The Application of Molecular POCT for Influenza and Strep Detection

Challenges and Solutions

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Disclosures

- Provided educational talks and received honoraria from Abbott, BioFire, Cardinal health, Cepheid, Hologic, Luminex, Quidel

Objectives

- Introduce Point-of-Care Testing (POCT) for infectious diseases
- Review different molecular testing methodologies
- Focus on CLIA-waived molecular testing options for infectious diseases
- Review data from a Group A strep POC study

Testing for pathogens:

Testing in Clinic (Point of Care)

- Rapid antigen tests
- **Molecular nucleic acid amplification testing (POCT)**

Laboratory Testing

- Culture workup (e.g. Strep)
- Molecular nucleic acid amplification

Point-of-care testing (POCT)

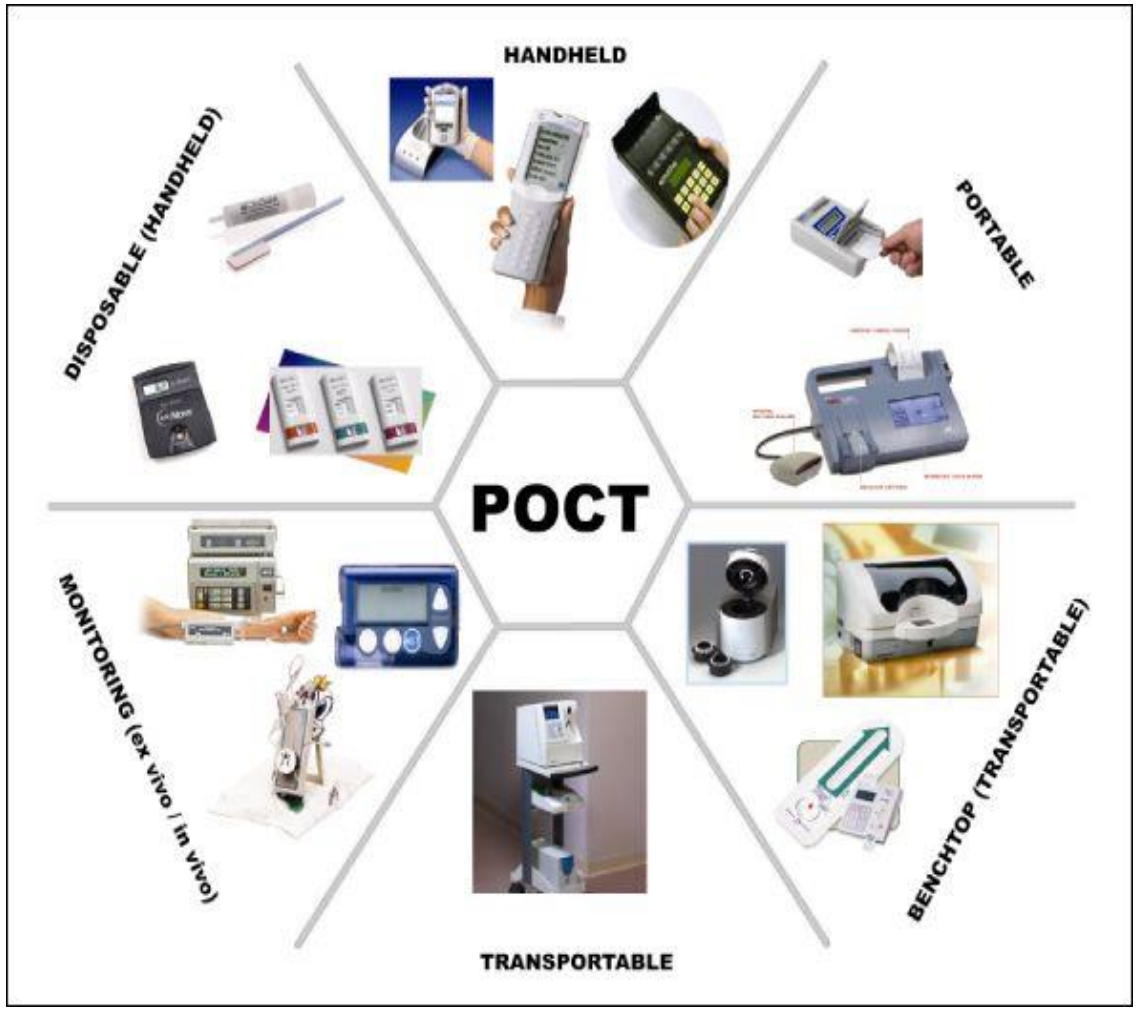
Testing performed <u>while</u> patient care is occurring

Main advantage is time gained

Therapeutic choices in real time

- Identify treatment to administer
- Avoid unnecessary drugs/treatments

Requires simple platforms with accurate results



https://i.pinimg.com/736x/0c/26/a9/0c26a969cd5be705139c9a71f39e3665--point-of-care-testing-lab-tech.jpg

Barriers to POCT and Solutions

Problems

- Not accurate enough for definitive diagnosis
  - E.g. rapid strep and flu tests
- Too difficult to perform at point-of-care
  - E.g. molecular testing
- Too Expensive

