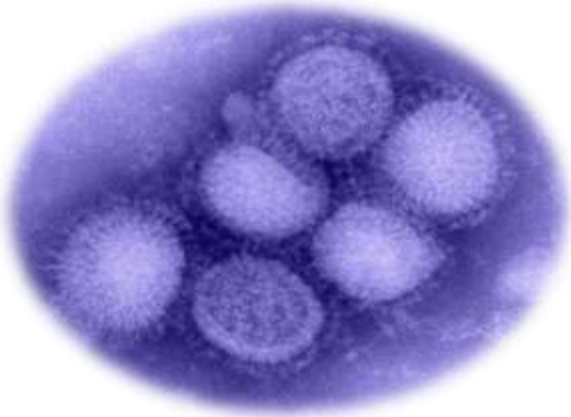
Molecular Diagnostics for Influenza & Other Respiratory Viruses



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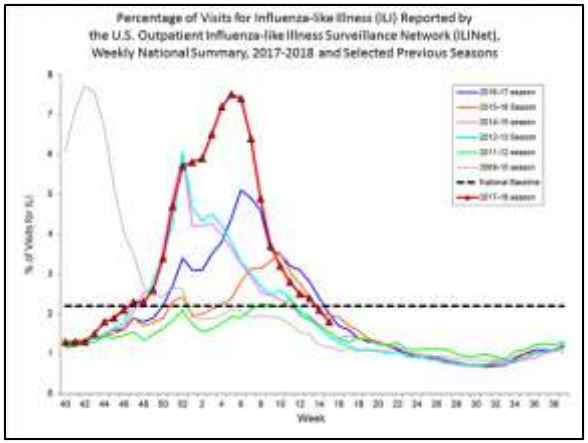
Disclosures

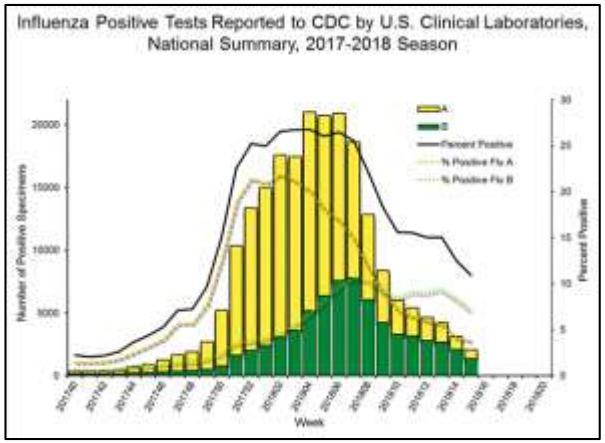
Nothing to Disclose

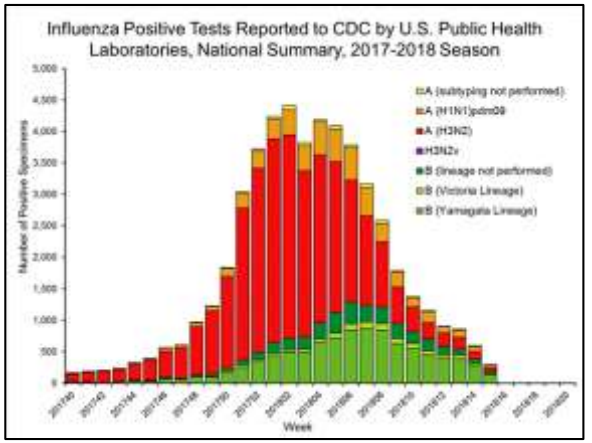
Objectives

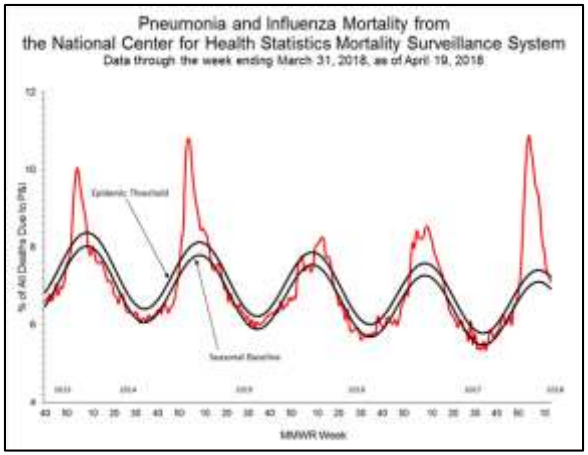
- Discuss the newest molecular technologies available for the rapid detection of influenza viruses and other respiratory viruses
- Explain the advantages and disadvantages of these rapid nucleic acid amplification tests
- Discuss how the newest molecular technologies may change the clinical and diagnostic paradigm in the care and management of individuals with respiratory illnesses

