

Molecular Diagnostics at Point of Care

It's The Future Already. Ack!

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Learning Objectives

- ▶ Participants should be able to:
 - ▶ Describe the basic work-flow of molecular diagnostic testing.
 - ▶ Describe some major amplification and detection methods.
 - ▶ Recognize the properties of analytes that make them candidates for molecular testing.
 - ▶ Recognize the molecular diagnostic platforms with CLIA-waived analytes.
 - ▶ Assess platforms for molecular influenza testing in the context of POCT.
 - ▶ Describe unique quality issues in molecular diagnostics which impact their use at point of care.

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.



Why Amplify?



► Sensitivity

- can detect small numbers of organisms
- can even detect dead or damaged organisms
- can detect unculturable organisms



► Speed

- As little as 15 min turnaround
- inoculum independence

Why Amplify, continued

► Targets

- Test for things there's no other way to test

- Uncultivable bugs

► Genetics

- Pharmacogenomics

- Prenatal testing

- Hypercoagulability, etc.

► Oncology

- Hematologic malignancies

- Diagnostic markers

- Minimal residual disease



Why Not Amplify?

- Clinical significance?
- Technical problems
 - Contamination
 - Inhibition
- Cost

Molecular Diagnostic Testing

•Specimen

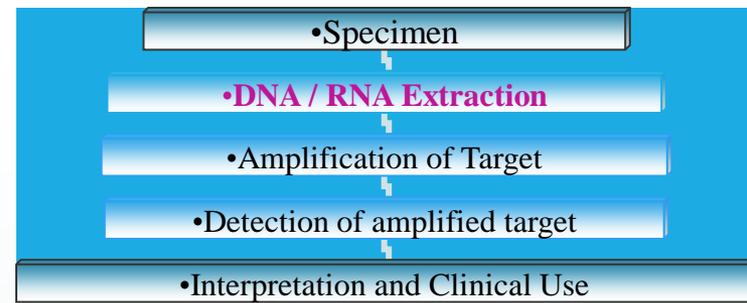
•DNA / RNA Extraction

•Amplification of Target

•Detection of amplified target

•Interpretation and Clinical Use

Extraction



➤ DNA/RNA Extraction

➤ Depends on:

➤ Specimen source (blood, CSF, stool)

➤ Target organism (human tumor, CMV, M. tuberculosis)

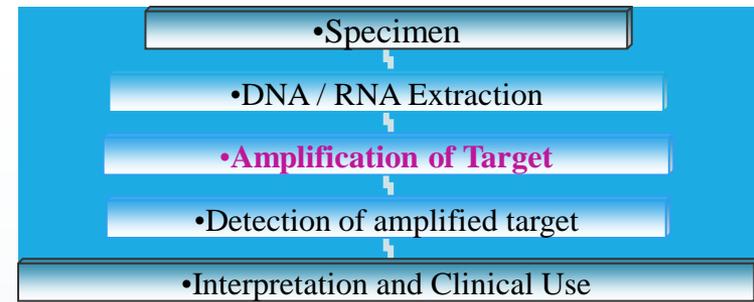
➤ Target nucleic acid (DNA, RNA)

➤ 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!

Amplification Enzymology

- ▶ DNA polymerase
 - ▶ makes DNA from ssDNA, requires priming



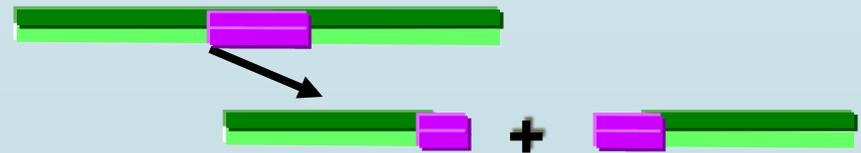
- ▶ RNA polymerase
 - ▶ makes RNA from dsDNA, requires specific start site