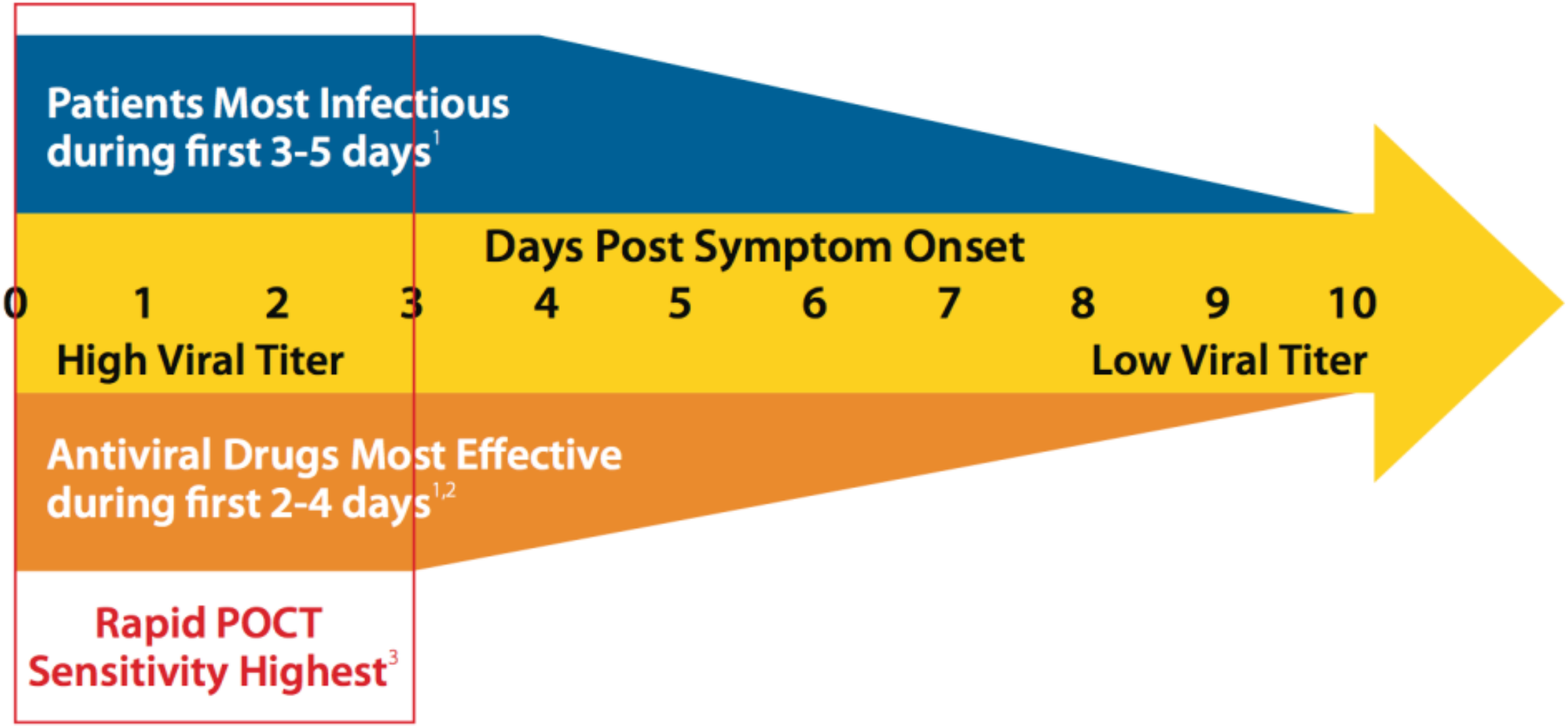
Solutions

- Increasing sensitivity and specificity
- Assays designed to be user-friendly and more error-proof
- Costs decreasing over time and reimbursement that matches test costs

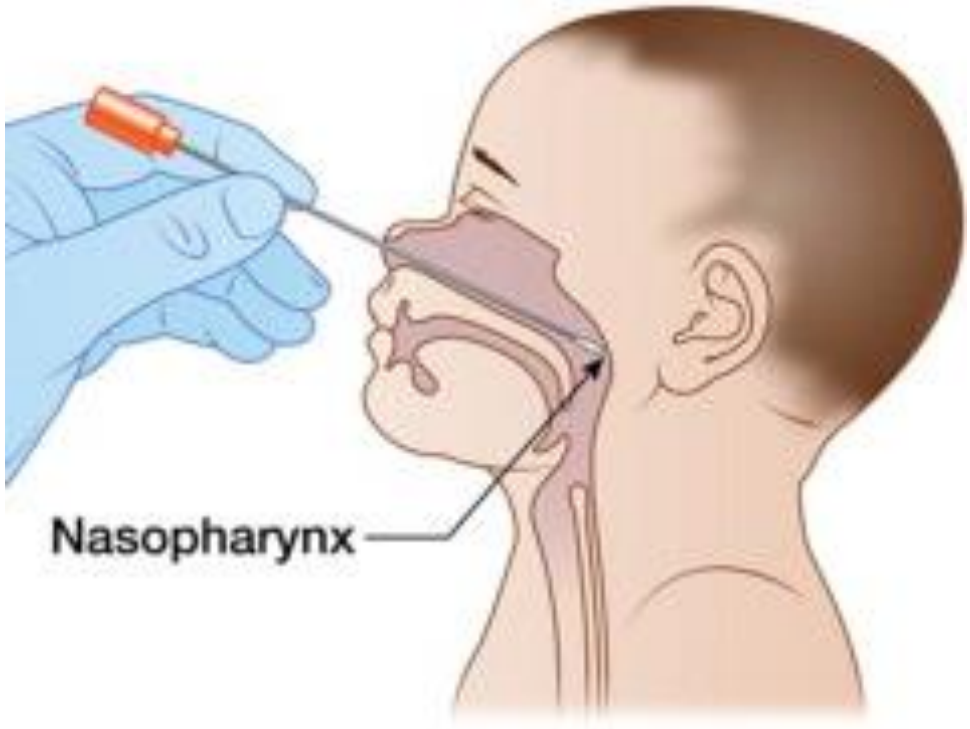
POCT in Infectious Disease Diagnostics

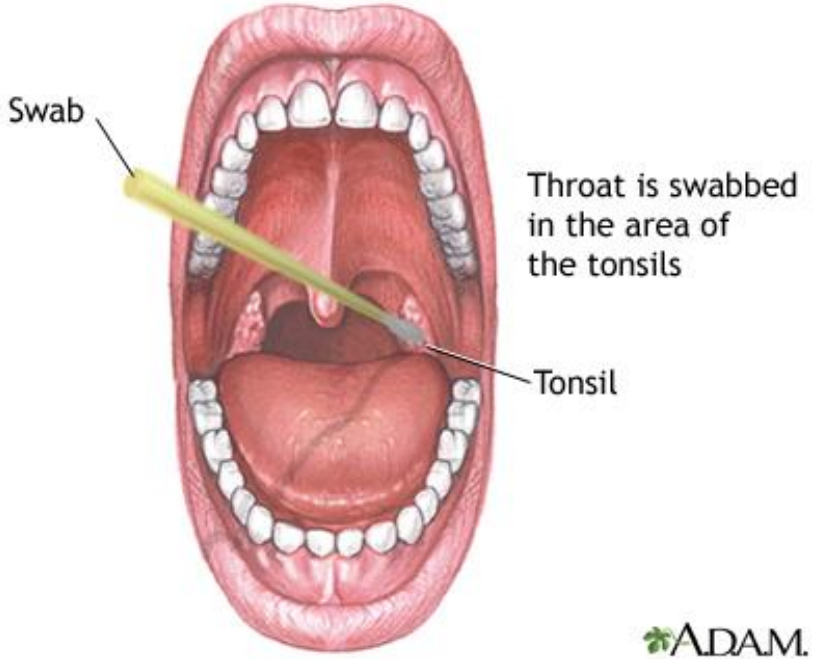
- CLIA waived tests that can be performed by facilities with a Certificate of Waiver
- Increasingly larger portion of infectious disease testing
- **Huge advantage of rapid answer for treatment decisions**
- QUALITY is key- results must approach the same sensitivity and specificity of laboratory tests

Timing is Everything!



