Marcus Zervos, MD

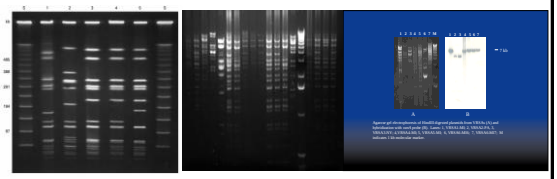
Are Clinical Labs Ready for Emerging Infectious Diseases

Professor of Medicine and Assistant Dean of Global Affairs,
Wayne State University School of Medicine
Division Head, Infectious Diseases, Henry Ford Health

The Big Picture

- Transformational advancements in field of diagnostics
- Where we are/where have we been
- Where are we going

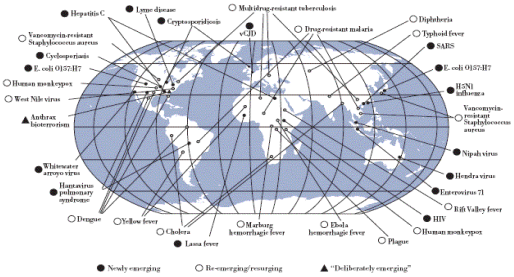
Where have we been/where are we



Zervos M 1987 Ann Int Med
https://doi.org/10.7326/0003-4819-106-5-687

Smith T NEJM 1999; 340:493-501
DOI: 10.1056/NEJM199902183400701

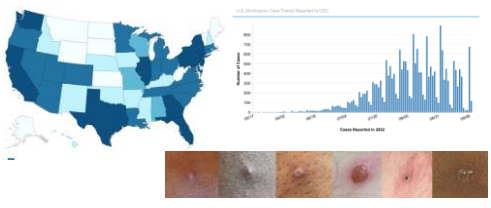
FIGURE 1 EXAMPLES OF EMERGING AND RE-EMERGING INFECTIOUS DISEASES THROUGHOUT THE WORLD.



Adapted from Morens, D.M., et al. 2004. The Challenge of Emerging and Re-emerging Infectious Diseases. *Nature* 430:242–49.

57,527 cases global, 21,894 USA

Monkeypox



https://www.cdc.gov/poxvirus/monkeypox/index.html accessed 9.9.2022

Poliovirus

- June 2022, poliovirus was confirmed in an unvaccinated immunocompetent adult resident of New York hospitalized with flaccid lower limb weakness.
- Vaccine-derived poliovirus type 2 was isolated from the patient and identified from wastewater samples in two neighboring New York counties.

Link-Gelles et al MMWR August 19,2022;71(33):1065-8

Where are we going

Outbreak investigation

Public health surveillance

Clinical applications

Genomics can provide powerful data in real time

- Informing diagnostics, vaccines and therapies
- Identifying variation across the genome
- Tracking and characterizing outbreak transmission
- Getting to functional mutations
- Informs countermeasures (prevention and therapy)

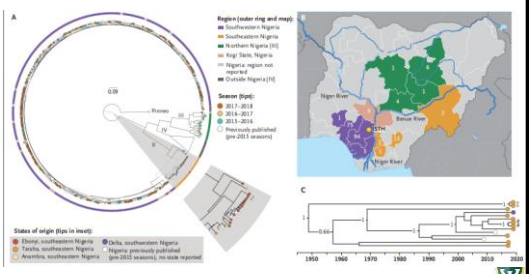
Molecular/new tests

- Detect:
  - what organisms are out there/tests for emerging diseases/known and novel pathogens
  - test for multiple agents
  - rapid
- Connect: information/data
- Empower: everyone in system/partner/training
  - The community to improve preparedness, response

Partnership

- Clinicians need to partner with laboratory:
  - What tests are implemented/available
  - Who to test
  - How to interpret/report comments
  - Who can order
  - Develop with lab algorithms and guidelines for use, restrictions on ordering

Outbreak investigation Lassa fever: Nigeria



Siddle KJ NEJM 2018:379;1745-53

Real-Time Genomic Surveillance

- 413 isolates at University of Pittsburgh
  - Detected 18 P. aeruginosa, pseudo outbreak of Serratia and a cluster of VRE
- CEID lab detected
  - cluster of NDM in a nursing home
  - in 89 S aureus cluster of daptomycin resistant S aureus
  - SARS-Co-V2 651 samples