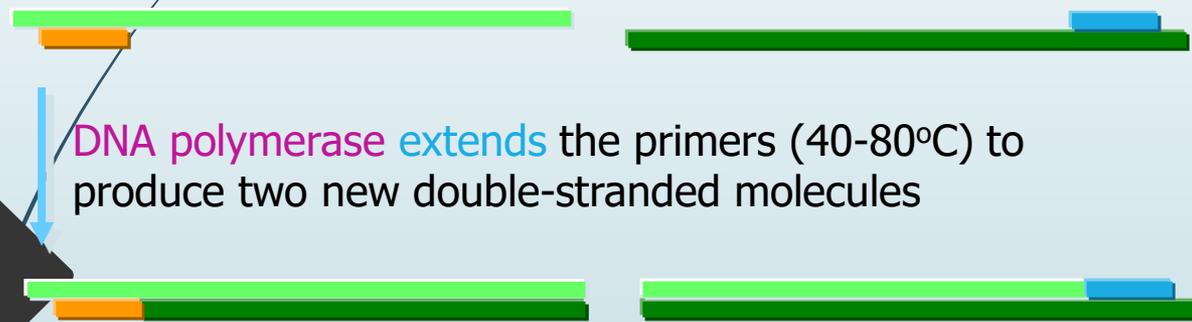
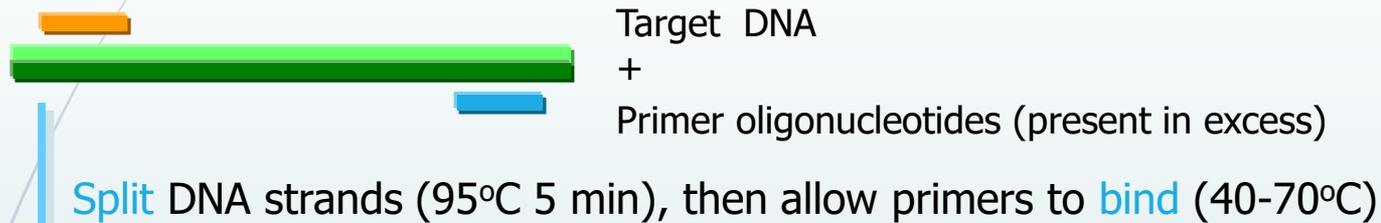
- ▶ Reverse transcriptase
 - ▶ makes DNA from RNA, requires priming



- ▶ Restriction endonucleases
 - ▶ cut DNA in a sequence specific manner



Polymerase Chain Reaction (PCR)



Repeat the split-bind-extend cycle



This 'short product' amplifies exponentially in subsequent split-bind-extend cycles, driven by the temperature changes in a 'thermal cycler'.

Reverse Transcriptase PCR (RT-PCR)



Target **RNA** +
Primer oligonucleotide

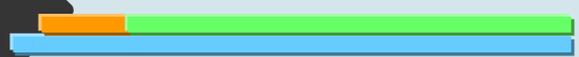
The diagram shows a horizontal blue bar representing the RNA target and a shorter orange bar representing the primer oligonucleotide positioned above it.

Primer binding (RT - 37°C)



The diagram shows the orange primer bar now bound to the blue RNA bar.

Reverse Transcriptase (RT) makes a DNA copy of the RNA target



The diagram shows a new green bar extending from the orange primer bar, representing the synthesis of a DNA copy. A black arrow points to this step.

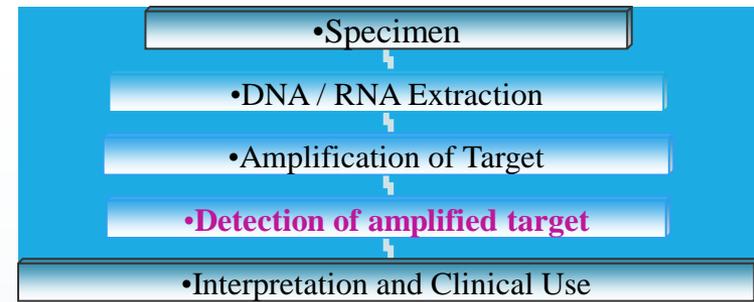
The DNA copy is used in a PCR reaction

PCR

Other Amplification Methods

- ▶ PCR isn't all there is!
 - ▶ Transcription-mediated amplification (TMA)
 - ▶ Loop-mediated isothermal AMPLification (LAMP)
 - ▶ Others
 - ▶ Isothermal technologies decrease the complexity of the instrument required.

