TECHNOLOGY TO DOWNSIZE AND SIMPLIFY MOLECULAR TESTING

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Richard L. Hodinka, PhD
To be announced
Molecular Diagnostic Technology Evolution

- Improved performance over traditional methods
- Faster turnaround time
- Impact on patient care and management
Molecular Testing Concerns

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

- Molecular ID testing is rapidly evolving and being downsized.
- Nanotechnology is growing by leaps and bounds and being aggressively applied to molecular testing.
- Advances in microelectronics, microfluidics and microfabrication have paved the way for new technologies and simplified molecular platforms.
- Ultimate goal is sample-in/answer-out testing for all laboratories regardless of size, resources, or capacity.
POC Molecular Testing

- Tremendous interest in using nanotechnology to provide simple, low-cost POC molecular diagnostics
- Explosion of lab-on-a-chip devices fabricated in wide range of materials using different fabrication techniques and forming diverse microfluidic systems
- Particularly true for HIV, TB, dengue fever, *E. coli* and malaria in high-burden, resource-poor settings
Integrated, Self-Contained Cassette for Isolation, Amplification, and Detection of NA

- Miniaturized fluidic network of reaction and incubation chambers, mixers, filters, membranes, valves, fluid actuators, conduits, and various interconnections all formed in a plastic substrate or chip
- Sample lysis, nucleic acid isolation, PCR, and detection of labeled PCR products on a lateral-flow strip
- To be used with companion instrument to drive the process or operated without instrumentation

Laboratory of Dr. Haim H. Bau, University of Pennsylvania Department of Mechanical Engineering and Applied Mechanics
LabChip Real-Time PCR for Influenza A

1. Isolate viral RNA from clinical samples.

2. RT (Reverse Transcriptase) reaction.
   - cDNA synthesis


4. Prepare Real-time PCR mixture by using NBS 2X SYBR Green Master Mix.
   - + cDNA
   - Distilled water
   - NBS 2x master mix

5. Inject 15 μl of reaction mix into each channel on Labchip.


   - Labchip case inserted into Real-time PCR instrument
   - Time to result, 15 min
Outcomes of New Molecular Revolution

- 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.
Selected Available Nanotechnologies

- Nanochips and Nanoarrays
- Gold and Silver Nanoparticles
- Centrifugal Microfluidics
- Actuated Microfluidics
- Quantum Dots
- Fluorescent Polymeric Nanoparticles
- Magnetic Nanoparticles
- Nanobiosensors
- Nanopores
So How Far Have We Come In Downsizing the Laboratory?
Selected Molecular Platforms Using Nanotechnology

- Cepheid GeneXpert
- IQuum Liat System
- Luminex xTAG Technology
- BioFire (ITI) FilmArray
- PrimeraDx ICEPlex System
- Nanosphere Verigene SP
- Autogenomics Micro Array
- GenturaDx Idbox System

- Focus Diagnostics Simplexa/3M Cycler
- Quidel/Biohelix BESI Cassette
- GenMark Dx eSensor
- BD Max System
- Meridian Illumigene
- Ion Torrent/Life Technologies
- Nanopore Technologies
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
GeneXpert Cartridge
Internal View of Cartridge

Actuated Microfluidics
Cepheid GenXpert Tests

**IVD**
- Xpert EV
- Xpert Flu
- Xpert van A
- Xpert *C. difficile*
- Xpert *C. difficile*/Epi
- Xpert MRSA
- Xpert MRSA/SA SSTI
- Xpert MRSA BC
- Xpert GBS
- Xpert SA Nasal Complete

**RUO**
- Xpert MTB/RIF

(endorsed by WHO to facilitate diagnosis of MDR-TB in high-burden, low-resource settings)
IQuum Lab-in-a-tube (Liat) Approach

- Assay processing performed in flexible Liat Tube containing pre-packed reagents
- Fully automated sample-to-result
- Peristaltic manipulation by sample processing actuators
- Real-time multi-target detection
- Liat Analyzer and Workstation
Liat Automation

Liat platform couples a flexible reaction vessel with mechanical elements that move fluid and control temperature.

