Point of Care Diagnosis of Infections: Today and Tomorrow

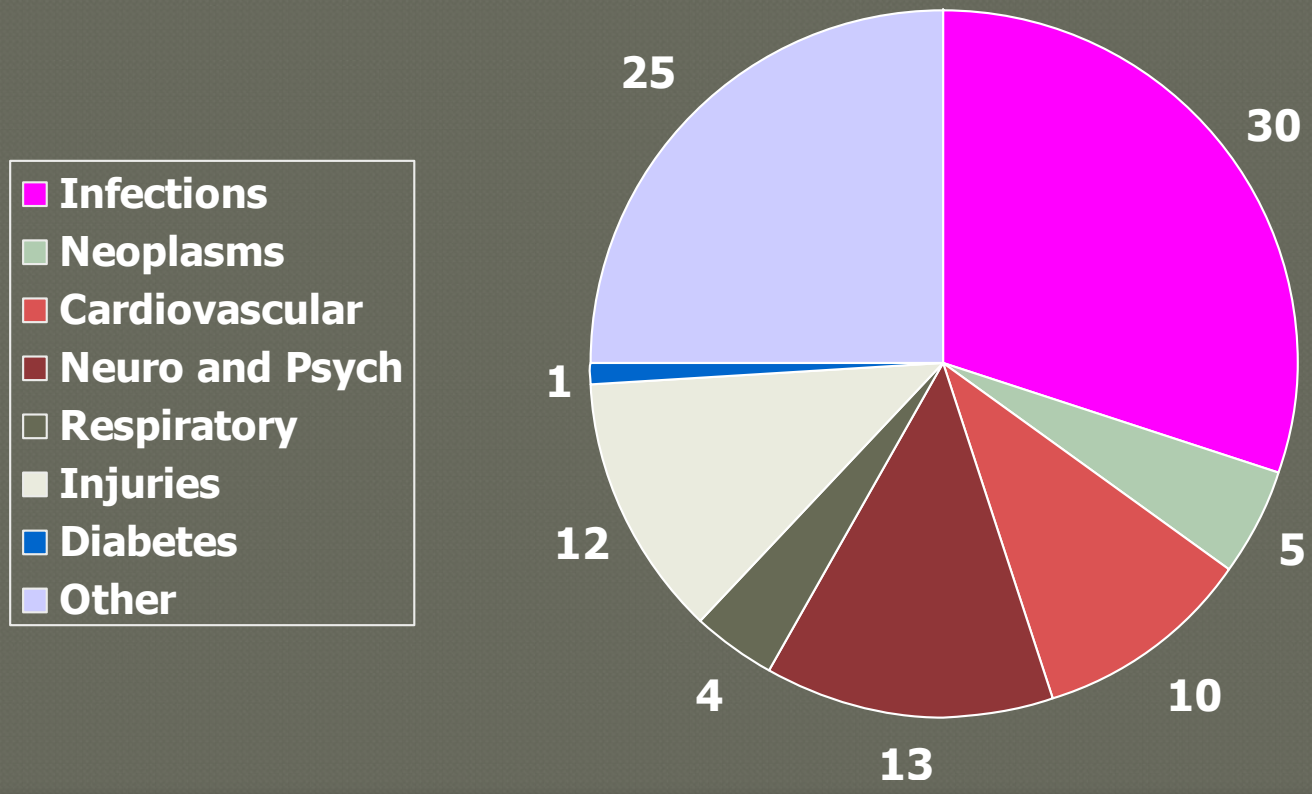
Sheldon Campbell M.D., Ph.D.

Pathology and Laboratory Medicine, VA Connecticut

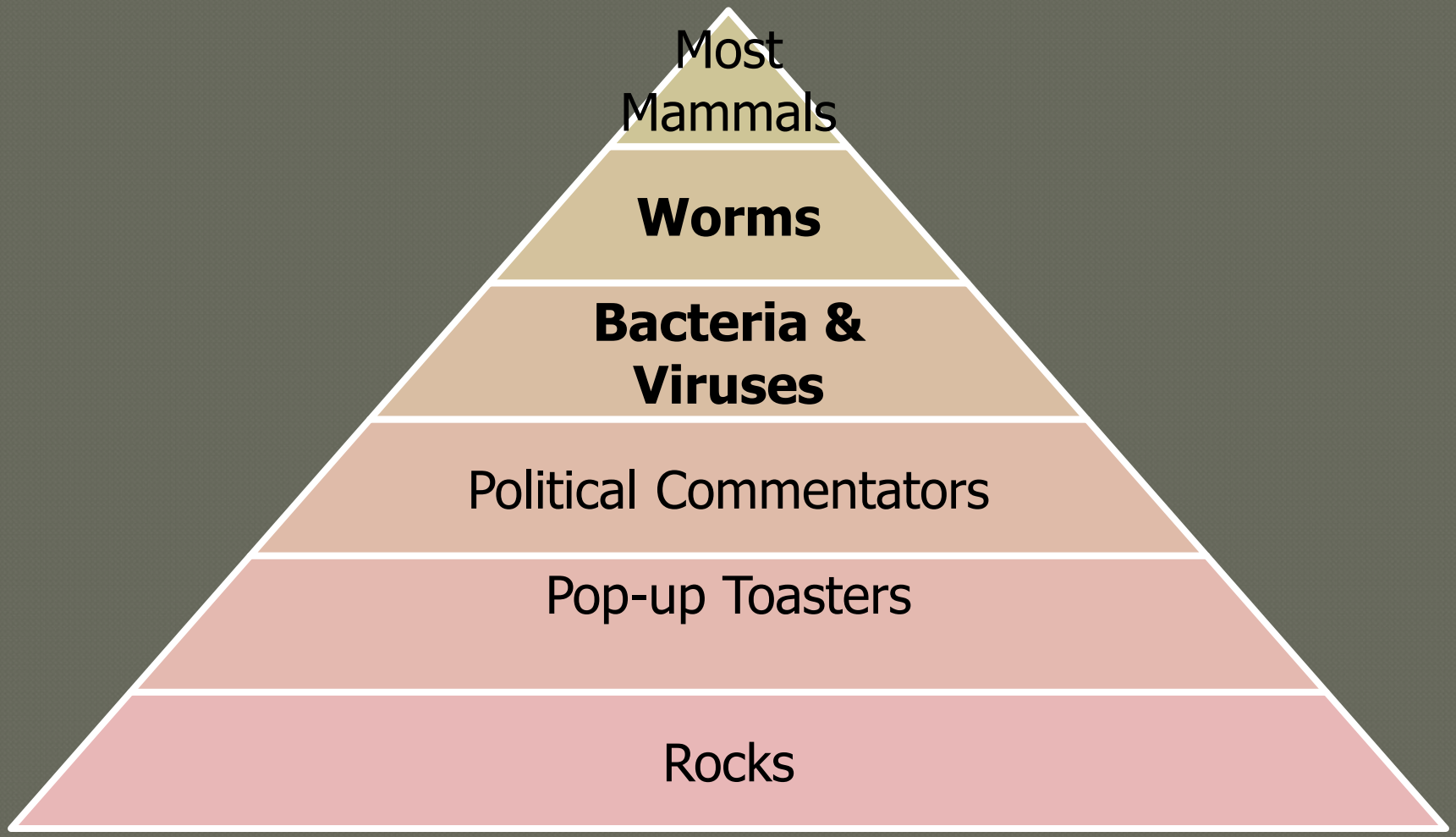
Department of Laboratory Medicine, Yale School of Medicine

Microbiology is Important!