So is Specimen Collection!





C. Satzke et al. / Vaccine 32 (2014) 165–179

The right specimen for the right test is key!

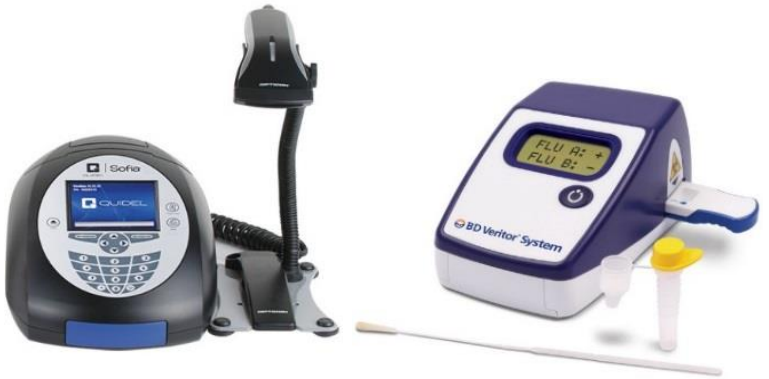
Types of POCTs available for Infectious Diseases

- Assays targeting detection of pathogens like flu A, flu B, RSV, Group A strep, HIV, HCV, *H. pylori*, syphilis, *T. vaginalis*, adenovirus, etc.

- Two basic types of tests
  - Rapid antigen detection tests
    - Detecting host antibodies produced against pathogen
    - Directly detecting antigens of pathogen
  - Molecular assays

Rapid antigen tests

- Available since the 1980s- Flu, GAS, RSV
- Most common first line of testing at clinic
- Immunoassays: detect pathogen-specific antigens
- Qualitative resulting
- Vary greatly in their sensitivity
  - Negative GAS results need culture confirmation



Notes from the Field

Group A Streptococcal Pharyngitis Misdiagnoses at a Rural Urgent-Care Clinic — Wyoming, March 2015

Alexia Harrist, MD, PhD[1,2]; Clayton Van Houten, MS[2]; Stanford T. Shulman, MD[3]; Chris Van Beneden, MD[4]; Tracy Murphy, MD[2]

Group A *Streptococcus* (GAS) is the most common bacterial cause of pharyngitis, implicated in 20%–30% of pediatric and 5%–15% of adult health care visits for sore throat (*1*). Along with the sudden onset of throat pain, GAS pharyngitis symptoms include fever, headache, and bilateral tender cervical lymphadenopathy (*1*,*2*). Accurate diagnosis and management of GAS pharyngitis is critical for limiting antibiotic overuse and preventing rheumatic fever (*2*), but distinguishing between GAS and viral pharyngitis clinically is challenging (*1*). Guidelines for diagnosis and management of GAS pharyngitis have been published by the Infectious Diseases Society of America (IDSA)* (*1*). IDSA recommends that patients with sore throat be tested for GAS to distinguish between GAS and viral pharyngitis; however, IDSA emphasizes the use of selective testing based on clinical symptoms and signs to avoid identifying GAS carriers rather than acute GAS infections (*1*). Therefore, testing for GAS usually is not recommended for the following: patients with sore throat and accompanying

The line list revealed nonadherence to IDSA guidelines in testing and treatment procedures. Ten of 34 (29%) patients aged ≥3 years who were tested for GAS reported no sore throat, the symptom that should prompt evaluation for GAS pharyngitis in patients aged ≥3 years (*1*). Two of these 10 were asymptomatic adult contacts of patients with diagnosed GAS pharyngitis; both asymptomatic contacts had positive RADT results and were prescribed an antibiotic. Of the 24 tested patients aged ≥3 years with sore throat, 19 (79%) reported cough or rhinorrhea, symptoms that suggest a viral rather than bacterial etiology (*1*). Although diagnostic testing of patients aged <3 years is not routinely recommended, testing of symptomatic children who are household contacts of persons with laboratory-confirmed GAS pharyngitis can be considered (*1*). Among the seven patients aged <3 years who were tested for GAS pharyngitis, five (71%) had GAS-positive family members indicated by shared surname included in the line list; however, all seven (100%) had cough, and five (71%) had rhinorrhea.

Four of six patients with negative RADT results received an antibiotic. The clinic practice was to send throat swabs from patients with negative RADTs to a commercial laboratory for back-up culture, but it is unknown whether the clinic obtained any GAS-positive throat cultures from RADT-negative patients

Investigation

- In March 2015, a rural urgent-care clinic serving a population of 5,000–7,000 reported a substantial increase in GAS pharyngitis infections since November 2014, with some infections nonresponsive to penicillin and amoxicillin to the Wyoming Department of Health (WDH).
- Findings:
  - Testing asymptomatic patients (no sore throat)
  - Testing of patients with viral illness symptoms
  - 86% positivity rate on rapid antigen tests performed
  - Clinic staff were reading tests at longer intervals than manufacturer's instructions- can lead to false positives
- Intervention
    - Cases declined, no resistance was found- likely viral illnesses misdiagnosed as GAS

Follow the FDA approved package insert!!!

