THE MINIATURIZATION OF MOLECULAR INFECTIOUS DISEASES TESTING

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Micro amigurumi crochet created by Su Ami
Objectives of Talk

- Recognize the latest advances in molecular technology for rapid and accurate viral and other infectious diseases testing
- Describe the advantages and issues surrounding these newly-developed specimen-to-result systems
- Discuss the overall impact of these types of test systems on patient care and health outcomes
Molecular Testing Concerns

- Still not for majority of clinical laboratories
- Issues of availability or accessibility
- Laboratories struggle to overcome hurdles of budgetary constraints, facility limitations, and need for highly trained personnel
- Technology still primarily for large academic medical centers and tertiary-care facilities
- Most tests remain as laboratory-developed tests (“home-brewed”)
- A definite need to enable even simpler technologies that all can use
TECHNOLOGY TO DOWNSIZE AND SIMPLIFY MOLECULAR TESTING
More Recent Molecular Advances

- Molecular ID testing is now being downsized
- Enormous growth in nanotechnology
- Being aggressively applied to molecular testing
- Advances in microelectronics, microfluidics and microfabrication have paved way for new technologies and simplified molecular platforms
- Sample-in/answer-out testing now a reality for all laboratories regardless of size, resources, or capacity; big push to extend to point of care
Drivers of POC Molecular Testing

- Reality of healthcare budgets; reduce healthcare spending
- Need to make healthcare more patient-centered
- Assess and treat patients in primary care or the community instead of secondary or tertiary hospitals
- Real-time test results can direct timely therapeutic interventions and improve clinical outcomes
- Facilitate earlier implementation of public health responses to outbreaks and hospital responses to HAIs
- Central laboratory concept may not be suitable
- POC testing may better meet physician/patient needs
- POC tests may improve healthcare in resource-poor settings (high infectious disease burden and diagnostic challenges)
Required Features for POC Devices: ASSURED Guidelines

- Affordable – for those at risk of infection
- Sensitive – minimal false negatives
- Specific – minimal false positives
- User-friendly – minimal steps to carry out test
- Rapid & Robust – short turnaround time and no need for refrigerated storage
- Equipment-free – no complex equipment
- Delivered – to end users; safe to use

The New Era of Molecular Testing

- Multiplex capabilities have greatly improved
- Variety of commercial platforms now cleared for U.S. and International markets
- Many targets from a single sample can be readily and simultaneously assessed
- Have great potential to:
  - Detect multiple agents from a single specimen
  - Can drive disease/syndrome-specific testing
  - Detect various genotypes/genetic variants
  - Detect agent and antimicrobial resistance genes
Outcomes of New Molecular Revolution

U.S. $18 billion dollar market by 2016 for POC

Assay Miniaturization

- Portability
- Lower Cost
- Less Sample
- Less Reagents
- Decentralize Testing
- Faster Turnaround
- Improved Healthcare
- Open/Expand Market

Desire is to have self-contained, fully integrated sample-to-report devices that accept raw, untreated specimens, perform all of the molecular steps, and provide interpreted test results in < 1 h

Photos: Sandia National Laboratories, CA
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Cepheid GeneXpert Platform

- Fully integrated sample prep, amplification and detection
- Fluidic extraction cartridge and I-CORE modules
- Unprocessed sample to result in less than 1 hour
Cepheid GeneXpert Systems

- First Molecular Test in a Box!
- 1, 2, 4, 16 or 48 modules
- Each module is operated and controlled individually
- Random access; individual cartridges can be run at any time
Gene Xpert Cartridge Video
Honeycomb Reaction Tube

- Over 1,000 wells for multiplex real-time PCR of 100s of targets
- Both qualitative and quantitative assays
Drive-In-Film and Test
Dean Street Express Video
GeneXpert Omni

- Small and portable
- Simple to use
- Proven cartridge technology
- Durable
- Solid state electronics
- Integrated battery
- 9.1” (23.1 cm) H, 3.0” (7.6 cm) W, 4.2” (10.6 cm) D
- 2.2 lbs (1.0 kg) Weight
- Results via WiFi or mobile phone in 15-30 min

Future Menu
- Flu/RSV
- Xpress Group A Strep
- Xpress Flu/RSV
- HPV
- Xpress Pertussis
- Xpress GBS Ultra
- Xpress CT/NG
- Xpress Vaginitis/Vaginosis
Desire to Use for POC Service