Detecting PCR Products in the Old Days



- Gel electrophoresis (\pm Southern blotting)
- Enzyme-linked assays
- Hybridization
Protection/chemiluminescent assay
- A multitude of formats available, to serve market and technical needs

Real-Time PCR

► 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

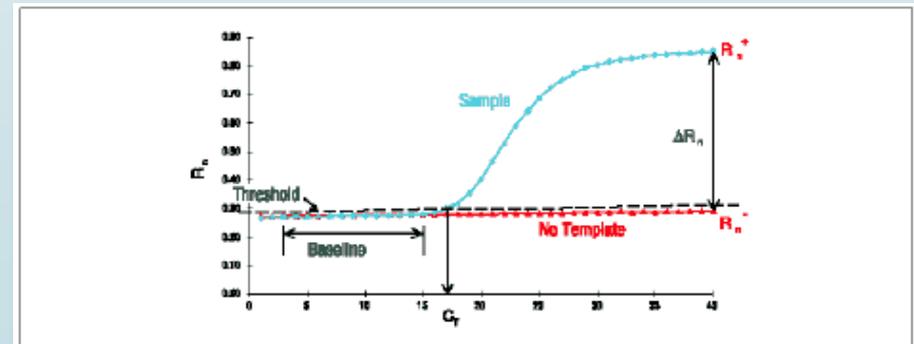
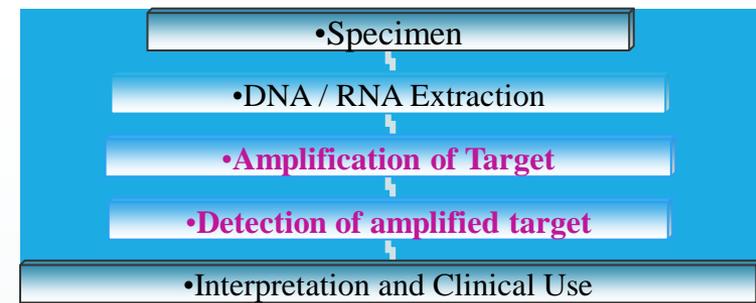


Figure 2. Model of a single amplification plot, showing terms commonly used in real-time quantitative PCR Figure from Applied Biosystems' DNA/RNA Real-Time Quantitative PCR bulletin).

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
- ▶ Chemical and Physical Inactivation

POC Molecular Diagnostics

➤ Infectious Disease

- Outpatient POC
 - GC / Chlamydia
 - **Group A strep**
 - HIV / HCV viral load
 - GI pathogens
- 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 pink actually exist in FDA-approved **waived** form. The rest are in active development.

What Does a Molecular POC Test Look Like?

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

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

Why Molecular: FDA Changed Classification of Rapid Flu Tests

- ▶ January 2017, FDA reclassified antigen-based RIDT systems into class II
- ▶ The **poor sensitivity** of some antigen-based RIDTs misdiagnosed cases.
- ▶ **Special controls** for antigen-based RIDTs for assuring a test's accuracy, reliability and clinical relevance.
 - ▶ Manufacturers of these tests had until January 12, 2018 to bring their tests into compliance with the new regulation.
- ▶ Require, among other things,
 - ▶ Minimum performance levels and analytical reactivity (inclusivity) testing for current circulating virus strains on an annual basis and in certain emergency situations.
 - ▶ The new minimum performance requirements for these tests are expected to lower the number of misdiagnosed flu infections by promoting the development of new, improved devices that can more reliably detect the virus.

Molecular Testing for Influenza

- ▶ Real-time methods can provide result in <1h.
- ▶ 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
- ▶ Multiple methods of waived to high complexity.
- ▶ Now clearly the ‘gold standard’
- ▶ Information sources:
 - ▶ <http://www.cdc.gov/flu/pdf/professionals/diagnosis/table1-molecular-assays.pdf>
 - ▶ CDC listing of waived molecular flu tests pending



CDC Guidance on Molecular Flu Testing

- ▶ Not necessary in every patient.
 - ▶ Outpatients with compatible syndromes during an outbreak may be presumed to have influenza.
 - ▶ Testing indicated for all inpatients.
 - ▶ If antiviral treatment is indicated and influenza testing isn't immediately available, do not delay treatment.
 - ▶ In institutions (e.g. LTC) early molecular testing may identify outbreaks.

Who to Test?