Laboratory testing

- Testing performed in centralized location
- Lab is licensed and accredited to perform patient testing
- Licensed laboratory personnel perform testing

http://www.genengnews.com/gen-articles/pcr-based-diagnostics-put-to-the-test/3894
https://visualsonline.cancer.gov/details.cfm?imageid=2230

Identification of GAS by culture

- Throat swab is collected and inoculated onto plates
  - Most laboratories are routinely identifying only GAS
    - Agar selective for strep, or BAP can be used with a Bacitracin (A) disk
      - GAS is SUSCEPTIBLE
    - Additional workup can be done if other pathogens suspected
      - e.g. blood agar for other streptococci (B, C, F, G) and A. haemolyticum, or modified Thayer-Martin for N. gonorrhoeae isolation
- Incubate in aerobic incubator with 5% CO_2
- Result: 24-48 hours

https://visualsonline.cancer.gov/details.cfm?imageid=2230

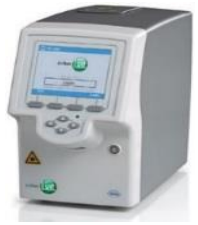
Molecular testing

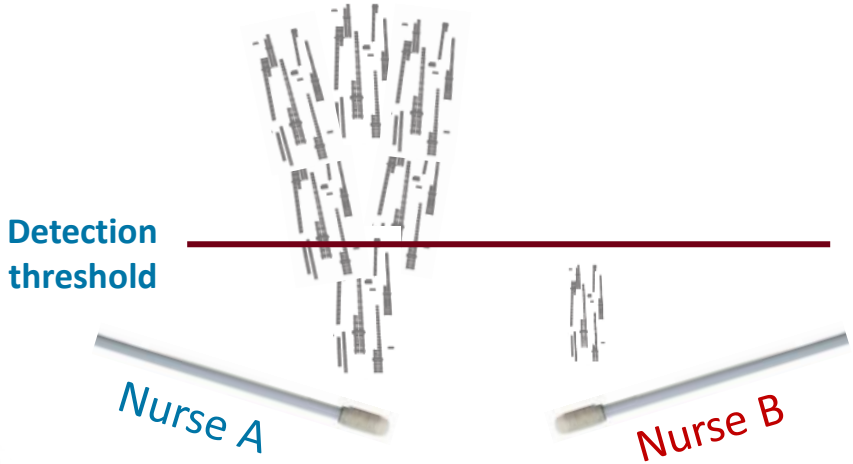
- In general, molecular methods are the most sensitive/specific testing
- Advantage for clinician - rapid turnaround time (hours, even minutes vs. 1-3 days)
- Downstream impact:
  - More rapid answer means **right therapeutic therapy** chosen up-front
  - Patient satisfaction

Traditional Molecular Laboratory testing platforms

- Highly sensitive/specific
- Performed in the laboratory by specialized personnel
  - **NOT POC-friendly**
- TAT can be 1-2 hours (**once received**, if NOT batched)
  - If batched, could be 12-24 hours, depending on how often testing is performed

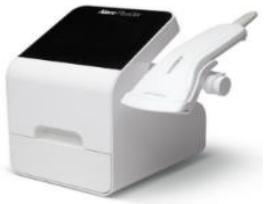
The quality and power of sample amplification

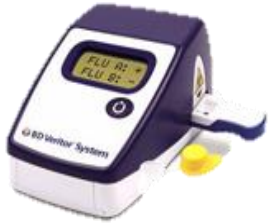




Amplified
Sample

Not Amplified
Sample





CLIA-waived Molecular POCT options

ID NOW™ (Abbott, formerly Alere™ i)

- 5-13 minutes to result for Flu
- ≤13 minutes to result for RSV
- 2-6 minutes to result for Strep A
- Isothermal Amplification
- Interpreted by instrument

Flu: CLIA-waived for use with nasal or nasopharyngeal swabs (direct and eluted in viral transport medium)

For *in vitro* Diagnostic Use
https://www.alere.com/en/home/product-details/id-now.htm
ID NOW™ Strep A 2 clinical trial data, held on file

Cepheid Xpert® Xpress Flu/RSV & Xpert Xpress Strep A

- 20-30 minutes to result Flu A/B, RSV
- 18-24 minutes to result Strep A
- RT-PCR
- Interpreted by instrument

Flu/RSV: CLIA-waived for use with nasal/nasopharyngeal swabs

For *in vitro* Diagnostic Use

http://www.cepheid.com/us/cepheid-solutions/clinical-ivd-tests/critical-infectious-diseases/xpert-xpress-flu

cobas® LIAT® - Lab In a Tube (Roche)

- 20 minutes to results Flu A/B, RSV
- 15 minutes to results Strep A
- RT-PCR
- Interpreted by instrument

Flu: CLIA-waived for use with nasopharyngeal swabs

For *in vitro* Diagnostic Use
https://diagnostics.roche.com/us/en/products/systems/cobas-liat-system.html

Silaris™ Influenza A&B Test (Sekisui)

30 minutes or less for flu A & B

RT-PCR amplification followed by hybridization and colorimetric visualization of amplified products on a test strip flu A & B

Results are interpreted visually by the operator

Flu: CLIA-waived for use with nasal swabs

For *in vitro* Diagnostic Use

https://www.sekisuidiagnostics.com/products-all/silaris-influenza-ab-test/

Molecular testing pros and cons

Pros	Cons
Can amplify genome	Typically costs more
Highly sensitive and specific	Takes longer