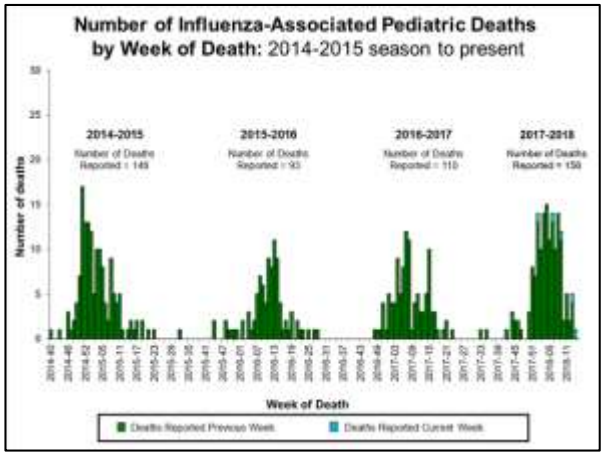
2017-18 Influenza Season

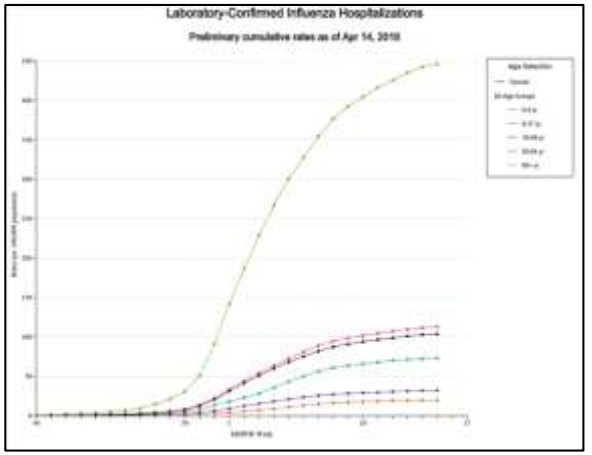




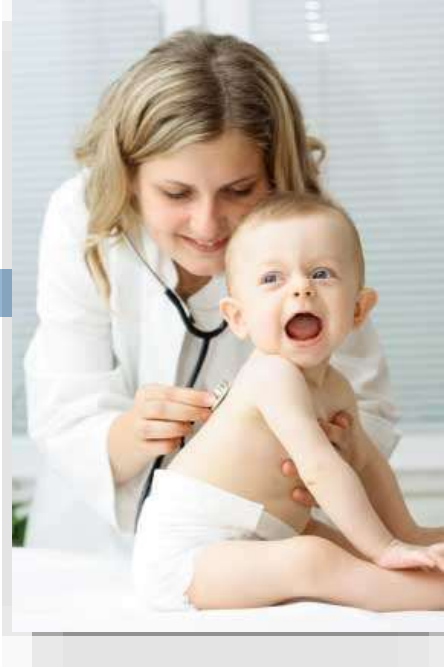








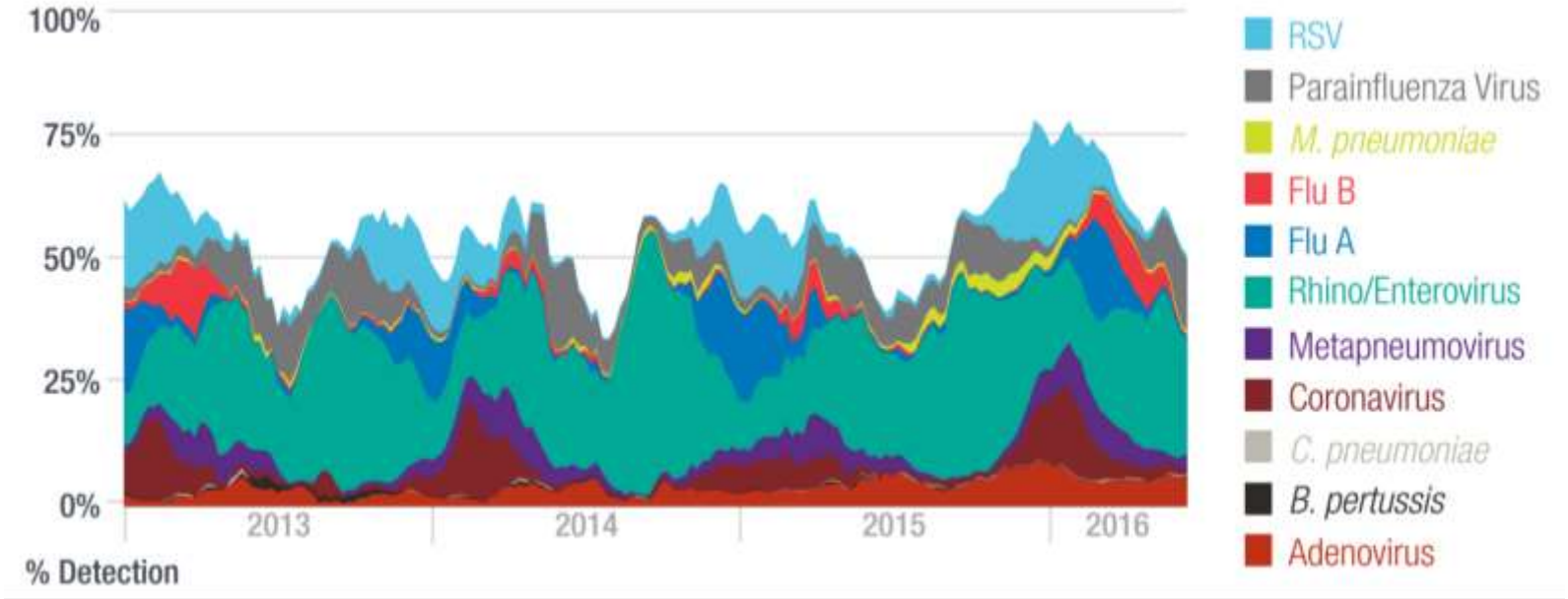
Clinical Diagnosis of RPs



- Can be difficult
- Wide array of pathogens with seasonal variation
- Signs and symptoms often overlap
  - May vary with age, underlying conditions, previous infection, and circulating type
  - Not always specific for any one organism
- Particularly true in children
- Laboratory needed

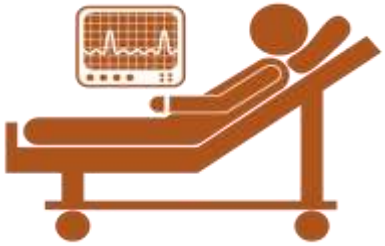
Who Is At Greatest Risk for RIs?

- The very young
- The elderly
- The chronically ill
- Those with immune compromise



Clinical and Economic Consequences of Respiratory Infections In the United States

25,000,000
family physician consultations[1]



34%
of 4.5 million primary ID hospital days/yr. due to LRTI between 1998 and 2006[2]

$40 billion
estimated annual cost of non-influenza–related viral respiratory tract infections[3]

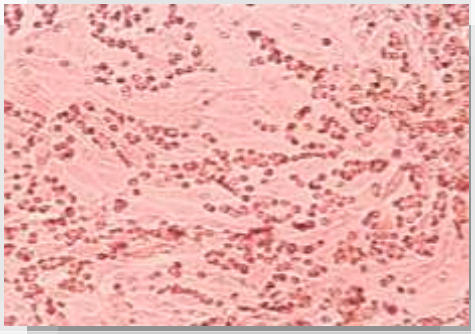
WHO estimates: 1.9-2.2 million childhood deaths annually and 20% of all hospitalizations in children <5 yrs. attributable to severe acute respiratory illness

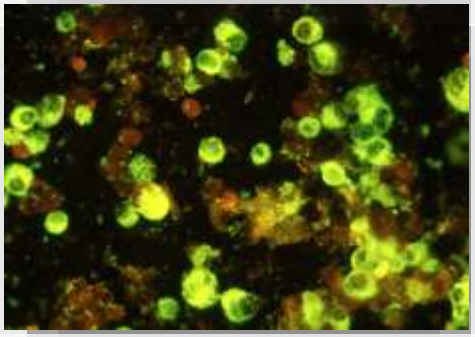
Heikkinen T, Järvinen A. *Lancet*. 2003;361:51-59.
Christensen KLY et al. *Clin Infect Dis*. 2009;49:1025-1035.
Fendrick AM et al. *Arch Intern Med*. 2003;163:487-494.

Laboratory Diagnosis of Influenza

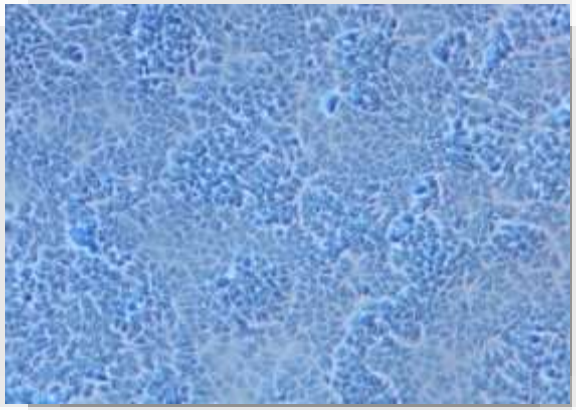
- Viral Culture
  - Conventional Tube Culture
  - Rapid Shell Vial/Plate Culture
- Rapid Antigen Detection
  - Solid-Phase Immunoassays (SPIA)
  - Immunofluorescence (IFA)
- Detection of Nucleic Acid
- Serology

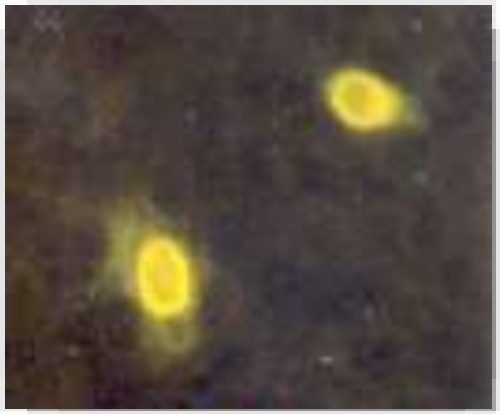
Traditional Tube Cultures



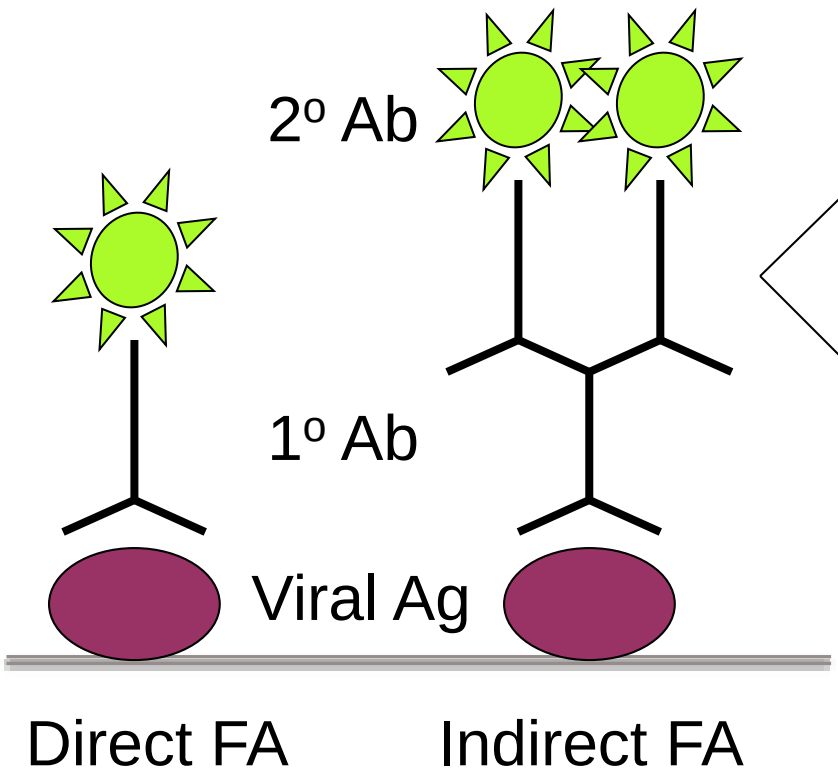


Rapid Cell Culture Systems

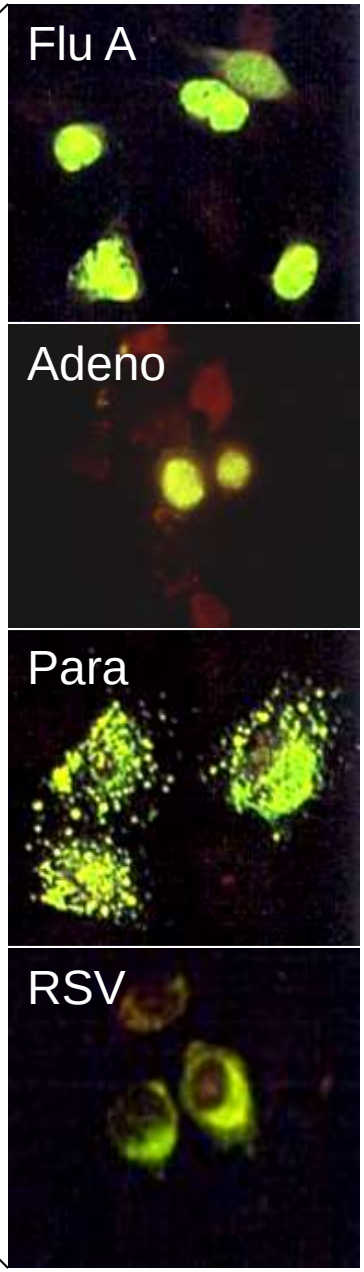




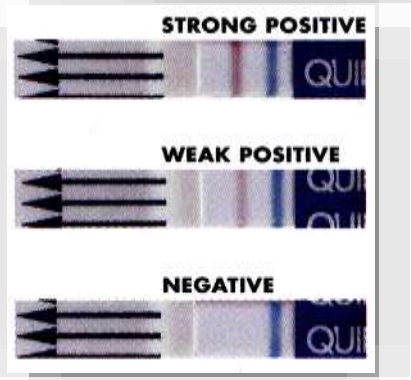
Immunofluorescence



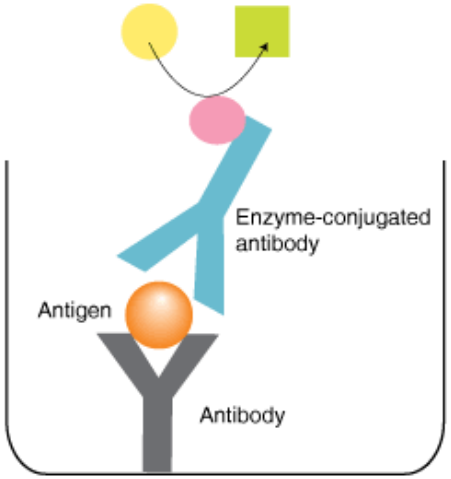
- Monoclonal Abs
  - Cocktail
  - Individual
- DFA: 15-30 min
- IFA: 30 min/Ab