Worldwide Lost Disability Adjusted Life Years, % of Total
Source: WHO Data 2002



The Grand Scale of Intelligence



Outline

- **The Targets**
  - Critical care
  - Common OP problems
  - Surveillance
  - Resource-Limited Environments
- **Today:** POCT Tests for Infections
  - Waived Methods
  - Example: HIV
  - Example: Rapid Flu
- **Tomorrow:** A Brief Glimpse
  - Molecular diagnostics at POC
- **A New Resource**
- **A Reflection**

Learning Objectives

- Participants should be able to:
  - Characterize important infections for which POC diagnosis ought to be valuable.
  - Describe current POCT tests for infections
  - Recognize critical issues in rapid HIV and influenza testing.
  - Describe the basic work-flow of molecular diagnostic testing.
  - Describe unique quality issues in molecular diagnostics which will impact their use at point of care e.g. contamination, inhibition, sampling

The Targets: General Principles

- Rapid testing
  - May be POC or lab-based
  - POC
    - Decisions on the order of minutes
      - Treatment
      - Admit versus discharge
    - Problems with follow-up
  - Lab-based
    - Treatment decisions on the order of hours
    - Captive populations

Targets: Critical Care

- For time-critical problems where *immediate* treatment decisions affect outcome
  - Meningitis and other CNS infections
  - Respiratory Infections; flu and others
  - Sepsis
    - Common bacteria
    - Biomarkers such as procalcitonin, soluble TREM-1 (triggering receptor expressed on myeloid cells)
    - Host response profiling (far-future)
  - Labor and delivery: HIV and group B strep

Targets: Common Outpatient Infections

- For ease of follow-up and patient convenience
  - Chlamydia, gonnorhoea / STDs
    - Follow-up is often a problem
  - Respiratory pathogens
  - Group A strep
  - Urinary tract infections
- For population screening
  - HIV
  - HCV

Targets: Surveillance and HAIs

- for TJC
  - MRSA
  - VRE
  - *Clostridium difficile*
- In many cases will be central-lab rather than POC
  - Exception – LTC facilities with minimal on-site lab support.

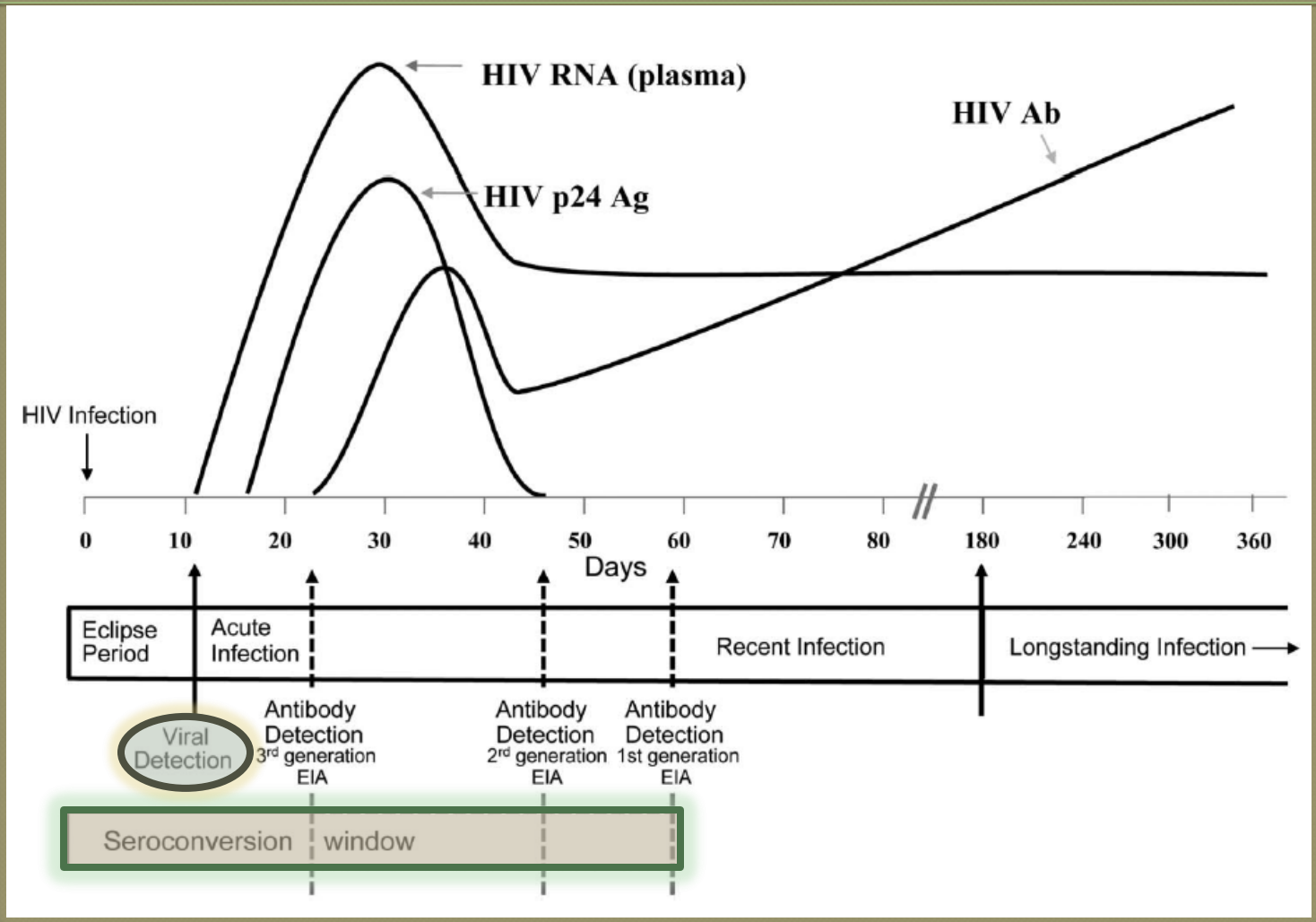
TODAY: Waived ID Tests

- Infectious Disease
  - Adenovirus
  - Helicobacter pylori Ab
  - **HIV-1&2 Ab**
  - HCV
  - **Influenza A/B**
  - Lyme Ab screen
  - Monospot
  - RSV
  - RSV Ab
  - Strep group A
  - Trichomonas
- Urinalysis & Microscopy
  - Dipstick UA
  - Fern test
  - Semen analysis (qual)
- Oncology
  - Bladder tumor-associated antigen
- Chemistry
  - ALT, AST
  - Microalbumin
  - DAU, ethanol, nicotine
  - Cholesterol, HDL, Triglycerides
  - Creatinine
  - N-telopeptide
  - FSH, LH
  - Glucose, Fructosamine, Hgb A1c
  - HCG
  - Ketones
  - Lactate
- Hematology
  - ESR
  - Fecal/Gastric Occult Blood
  - Hematocrit / Hemoglobin
  - Prothrombin time

HIV – Why There's a Problem

- ***Analytical:*** HIV serodiagnosis is pretty good, sensitive & specific, known 'window period.'
- ***Preanalytical:*** Annual incidence and death rate from AIDS stable since 1998
  - In 2000, 2 million CDC-funded HIV tests, 18,000 new diagnoses
  - Of 2,261 young MSM studied in 5 cities, **25%** infected with HIV, ***48%*** unaware they were infected
  - Access to testing an issue
- ***Postanalytical:*** Of persons with new (+) tests, 31% did not return to receive the result
  - 39% of persons with (-) results
  - Clinical use of the results an issue
  - Timely results when immediate management is an issue
- If African-Americans were a separate nation, they'd have the sixth-worst HIV problem in the world.
- Therefore, rapid testing.