- Doctor’s offices
- Drugstore clinics
- In the field
- At home

Not confined to regulated laboratory environments

Accelerated turnaround times for results
GeneXpert: Results While You Ride
IQuum Lab-in-a-tube (Liat) Approach

- Designed for fast, simple, as-needed, near-patient testing
- Assay processing performed in **flexible Liat tube containing pre-packed reagents**
- Fully automated **sample-to-result in ≤ 20 minutes**
- Peristaltic manipulation by sample processing actuators; **closed system**
- Real-time multi-target detection
- Liat Analyzer and Workstation
IQuum Liat System Operation

1. Add Sample
2. Scan Barcode
3. Insert Tube

Results in 20 min
Roche cobas Liat

Weighs 8.3 lbs; ~$12,000

- Influenza A/B Assay* (CLIA waived)
- Strep A Assay* (CLIA waived)
- Influenza A/B + RSV Assay*
- MRSA/SA Assay
- C. difficile Assay
- HIV Quant Assay
- HIV Qual Assay

*FDA cleared
Alere i System

“Molecular in Minutes”

- Small footprint
- Streamlined workflow; rapid throughput
- Isothermal amplification; detection using fluorescent molecular beacons
- Visual touch screen
- Easy to use in any setting
- First CLIA waived molecular tests for Influenza A & B and Group A Strep
- <15 minutes to complete test; can be used in laboratory or at point-of-care

Weighs 6.6 lbs, ~$5,000
Alere q HIV 1/2
Cartridge and Instrument

- Portable point-of-care device
- Touch screen: Intuitive and easy to use
- Built in battery; light weight (17.2 lbs)
- Multiplex capability
- Easy to use without laboratory skills or training
Alere q HIV 1/2 Specimen Collection
## Alere™ q HIV-1/2

### Intended Test Use
Test for Diagnosis of HIV Infection and Monitoring of Viral Load in HIV+ patients

### Goal of Test
Detection of HIV-1 AND/OR HIV-2 RNA in whole blood OR EDTA plasma; fully quantitative viral load measurement of HIV-1 M, N and HIV-2 LOD 100cp / test

### Analyzer Instrument
Portable device; app 5kg weight; no maintenance or calibration required

### Quantitation
Quantitative; HIV-1 and HIV-2 separately reported

### Precision
0.3 log

### Sample Specimen
25 µL of finger prick blood (25µL) or EDTA plasma

### # steps required to run the test
Test does not require any sample handling or pre-treatment by user
Alere q TB - Test

- Sputum liquification, homogenization, and filtration
- Nucleic acid purification and TB DNA enrichment
- Reagent reformulation and reaction setup
- Isothermal amplification
- Real-time fluorescence signal detection
Luminex ARIES Platform

- Fully automated, sample-to-result
- **Single use cassette with all required reagents on board**
- Cassette designed to accept different sample types, various assay protocols, and multiple analyte detection
- Runs in batch (6/slot) and single mode; 1-12 tests/box, scalable to 48 tests with 4 units
- 6 color optics for multiplexing
- Plug-and-play technology
- HSV 1/2; Influenza A, B & RSV Assays
Enigma ML (MiniLab) Platform/Cartridge
Janssen Diagnostics Biocartis Idylla

- Fully automated
- Real-time PCR
- <2min hands-on time
- Sample-to-result
- Both RNA and DNA
- Wide sample types
- Oncology, ID

CE-IVD marking for Respiratory IFV-RSV Panel
Luminex xTAG Technology

- Multiplexed nucleic acid-based test
- Couples PCR and flow cytometry to simultaneously detect multiple viral strains and subtypes
- Uses a solution-based microarray capable of combining any set of 100 single DNA tests and performing them in a single reaction
- Lasers are used to read color-coded, 5.6 micron microspheres (beads) that attach to specific nucleic acid sequences; beads are internally dyed with varying concentrations of two fluorophores to differentiate them
- xTAG RVP first product cleared by U.S. FDA for multiplex detection of viral nucleic acids. They now have the xTAG RVP Fast and the xTAG GGP FDA-cleared as well
xTAG RVP

I. Multiplex PCR
II. Exo/SAP Treatment
III. TSPE Universal Tag
IV. Tag-Anti-Tag Hybrid On Colored Beads
V. Liquid Array Detection