- **Moveable clamp**: controls direction of inter-compartment seal bursting
- **Flexible reaction vessel**: unit-dose reagents separated by breakable seals
- **Moveable plunger**: fluid motion and temperature control
- **Stationary thermal controllers**: temperature control
IQuum Liat System Operation

1. Add Sample
2. Scan Barcode
3. Insert Tube
   Results in 20 min

Done
Liat Influenza A/B Assay

- **Assay target:** Influenza A & B
- **Sample to result time:** ~20 min
- **Sample collection:** NP swab
- **Assay chemistry:** silica magnetic bead extraction & multiplex real-time PCR detection
- **Internal control:** RNA process control to minimize likelihood of a false negative result

<table>
<thead>
<tr>
<th>Assay</th>
<th>Viral Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
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<tr>
<td>Liat</td>
<td>Positive</td>
<td>34</td>
<td>13</td>
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<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>388</td>
<td>388</td>
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<tr>
<td></td>
<td>Total</td>
<td>34</td>
<td>401</td>
<td>435</td>
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</tbody>
</table>

* Of 13 false positive samples, 8 were Influenza A positive, 4 were negative, and 1 was indeterminate by PCR/sequencing due to low sequence quality score.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Viral Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
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</thead>
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<tr>
<td>Liat</td>
<td>Positive</td>
<td>30</td>
<td>24</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>381</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>405</td>
<td>435</td>
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</table>

* Of 24 false positive samples, 13 were Influenza B positive, 3 were negative, and 8 samples were indeterminate by PCR/sequencing due to low sequence quality score.

<table>
<thead>
<tr>
<th>Assay</th>
<th>%</th>
<th>95% CI</th>
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<tr>
<td>Liat</td>
<td>Sensitivity</td>
<td>100.0%</td>
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<tr>
<td></td>
<td>Specificity</td>
<td>96.8%</td>
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</table>

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<th>95% CI</th>
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<tbody>
<tr>
<td>Liat</td>
<td>Sensitivity</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>94.1%</td>
</tr>
</tbody>
</table>
Liat HIV Quantitative Assay

- Sample-to-result detection and quantification of HIV at POC
- **Sample:** plasma or whole blood
- **Assay chemistry:** silica magnetic bead extraction & multiplex real-time PCR detection
- **Assay time:** ~30 min
- **LOD:** 50-78 copies/ml
- **Dynamic range:** 10-10^6 copies/ml
- **Reactivity:** HIV-1 group M, clades A-H, group O
Clinical Evaluations of Liat HIV Quant

University of North Carolina
April 2010, 80 clinical samples

University of Washington
June 2010, 120 clinical samples

Roche Amplicor HIV Monitor 1.5
Abbott m2000 HIV Assay

\[ y = 0.98 + 0.12 \]
\[ R^2 = 0.92 \]
GenturaDx Idbox System

- Fully automated, sample-to-result (22 inches (1/2 meter) square
- Single use cassette with all required reagents on board
- Cassette designed to accept different sample types, various assay protocols, and multiple analyte detection
- Idbox runs in batch (6/slot) and single mode; 1-12 tests/box, scalable to 48 tests with 4 units
- 6 color optics for multiplexed real-time qualitative and quantitative detection
- Plug-and-play technology
- Assays
  - HSV 1, 2
  - Influenza A, B and RSV
- Recently acquired by Luminex
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
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

Analysis

xTAG RVP
xTAG RVP FAST
xTAG GPP

Bead and Target Detection

I. Multiplex Tagged PCR
II. Hybridization & Detection

TAT ~5 h
HOT 1.5 h
BioFire (ITI) FilmArray System

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

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

- Sample Injection Port
- Cell Lysis
- DNA/RNA Purification
- PCR I
- PCR II
- Water Injection Port
- Reagent Storage (freeze dried, stable @ RT)
- Chemical Circuit Board