Timing of Diagnostic Events in HIV Infection



From Branson B: J Acquir Immune Defic Syndr 2010;55:S102–S105

HIV Antibody Tests

- Screening EIAs
  - 1st Generation: viral lysates
  - 2nd Generation: recombinant antigens, ↑ specificity
  - 3rd Generation: recombinant antigens, sandwich format with improved IgM detection, ↑ sensitivity in early infection.
  - 4th Generation: includes antigen detection capability for even earlier detection

Performance of Rapid HIV in Early Infection

- Panel of antibody tests from recently infected individuals tested with rapids, lab-based EIA, and NAAT.
- 42 specimens negative for Ab by at least 1 screening test but NAAT (+).

Rapid HIV in Early Infection

- Performed on stored plasma
- Lab-based
  - 14/42 (+) with Genetic Systems HIV-1/HIV-2 PLUS O EIA (3rd-gen test)
  - 0/42 reactive with Vironostika HIV-1 Microelisa (1st-gen test)
- Rapids
  - 1/42 (+) by either Oraquick or Clearview
  - 11/42 (+) by Uni-Gold
  - 7/42 (+) with Multigent

Rapid HIV Follow-up

- 30/42 patients had follow-up samples available.
- 30/30 (+) by Uni-Gold and Multigent
- 26/30 (+) by Oraquick; 29/30 by Clearview.
- Uni-gold uses sandwich-capture and may detect IgM better than other rapids; also uses more blood.
- Louie B et al (2008) Assessment of rapid tests for detection of human immunodeficiency virus-specific antibodies in recently infected individuals. J. Clin. Microbiol. 46:1494-97.
- Remember pooled PCR does better than any serological test

Relationship of Confirmatory Methods to Window Period

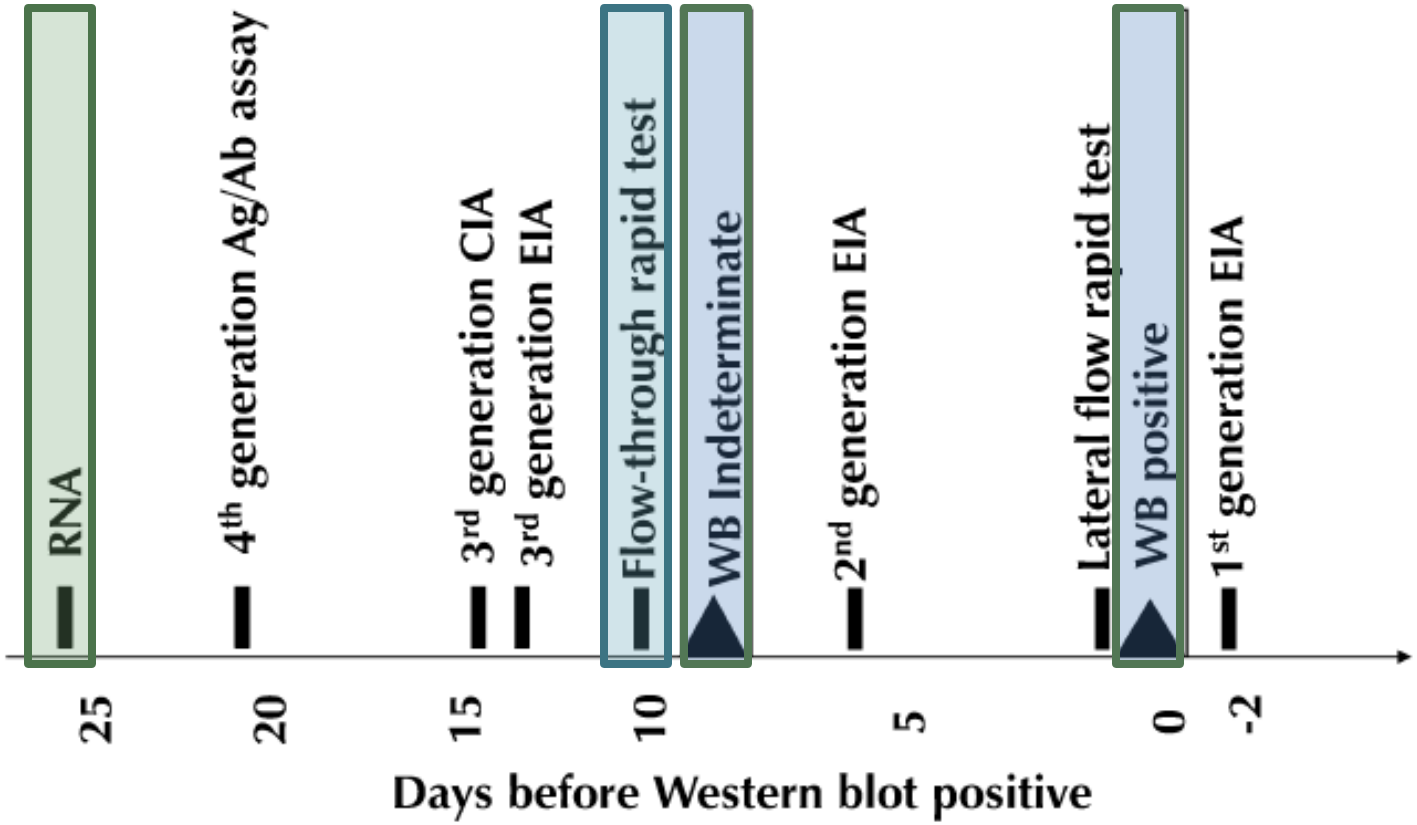
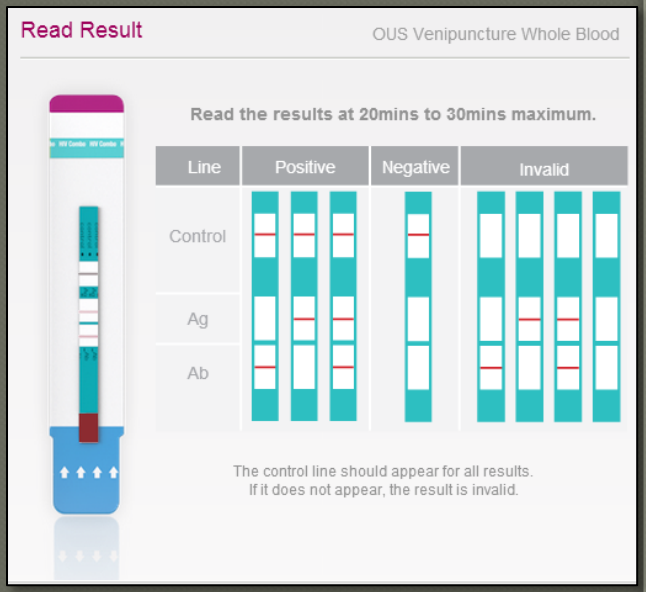


FIGURE 2. Reactivity of FDA-approved assays for HIV-1 compared with Western blot.

From Branson B: J Acquir Immune Defic Syndr 2010;55:S102–S105; highlights added

Fourth-Generation POC HIV?

- Alere Determine HIV Ag/Ab combo test.
- Analysis in 17 seroconverters placed Determine Combo (Ag+/Ab-, Ag+Ab+, Ag-/Ab+) and Ab-component reactivity at 15.5 and 7 days before WB positivity, respectively.
- In 26 seroconverters, Determine Combo was reactive in 99.0% and 92.5% of 3rd and 4th generation IAs-reactive specimens, respectively.
- Based on previous results with the same seroconversion panels, combined Ag/Ab reactivity of the Determine Combo appears between FDA-approved 4th and 3rd generation laboratory IAs.
- S. Masciotra, W. Luo, A.S. Youngpairoj, M.S. Kennedy, S. Wells, K. Ambrose, et al. J Clin Virol, 58 (Suppl. 1) (2013), pp. e54–e58



Question!