TAT ~7-8 h
HOT 2-2.5 h

xTAG RVP
xTAG RVP Fast
xTAG GPP

xTAG RVP Fast

I. Multiplex Tagged PCR
II. Hybridization & Detection

TAT ~5 h
HOT 1.5 h

Bead and Target Detection
BioFire FilmArray System

- Closed system for sample preparation, nested multiplex PCR, and analysis
- Chemical circuits in a pouch; sample to result in ~65-70 min
- Fully automated instrument; integrated electropneumatic systems
The FilmArray Reaction Pouch

- **Silica bead beating to release nucleic acids**
- **Magnetic bead NA extraction**
- **RT for RNA Targets**
- **Reagent Storage (freeze dried, stable @ RT)**
- **Chemical Circuit Board**
- **1st stage multiplex PCR**
- **2nd stage nested PCR**

High density array with >100 individual 2nd stage PCR wells; each well contains one reaction and results are generated from analysis of melt curves.

- **Bocavirus**
- **N2**
- **Influenza A**
- **H3**
- **Matrix**
- **NP**
FilmArray Run Set-up

1. Load Pouch
2. Inject Hydration Solution
3. Add Sample to Buffer
4. Inject Sample
5. Load Pouch in FilmArray
6. Enter Pouch/Sample ID, User Info, Press Go!
Pneumatic Bladders

Bladders inflate over blisters to push liquid

Air Channels

Pistons close channels

Pneumatic Bladders
BioFire FilmArray 2.0

72 inches wide

36 inches high

24 inches deep
BioFire FilmArray Panels

Available (US-IVD, Health Canada IVD, CE-IVD Europe)

- Respiratory Panel – 20 targets; viruses, bacteria
- Blood Culture ID Panel – 27 targets; Gram +/Gram- bacteria, yeast (Candida spp.), antibiotic resistance genes
- GI Panel – 22 targets; bacteria, protozoa, viruses
- Meningitis/Encephalitis Panel – 14 targets; bacteria, viruses, yeast

Future Applications

- BioThreat Panel – various bacterial and viral biothreat agents of significance
GenMark ePlex Sample-to-Answer System

- Fully integrated
- **Single use, self-contained cartridge**
- Multiple sample types
- Automated, extraction, amplification and detection
- Random and continuous access
- Modular and scalable configuration; 1-4 towers
- <2 min hands-on time
- Sample to result in 60 to 90 minutes
- 80 capture electrodes
Electrowetting Technology

- **Electronic fluid movement**
- Fixed temperature PCR heaters eliminate ramp rate delay
- Independent PCR lanes to optimize assay performance
- **Enables rapid thermal cycling for fast turnaround time**
- Uses same eSensor technology to read results
GenMark Dx eSensor Technology

Capture probe and signal probe complimentary to different segments of target DNA

Form complex at surface of electrode

Electrochemically active label
ePlex Menu of Tests

**Respiratory Pathogen Panel (RP)**
Viral and bacterial targets from nasopharyngeal swab

**Blood Culture Identification**
**Gram-Positive Panel (BCID-GP)**
Bacterial & resistance targets from positive blood culture

**Blood Culture Identification**
**Gram-Negative Panel (BCID-GN)**
Bacterial & resistance targets from positive blood culture

**Gastrointestinal Pathogen Panel (GI)**
Bacterial, viral, and parasitic targets from stool samples

**HCV Genotyping Panel (HCVg)**
Typing and subtyping of HCV 1a, 1b, 2a/c, 2b, 3, 4, 5, and 6 from plasma or serum

**Central Nervous System Panel (CNS)**
Bacterial, viral, and fungal targets from cerebrospinal fluid samples

**Fungal Pathogen Panel (FP)**
Fungal targets associated with bloodstream infections from positive blood culture
Nanosphere Verigene SP System

- Verigene Reader and Processor
- **Gold nanoparticle technology**
- Microarray-based detection platform
- One user pipetting step
- <5 min hands-on time
- Sample-to-result automation
- **Random access**
- TAT of ~3.5 h

Functionalized with sequence-specific oligos

- RV+
- BC-GP
- Test Cartridges
- Reader
- Processor
Nanosphere Verigene System

Consumables

- Loading Reader SP
- Loading Processor SP

- Test Cartridge
- Tip Holder
- Extraction Tray
- Amplification Tray
Nanosphere Verigene Test Cartridge

- Test Cartridge
- Reagent Pack
- Substrate Holder
**Gold Nanoparticle Probe-Based Chip Assay**

- **Gold nanoparticle probe**
- **Signal Enhancement**
- **Target**
- **Target 1**
- **Target 2**
Verigene Clinical Microbiology Tests