Rapid Influenza Detection Tests



- Self-contained devices; MFT, LF, OIA
- Easy to use; moderate or waived complexity
- Can do point-of-care or near-patient testing
- Assay steps are minimal
- Rapid results (15-30 min)
- Built-in internal control
- Can batch or do one at a time



Accuracy of RIDTs

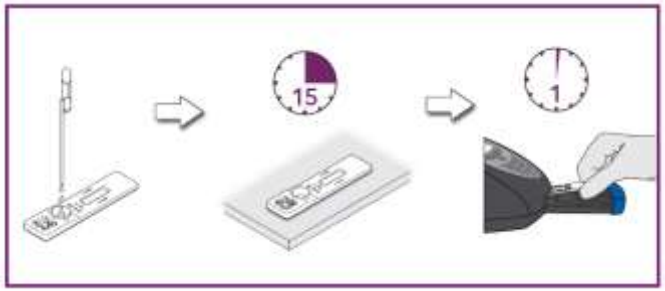
- False-negatives are highly likely (sensitivity normally ~40-80%; 10-70% for 2009 H1N1)
- Specificity generally good (85% to 98%), but false-positives will occur
- May vary by patient age, specimen type, specimen adequacy and storage, virus type/subtype, emergence of new strains
- PPV and NPV are highly dependent on prevalence
  - High: false + less likely; false – and true + more likely
  - Low: false + and true - more likely; false – less likely

FDA Reclassification of RIDTs

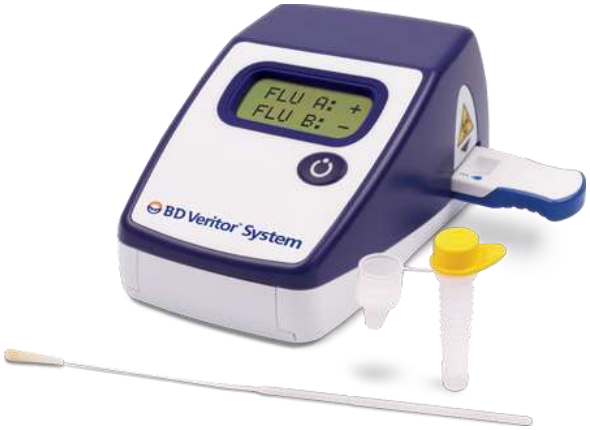
- In 2017, reclassified from Class I to Class II devices
- Must meet new minimum performance standards
- Requirement for annual reactivity (inclusivity) testing for current circulating virus strains
- On 12 January 2018, FDA began enforcement
- Many previously available RIDTs were removed from U.S. market; now only 6 devices

Newer Digital Immunoassays

Quidel Sophia



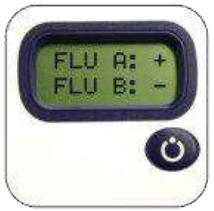
BD Veritor



Easy sample processing
Unitized tube containing the correct volume of process reagent facilitates workflow

Ready in minutes
Test device is ready to insert into reader 10 minutes after sample is added

Insert and read
Simple one-touch button readies the reader for test device insertion



Results delivered
Once the test device is inserted in the reader, an objective, digital test result is diplayed in 10 seconds

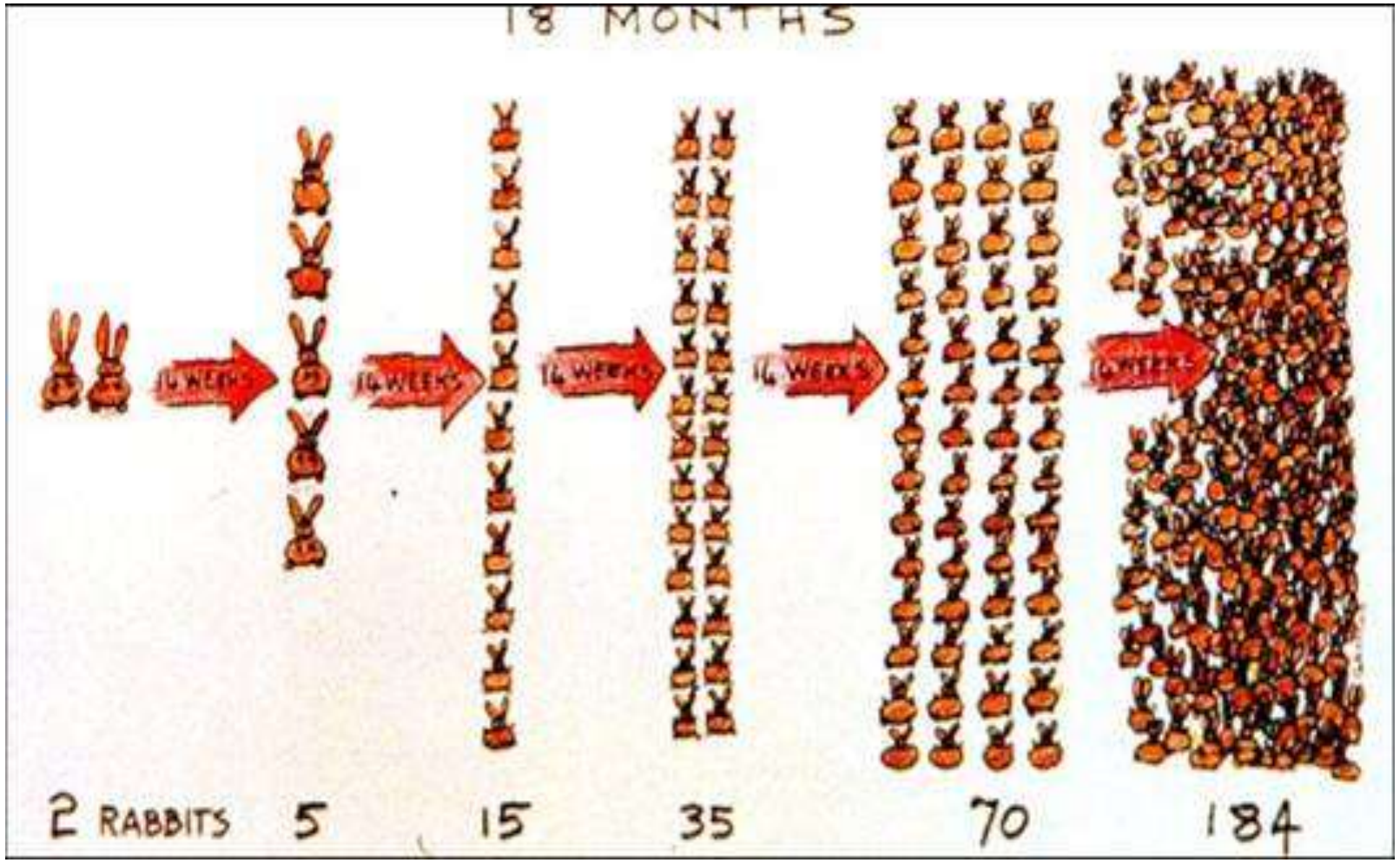
Newer Digital Immunoassays

- Developed to improve sensitivity and specificity of RIDTs
- Use instrument-based digital scan
- DIAs consistently outperform visually read RIDTs; false-positives have been reported
- Offer objective results with reduction in reader variability
- Are less sensitive than molecular assays

Bundling of RV Tests

- Over the years, **bundled tests for broad coverage** and increased sensitivity
- Rapid solid phase immunoassays
- Immunofluorescence Assays
- Rapid cell culture systems
- Comprehensive tube viral cultures

Nucleic Acid Amplification in Virology



Molecular Testing Over The Years

- **Extensive growth** and development over several decades
- **Significant advantages** over more conventional methods
- Industry is totally driven by technology
- Steady growth fueled by **new technologies, automation, innovations, expanded test menus**
- Device manufacturers have invested in clinical trials and pursued CE-IVD and FDA clearance