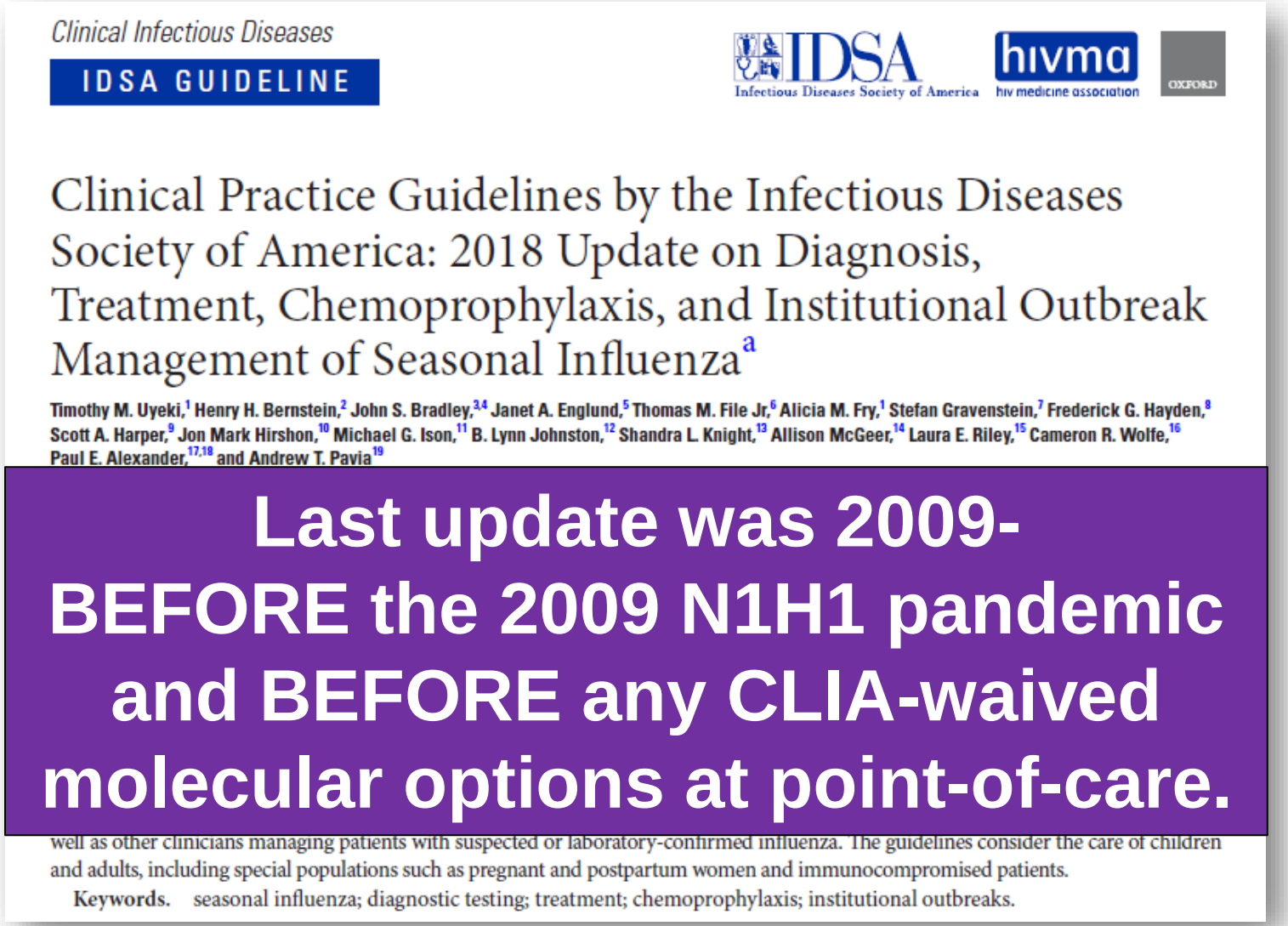
Comparison of Methods

	POCT Rapid Antigen	Culture	Laboratory Molecular	POCT Molecular
Fast	X			X
Convenient	X			X
Actionable Results	X	X	X	X
POCT- Friendly	X			X
Little/No Subjectivity		X	X	X
LIS/EMR Interfaced			X	X
High Sensitivity/Specificity		X	X	X
Low Cost	X	X		

Specific Benefits of POC Molecular Testing in a resource-limited setting:

- Drastically improved TAT- no need to send to a reference lab for confirmation (e.g. GAS culture NOT needed)
  - Getting results in real-time to act upon them
- Same testing SOC that would be found at tertiary center
  - clinical confidence in test method/results for diagnosis
- Clinic can perform testing that could previously only be done by specialized testing staff
- Test accuracy and healthcare efficiency/lab stewardship
- Offering new technology for community and outreach

Another big change has happened lately….



Clinical Infectious Diseases

IDSA GUIDELINE

IDSA Infectious Diseases Society of America

hivma hiv medicine association

OXFORD

Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenza[a]

Timothy M. Uyeki,[1] Henry H. Bernstein,[2] John S. Bradley,[3,4] Janet A. Englund,[5] Thomas M. File Jr,[6] Alicia M. Fry,[1] Stefan Gravenstein,[7] Frederick G. Hayden,[8] Scott A. Harper,[9] Jon Mark Hirshon,[10] Michael G. Ison,[11] B. Lynn Johnston,[12] Shandra L. Knight,[13] Allison McGeer,[14] Laura E. Riley,[15] Cameron R. Wolfe,[16] Paul E. Alexander,[17,18] and Andrew T. Pavia[19]

Last update was 2009- BEFORE the 2009 N1H1 pandemic and BEFORE any CLIA-waived molecular options at point-of-care.

well as other clinicians managing patients with suspected or laboratory-confirmed influenza. The guidelines consider the care of children and adults, including special populations such as pregnant and postpartum women and immunocompromised patients.

Keywords. seasonal influenza; diagnostic testing; treatment; chemoprophylaxis; institutional outbreaks.

IDSA Influenza Clinical Guidelines 2018 • **CID 2018- p.5**

What Test(s) Should Be Used to Diagnose Influenza?

Recommendations

10. Clinicians should use rapid molecular assays (ie, nucleic acid amplification tests) over rapid influenza diagnostic tests (RIDTs) in outpatients to improve detection of influenza virus infection *(A-II)* (see Table 6).
11. Clinicians should use reverse-transcription polymerase chain reaction (RT-PCR) or other molecular assays over other influenza tests in hospitalized patients to improve detection of influenza virus infection *(A-II)* (see Table 6).

15. Clinicians should not use RIDTs in hospitalized patients except when more sensitive molecular assays are not available *(A-II)*, and follow-up testing with RT-PCR or other molecular assays should be performed to confirm negative RIDT results *(A-II)*.

IDSA Influenza Clinical Guidelines 2018 • **CID 2018- p.13**

Table 6. Influenza Diagnostic Tests for Respiratory Specimens

Testing Category	Method	Influenza Viruses Detected	Distinguishes Influenza A Virus Subtypes	Time to Results	Performance
Rapid molecular assay	Nucleic acid amplification	Influenza A or B viral RNA	No	15–30 minutes	High sensitivity; high specificity
Rapid influenza diagnostic test	Antigen detection	Influenza A or B virus antigens	No	10–15 minutes	Low to moderate sensitivity (higher with analyzer device); high specificity;
Direct and indirect immunofluorescence assays	Antigen detection	Influenza A or B virus antigens	No	1–4 hours	Moderate sensitivity; high specificity
Molecular assays (including RT-PCR)	Nucleic acid amplification	Influenza A or B viral RNA	Yes, if subtype primers are used	1–8 hours	High sensitivity; high specificity
Multiplex molecular assays	Nucleic acid amplification	Influenza A or B viral RNA, other viral or bacterial targets (RNA or DNA)	Yes, if subtype primers are used	1–2 hours	High sensitivity; high specificity
Rapid cell culture (shell vial and cell mixtures)	Virus isolation	Influenza A or B virus	Yes	1–3 days	High sensitivity; high specificity
Viral culture (tissue cell culture)	Virus isolation	Influenza A or B virus	Yes	3–10 days	High sensitivity; high specificity

Negative results may not rule out influenza. Respiratory tract specimens should be collected as close to illness onset as possible for testing. Clinicians should consult the manufacturer's package insert for the specific test for the approved respiratory specimen(s). Most US Food and Drug Administration (FDA)–cleared influenza diagnostic tests are approved for upper respiratory tract specimens but not for sputum or lower respiratory tract specimens. Specificities are generally high (>90%) for all tests compared to RT-PCR. FDA-cleared rapid influenza diagnostic tests are Clinical Laboratory Improvement Amendments (CLIA)–waived; most FDA-cleared rapid influenza molecular assays are CLIA-waived, depending on the specimen.