Sundermann A Clin Infect Dis 2021:doi10.1093/cid/ciab946

Clinical applications

- Need technologies that are pathogen specific and agnostic, POC, applicable to multiple hosts
  - No pathogen identified in 40-60% of meningoencephalitis and 25-60% of pneumonia
  - Miss or delay diagnosis results in adverse outcomes mor expensive care
- Multiplex
- Rapid, cost effective
- Cant test for everything

Rapid clinical molecular tests for diagnosis

- PCR diagnostics
  - Organism identification: multiplex GI, meningitis and respiratory panels; can detect virus/bacteria/parasites/yeast
  - Resistance gene identification
  - Mutations with clinical importance (eg HIV)
- Magnetic resonance (T2) use in candida mostly
  - The sensitivity of T2Candida, blood culture in proven cases of invasive candidiasis is **55%, 45%**

Arendrup ML Open Forum Infect Dis 2019:6;136

FDA approved multiplex

Parameter	FilmArray	Verigene	x-TAG RVP	x-TAG RVP FAST	NxTAG-RPP	eSensor RVP	ePlex
Analysis platform	FilmArray system or Biofire system	Verigene system	Luminex 100/200	Luminex 100/200	Luminex MAGPIX	eSensor	ePlex system
Acceptable specimens	NP swab	NP swab	NP swab	NP swab	NP swab	NP swab	NP swab
Number of targets	26	16	12	8	20	14	17
Pathogens							
Viruses							
Adenovirus	✓	✓	✓	✓	✓	✓ (differentiates B/E from C)	✓
Coronavirus							✓
Coronavirus HKU1	✓				✓		
Coronavirus NL63	✓				✓		
Coronavirus 229E	✓				✓		
Coronavirus OC43	✓				✓		
SARS-CoV-2	✓						
Human bocavirus					✓		
Human metapneumovirus	✓	✓	✓	✓	✓	✓	✓
Influenza A	✓	✓	✓	✓	✓	✓	✓
Subtype H1	✓	✓	✓	✓	✓	✓	✓
Subtype H3	✓	✓	✓	✓	✓	✓	✓
Subtype 2009 H1N1	✓					✓	✓
Influenza B	✓	✓	✓	✓	✓	✓	✓
Parainfluenza 1	✓	✓	✓		✓	✓	✓
Parainfluenza 2	✓	✓	✓		✓	✓	✓
Parainfluenza 3	✓	✓	✓		✓	✓	✓
Parainfluenza 4	✓	✓			✓		✓
Respiratory syncytial virus	✓	✓		✓	✓		✓
Respiratory syncytial virus A		✓	✓		✓	✓	✓
Respiratory syncytial virus B		✓	✓		✓	✓	✓
Rhinovirus/enterovirus	✓	✓	✓	✓	✓	✓	✓
Bacteria							
Chlamydophila pneumoniae	✓				✓		✓
Mycoplasma pneumoniae	✓				✓		✓
Bordetella pertussis	✓	✓					
Bordetella parapertussis	✓						
B. parapertussis/Bordetella bronchiseptica		✓					
Bordetella holmesii		✓					
Throughput	Scalable	Low	Medium	Medium	High	Medium	Scalable
Time to result	1 hour	~2.3 hours	~6 hours	~6 hours	~6 hours	~6 hours	~1.5 hours

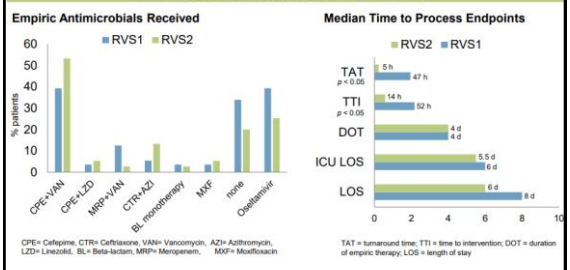
Ramanan et al Clin Micro Rev 2018;31:1-28

Utility of panels

- Variability in studies showing change in resource utilization including treatment and isolation
- Observational studies of inpatients demonstrated that PCR testing was associated with less antibiotic use in both pediatric and adult study populations, as well as increased appropriate antiviral use, compared to patients with negative test results
- The IDSA's "Guideline for the Implementation of an Antibiotic Stewardship Program" suggests the use of rapid viral testing for respiratory pathogens to reduce the use of inappropriate antibiotics. However, no recommendation is given regarding the specific viral targets
- In a study by Malachira et al. , the mean duration of contact isolation in the panel group (n=70 participants) was 1.9 days compared to 2.7 days in the conventional group (n=70 participants) (p<0.001). This difference was due to differences between groups in stool pathogen ID