The New Era of Molecular Testing

- More technological breakthroughs
- **Major change** in molecular testing landscape
- Our **multiplex capabilities** have **greatly improved**
- **Multiple commercial platforms** now licensed for U.S. and International markets
- Redefining the diagnosis of infectious diseases
- **Have great potential to**:
  - Detect multiple agents from a single specimen
  - **Drive disease/syndrome-specific testing**
  - Detect various genotypes/genetic variants
  - Detect antimicrobial resistance genes
- **Multiplex assays now increasingly used in everyday clinical practice and at POC**

Molecular Multiplex RP Panels

- Have reached the greatest maturity over years
- Now have multiple commercial assays/platforms
  - **Highly multiplex assays** for broad detection of many pathogens on large scale
  - **Low-density assays** designed to detect smaller and more focused number of pathogens
  - More **simplified CLIA-waved tests** for specimen-to-result analyses
- **Predominantly for viruses**; small number of bacteria

Highly Multiplexed PCR Platforms

- **Syndrome-Based Diagnostics**
- One sample-multiple results
- **One-size-fits-all**
- **Comprehensive panels** of probable pathogens causing a particular syndrome
- Currently designed to test for respiratory, bloodstream, central nervous system, GI, and sexually transmitted infections and infections in transplant recipients

Highly-Multiplex Molecular RP Tests

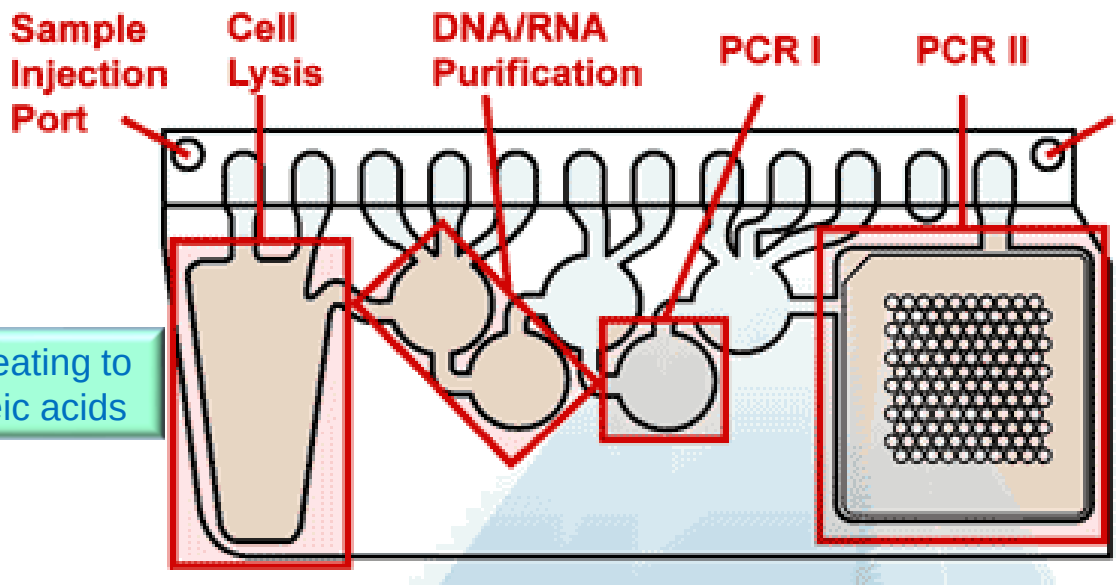
Platform	No. of Targets	Technology	Time to Result	Status
BioFire FilmArray RP	20	Nested real-time PCR on microarray with melt curve analysis	65 min	MC
BioFire FilmArray RP2	21		45 min	MC
BioFire FilmArray RP EZ	14		65 min	W
GenMark XT-8	14	PCR with electrochemical sensor detection	8h	HC
GenMark ePlex	21		1.5 h	MC
Luminnex xTAG RVP	12	End-point PCR with bead-based flow cytometry detection	7-8 h	HC
Luminex xTAG RVP FAST	9		5-6 h	HC
Luminex NxTAG	20	End-point PCR with magnetic bead fluorescent-based detection	5 h	MC
Luminex Verigene RP Flex	16	End-point PCR with microarray gold nanoparticle detection	2 h	MC
STAT Dx DiagCORE (CE-IVD)	22	Real-time PCR with fluorescence-based detection	1 h	MC

W, CLIA-waived; MC, moderate complexity; HC, high complexity

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



Reagent Storage (freeze dried, stable @ RT)

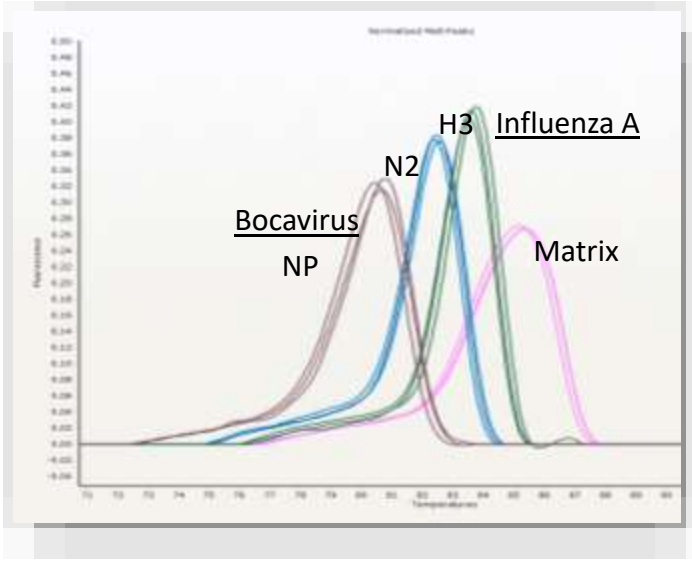
Chemical Circuit Board

Silica bead beating to release nucleic acids

1st stage multiplex PCR
2nd stage nested PCR

Magnetic bead NA extraction

RT for RNA Targets



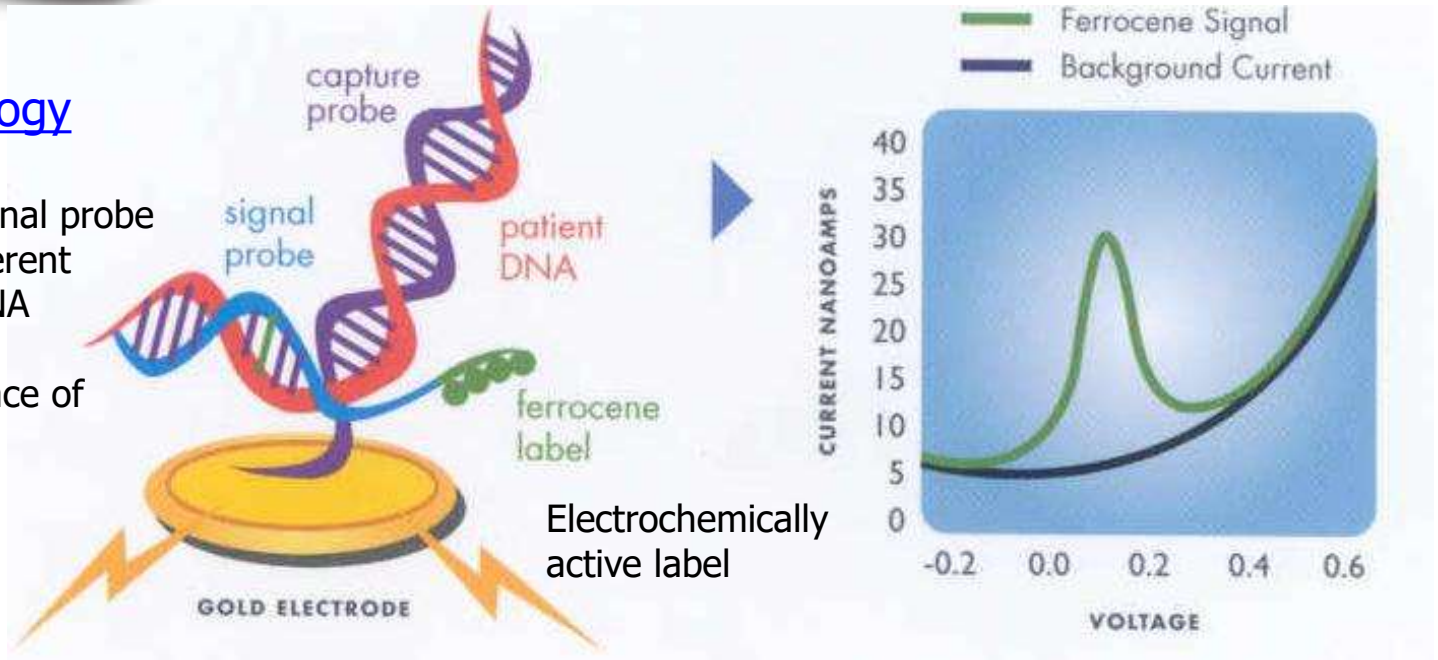
High density array with >100 individual 2nd stage PCR wells; each well contains one reaction and results are generated from analysis of melt curves

GenMark ePlex Sample-to-Answer System

[eSensor Technology]