- Silica bead beating to release nucleic acids
- Magnetic bead NA extraction
- RT for RNA Targets

- Bocavirus
- N2
- Influenza A
- H3
- Matrix

1\textsuperscript{st} stage multiplex PCR
2\textsuperscript{nd} stage nested PCR
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!
Bladders inflate over blisters to push liquid

Pneumatic Bladders

Air Channels

Pistons close channels
BioFire FilmArray Panels

● **Available** *(US-IVD, Health Canada IVD, CE-IVD Europe)*
  - Respiratory Panel – 20 respiratory pathogens (17 viruses; 3 bacteria)

● **Future Applications**
  - Blood Culture ID Panel – Gram +/Gram- bacteria, fungi *(Candida spp.)*, antibiotic resistance
  - GI Panel – bacteria, diarrheagenic *E. coli/Shigella* spp., protozoa, viruses
  - BioThreat Panel – various bacterial and viral biothreat agents of significance
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+

Test Cartridges

BC-GP

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 Based Chip Assay

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
- **BC-GP** – *Staphylococcus, Streptococcus, Enterococcus, Micrococcus, Listeria*, resistance genes (mec A, van A, van B)

**Future Applications**

- **BC-GN** – various Gram- bacteria, resistance genes (KPC, NDM, CTX-M, VIM, IMP, OXA)
- **CDF** – tcdA gene, tcdB gene, BI/NAP₁/027 hypervirulent strain differentiation
- **EP** – various bacteria, stx1, stx2, viruses
- **RV-XP** – Full panel of respiratory viruses and H275Y resistance gene
GenMark Dx eSensor XT-8 System

Capture Probe
Electrodes are coated with a capture probe specific for a viral target; 72 distinct electrodes

Sample Amplicon Loading Reservoir

Pneumatic Pump Membrane
Pumps sample solution through the cartridge chambers

EEPROM
Pre-programmed memory chip contains the test protocol, lot number, and expiration date

Assay based on traditional PCR; TAT ~5.5 h, 40 min HOT
Multiplex detection using biosensor cartridge
Cartridges are self-contained and configured for a given test
XT-8 instrument has modular design consisting of base module and up to three test cartridge-processing towers of eight cartridge slots each
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

eSensor RVP (FDA-licensed)
Focus Dx Simplexa/3M Cycler

- Microfluidic/Microelectronic Centrifugation Platform
- 3M Integrated Cycler
  - Universal Discs
  - Direct Amplification Discs
- Real-time PCR assays
3M Integrated Cycler

- PCR on CD-sized compact disc; similar to 96-well plate
- Extracted NA and reagents into sample wells
- Centrifugal force to mix reagents and move fluids to periphery for PCR reaction
- Uniform infrared heating, high velocity fan cooling and optical detection; 20 sec cycle times
- Small footprint – 12” (31 cm) H x 12”(31 cm) D x 8” (21 cm) W; 15 lb (7 kg)
- 96 samples in 30-75 min; run user-developed tests
- Easy integration into all laboratories
Direct Amplification Disc

- 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
- 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
Quidel AmpliVue System

- Quidel and Biohelix Corporation Partnership
- Simple Sample Preparation-No thermal cycling
  - Sample Prep – Heat lysis-95°C, 10 min; can tolerate crude samples (e.g., blood, fecal material)
  - Amplification – Isothermal, single temperature (64°C) for 60 min
- No large equipment, no capital expenditure; only requires a heat block or water bath
- Uniform pipetting volumes; lyophilized reagents
- Simplified, lateral flow, self-contained detection
- Product Pipeline – Assays for C. difficile, S. aureus, MRSA, HSV 1 & 2 (IsoAmp HSV), HIV
Quidel AmpliVue System

Helicase Dependent Amp

BESSt Cassette Detection

Step 1. Helicase unwinding and primer binding

Step 2. DNA polymerization

Step 3. Target DNA amplification

Biohelix Express Strip (BESSt)
BESt Cassette Detection

Step 1: Amplicon Cartridge

Step 2

Step 3

Detection Chamber

Step 4

Step 5
Meridian Bioscience Illumigene

- Sample Preparation – Heat lysis-95°C, 10 min; can tolerate crude samples (e.g., blood, fecal material)

