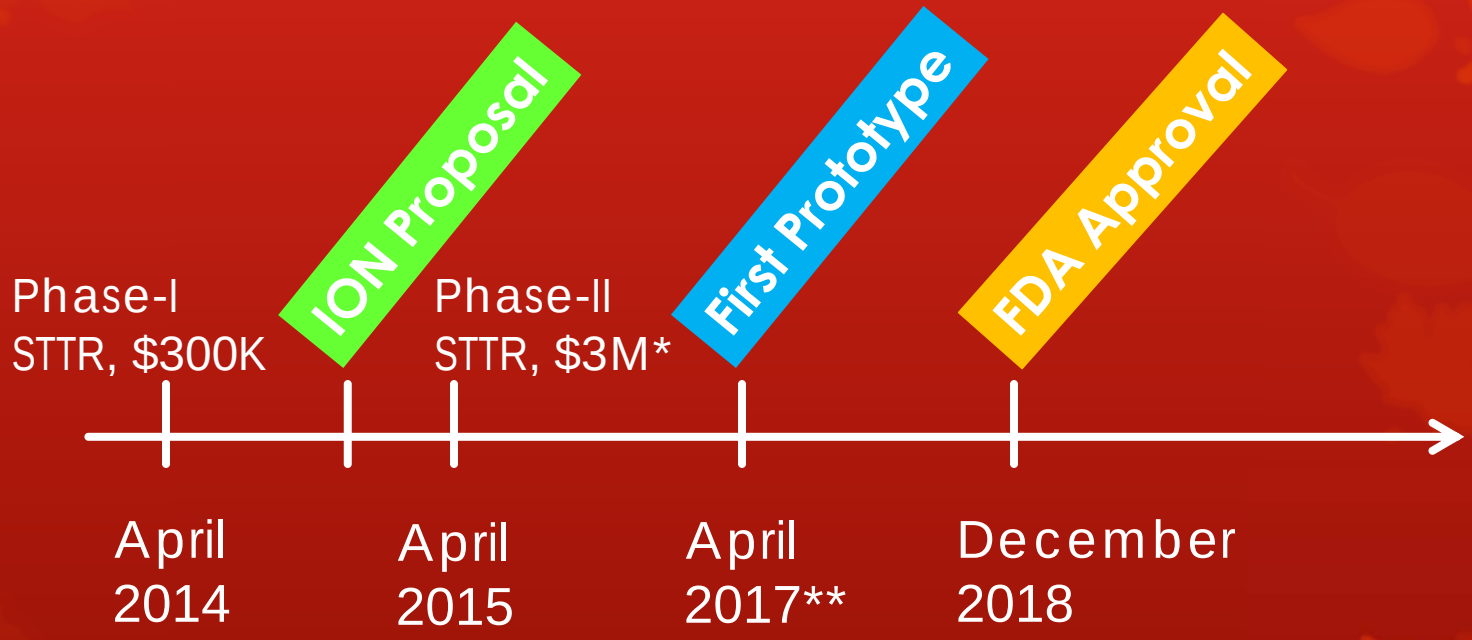
Other Applications

- Infectious Disease
  - MRSA
  - Antibiotic susceptibility
  - Blood infections
  - Meningitis
- Oncology
  - Blood test for cancer mutation markers
    - For example, T790M mutation test for **AQUIRED resistance to TKI treatment in EGFR-positive lung cancer** and other EGFR-positive cancer
  - Blood test for glycoprotein cancer markers
    - For example, Serum Tn antigen and Anti- Tn antigen **malignancy test to accompany imaging tests**

Capital Formation Plan-Early On, Non-Dilutive Funding

- Signed an option agreement with Drexel
- Actively pursuing NIH STTR/SBIR grants as part of the funding strategy
- A Phase-I STTR of $300K on CDI detection has started April 15, 2014.
- Phase II STTR grant up to $3M on product development of CDI detector is planned on August 5th.
- 2nd Phase-I STTR on blood malignancy test to prescreen lung cancer will be submitted on August 5th.