- If you perform rapid HIV testing, do you test a population with high risk for early infection?
- How do you inform patients of the risks of the 'window period'?

Don't Count The Lab Out!

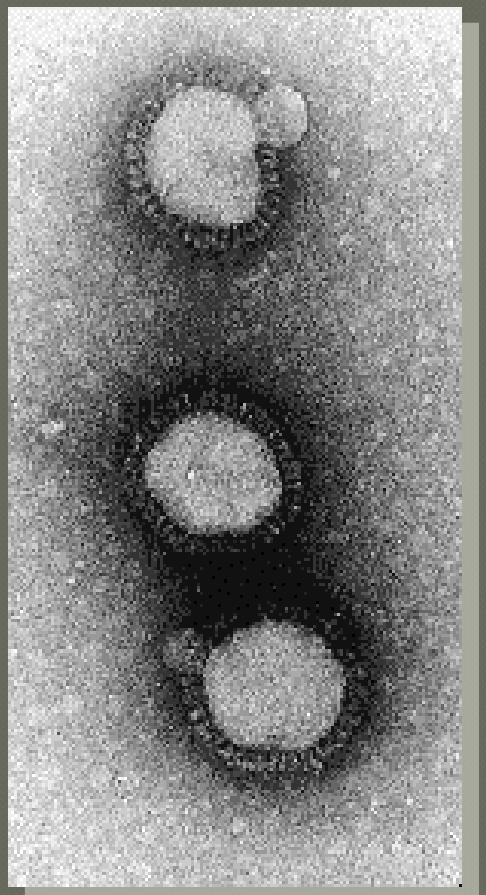
- Labs need to reduce TAT from lab-based HIV testing
  - Random-access HIV
    - Avail now on several platforms
    - Goal should be to give HIV in same time frame as Troponin
  - Preliminary reporting of positives prior to confirmation
    - If it can be done with rapids, why not with lab-based tests?

Best Practices

- Do rapid HIV testing with oversight from a clinical laboratory to assist in good laboratory practices.
- Monitor quality; false-positive rates are the easiest to check, but sensitivity may be an issue as well.
- Be aware of laboratory-based alternatives to POCT and be intentional about which best serves patients.
- Examine outcomes in *your* population.

Rapid Flu Testing

- Diagnostic approaches to influenza
- Rapid flu tests
- Impact and assessment of rapid flu testing



Influenza – It's not Just 'the Flu'

- In the US 5-20% of the population gets influenza each year
  - 200,000 hospitalizations, 36,000 deaths
- Worldwide, 3-5 million severe cases
  - 250-500,000 deaths per year
- That's an average; a pandemic would greatly increase these numbers
  - And also drive us all berserk with no notice as in April-May 2009.

Symptoms of Influenza

- Sudden onset of fever
- Headache
- Extreme Tiredness
- Dry cough
- Sore throat
- Runny nose
- Muscle aches
- GI symptoms (more common in children)
  - More common with 2009 pandemic strain – seen in 25% of cases

Flu Testing Methods

- Culture
  - Newer shell vial methods 24-48h
  - 'Gold standard'
- DFA
  - Rapid (1-2h)
  - Sensitivity from top centers approaches culture.
  - Requires fluorescent scope and highly trained technical staff.
- Molecular Diagnostics
  - Real-time methods can be ~1h or so.
  - In some cases real-time PCR methods exceed culture in sensitivity (probably due to viral loss in transport)
  - High skill and equipment requirements
  - Emerging 'gold standard'
- Rapid antigen tests
  - 50-70% sensitivity, despite occasional publications to the contrary
    - Possibly lower in adults
  - Rapid, simple, no equipment needed.

Decisions Based on Flu Testing

- Positive Test
  - Do not start antibacterials
  - Start antivirals – within 48h of onset for best outcome
  - Droplet precautions
  - Don't do other tests
- Consequences of error: potentially severe
- Speed helps.
- Specificity is essential!
- A vs B sometimes matters

- Negative Test
  - Consider antibacterial therapy
  - Do not start antivirals
  - No droplet precautions
  - Further diagnostic studies
- Consequences of error: moderate
- Sensitivity would be nice, too.

Adapted from Storch GA (2003) Curr. Opinion Ped. 15:77-84

Rapid Flu vs Other Methods

- Culture, DFA, Molecular
  - All are much more sensitive than rapids
  - TAT a few to 48h, depending on batching and shift coverage and other workflow issues.
- Rapid flu
  - Fast
  - Insensitive
- While some labs might use a rapid for an initial test, poor performance relative to other methods.

Test Performance vs Novel 2009 Influenza

- Ginocchio et al J Clin Virol 2009:45:191-5
- 6090 samples over 5 weeks
- Tested by various combinations of PCR, DFA, and 2 different rapid tests

Test	Sens%	Spec%	PPV%	NPV%
PCR	97.8	100	100	97.3
R-mix Culture	88.9	100	100	97.3
DFA	46.7	94.5	91.3	58.9
BinaxNow A&B	9.6	93.6	77.4	47.9
3M Rapid A+B	40			

Rapid Flu vs *Nothing*

- VA study reviewed use of rapid antigen test in older adults
  - An unfavorable population
- Eighty-four adults positive for influenza.
- Adding rapid flu to symptoms enhanced the ability to diagnose influenza in the acute setting.
  - Positive predictive value of fever plus cough increased from 32% to 92% with a positive rapid flu.
- Appropriate therapy
  - 20/22 (91%) patients with a positive rapid and symptoms < or =48 h received antiviral treatment
  - 1/12 (8%) of patients with a negative rapid and a positive culture.
- D'Heilly SJ, Janoff EN, Nichol P, Nichol KL. Rapid diagnosis of influenza infection in older adults: influence on clinical care in a routine clinical setting. Journal of Clinical Virology. 42(2):124-8, 2008 Jun.
- If the choice is between rapid antigen test and no test at all, there's data to support rapid antigen.

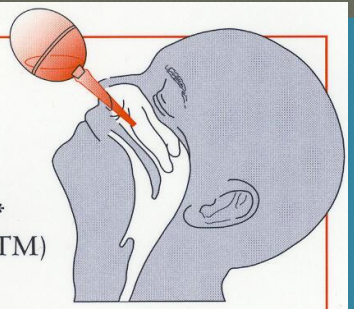
Best Practices & Tidbits

- Best Practices
  - Educate providers on specimen collection
  - Test during the ‘season’ only
  - Test early in illness
  - Provide guidance on interpretation of results

Influenza Specimen Collection

Specimen collection is probably *the* critical step in influenza testing

Nasopharyngeal Wash: Bulb Method

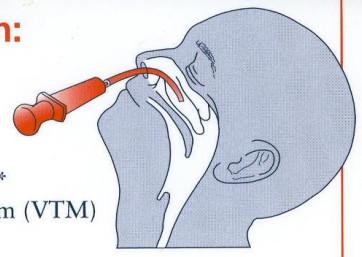


Materials: Saline
1-2 oz. tapered rubber bulb*
Viral Transport Medium (VTM)
Specimen container

1. Suction 3-5 ml saline into a new sterile bulb.
2. Insert bulb into one nostril until nostril is occluded.
3. Instill saline into nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
4. Empty bulb into suitable dry, sterile specimen container or one containing VTM, according to virology laboratory requirements.

* Length and diameter of bulb as appropriate for infant, child or adult.