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

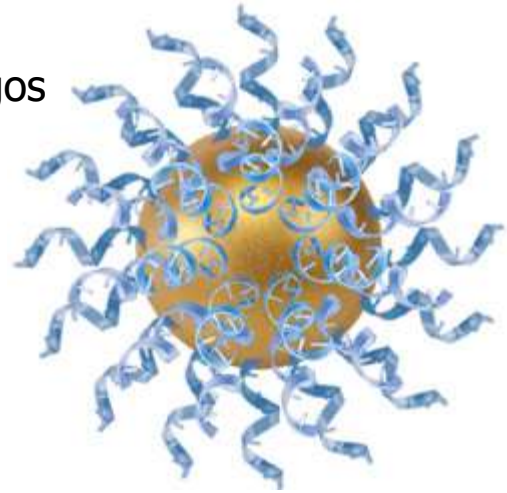
Form complex at surface of electrode



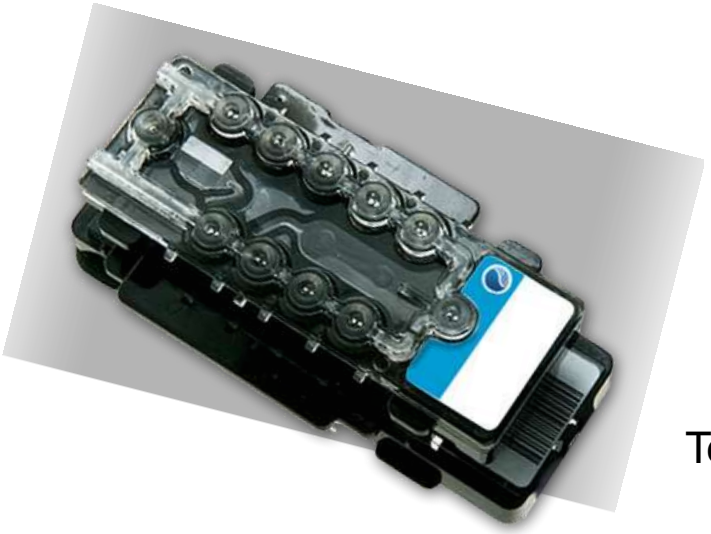
Electrochemically active label

Luminex Nanosphere Verigene SP System

Functionalized with sequence-specific oligos



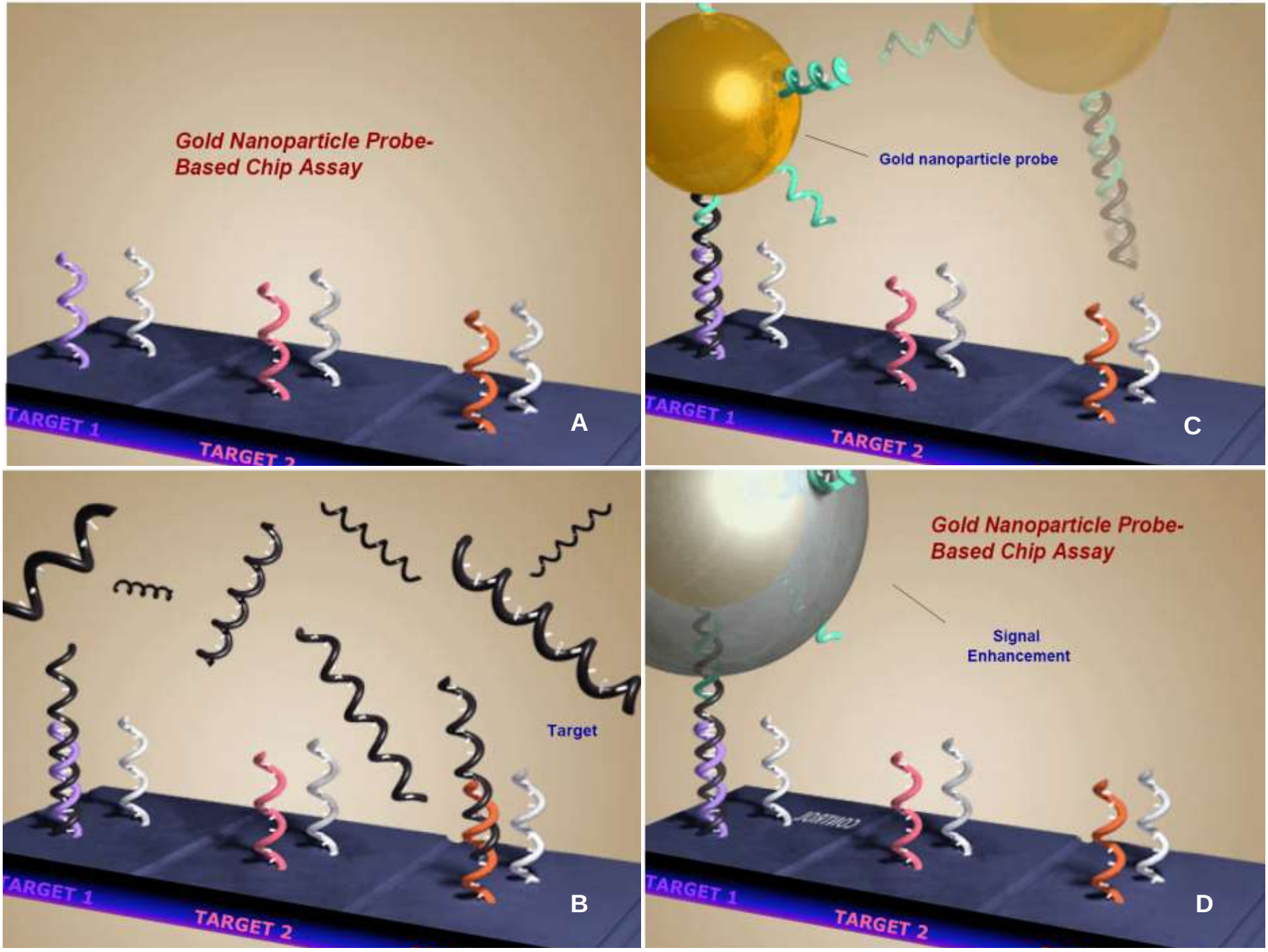
- Verigene Reader and Processor
- **Gold nanoparticle technology**
- **Microarray-based detection platform**
- One user pipetting step
- <5 min hands-on time
- Sample-to-result automation
- **Random access**
- TAT of ~3.5 h



Test Cartridge

Reader

Processor


Gold Nanoparticle Probe-
Based Chip Assay
TARGET 1
TARGET 2
A
Target
B
Gold nanoparticle probe
C
Signal
Enhancement
D

Luminex NxTAG System

- Closed system
- High throughput runs
- 1-96 samples
- Customize selection of targets
- Up to 20 pathogens in single test

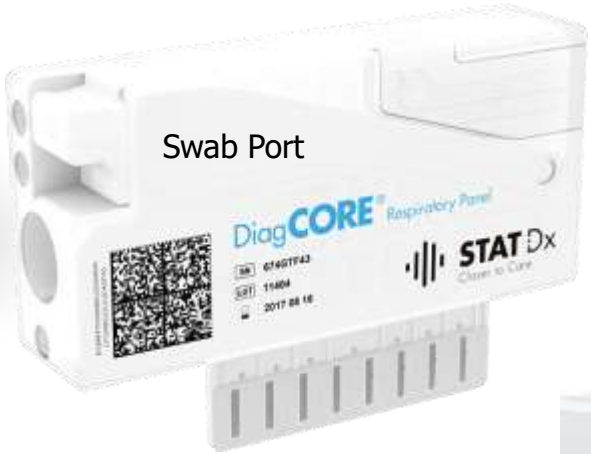
Step 1
Add 1-96 extracted samples to pre-plated test wells

Step 2
Integrated multiplex PCR and bead hybridization

Step 3
Read on MAGPIX®

Qiagen STAT Dx DiagCORE System

- Extraction, amplification, detection all in one cartridge
- All reagents on board
- <1 minute hands on time
- Sample to result in ~1 hour



Liquid Samples
up to 1.5 ml

8 PCR Reaction
Chambers
Each 6-Plex Capability

Pathogens in Highly Multiplex Panels

Pathogens	FilmArray			GenMark		Luminex				STAT Dx
	RP	RP2	RP EZ	XT-8	ePlex	xTAG	xTAG F	Nx TAG	V Flex	DiagCORE
Adenovirus	●	●	●	●	●	●	●	●	●	●
RSV (No Group Differentiation)	●	●	●				●			●
Groups A & B				●	●	●		●	●	
Influenza A (No Type Differentiation)	●	●	●	●	●	●	●	●	●	●
Influenza A Subtypes H1 & H3	●	●	●	●	●	●	●	●	●	●
Influenza A Subtype 2009 H1N1	●	●	●	●	●			●		●
Influenza B	●	●	●	●	●	●	●	●	●	●
Parainfluenza (No Type Differentiation)			●							
Parainfluenza Types 1, 2, 3	●	●		●	●	●		●	●	●
Parainfluenza 4	●	●			●			●	●	●
Metapneumovirus	●	●	●	●	●	●	●	●	●	●
Rhinovirus/Enterovirus	●	●	●	●	●	●	●	●	●	●
Coronavirus (No Type Differentiation)			●							
Coronavirus NL63, HKU1, 229E, OC43	●	●			●			●		●
Bocavirus								●		●
Chlamydophila pneumoniae	●	●	●		●			●		
Mycoplasma pneumoniae	●	●	●		●			●		●
Bordetella pertussis	●	●	●						●	●
Bordetella (other species)		●							●	
Legionella pneumophila										●