Does the patient have signs and symptoms suggestive of influenza, including atypical clinical presentation, or findings suggestive of complications associated with influenza?^{2,3}

Yes

No

Is the patient being admitted to the hospital?

Yes

No

Will influenza testing results influence clinical management?⁴

Influenza testing probably not indicated; consider other etiologies

Test for influenza; start empiric antiviral treatment for hospitalized patients while results are pending (molecular assays should be used for influenza testing of hospitalized patients.)^{4,5,6,7,8} Proper interpretation of testing results is important.

Yes

No

Influenza clinically diagnosed; start empiric antiviral treatment if the patient is in a high-risk group for influenza complications^{7,8}, or has progressive disease, advise close follow-up if worsening

Factors Impacting Results

- Time from onset of illness (ideally <4d)
- Source of specimen (usually NP)
- Lower respiratory tract specimens in severe / prolonged illness
 - Not FDA-approved sample type.
- Proper storage and rapid transport of samples.
- Careful attention to manufacturer's directions.

FDA-approved Waived Molecular Influenza (and sometimes more!) Tests

- ▶ Alere i
 - ▶ Influenza A and B
 - ▶ RSV
 - ▶ Group A strep
- ▶ BioFire FilmArray EZ
 - ▶ Respiratory Panel
- ▶ Cepheid Xpert Xpress
 - ▶ Flu A/B/RSV assay
- ▶ Mesa Biotech. Inc. Accula Dock
 - ▶ Flu A/Flu B Test
- ▶ Roche LIAT
 - ▶ Influenza A/B
 - ▶ Influenza A/B/RSV
 - ▶ Group A Strep

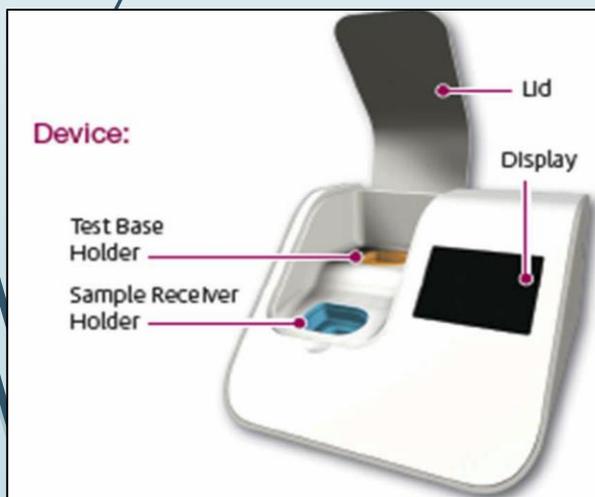
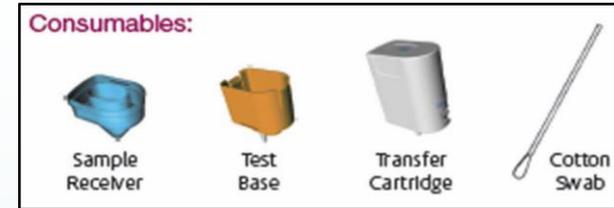
Alere I

► Analytes

- Influenza A&B; RSV; Group A Strep

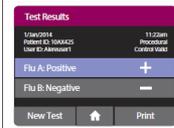
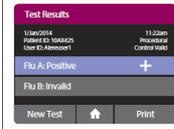
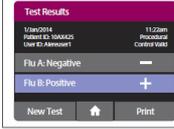
► Procedure

- 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.

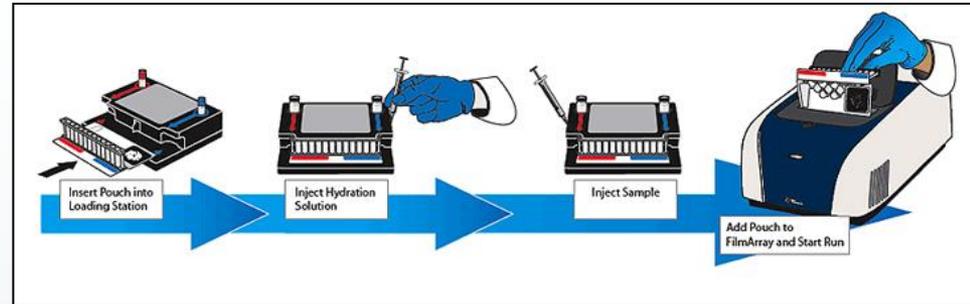


RESULT INTERPRETATION

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
	<p>Flu A Viral RNA Detected; Flu B Viral RNA Not Detected.</p> <p>This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.</p>
	<p>Flu A Viral RNA Detected; The presence or absence of Flu B Viral RNA cannot be determined.</p> <p>This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.</p>
	<p>Flu B Viral RNA Detected; Flu A Viral RNA Not Detected.</p> <p>This result does not rule out co-infections with other pathogens or identify any specific influenza B virus lineage.</p>