Pinsky BA J Clin Micro 2019;57:e00373-19
Malachira et al Open Forum Infect Dis 2018; 224
Clin Infect Dis 2016;62:e51–e77

Antimicrobial Therapy and Primary Endpoints



https://onlinelibrary.wiley.com/doi/full/10.1111/tid.12854

Utility of Panels: INHALE WP3

- A POC PCR test helped diagnose HAP or VAP but did not lead to a higher cure rate compared with usual care.
- 550 ICU patients with HAP or VAP were randomized to testing with the FilmArray Pneumonia Panel PCR test or to usual care. 92 children and 183 patients with Covid-19 at enrollment were included.
- Significantly more assay-tested patients received "appropriate" antibiotic therapy at 24 and 72 hours (77% vs. 56% at 24 hours; 73% vs. 59% at 72 hours).
- Clinical cure rates at 14 days were nonsignificantly lower in the assay group (57% with intervention vs. 65% with usual care).
- The groups had similar mortality rates through 28 days, median ventilator-free days by day 21, and median length of stay in ICU.

Enne V et al IDWeek 2022

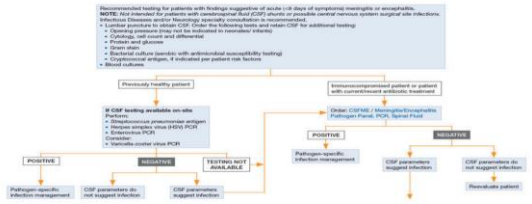
Molecular methods to detect and differentiate MSSA and MRSA .

- Is becoming a useful tool for rapid detection of bacteremia caused by MSSA and MRSA.
- Requires an active stewardship team
  - The median time to appropriate therapy was significantly shorter in the post-RDT group (15 versus 0 h, $P < 0.001$), and the mean length of stay for patients with coagulase-negative staphylococcus was significantly shorter (10.5 versus 7.7 days; $P = 0.015$).
- Useful for infection control

Bukowski PM 2018 62(11) e01334-18

MAYO CLINIC

Meningitis/Encephalitis Panel Algorithm



The Big Picture

- Transformational advancements in field of diagnostics
  - WGS
  - Sanger sequencing
  - Metagenomics, next gen sequencing
  - CRISPR
  - Biomarkers
  - Multiplex biosensing technologies

WGS

- Allows opportunity to study the evolution in real time
- How selective pressures vary over time and affect vaccine and treatment, transmission virulence, immunity especially in immune compromised
- Use for surveillance and outbreaks/impacts public health

COVID-19 highlights the need for testing and genomic capacity

- Establish testing sites
- Scaling up sequencing capacity
- Develop cloud based analytics
- Investigating transmission, variants associated with transmission, vaccine and treatment failure

WGS application to SARS-CoV-2

- Study of the ability of the virus to evolve, the traits that have been selected and the genomic regions responsible for them.
- How selective pressures vary over time, as immunity from vaccines or prior infection accumulates in the population
- The importance of monitoring long term infections in immunocompromised patients.

COVID-19 What have we learned

- We were not prepared: erosion of public health infrastructure
- Public health cannot just respond to emergencies
- Health inequity
- Build laboratory capacity
- Standardize and improve data systems/analytics/big data
- Translate research into programs/learn from response
- Improve communication/utilize media
- Build public health staffing/workforce/collaborations
- Living guidance for clinical management

We were not prepared: early testing

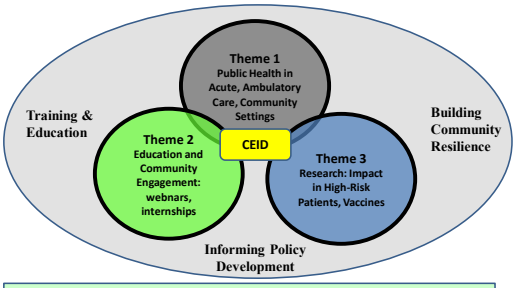
Sanchez et al MMWR 2020;69;27:882-6

Transitioning to Endemic State: The Pandemic is not over

- Many similarities to pandemic influenza. Over time, influenza became a manageable illness with vaccines and effective therapies
- We can optimize our public health response by:
  - Building up laboratory surveillance
  - Enhancing ability to provide rapid testing
  - Conducting prompt contact tracing and outbreak investigation and control
  - Addressing vaccine hesitancy and delivery issues