Nasopharyngeal Wash: Syringe Method



Materials: Saline
3-5 ml syringe*
2" 18-20 gauge tubing*
Viral Transport Medium (VTM)
Specimen container

1. Fill syringe with saline; attach tubing to syringe tip.
2. Quickly instill saline into nostril.

3a. Aspirate the recoverable nasal specimen. Recovery must occur immediately, as the instilled fluid will rapidly drain.

3b. *(Alternate)* In appropriate cases, patients may tilt head forward to allow specimen to drain into suitable sterile container.

4. *(If aspirated)* Inject aspirated specimen from syringe into suitable dry, sterile specimen container or one containing VTM, according to virology laboratory requirements.

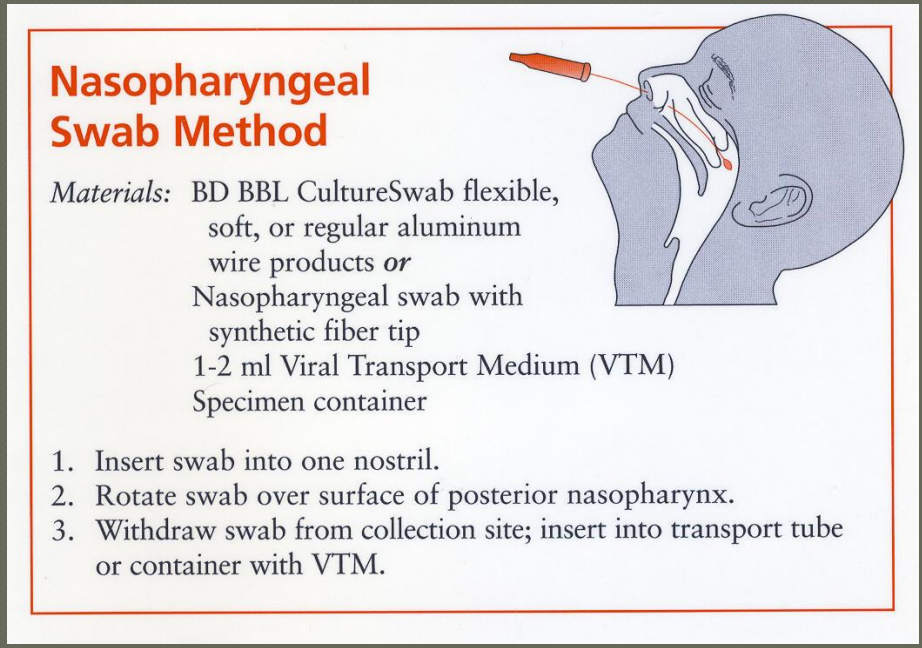
* Length and diameter of syringe and tubing as appropriate for infant, child or adult.

Washes are some-what better than swabs*

***A general but not-quite universal rule of microbiology: swabs are evil**

Specimen Collection cont.

- Important to get ciliated epithelial cells
- Children shed more virus than adults; tests tend to be more sensitive in kids



When to test?

- Remember – false-positives have potentially severe consequences, e.g. non-treatment of a serious bacterial infection.
- Test during the flu season, ***only***.
- Potential strategies:
  - Seasonal: test Oct-Dec→March or so.
    - Early season – retain specimen for confirmatory testing!
  - Incidence-based testing – monitor regional influenza per CDC and State systems, begin testing only when influenza reported in the area.
- Remind providers to test *early in illness*; the best therapeutic results are when drugs are started within 48h of onset.

Interpreting Results of Rapid Flu

- A comment might be a good idea:
  - Remind providers that rapid tests are insensitive.
  - Remind providers that out-of-season false-positives outnumber true-positives.
  - Remind providers of known sources of error; e.g. bloody samples.
- Provide supplementary testing at least for selected patients and off-season or early-season positives.

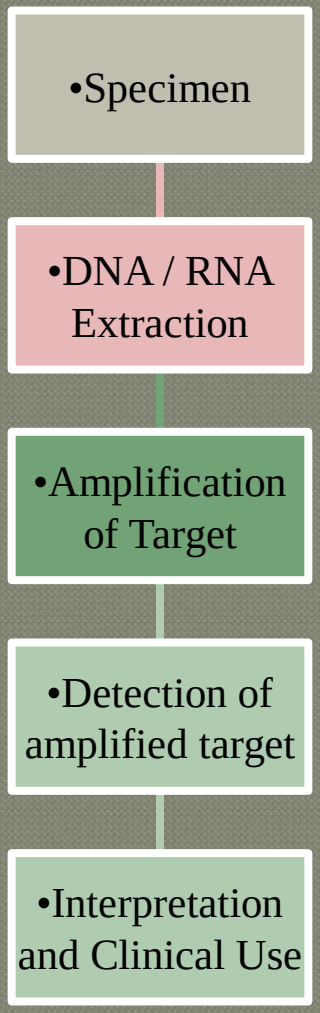
Tomorrow: Molecular vs non-Molecular

- Non-molecular for…
  - Serologic diagnosis
    - HIV, viral hepatitis,
  - Host factors
    - Sepsis markers
  - Things where antigen performs well
    - Intestinal protozoa (not usually POC)
- Molecular for…
  - ***Most things***

What is Molecular Diagnostics?

- Analysis of DNA or RNA for diagnostic purposes. Molecular diagnostics have found widespread application with the advent of ***amplification methods*** (PCR and related approaches).
- Huge scope
  - From single-target molecular detection of pathogens…
  - To pharmacogenomic analysis of metabolism genes for drug dosing…
  - To whole genome sequencing for disease susceptibility and God knows whatall.

Molecular Diagnostic Testing



Extraction

- **DNA/RNA Extraction**
  - Depends on:
  - Specimen source (blood, CSF, stool)
  - Target organism (human tumor, CMV, M. tuberculosis)
  - Target nucleic acid (DNA, RNA)
- Increasing automation
  - Magnetic or other separation methods.
  - REQUIRED for POC

Amplification

- Nucleic Acid Amplification means taking a small number of targets and copying a specific region many, many times.
- NAAT, NAT, etc; commonly-used abbreviations
- PCR is the most common amplification scheme, but there are others!
  - TMA, SDA, LAMP

Real-Time Amplification

- Combination
  - Detection
  - Amplification
- RT-PCR Instruments monitor product formation by detecting change in fluorescence in a tube or well during thermal cycling.
- Frequently use PCR for amplification
  - Robust
  - Off-patent

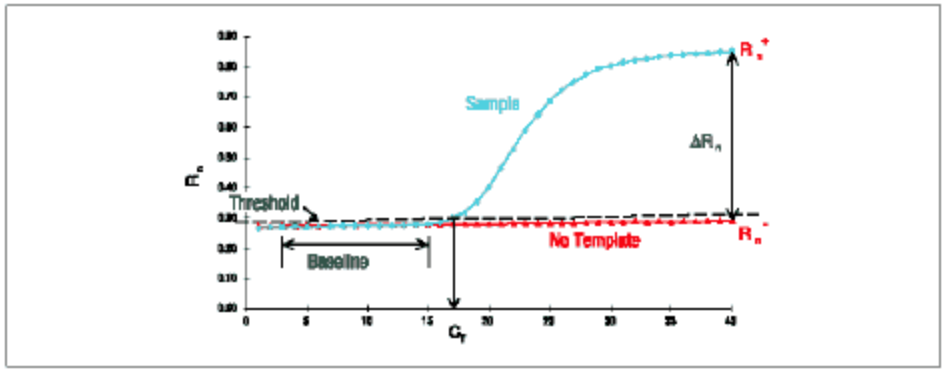


Figure 2. Model of a single amplification plot, showing terms commonly used in real-time quantitative PCR Figure from Applied Biosystems' DNA/RNA Real-Time Quantitative PCR bulletin).

Contamination!