BioFire FilmArray Respiratory Panel



- ▶ Detects: Influenza A and B (discriminates H1, H3, 2009 H1), Respiratory Syncytial Virus, Parainfluenza virus, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Coronavirus, *Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*
- ▶ Sample to answer ~1h



Cepheid Xpert XPress



- Analytes
 - Flu A and B
 - Flu + RSV
 - Group A Strep
- Sample to answer
~20 min



1

Transfer 300µl of prepared sample into the large hole



2

Dispense binding reagent into small hole



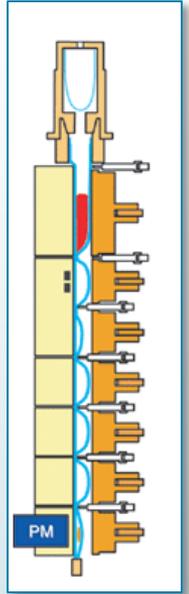
3

Insert cartridge and start assay



Roche LIAT Influenza A/B Assay

- ▶ LIAT stands for Lab-In-A-Tube
- ▶ Tests
 - ▶ Influenza A&B, Flu A/B/RSV, group A strep
- ▶ Sample to answer .5h



cobas® **Liat** PCR system



STEP 1.
Add sample



STEP 2.
Scan barcode



STEP 3.
Insert tube



Done!
Results in 20 minutes

Mesa Biotech Dock (FDA approved, not yet available in US)



Mesa Biotech Dock

The Mesa Biotech Dock senses the test cassette that has been inserted into the Dock, automatically selects the optimized protocol for each test, and provides step-by-step instructions to the user.



Flu A/Flu B Test Cassette (Disposable)

The Flu A/Flu B Test Cassette is a single use disposable that hermetically seals after sample addition and prior to nucleic acid amplification to ensure test accuracy. Each cassette is packaged with nasal swab buffer, nasal swabs, and disposable pipettes to provide all components required to run a test. Stabilized dried reagents within the cassette eliminate the need for refrigerated storage and transportation.

Are All Molecular Tests The Same?

- ▶ **Of course not.** That would be too simple.
- ▶ Numerous, rather confusing studies.
 - ▶ There are few comparisons of multiple methods. Sorry.
 - ▶ Don't take this as a comprehensive assessment of both assays; neither performed as well as the authors' homebrew RT-PCR.
- ▶ Performance **DOES** vary within the molecular tests.
- ▶ Pay attention not only to sensitivity / specificity numbers, but also to **comparator method**.
 - ▶ Comparisons with culture make a method look better; comparisons with a highly optimized molecular method or with a panel of different methods is a more stringent comparison.

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

A POC Example



Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Virology

Prospective evaluation of the Alere i Influenza A&B nucleic acid amplification versus Xpert Flu/RSV

J.C. Nguyen Van ^{a,*}, F. Caméléna ^{a,1}, M. Dahoun ^{a,1}, B. Pilmis ^b, A. Mizrahi ^a, J. Lourtet ^a, S. Behillil ^c, V. Enouf ^c, A. Le Monnier ^a

^a Unité de Microbiologie Clinique, Groupe Hospitalier Paris Saint Joseph, Paris, France
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^c National Influenza Center (Northern France), Institut Pasteur, Paris, France



► Comparison of Alere i and lab-based Xpert

► 96 respiratory swabs, 86 adult, 10 children

Table 1
Performance of the Alere i Influenza A&B assay versus Xpert Flu/RSV assay.