**Available (US-IVD; Outside US)**

- **RV+** – RSV A/B, influenza A/B w typing (AH1, AH3, A2009 H1), H275Y resistance gene
- **RV-Flex** – Full panel of respiratory bacteria and viruses
- **BC-GP** – *Staphylococcus, Streptococcus, Enterococcus, Micrococcus, Listeria*, resistance genes (mec A, van A, van B)
- **BC-GN** – various Gram- bacteria, resistance genes (KPC, NDM, CTX-M, VIM, IMP, OXA)
- **CDF** – tcdA gene, tcdB gene, PCR ribotype 027 hypervirulent strain differentiation
- **EP** – various bacteria, stx1 & stx2 toxin genes, viruses

**Future Applications**

- **BC-Y** – broad panel of most common yeasts in blood cultures
Great Basin Portrait System

- Sample in-result out cartridge (Helicase-Dependent Isothermal Amplification)
- All reagents on board; one-time use
- Minimal hands-on time, few steps, insert and go
- Chip-based detection; multiplex up to 64 targets/assay
- FDA clearance for GBS, Shiga Toxin Direct Test, Toxigenic *Clostridium difficile*, and Staph ID/R Blood Culture Panel; in development - CT/NG Test, *Bordetella* Direct Test, Nasal SA Pre-Screen, *Candida* Blood Infections Panel, Stool Bacterial Pathogens Panel
Quad Card

Sample Application
Nucleic Acid Purification
PCR
Endpoint detection

EncompassMDx Optimum

Fully automated, sample in-result out
Open system – can use own reagents

Small, Tecan Liquid Handler
Rheonix Card Cartridge Video
A Rapid, Self-confirming Assay for HIV: Simultaneous Detection of Anti-HIV Antibodies and Viral RNA

Zongyuan Chen¹, Hui Zhu¹, Daniel Malamud²,³, Cheryl Barber², Yhombi Yvon Serge Ongagna², Rubina Yasmin¹, Sayli Modak², Malvin N. Janal², William R. Abrams², and Richard A. Montagná¹,*
¹Rheonix, Inc., Ithaca, New York, USA

Rheonix's Dual Immunoassay, NAAT Zika Test Wins Supplemental SBIR Funding

Apr 19, 2016
NEW YORK (GenomeWeb) – "The Zika virus appears to disappear from blood in six to 10 days, but is still detectable in saliva and urine," Daniel Malamud, Rheonix's collaborator at NYUCD said in a statement. "Anti-Zika antibodies can be detected several days after infection. A combined RNA and antibody test will enable detection of both early and late Zika virus infections."
Focus Dx Simplexa/3M Cycler

- **Microfluidic/Microelectronic Centrifugation Platform**
- **3M Integrated Cycler**
  - Universal Discs (96 wells)
  - Direct Amplification Discs (8 wells)
- **Real-time PCR assays**

12” (31 cm) H x 12” (31 cm) D x 8” (21 cm) W; 15 lb (7 kg)
FocusDx Simplex Direct Assays

- 8 well plate
- Built-in extraction reagents
- Add sample and PCR reagents
Simplexa Assays

**U.S. Market**
- Flu A/B & RSV Direct
- Flu A/B & RSV
- HSV 1 & 2 Direct
- Influenza A H1N1 (2009)
- *C. difficile* Universal Direct

**International**
- BKV
- *Bordetella* Universal Direct
- *C. difficile* Universal Direct
- CMV
- Dengue
- EBV
- Flu A/B & RSV
- Flu A/B & RSV Direct
- Influenza A H1N1 (2009)
- Group A Strep Direct
- HSV 1 & 2 Direct
BD Max System

- Fully automated, random access platform
- Easy to use; 1-2 pre-processing steps
- Rapid turn-around time; 45-90 min
- Moderate throughput; ~150 results/shift
- Small footprint for instrumentation
- IVD Products – GBS, MRSA, MRSA XT, C. difficile, StaphSR, Enteric Bacterial Panel, Enteric Parasite Panel
- Open System Configuration - LDAs
BD MAX System

Sample Vial

Unitized Reagent Strip

PCR Cartridge

Instrument
Cartridge and Miniature Thermal Cycler

- Sealed amplification/detection chambers
- Dedicated 2-color optics per PCR lane; LED excitation, photodetectors
- Microvalve
- PCR Reactor (4 µl)
- Inlet Hole
- 24 lane cartridge
- 12 Independent PCR Lanes
- 2 x 24 NanoValves
- Microheaters
- Temperature Sensors
- Reaction Zone