- What happens when you make 10^6 copies of a single short sequence in a 100ml reaction?
  - You end up with 10^4 copies/ul
  - What happens when you pop the top off a microcentrifuge tube?
    - …or pipet anything
    - …or vortex anything
    - …or…
- You create aerosols
  - Droplet nuclei with diameters from 1-10 μm persist for hours/days
  - Each droplet nucleus contains amplified DNA
  - Each amplified molecule can initiate a new amplification reaction

Ways to Prevent Contamination

- *Meticulous technique*
  - Hoods, UV, bleach, physical separation of work areas
- *Assay design*
  - avoid opening tubes for reagent addition, etc.
  - reactions that produce RNA products
  - negative controls
  - **real-time assays with closed-tube detection**

POC Molecular Diagnostics

- Infectious Disease
  - Outpatient POC
    - GC / *Chlamydia*
    - Group A strep
    - HIV / HCV viral load
  - Acute-care POC – Lab vs POC
    - Respiratory pathogens
    - CNS pathogens
  - Nosocomial / Screening
    - MRSA / VRE
    - *C. difficile*
  - Biopreparedness
    - Military development and applications
  - Diseases of Under-resourced populations
    - Tuberculosis incl drug-resistance
- Others
  - Pharmacogenetics
  - Hypercoagulability
  - Other genetic diseases
  - Oncology
    - Lower priority for POC
    - Large number of diseases
    - Solid tumors – need tissue
    - Generally easier follow-up.
- NOTE: the ones in red actually exist in some form (mostly pre-approval). The rest are guesses.
- Slow introduction due to cost, mostly.

Why Molecular? Rapid flu versus Other Methods

Influenza A Rapid Test Performance¶

Rapid Test	Sens%	Spec%	Compared With	Comments	Reference
Directigen	**58.8**	99.2	Molecular	A&B performance combined	Liao et al JCM 47(3):527-32, 2009 Mar
3M QuickVue BinaxNow	**75** **73** **55**	98 99.5 100	Culture	Archived specimens	Dale et al JCM 46(11):3804-7, 2008 Nov
BinaxNow	**53**		RT-PCR	2 of 237 samples were flu B pos by RT-PCr but flu A by NOW.	Landry et al JCV. 43(2):148-51, 2008 Oct
BinaxNow	**61**	100	RT-PCR	DFA was 81% sensitive	Rahman et al Diag Micro Infect Dis 62(2):162-6, 2008 Oct
RemelXpect BinaxNow	**47.7** **78.3**	98.7 98	Culture	20.3/99.8 Flu B 35.9/99.9 Flu B	Cruz et al JCV 41(2):143-7, 2008 Feb
BinaxNow	**52**		RT-PCR	70% in days 1-3 of disease	Nilsson et al Inf Cont & Hosp Epi 29(2):177-9, 2008 Feb
Directigen	**42**	96	Culture		Rahman et al Diag Micro Infect Dis 58(4):413-8, 2007 Aug
BinaxNow Directigen QuickVue	**73** **69** **67**	99 100 100	RT-PCr	Sensitivity only 30% vs flu B for all	Hurt et al JCV 39(2):132-5, 2007 Jun
Quickvue	**85**	97	RT-PCR		Mehlmann et al JCM 45(4):1234-7, 2007 Apr.
Directigen + Quickvue + BinaxNOW	**63**	97	RT-PCR	Data pooled from all rapids;	Grijvala et al Pediatrics. 119(1):e6-11, 2007 Jan

Convenience sample of recent literature; selected by Medline search + fit to single page

What Will a Molecular POC Test Look Like?

- Automated, fully integrated
  - Sample preparation
  - Amplification and detection
  - Reproducibility
  - Reliability
  - Such systems are emerging
- Quality need not be compromised for POC molecular tests
  - Unlike most of the antigen tests versus lab-based methods

What to think about

- All the usual QC and QA, plus:
- Interferences
  - Extraction efficiency
  - **Inhibition** by:
    - Blood
    - DNA
  - Internal amplification / extraction controls
- Contamination
  - Extraordinarily sensitive methods
  - **Specimen** cross-contamination
    - Native material transferred from a positive to a negative specimen
    - Collection devices
    - Ports, racks, hands
  - **Amplicon** contamination
    - From amplified material
    - How well is the product contained?
    - Waste disposal
  - Carry-over studies

Future Developments

- Technological advances
- - performance
- - speed
- - footprint
- Expanded test menus
- - quantitative assays
- Resource limited settings

Molecular Testing for Influenza – Moving Toward POCT

- Real-time methods can provide result in ~1h or so.
- Molecular methods as a class exceed culture in sensitivity (probably due to viral loss in transport)
- Detection properties do vary from system to system – do your homework!
- Moderately to very expensive equipment
- Moderate to high complexity (no CLIA-waived tests yet).
- Now clearly the 'gold standard'
- Information sources:
  - http://www.cdc.gov/flu/pdf/professionals/diagnosis/table1-molecular-assays.pdf
  - CAP Website for some price information
  - Manufacturer's web sites and PubMed for pictures, workflow and other information.

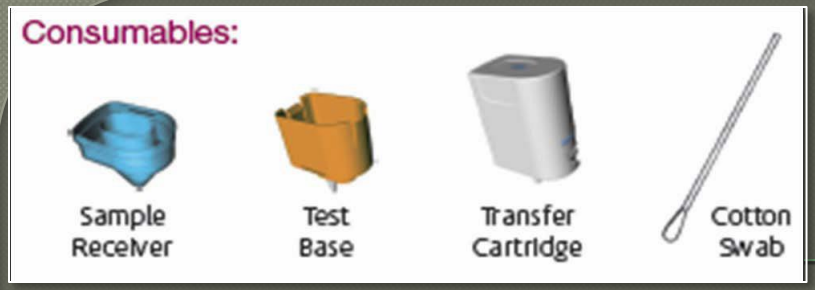
FDA-approved Molecular Influenza Tests

- Alere I Influenza A/B
- Cepheid Xpert Flu Assay
- eSensor Respiratory Viral Panel
- FilmArray Respiratory Panel
- Ibis PLEX-ID Flu (seems to be off the market)
- Iquum LIAT Influenza A/B Assay
- Prodesse PROFLU and PROFAST
- Quidel Molecular Influenza A+B Assay
- Qiagen Artus Influenza A/B Rotor-gene RT-PCR kit
- Simplexa Flu A/B & RSV and Flu A/B & RSV Direct and Influenza A H1N1 (2009)
- Verigene Respiratory Virus Nucleic Acid Test and RV+ Test
- X-TAG Respiratory Viral Panel and RVP-FAST

More on the way!!

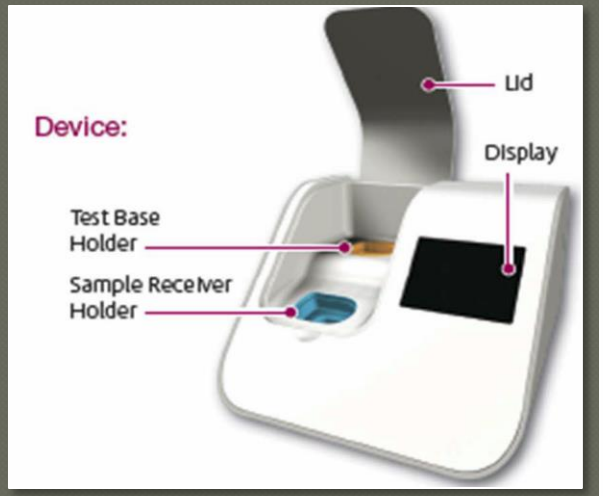
Alere I Influenza A and B

- Recently approved (6/16/2014)
- **CLIA Waived**; 15 min to result



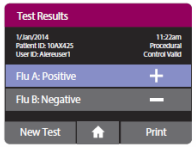
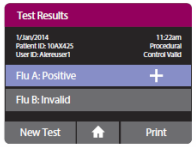
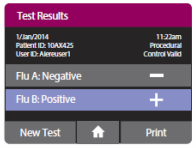
Alere I Workflow

- Bring supplies to room temperature.
- Put test base and sample receiver on instrument; allow to warm.
- Place swab in sample receiver, mix.
- Apply transfer cartridge to sample receiver.
- Move transfer cartridge to test base.
- Close lid; test runs 10 minutes.