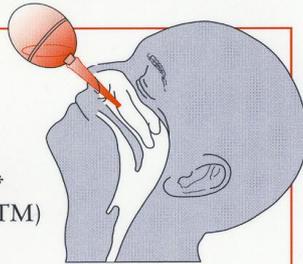
Virus	No. of samples Alere versus Xpert				Sensitivity, % (95% CI)	Specificity, % (95% CI)	NPV	PPV
	+/+ (true positive)	+/- (false positive)	-/+ (false negative)	-/- (true negative)				
Influenza virus A	30	0	2	56	93.8 (79.2–99.2)	100 (93.6–100)	97.0	100
Influenza virus B	8	0	0	56	100 (63.1–100)	100 (93.6–100)	100	100
Influenza virus A + B	38	0	2	56	95 (88.2–100)	100 (95.0–100)	96.5	100

Influenza Specimen Collection

- Specimen collection is probably *the* critical step in influenza testing
 - Good test on a bad specimen = bad test

Nasopharyngeal Wash: Bulb Method

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

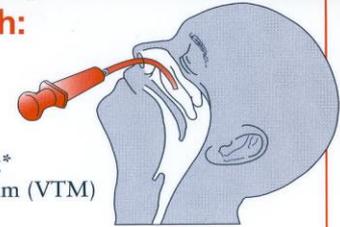


1. Suction 3-5 ml saline into a new sterile bulb.
2. Insert bulb into one nostril until nostril is occluded.
3. Instill saline into nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
4. Empty bulb into suitable dry, sterile specimen container or one 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 somewhat better than swabs*

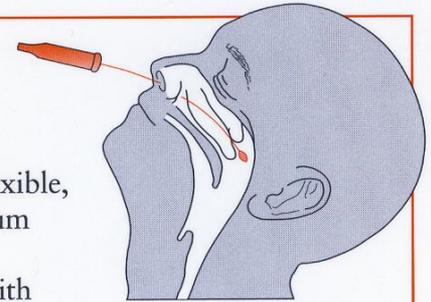
*A general but not-quite universal rule of microbiology: swabs are evil

Specimen Collection – The NP Swab

- ▶ NOT A THROAT SWAB. NOT A NASAL SWAB. A NASOPHARYNGEAL SWAB.
 - ▶ Except for tests where that's not the specimen...
- ▶ Important to get ciliated epithelial cells – this is a cell-associated virus
- ▶ Test early; more virus is shed early than later in disease.
 - ▶ A test a week after onset of symptoms is useless.
- ▶ Children shed more virus than adults
 - ▶ Tests tend to be more sensitive in kids

Nasopharyngeal Swab Method

Materials: BD BBL CultureSwab flexible, soft, or regular aluminum wire products *or* Nasopharyngeal swab with synthetic fiber tip
1-2 ml Viral Transport Medium (VTM)
Specimen container



1. Insert swab into one nostril.
2. Rotate swab over surface of posterior nasopharynx.
3. Withdraw swab from collection site; insert into transport tube or container with VTM.

When to test?

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
		RHINOVIRUS									
CORONAVIRUS					ENTEROVIRUS						
ADENOVIRUS											
		PIV-3					PIV2,3				
RSV											RSV
INFLUENZA											
MPV											
GROUP A STREPT											

- Remember – false-positives have potentially severe consequences, e.g. non-treatment of a serious bacterial infection.
- Test during the flu season.
 - This is the conventional wisdom, to be modified in travelers and people with contacts who are travelers. Note that other viruses don't have influenza's striking seasonality.
 - Molecular tests may have higher specificity than the old antigen tests, but still; question off-season positives.
- 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.

Who to Test?

- Expensive molecular flu tests may be best deployed selectively.
- Consider testing:
 - Patients destined for hospital admission.
 - Compromised patients at high risk likely to benefit from treatment.
- Consider not testing:
 - Otherwise healthy people who probably don't need anything but reassurance and good hydration.
- Remember that influenza and bacteria can and often do co-infect.
 - Really sick patients may have a bacterial superinfection facilitated by the virus.

(Potential) Benefits of Flu Testing

38

► For positives...

- Rapid treatment.
- Avoidance of antibiotics and costs and complications thereof.
 - We all know what a large fraction of antibiotics are used for viral infections.
- Avoidance of further workup / admission in some cases.
 - How much will test impact this versus clinical condition of the patient?
- Infection control – inpatient and outpatient.
- Patient flow in outpatient settings:
 - diagnosis – disposition/treatment – onward.
- **All these depend on a result provided within the encounter time or shortly thereafter.**

► For negatives...