Time Line



*Based on the fact that current results had exceeded the milestone set for Phase-I grant

**With additional funding this date can move up substantially

Summary

- PEPS technology brings the precision of genetic testing to the field
- CDI is a serious health care infection. Early detection and correct treatment is critical to reduce mortality, morbidity, as well as financial burden
- Although CDI is the initial focus, this platform technology has wide applications
- Performance, speed, and relatively low cost appears to be attractive to hospitals
- Our data provides confidence that the sensor works, it's simply a question of developing it into a working prototype, and validating on the instrument
- FDA pathway is straightforward
- A strong core team is in place to take it to the next level

Thank you

Appendix

PEPS Target Product Profile Background

- *Clostridium difficile* (CD), a bacterium causing diarrhea and other intestinal problems with high mortality of 18-30% that links to 14,000 annual deaths in the US. CDI is an antibiotic-associated infection as well as a health-care-associated infection.
- Current CDI diagnosis relies on CD toxins enzyme immunoassay (EIA) together with antigen (GDH) EIA. However, the sensitivity of stool toxin EIA is only 60%.
- Nucleic acid amplification test (NAAT) using quantitative real-time polymerase chain reaction (qPCR) or loop-mediated isothermal amplification (LAMP) to detect the toxin gene tcdB or tcdA is sensitive and specific but qPCR and LAMP requires expensive fluorescent probes ($30-$58 per kit).
- Neither qPCR nor LAMP are widely available as rapid qPCR requires expensive equipment (>$150K) and LAMP requires users to isolate DNA prior to LAMP amplification.

PEPS Target Product Profile

Profile	• Platform technology features a piezoelectric plate sensor (PEPS), that permits genetic testing without amplification, that is low cost (less than $3K for instrument, less than $10 for test), rapid (40 minutes), and point of care. Been in development for 10-years • PEPS detection is different from other non-amplified DNA detection. Those sensors still need the steps of DNA extraction and denaturation before detection and they can only detect purified, denatured DNA in high concentrations in PBS. In comparison, PEPS can detect the DNA of bacteria directly in stool at 60 copies/ml all within a continuous flow system in 40 min without the need to extract the DNA or amply the DNA. • One-step, multiplexed test detects multiple bacterial genes (Toxin B (tcbB) gene and binary toxin gene cdtB-associated with severity and recurrence) from stool to diagnose CDI and assess the severity and risk of recurrence at the point of care.
Specimen type	• Serum, sputum, stool, urine. 1 ml.
Time for results	• 40 minutes
Sensitivity	• Sensitivity 95%; specificity 95%. As good or better than PCR
Through-put	• 36 samples per day/per module
Portability	• 8” by 6” by 6”
Data Management	• PC connection. Upload to existing system
Value Proposition	• This one-step, multiplexed test detects multiple bacterial genes (Toxin B (tcbB) gene and binary toxin gene cdtB-associated with severity and recurrence) from stool to diagnose CDI and assess the severity and risk of recurrence at the point of care. This would allow earlier and better treatment decisions, and minimize the mortality rate and recurrence risk, as well as prevent CDI from spreading. • This rapid, accurate, quantitative, and low-cost CDI test does not require highly trained personnel and can be widely available at point of care such as small- and medium-size hospitals, outpatient clinics, and nursing homes for rapid and accurate CDI diagnoses.

Clostridium difficile Infection (CDI)

- CDI is a common cause of antibiotic-associated diarrhea (AAD).
  - It accounts for 25% of all AAD
- CDI can lead to
  - pseudomembranous colitis,
  - toxic megacolon,
  - perforations of the colon,
  - sepsis, and
  - death.
- Symptoms of CDI include
  - watery diarrhea, fever, nausea, loss of appetite, abdominal pain
- CDI risk factors include
  - antibiotic exposure, proton pump inhibitors,
  - gastrointestinal surgery/manipulation,
  - long stay in healthcare settings,
  - a serious underlying illness,
  - immunocompromising conditions,
  - advanced age