Microthermal circuit wafer enables rapid thermal cycling - ~20 sec/cycle
Operating a BD Max System

One Manual Pipetting Step

Load Reagents and Specimens

Place Rack in BD Max

Load PCR Cartridge

Place on BD Max

Create Worklist and Close Door to Initiate Run
Tetracore T-COR 8 Instrument

- Real-time and isothermal assays
- 8 independent wells; random access
- Touch screen (10.4 in) with virtual keyboard
- 4 dye channels; expandable to 6; multiplex capable
- Small footprint, lightweight design (9.9 lbs)
- **Battery powered with power backup** (4 h thermocycling, 8-10 h in isothermal mode; no system downtime)
- Integrated barcode scanner
- **Cloud ready via WiFi or Ethernet**
- Designed for CLIA waiver
4-Steps for Flu and Strep Assays: **SLyDR** Method

- **Swab**
- **Lyse**
- **Drop**
- **Run**
Software: **PCR Hosted Online Gateway** or “**PHOG**”

- **PHOG** – PCR Hosted Online Gateway
- Software that monitors every instrument on a given network
- **Instruments report up into the PHOG in real-time**
- Interactive Dashboard allows simple view on network
- Information in the PHOG can be “pushed” to e-mail/text
  - Alerts can be automatically sent to specified recipients
  - LIS/LMR/EMR interfaces communicate with PHOG
- **Applications include:**
  - Epidemiology
  - Patient management
  - Resource management
  - Supply chain management
  - Quality reporting
Software: PCR Hosted Online Gateway or “PHOG”
Turn Your iOS Device Into a Disease-Detecting Mobile DNA Lab

- Biomeme
- **Smartphone centric PCR machine**
- Connected wirelessly via Bluetooth
- **Slot into portable PCR instrument; use app**
- iPhone camera used to detect amplified product
- ~$1,000 cost
- Ultimate goal is to change how we think about molecular testing
Fluorescentric Handheld Device

- Uses smartphone for optics, data collection, telemetry
- XCR – extreme chain reaction; “blazing” fast amplification (5 min)
- Integrated fluorescence detection
- Extremely low power requirement; no moving parts, no fluidic movement
- Low cost portable instrument
- Low cost reagents
- Compatible with existing PCR
QuantumDx

- A startup company has invented a DNA analyzer that could potentially revolutionize healthcare in the developing world.
Study Describes ‘World’s Smallest’ qPCR Platform – genomeweb, December 23, 2015

Lab on a Chip

- Report on one of smallest fully autonomous real-time PCR systems to date
- Weighs 90 grams and measures 10 cm long, 6 cm wide, and 3.3 cm tall
- Uses a 12V battery for power
- Process two samples + 1 positive and 1 negative control using 200 nl of sample placed in a virtual reaction chamber
- Uses standard 40-cycle PCR in <35 min
- Captured data is stored on device and exported via USB port
Lab-On-A-Drone

Drone-based mobile bioanalysis

Inexpensive, low power

In-flight PCR

Ebola  S. aureus

USB  USB  hand
battery  battery  crank

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analytical chemistry

Lab-on-a-Drone: Toward Pinpoint Deployment of Smartphone-Enabled Nucleic Acid-Based Diagnostics for Mobile Health Care

Aashish Priye,† Season Wong,‡ Yuanpeng Bi,†,‡ Miguel Carpio,†,‡ Jamison Chang,†,‡ Mauricio Coen,†,‡ Danielle Cope,†,‡ Jacob Harris,†,‡ James Johnson,†,‡ Alexandra Keller,†,‡ Richard Lim,†,‡ Stanley Lu,†,‡ Alex Millard,†,‡ Adriano Pangelinan,†,‡ Neal Patel,†,‡ Luke Smith,†,‡ Kamfai Chan,‡ and Victor M. Ugaz*†,‡

Anal Chem 2016. 88:4651-4660
Conclusions

- Miniaturization and simplification of highly complex molecular procedures is a reality.
- Will see more high performance, easy-to-use, fully integrated, specimen-to-result, multiplexed molecular platforms.
- Should extend availability of molecular diagnostics to every laboratory and even to point-of-care and non-traditional testing sites.
- Will shape future of pathogen identification, monitoring of drug susceptibility and disease progression, and surveillance.
- May need to re-think our approach to testing.
LET'S HAVE ONE MORE
AND THEN WE'LL GO!!

Any questions?