Other species - B. parapertussis(RP2, V Flex)/B. bronchiseptica (V Flex)/B. holmesii (V Flex)

Molecular Multiplex RP Panels

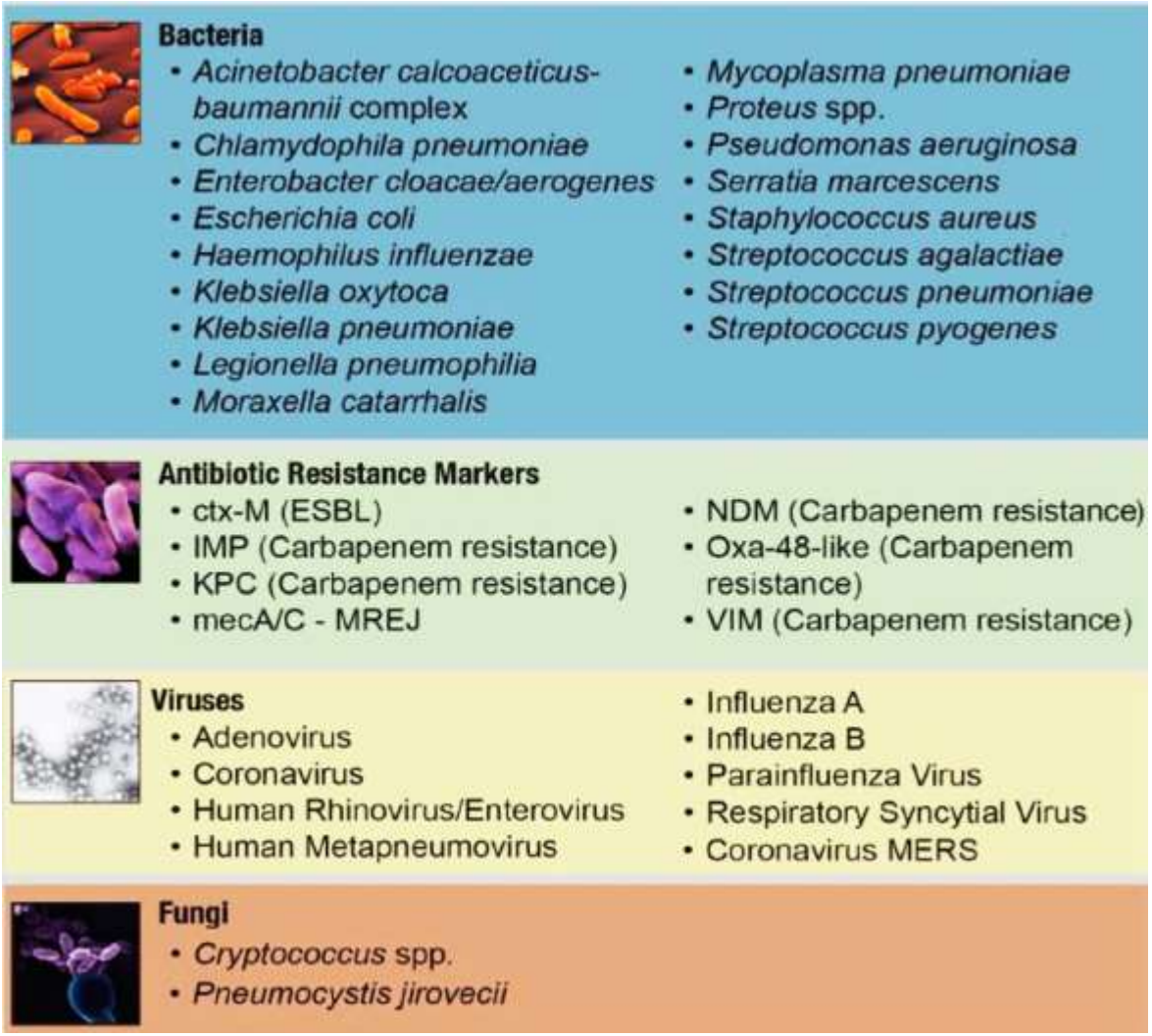
- **Comparable performance characteristics** seen from one manufacturer to another
- **Some differences** in sensitivity and specificity **for specific pathogens**
- Normally not as sensitive as single-target LDTs
- No single multiplex test covers continuum of respiratory pathogens
- **Technical differences** in number and types of pathogens detected, throughput, turnaround time, specimen source, ease of use, automation, versatility, cost

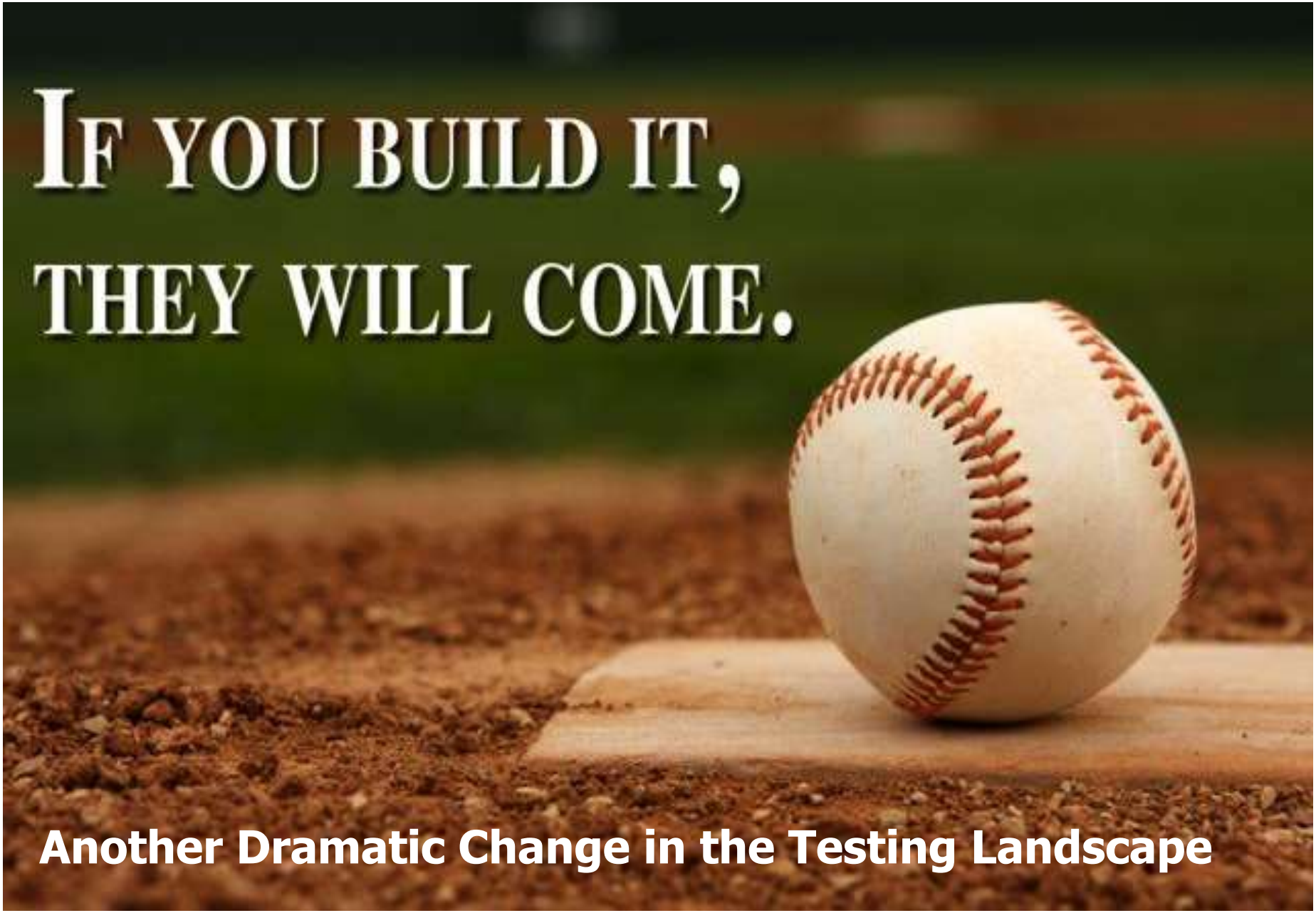
FilmArray Respiratory Panel EZ

- **14 respiratory viral and bacterial targets**
- CLIA-waived version of CE-IVD, FDA-cleared respiratory panel
- **Performed in ~1 hour**
- Sample type: nasopharyngeal swab
- Designed to run on a single computer/instrument configuration of FilmArray 2.0 System
- **Currently not available outside U.S.**

LRTI Molecular Multiplex Panel

- BioFire FilmArray
- **17** bacterial targets
- **9** viral targets
- **2** fungal targets
- **7** select resistance gene markers
- Sputum and BALs




IF YOU BUILD IT,
THEY WILL COME.
Another Dramatic Change in the Testing Landscape