RESULT INTERPRETATION

When the test is complete, the results are clearly displayed on the instrument screen. An individual result for both influenza A and influenza B will be provided.

Instrument Display	Interpretation of Results and Follow-up Actions
	**Flu A Viral RNA Detected; Flu B Viral RNA Not Detected.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Flu A Viral RNA Detected; The presence or absence of Flu B Viral RNA cannot be determined.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Flu B Viral RNA Detected; Flu A Viral RNA Not Detected.** This result does not rule out co-infections with other pathogens or identify any specific influenza B virus lineage.

Cepheid Xpert Flu Assay

- From Cepheid
- Detects Flu A and B; discriminates 2009 H1N1.
- Approved for nasopharyngeal swabs, nasal aspirates, and nasal washes.
- Moderately complex
- List price ~$50/cartridge, instruments $24,900–$174,400 depending on capacity
- Sample to answer ~1h

Xpert Flu Workflow

1

Transfer 300µl of prepared sample into the large hole

2

Dispense binding reagent into small hole

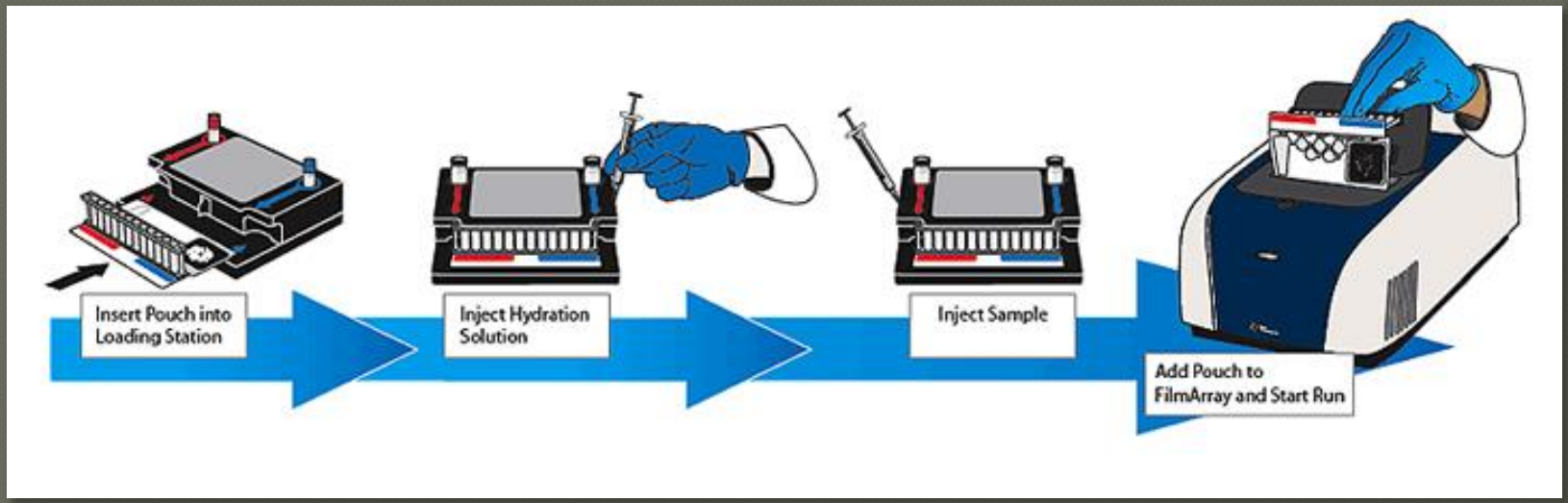
3

Insert cartridge and start assay

FilmArray Respiratory Panel

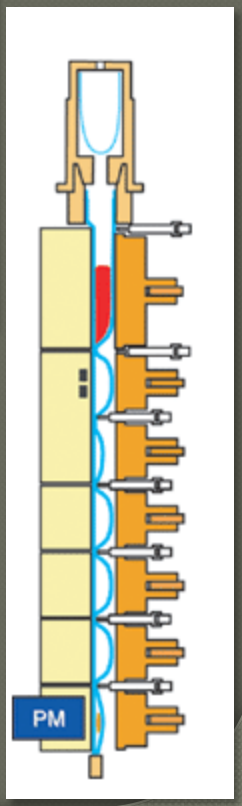
- From: Biofire, in the process of being acquired by BioMerieux
- Detects: Influenza A and B (discriminates H1, H3, 2009 H1) Respiratory Syncytial Virus, Parainfluenza 1, 2, 3 and 4 virus, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, 4 Coronavirus variants, *Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*
- Approved for NP swabs
- Moderately complex
- List price: $129/sample; instruments $39,500 each
- Sample to answer ~1h

Filmarray Workflow



Roche LIAT Influenza A/B Assay

- From Iquum (recently acquired by Roche); LIAT stands for Lab-In-A-Tube
- Detects Influenza A&B
- Approved for NP swabs
- CLIA-waived 9/2015.
- List price N/A
- Sample to answer .5h



LIAT Workflow

STEP 1.
Add sample

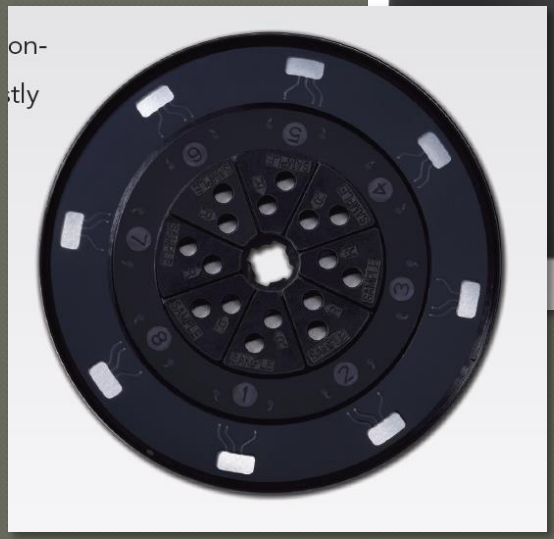
STEP 2.
Scan barcode

STEP 3.
Insert tube

Done!
Results in 20 minutes

Simplexa Flu A/B & RSV and Flu A/B & RSV Direct and Influenza A H1N1 (2009)

- From Focus Diagnostics / 3M
- Detects Influenza A&B and RSV; a separate test discriminates 2009 H1N1
- Approved for NP Swabs
- Highly complex (Direct version is Moderately complex)
- List price: $49 reagents, requires Focus/3M Cycler
- Sample to answer ~4h, ~2h for Direct



Not All Molecular Tests Are The Same

- Numerous, rather confusing studies; I picked one simple example.
- Don't take this as a comprehensive assessment of both assays; neither performed as well as the authors' homebrew RT-PCR.