- Save cost of antiviral therapy.
- Save isolation cost / inconvenience
- Continue diagnostic workup if patient's condition warrants it.

Does it Make Sense to Test?

INFECTIOUS DISEASE/ORIGINAL RESEARCH

Cost-Utility of Rapid Polymerase Chain Reaction-Based Influenza Testing for High-Risk Emergency Department Patients

Andrea Freyer Dugas, MD; Sara Coleman, MPH, MBA; Charlotte A. Gaydos, DrPH, MPH; Richard E. Rothman, MD, PhD; Kevin D. Frick, PhD, MA

- Cost-effectiveness studies are tricky.
- Assuming a \$50,000 per quality-adjusted life-year willingness-to-pay threshold, the most cost-effective treatment option is treatment according to provider judgment from 0% to 3% prevalence, treatment according to a PCR-based rapid influenza test from 3% to 7% prevalence, and treating all at greater than 7% prevalence.
 - ...but this ignored induction of antiviral resistance, transmission of flu, and cost avoidance in tested patients; only treatment cost and effect was counted.
 - “Patients who did not have influenza were not evaluated further because influenza testing or treatment would have no further effect on their care or outcomes.”
 - Ann Emerg Med. 2013;62:80-88

Managing POC Molecular

- 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**

Molecular ≠ Magic



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MINIREVIEW



The Drift in Molecular Testing for Influenza: Mutations Affecting Assay Performance

Kathleen A. Stellrecht^a

^aDepartment of Pathology and Laboratory Medicine, Albany Medical Center Hospital and Albany Medical College, Albany, New York, USA

- Influenza is a moving target.
 - WHO recommended targeting highly conserved M gene region 144-251.
 - CDC used a slightly different region of the same gene.
 - But strains mutated in those areas have been isolated.

TABLE 1 WHO and CDC M1 gene PCR target region patterns^a

Organization and pattern	Sequence (gene region 144–251)
WHO	<u>AAGACCAATCCTGTCACTCTGA</u> CTAAGGGGATTTTGGGG TTTGTGTTACGCTCACCGT GCCCGAGTGAGCGAGGACTGCAGCGT TAGACGCTTTGTCCAAAATGCCCT
CDC	-- <u>GACCRATCCTGTCACTCTGA</u> ----- CGTGCCCGAGTGAGCGAGGACTGCA --- <u>TAGACGCTTTGTCCAA</u> RAATGCCCT
A	-----T-----
B	-----T-----A-----
C	-----T-----A-----T-----
D	-----T-----T-----A-----T-----
E	-----T-----T-----A-----T-----A-----

^aUnderlined bases represent the recommended forward and reverse primer sequences; bold font bases represent the recommended probe sequences. Nucleotides in shaded font represent divergence from the reference sequence [A/Wyoming/01/2003(H3N2); GenBank accession number [DQ849011.1](#)].

Mutant Viruses Arise...