Compact Specimen-to-Result MDx Tests

- **Further downsizing of processes and platforms**
- **Designed to be used at point-of-care** in same settings as rapid antigen tests
- Physicians' offices, hospital ED/ICU, walk-in clinics, drugstores, at home, in the field
- **Small, fast, simple-to-use, accurate**
- Results available at time of patient-physician interaction
- **Performance shown to be similar to other available molecular-based laboratory assays**
- **Paradigm shift towards decentralized testing**

Key Features of Systems

- Self-contained products and instruments
- Utilize unprocessed samples
- No sophisticated operation requirements or training
- No intervention between steps
- Little to no need for equipment maintenance
- No manual result analysis

Compact Specimen-to-Result MDx RP Tests

Platform	Targets Detected	Time to Result	Status
Alere i System	Influenza A & B; RSV (separate kits)	<15 min	W
	Influenza A & B 2 (second generation)	<5 min	Seeking W
Cepheid GeneXpert	Flu A & B; Flu A/B & RSV (separate kits)	60-75 min	MC
Cepheid GeneXpert Omni	Flu A & B; Flu A/B & RSV (separate kits)	20 min	W
Focus Dx Simplexa	Flu A/B & RSV	30 min	MC
Janssen Diagnostics	Flu A/B & RSV, discriminates between H1, H3, 2009 H1, H275Y oseltamivir resistance mutation	50 min	MC
Luminex AIRES	Flu A/B & RSV	<2 h	MC
Mesa Biotech Accula	Flu A/Flu B Test	30 min	W
Quidel Solana	Influenza A & B, Respiratory Viral Panel (Flu A/B+RSV+hMPV), RSV+hMPV	40 min	MC
Roche cobas LIAT	Flu A & B; Flu A/B & RSV (separate kits)	20 min	W

W, CLIA-waived; MC, moderate complexity

Compact Specimen-to-Result Systems

Cepheid GeneXpert

Cepheid GeneXpert Omni

Roche Cobas Liat

Alere i System

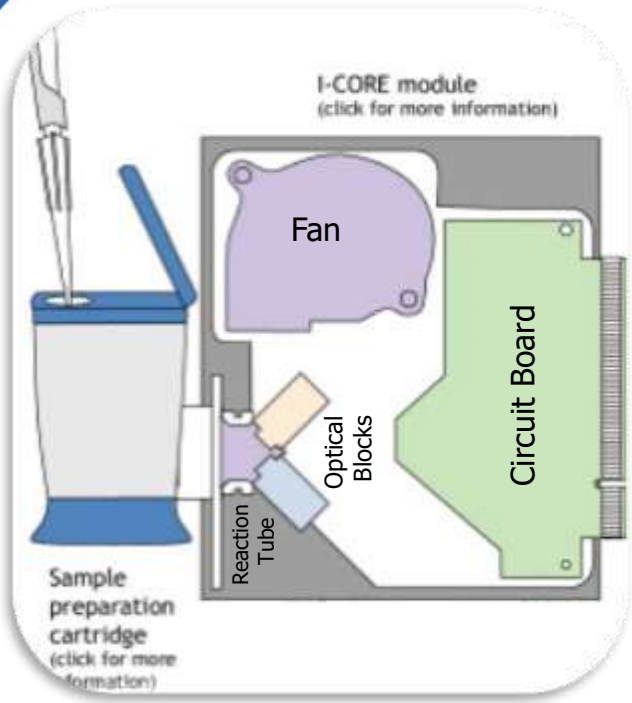
Luminex ARIES

Focus Dx

Janssen Dx

Quidel Solana

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

GeneXpert Cartridge Inner Workings

Processing Chamber

Contains reagents, filters, and capture technologies necessary to extract, purify, and amplify target NA

Reaction Tube

Allows for performance of rapid thermal cycling and optical excitation/detection

Valve Body

By turning, it directs fluids into different chambers and PCR tube

Cepheid GeneXpert Systems

- First Molecular Test in a Box!
- 1, 2, 4, 16, 48 or 80 modules
- Each module is operated and controlled individually
- Random access; individual cartridges can be run at any time

Infinity-48s/80

I

II

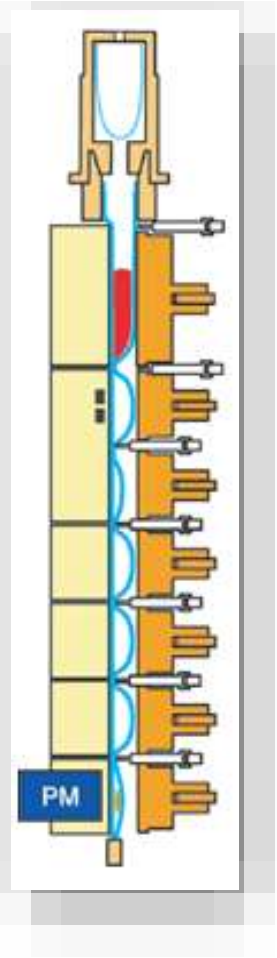
IV

XVI

GeneXpert Omni Xpress System

- Point-of-care system
- 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 Wi-Fi on mobile phone in 15-30 min

Roche Cobas Liat (Lab-in-a-tube)



- Flexible Liat tube
- Pre-packed reagents
- Fully automated
- Closed system
- Processing actuators for peristaltic manipulation
- Real-time PCR

Weighs 8.3 lbs.; ~$12,000

Alere i System

Materials Required to Run a Test:

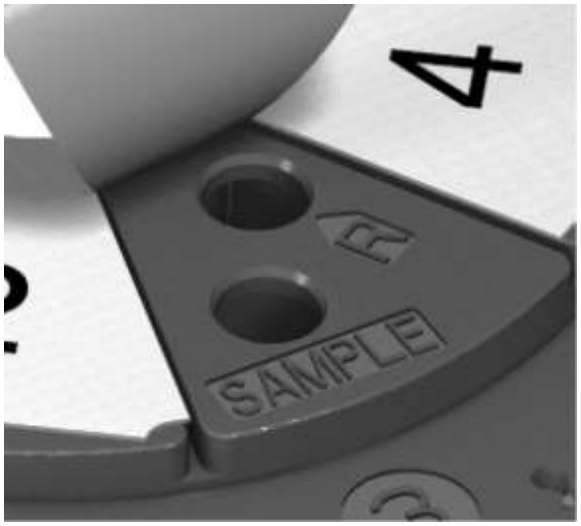
"Molecular in Minutes"

Instrument:

Weighs 6.6 lbs., ~$5,000

- **Small footprint**
- **Streamlined workflow; rapid throughput**
- **NEAR (Nicking Enzyme Amplification Reaction)**
- One constant temperature (**Isothermal**); detection using fluorescent molecular beacons
- Visual touch screen
- Easy to use in any setting; **can be used in laboratory or at point-of-care**

FocusDx Simplex Direct Assays



- 8 well plate
- Built-in extraction reagents
- Add sample and PCR reagents
- **Flu A/B & RSV Direct**

Lab Benefits of Multiplex RP Panels

- **Enhanced detection** rates compared to conventional methods
- Allows for **rapid turnover of results**
- **Access** to routine testing for pathogens that have previously been difficult to detect
- Allows for **consolidation** of testing methods
- **High throughput** automated testing
- **Enhances operational efficiency** and **improves cost effectiveness** of testing

Clinical Benefits of Multiplex RP Panels

- **More definitive diagnosis** allow clinicians to provide higher quality of care to their patients
- **Simplifies** the testing algorithm
- **Reduces** the number of tests and specimens to be ordered
- Obtain results in **clinically relevant and actionable timeframe**
- Potential for **reduction** in overall health care costs
- **Improved** patient care and patient/provider satisfaction

Clinical Benefits of Rapid and Accurate RP Diagnosis

- Informing timely and effective antibiotic or antiviral therapy

- Preventing secondary spread of infection
- Shortening hospital stays

- Reducing costs of unnecessary tests

- Provide a specific diagnosis; early informed decision making
- Help manage high-risk patients (e.g., cancer, transplant, HIV, those in ICU, those with underlying co-morbidity)
- Education and clinical awareness
- Rapid outbreak ID at local, regional, national, and global levels

Will Molecular Testing Result in Improved Outcomes?

Publication	Outcome (Peds)	Conclusion
Mahoney et al., 2009	**Lower Costs ~ $291 less/case, $529,620/yr saved decrease length of stay (>90% of costs)**	**YES**
Dundas et al., 2007	**Offers significant cost savings from reduced labor, greater efficiency, and potential revenue from referral testing**	**YES**
Wishaupt et al., 2011	**No significant difference in hospital admissions, length of stay and antibiotics used**	**NO**
Van de Pol et al., 2011	**Antibiotic prescribing practices did not change**	**NO**
Doan et al., 2012, Cochran Review	**Evidence insufficient to support routine RV diagnosis as means to reduce antibiotic use; rapid RV testing does reduce the rate of chest x-rays in the ED**	**NOT AT THIS POINT**
McCulloh et al, 2014	**Improved appropriate osletamivir treatment; negative patients had more antibiotics started, positive patients saw modest D/C in antibiotics; RVP enhanced physician decision-making**	**YES**
Rogers et al, 2014	**Multiplex testing improved antibiotic usage, shortened length of stay, and reduced amount of time patients spent in isolation**	**YES**
Nelson et al., 2015	**Cost effectiveness model found molecular testing to be most effective approach for evaluation acute respiratory infections for hospitalized patients**	**YES**

Will Molecular Testing Result in Improved Outcomes?