Abbreviation: RT-PCR, reverse-transcription polymerase chain reaction.

IDSA Influenza Clinical Guidelines 2018 • **CID 2018- p.13**

Table 7. Interpretation of Influenza Testing Results on Respiratory Specimens

Test and Characteristics	Low Influenza Activity[a]		High Influenza Activity[b]	
Rapid influenza diagnostic test (antigen detection: immunoassay or immunofluorescence assay) • Low to moderate sensitivity • High specificity ➢ Should not be used for testing of patients with progressive illness and hospitalized patients	*Negative result* NPV is high: ➢ Likely to be a true-negative result if an upper respiratory tract specimen was collected <4 days after illness onset ➢ If epidemiologically linked to an influenza outbreak, consider confirming with molecular assay	*Positive result* PPV is low: ➢ Likely to be a false-positive result ➢ Confirm with molecular assay	*Negative result* NPV is low: ➢ May be a false-negative result, especially if upper respiratory tract specimen was collected >4 days after illness onset, cannot exclude influenza virus infection ➢ Do not withhold antiviral treatment if clinically indicated ➢ Confirm with molecular assay	*Positive result* PPV is high: ➢ Likely to be a true-positive result

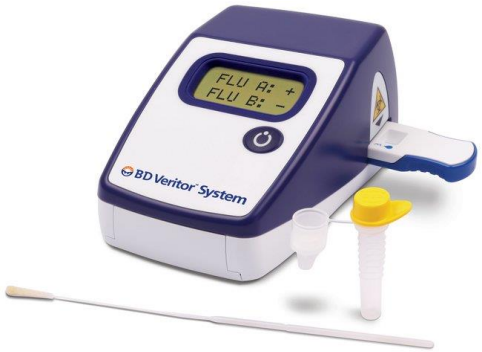
During low flu activity positive RIDTs should be confirmed by molecular
During high flu activity negative RIDTs should be confirmed by molecular

Group A Streptococcus Study

GAS study goals

Compare the BD Veritor™, Alere i™, and culture for detection of GAS

BD Veritor™



RIDT with reader

ID NOW™

Isothermal amplification

Culture

Evaluate the hypothetical impact of results on antibiotic utilization

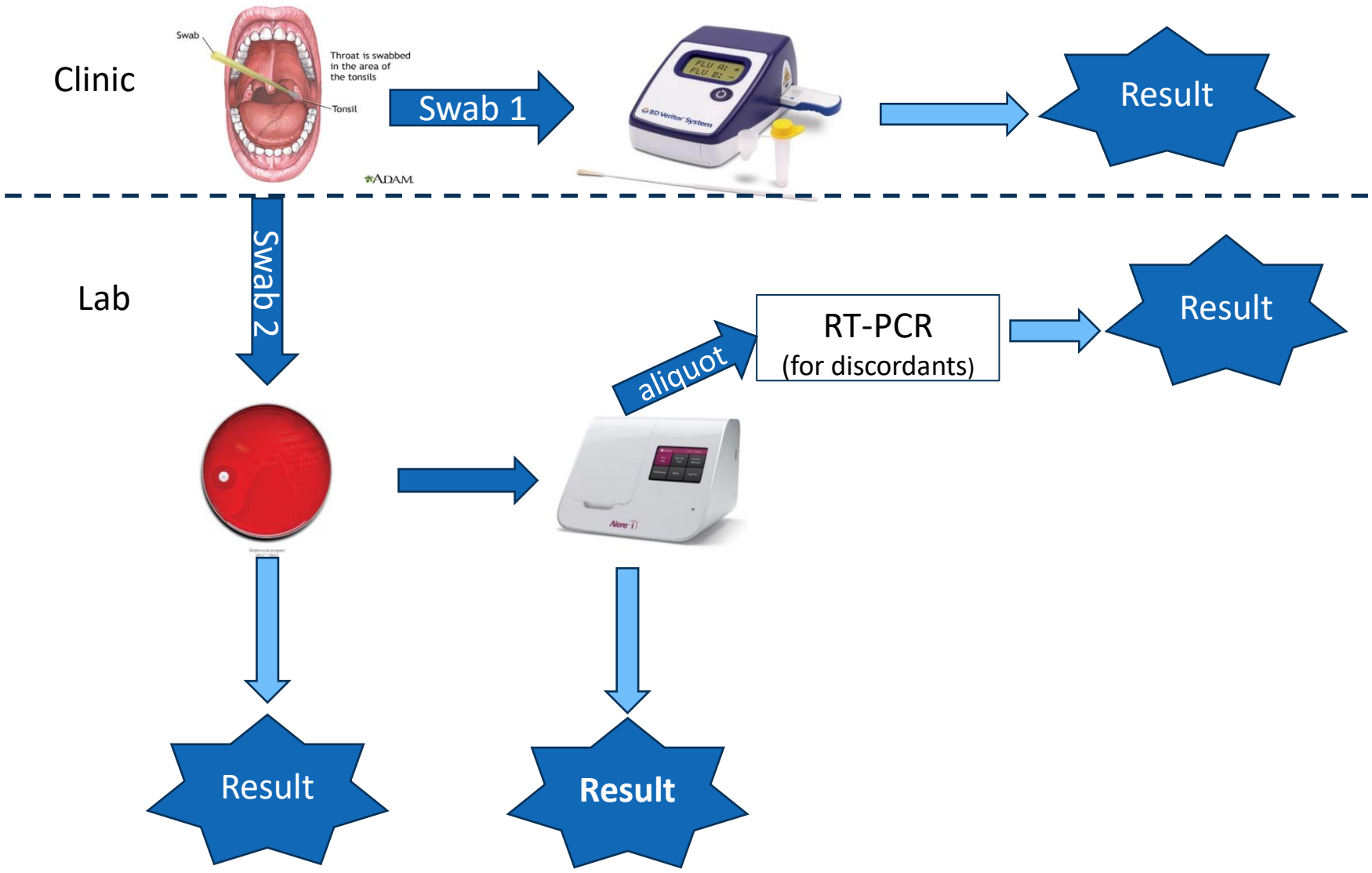
Berry et. al, J. Clin. Microbiol., 2018

Study design

- Prospectively tested 216 clinical throat samples that were collected during the months of May and June of 2016 for routine strep throat testing from two predominantly pediatric outpatient clinics within our hospital system.
- Routine patient testing (BD Veritor™ with reflex to group A strep culture) was performed and compared to results obtained on the ID NOW™ (formerly Alere™ i) system.
- Inclusion criteria was a strep throat test ordered by a clinician. Pediatric cases (<18 years of age) accounted for 199 (92.1%) of the specimens, while adults (≥18 years of age) accounted for 17 (7.9%) of the specimens.
- Each patient was subjected to two Rayon throat (posterior oropharynx) swabs as a part of their routine strep throat workup in the clinic. BD Veritor™ testing was performed in the clinic where patients were initially seen.