Genomic Surveillance Laboratory: MI-SAPPHIRE

- CEID received $4.3 million in federal funding to expand sequencing for COVID-19 and other infectious diseases
- CEID will collect and analyze genomic data to address emerging infectious disease threats and enhance the city, state and region's ability to respond to those threats
- CEID will increase lab and sequencing capacity in Detroit it starting with SARS-CoV-2 and then other infectious disease threats with the potential for broad community spread.
- Funding for the Michigan Sequencing Academic Partnership for Public Health Innovation and Response (MI-SAPPHIRE) is through a CDC Epidemiology and Laboratory Capacity grant MDHHS received.



Platforms: Building public health and research laboratory capacity, testing, vulnerable populations, bioinformatics, clinical trials, community-based participatory research, electronic health records, genomics, mobile health, practice-based research networks, surveillance, outbreak prevention

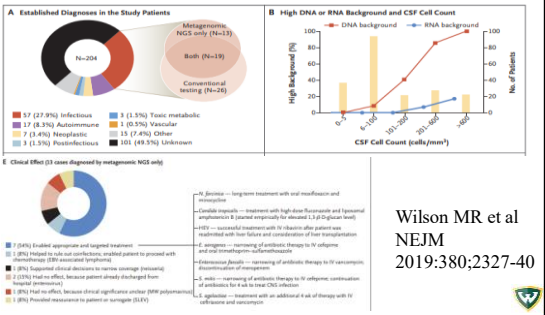
Metagenomics

- Metagenomic next-generation sequencing (mNGS) is a shotgun sequencing approach in which all of the nucleic acid (DNA and RNA) in a clinical sample is sequenced at a very high depth, 10-20 million sequences per sample.
- mNGS can be performed for any type of clinical sample, including cerebrospinal fluid, plasma, respiratory secretions, saliva, urine, stool, or tissue

Metagenomics

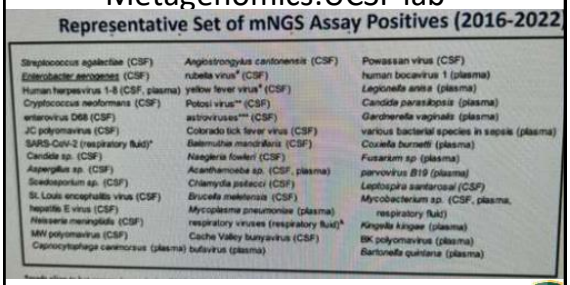
- Can be used for novel pathogen discovery/public health surveillance
- Clinical use CLIA validated
- Amenable to POC
- Relatively rapid
- ID by host response to particular condition or pathogen (eg West Nile not detected by PCR, febrile illness infection vs autoimmune)

Clinical Application Metagenomics



Wilson MR et al NEJM 2019:380;2327-40

Metagenomics:UCSF lab

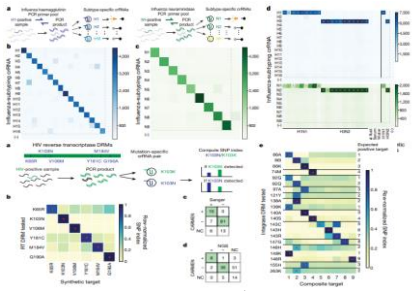


For Providers (ucsf.edu)

CRISPR

- Repeat sequences/spacers transcribed into RNA or DNA, which are complimentary to viral genome
  - CRISPR allows bacteria to defend themselves vs phages
- Cas13 for RNA viral diagnostics
- Amenable to microassay, multiplex, POC, relatively rapid
- Doesn't need informatics

CRISPR detection using cas13



Influenza subtype discrimination

HIV reverse transcriptase mutations

Ackerman C Nature 2020;582:277-82

Conclusion

•We have the power to prevent and defeat infectious diseases, but only we step up the fight

•We must pool our greatest resources—our imagination and intellect—to fight this collective fight. For as Joshua Lederberg noted, "Pitted against microbial genes, we have mainly our wits."

•We need a vision a strategy and a plan for diagnostics that can meet the challenges of the 21st century