- Loop-mediated isothermal amplification (LAMP) – single temperature (65°C) for 40 min

- No large equipment; vortex, pipette, heat block and provided heater/reader

- Simplified detection

- LAMP applied to many different microorganisms

- *C. difficile*, GAS, GBS Assays
Great Basin Portrait PA5000 System

Helicase-dependent amplification with biotin-anti-biotin HRP detection
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
- 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

24 lane cartridge

Microvalve
PCR Reactor (4 µl)
Inlet Hole

2 x 24 NanoValves
12 Independent PCR Lanes
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
Benchtop Sequencing Systems

Roche 454 GS Junior

Life Technologies Ion PGM

Illumina MiSeq

Life Technologies Ion Proton
## Benchtop Sequencing Systems

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Chemistry</th>
<th>Read Length (bases)</th>
<th>Run Time (h)</th>
<th>~ List Price</th>
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<tbody>
<tr>
<td>454 GS Junior</td>
<td>Pyrosequencing</td>
<td>400</td>
<td>10</td>
<td>$100,000</td>
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<tr>
<td>Ion PGM</td>
<td>Proton Detection</td>
<td>35-200</td>
<td>0.5-4.5</td>
<td>$80,000</td>
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<tr>
<td>Ion Proton</td>
<td>Proton Detection</td>
<td>Up to 200</td>
<td>2-4</td>
<td>$145,000 + $75,000 for compulsory server</td>
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<tr>
<td>MiSeq</td>
<td>Reversible Terminator</td>
<td>36-250</td>
<td>4-39</td>
<td>$125,000</td>
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</table>

*Cost per run varies by instrument but ≤$1,000*
Ion Semiconductor Sequencing

First post-light sequencer

Personal Genome Machine (PGM)

Powered by Scalable Silicon Chips

Torrent Server and Databases
Simple Natural Chemistry
Ion PGM: Fast Direct Detection

DNA → Ions → Sequence

- Nucleotides flow sequentially over Ion semiconductor chip
- One sensor per well per sequencing reaction
- Direct detection of natural DNA extension
- Millions of sequencing reactions per chip
- Fast cycle time, real time detection
- Directly translates chemical signals into digital information
Nanopore Sequencing

Oxford Nanopore Technologies, U.K.

Involves threading long, unbroken strands of DNA or RNA through a membrane pore using an enzyme, and measuring resultant changes in electrical current as nucleotide bases pass through it.

Nanopore chips can process DNA at a rate of 20-400 bases/second/pore.

No sample amplification necessary.
Nanopore Sequencers

500 nanopores

GridION Node
2,000 nanopores
Digital PCR – Next Generation PCR

Clinical Utility

- Quantification with no standard curve
- Detect virus at extremely low levels
- Use low amounts of sample and reagents
- High multiplex capability
- Sequencing
- Variant ID

Select Manufacturers

- Fluidigm Access Array
- Life Technologies OpenArray
- Bio-Rad Droplet RainDance RainDrop
Palm PCR – Any Where, Any Time

- Palm-sized, portable, stand-alone thermal cycler; 3 x 5 x 2 inches and ~3/4 of a lb (350 g)
- Powered by a Li+ battery; 4 h on single charge
- Use indoors and outdoors
- Complete cycling in 18-39 min; no temperature ramping
- 400 bp amplicons in 18 min with sensitivity below 10 copies/rxn
- End-point PCR
- Commercially available for <$10,000
Conclusions

- Miniaturization and simplification of highly complex molecular procedures is a reality
- Will see more high performance, easy-to-use, specimen-to-result, multiplexed molecular platforms
- Should extend availability of molecular diagnostics to every laboratory and even to point-of-care
- Will shape future of pathogen identification, monitoring of drug susceptibility and disease progression, and surveillance
- May need to re-think our approach to testing