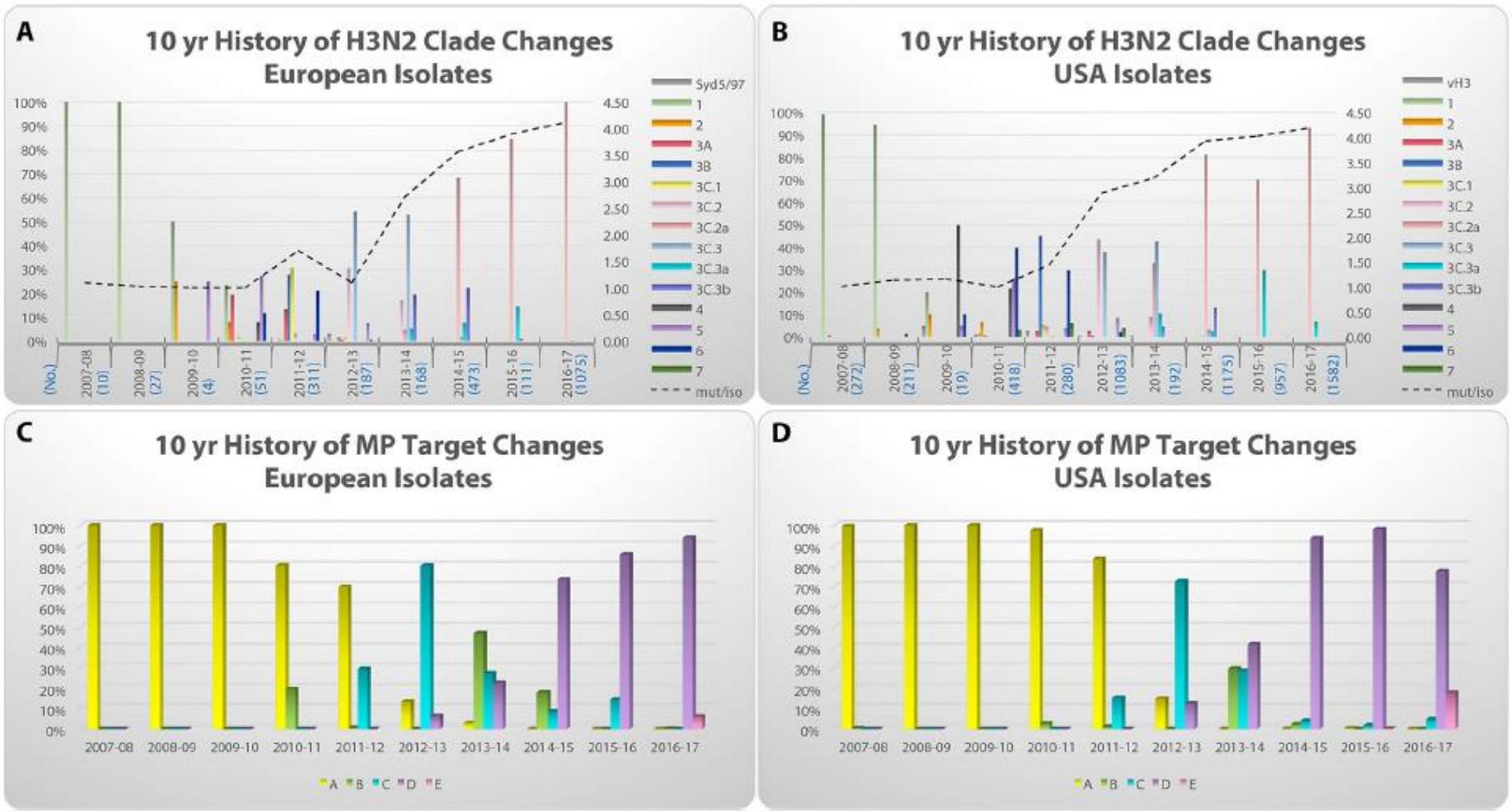


FIG 1 Ten-year history of annual trends in H3N2 clades and MP PCR target region patterns based on GISAID data (<https://www.gisaid.org/>). (A and B) Clade changes for (A) European and (B) USA 2014–2015 isolates along with the number of mutations observed in the MP PCR target region between nucleotides 144 and 251. (C and D) MP PCR target region pattern changes for (C) European and (D) USA isolates. Patterns are coded by letter based on Table 1 data.

Impact of Drift in Molecular Targets

TABLE 2 Sensitivities of commercial PCR assays during the 2014/15 respiratory virus season

Assay	<i>n</i>	% sensitivity	Reference
cobas Influenza A/B test	275	92	19
FTD21 ^a	563	91	18
Prodesse ProFAST+	275	100	19
Prodesse ProFlu+	135	76	19
RespiFinder RG	166	51	17
FilmArray Respiratory Panel	275	97	19
Xpert Flu	275	85	19
Xpert Flu/RSV XC assay	102	100	18

^aFTD21, Fast Track Diagnostics Respiratory Pathogens 21.

- Changes in viral sequences impacted sensitivity of some test-systems.
- This is likely to continue; even molecular tests will need to be monitored for loss of sensitivity.
- Regulatory agencies may need to adapt to the need for rapid changes to test formulation.



False-negative RDT results and implications of new reports of *P. falciparum* histidine-rich protein 2/3 gene deletions

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INFORMATION NOTE

- To the extent that diagnostics impact disease control, diagnostics, as well as anti-infectives, will exert selective pressure on pathogens.



Future Developments

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

Involve your microbiologists!

- “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>