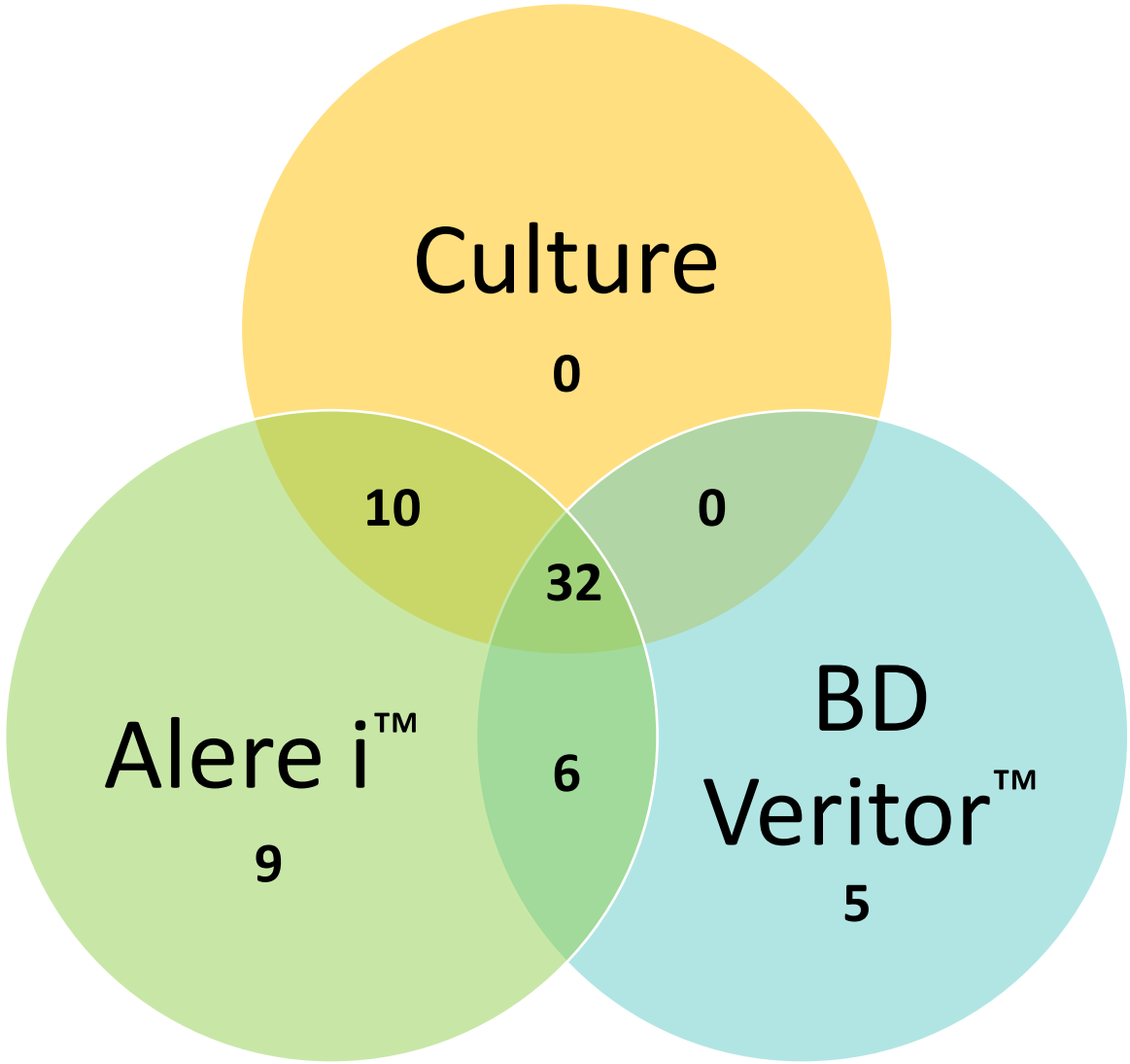
Berry et. al, J. Clin. Microbiol., 2018

Study Design



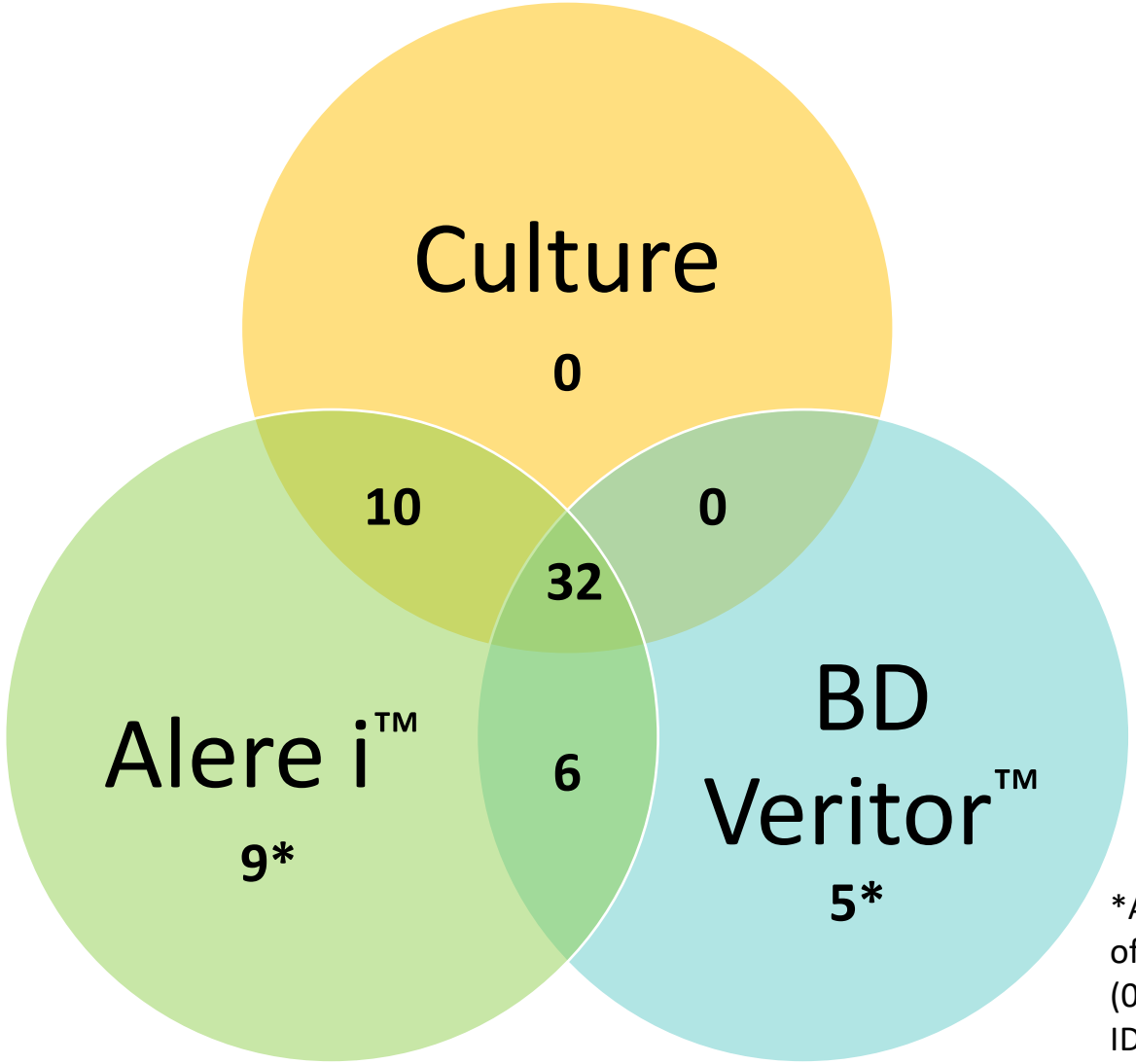
Berry et. al, J. Clin. Microbiol., 2018

Distribution of positive results



Berry et. al, J. Clin. Microbiol., 2018

Distribution of positive results



*Assay adjudication was done for each of the single-assay positive results 0/5 (0%) of BD Veritor™ and 8/9(89%) of the ID NOW™, were confirmed by RT-PCR

Berry et. al, J. Clin. Microbiol., 2018

Table 1: Sensitivity, Specificity, Accuracy, and Kappa Index analysis of each assay

		Culture - Gold Standard		
Assay		**POSITIVE**	**NEGATIVE**	**Total**
Alere i™	Positive	42	15	57
	Negative	0	158	158
	Total	42	173	215
	Sensitivity (95% CI) (%)			100.0 (91.6, 100.0)
	Specificity (95% CI) (%)			91.3 (86.1, 95.1)
	Accuracy (95% CI) (%)			93.0 (88.8, 96.0)
	Kappa Index			0.805 (0.711, 0.898)
	Kappa Index P-value			<.0001
Veritor™	Positive	32	11	43
	Negative	10	162	172
	Total	42	173	215
	Sensitivity (95% CI) (%)			76.2 (60.5, 87.9)
	Specificity (95% CI) (%)			93.6 (88.9, 96.8)
	Accuracy (95% CI) (%)			90.2 (85.5, 93.9)
	Kappa Index			0.692 (0.569, 0.815)
	Kappa Index P-value			<.0001

Berry et. al, J. Clin. Microbiol., 2018

Table 2: Sensitivity, Specificity and Accuracy of RT-PCR Adjudicated Results

		Culture + RT-PCR Positive		
Assay		**POSITIVE**	**NEGATIVE**	**Total**
Alere i™	Positive	56	1	57
	Negative	0	158	158
	Total	56	159	215
	Sensitivity (95% CI) (%)		→	100.0 (93.6, 100.0)
	Specificity (95% CI) (%)		→	99.4 (96.6, 99.9)
	Accuracy (95% CI) (%)			99.5 (97.4, 99.9)
Veritor™	Positive	37	6	43
	Negative	10	162	172
	Total	47	168	215
	Sensitivity (95% CI) (%)		→	78.7 (64.3, 89.3)
	Specificity (95% CI) (%)		→	96.4 (92.4, 98.7)
	Accuracy (95% CI) (%)			92.6 (88.2, 95.7)