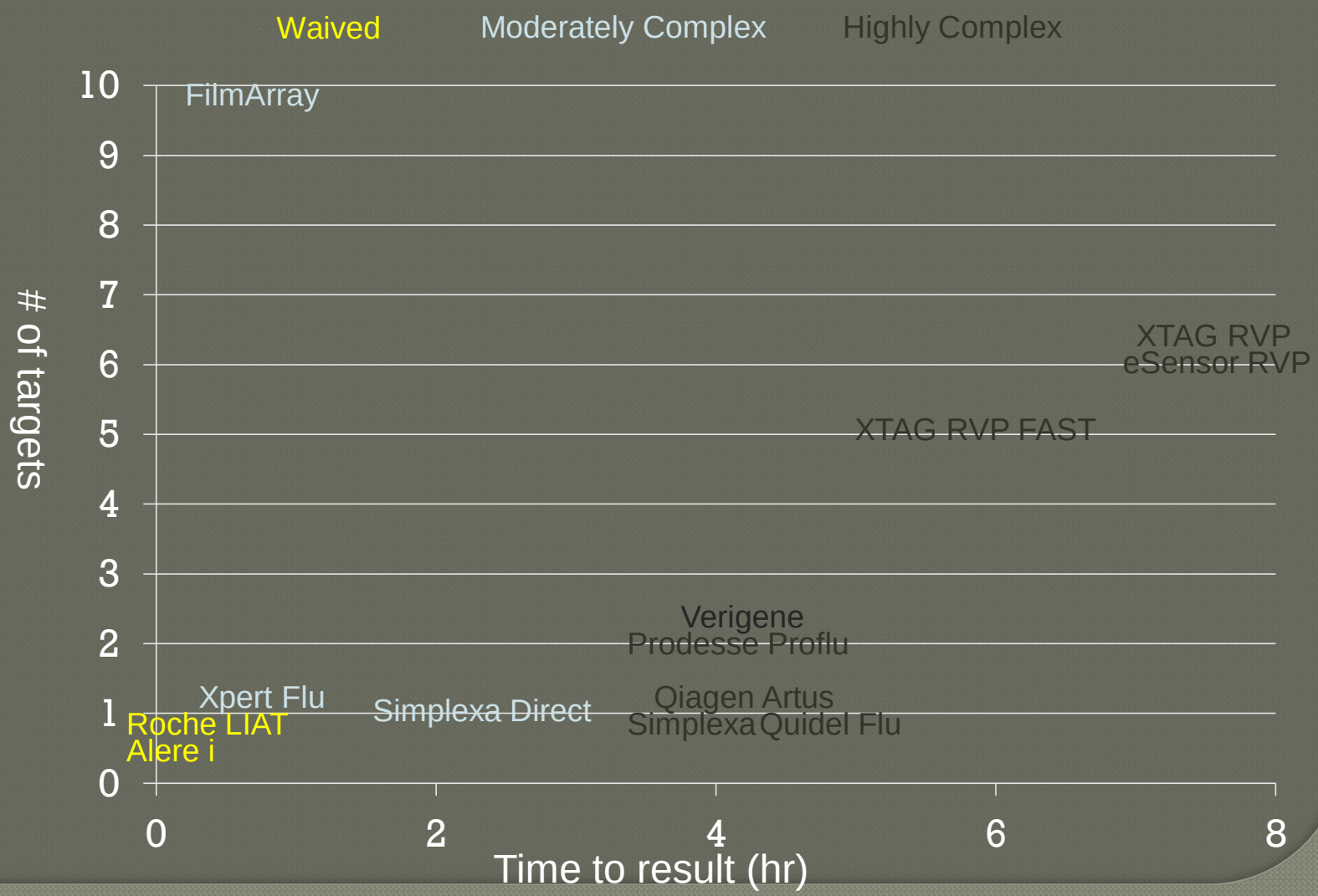
TABLE 1

Sensitivity of the Verigene RV+ test and the Simplexa Flu A/B & RSV kit by virus (n = 350)

Test	% Sensitivity for[a]:		
	Influenza A virus	Influenza B virus	RSV
Verigene RV+	96.6 (56/58)	100 (21/21)	100 (93/93)
Simplexa	82.8 (48/58)	76.2 (16/21)	94.6 (88/93)

Comparative Evaluation of the Nanosphere Verigene RV+ Assay and the Simplexa Flu A/B & RSV Kit for Detection of Influenza and Respiratory Syncytial Viruses; Kevin Alby, Elena B. Popowitch and Melissa B. Miller, J. Clin. Microbiol. January 2013 vol. 51 no. 1 352-353

Speed and Multiplexing and Complexity



Parsing Cost

- Cost per test depends on reagent + instrumentation + labor.
  - How many single-test modules do you need?
- Make sure to count in instrumentation for extraction, if needed.
- Reimbursement is a moving target; ask an expert.
- Potential for savings elsewhere in the system, if your bean-counters are sophisticated.

Next-Generation Technologies

- Non-amplification-based nucleic acid detection
- Mass-spectroscopy-based detection of organisms
- Nanotechnology-based detection and analysis of microbes and molecules

LETTERS
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nature nanotechnology

Label-free biomarker detection from whole blood

Eric Stern[1], Aleksandar Vacic[2], Nitin K. Rajan[2], Jason M. Criscione[1], Jason Park[1], Bojan R. Ilic[3], David J. Mooney[4], Mark A. Reed[2,5]* and Tarek M. Fahmy[1,6]*

NATURE NANOTECHNOLOGY DOI: 10.1038/NNANO.2009.353 LETTERS

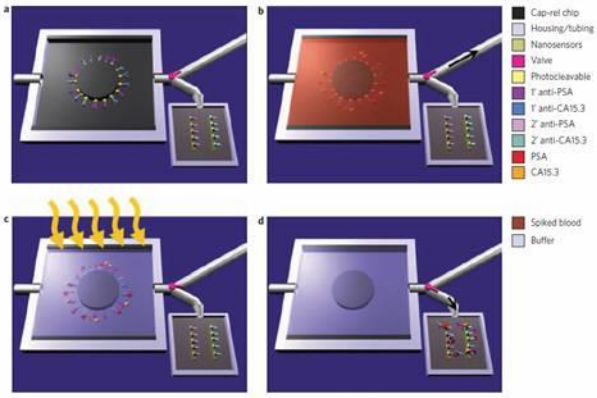

Figure 1 | Schematic of MPC operation. a, Primary antibodies to multiple biomarkers, here PSA and carbohydrate antigen 15.3 (CA15.3), are bound with a photocleavable crosslinker to the MPC. The chip is placed in a plastic housing and a valve (pink) directs fluid flow exiting the chip to either a waste receptacle or the nanosensor chip. b, Whole blood is injected into the chip with the valve set to the waste compartment (black arrow shows the direction of fluid flow) and, if present in the sample, biomarkers bind their cognate antibodies. c, Washing steps follow blood flow, and the chip volume (5 μl) is filled with sensing buffer before UV irradiation (orange arrows). During UV exposure, the photolabile crosslinker cleaves, releasing the antibody–antigen complexes into solution. d, The valve is set to the nanosensor reservoir (black arrow shows the direction of fluid flow) and the 5 μl volume is transferred, enabling label-free sensing to be performed to determine the presence of specific biomarkers.

Reflection

- "Point-of-care testing, especially those analyses that are conducted at the patient's bedside, in a physician's office, or in a clinic, is a growing trend in health care, and clinical microbiology professionals should prepare for this future reality. Clinical microbiologists must ensure that the individuals who perform point-of-care testing understand how to interpret the results. Clinical microbiologists should be called upon to help select the assay targets, advise on test formats, and participate in clinical trials."
- From "Clinical Microbiology in the 21st Century: Keeping the Pace". American Academy of Microbiology, 2008. Available on-line at: http://www.asm.org/academy/index.asp?bid=58445