Publication	Outcome (Adults)	Conclusion
Oosterheert, et al, 2005	**No statistical difference in reduction of antibiotic treatment; increased treatment and diagnostic costs**	**NO**
Brittan-Long et al, 2011	**Associated with with decrease in unnecessary antibiotic use**	**YES**
Blaschke et al, 2013	**For influenza diagnosis, decreased antibiotic treatment and ancillary tests, Improved antiviral prescriptions; rapid results may result in more efficient & appropriate care**	**Yes**
Hernes et al, 2014	**No statistical difference in reduction of antibiotic treatment or length of hospital stay**	**NO**
Rappo et al., 2016	**Decrease in unnecessary antibiotics, ED length of stay, need for hospital admissions, number of chest radiographs**	**YES**
Green et al., 2016	**For adult outpatients, testing positive for influenza was associated with receiving fewer antibiotic prescriptions; no such effect seen for non-influenza viruses**	**YES/NO**
Gadsby et al., 2016	**Significantly improved of pathogen detection in CAP, particularly in antimicrobial-exposed patients; also may enable early de-escalation from broad-spectrum empirical antimicrobials to pathogen-directed therapy**	**YES**
Lowe et al., 2017	**Targeted antimicrobial stewardship intervention facilitated reduction in duration of antibiotic treatment**	**YES**
Brendish et al., 2017	**Point-of-care molecular testing was associated with reduced length of stay, improved influenza detection and antiviral use, and use of single doses or brief courses of antibiotics**	**YES**

Issues/Obstacles for Multiplex RP Panels

- **Cost containment** (e.g., capital expense, annual service contracts, cost/test)
- **Cost-benefit analysis** – paucity of outcome-based studies demonstrating direct benefit to patient care; reality is such studies are exceedingly difficult to perform
- **Reimbursement** for testing
- **Limited flexibility** – fixed panels at fixed costs
- **Limited clinical experience** with certain pathogens, asymptomatic shedding, and co-detections
- Like all NAATs, persistence of residual nucleic acid may confound result interpretation

Commercial Payer Coverage

- **Largely silent** on whether they will cover costs of molecular multiplex RP
- **No specific mention** of molecular multiplex RP in most health plan coverage policies
- **Language** that does exist **is fairly vague**
  - "Will be reviewed for medical necessity on case-by-case basis"
  - "Based on review of available data, may consider eligibility for coverage
- Payment most likely **associated with coding** without specific coverage policy in place

Reimbursement for Multiplex RP

Procedure	CPT Code	2017 Medicare National Limit
Prior to 2013		
Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism	87798	$48.14
Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, reverse transcription and amplified probe technique, each type or subtype	87501	$70.39
After 2013 – new codes for multiplex RP		
Infectious agent detection by nucleic acid (DNA or RNA); respiratory viruses, includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, **3-5 targets**	87631	$175.98
…**6-11 targets**	87632	$298.77
…**12-25 targets**	87633	$571.72

Palmetto GBA MolDX LCD

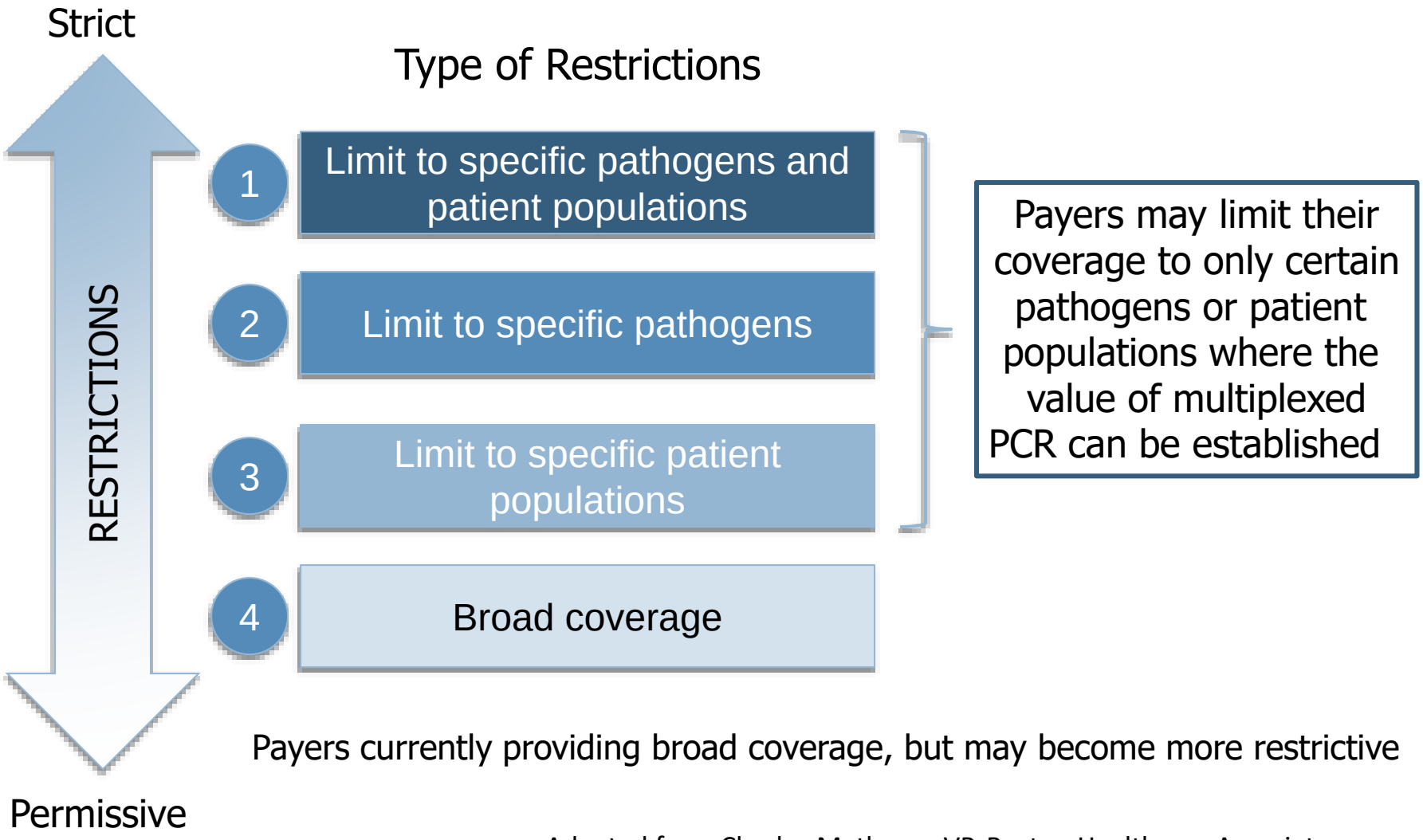
- Proposed local **non-coverage determination** for molecular multiplex RVP
- **Rationale**:
  - Pathogens detected often do not share overlapping symptoms
  - Lack of clarity on performance (sensitivity and specificity)
  - No clinical utility studies demonstrate that rapid, accurate multiplexed NAAT tests decrease use of empirical antibiotics and allow for more targeted approach to using antivirals

"The multiplex PCR respiratory viral panels are effectively a "one size fits all" diagnostic approach, and do not meet Medicare's reasonable and necessary criteria"

"The use of highly multiplexed NAAT tests as frontline diagnostics cannot be justified at the current time. A panel that includes pathogens that are very rare, or a panel in which all pathogens do not cause overlapping clinical syndromes…is not reasonable or necessary"

Adapted from Charles Mathews, VP, Boston Healthcare Associates

Payer Approaches to Multiplex Panels



Adapted from Charles Mathews, VP, Boston Healthcare Associates

What Drives Ordering Patterns

- Base primarily on **clinical presentation**
- **Needs vary** by season, geography, and even from patient to patient
- **Patient demographics**
  - Inpatient vs. outpatient
  - Underlying conditions
  - Children, the elderly
  - Otherwise healthy adults
- **Hospital committee decisions** – infection control
- Desired **turnaround time** (TAT)

Options for Molecular RP Testing

- **Single target** – Serial one-opt; hunt-and-peck
- **One-size-fits-all** – large multiplex panels
- **Smaller panels** for specific pathogens
- **Coupling** of smaller panels AND one-size-fits-all strategy

Perspective on Flexible Testing

- Highly multiplexed “one-size-fits-all” panels can be costly and do not always meet diverse testing needs
- **Verigene RP *Flex* Test**
  - Broad panel of 16 viral and bacterial targets
  - Any combination of targets can be ordered
  - **Can tailor testing to specific needs** of each patient
  - **Masking of target results** not requested
  - After test completion, **additional results** not initially reported can be **reflexed instantly at extra cost**
  - **You pay only for targets used** and no delay in running added tests

Conclusions

- Molecular testing has been **downsized and simplified**
- Now have **many high performance**, easy-to-use, fully integrated, specimen-to-result, **multiplexed molecular platforms**
- **Extends availability** of molecular diagnostics to every laboratory and to point-of-care and non-traditional testing sites
- **One size most likely does not fit all**
- Get to know their **strengths and weaknesses**
- Small, but growing body of evidence that supports their **positive impact on patient care**


THANK
YOU