Alere i™: 14/15 confirmed by RT-PCR

Veritor™: 5/11 confirmed by RT-PCR

Berry et. al, J. Clin. Microbiol., 2018

Antibiotics chart review

73/215 (34%) patients given antibiotics at the time of clinic visit

26/73 (36%) treatment inappropriate- confirmed GAS negative result

- In 20/26 (77%) cases, ALL tests were negative

All 5 false positive BD Veritor™ results were treated with antibiotics

- 19% (5/26) of inappropriately treated cases

13/215 (6%) cases where the BD Veritor™ result was negative and antibiotics were not started at the time of the clinic visit, but that were subsequently detected by RT-PCR

- Alere i™ result was positive in 13/13 (100%) of these same cases
- In 6/13 (46%) cases, the antibiotics were started 2-6 days after the clinic visit, after receiving culture results

Berry et. al, J. Clin. Microbiol., 2018

Summary - GAS study

- The Alere i™ had higher sensitivity and specificity when compared to BD Veritor™
- RT-PCR showed that none of the 5 positives (0%) detected only by the BD Veritor™ confirmed, while 8/9 (89%) of positives detected by the Alere i™ confirmed
- 36% (n=26) of patients who were given abx had no GAS identified. Of this group 19% (n=5) had false-positive BD Veritor™ results

Berry et. al, J. Clin. Microbiol., 2018

Summary – GAS study (continued)

- 6% (n=13) of positive cases were missed by the BD Veritor™, while the Alere i™ detected all 13 (100%) cases.

- Antibiotics were started 2-6 days after the visit in 6 (46%) cases, with one patient lost to documented follow-up.

- The remaining 6 (46%) patients were culture negative and were therefore not treated, but were RT-PCR confirmed as positive. Use of the Alere i™ assay could have potentially led to these 6 (100%) missed patients being treated.

Berry et. al, J. Clin. Microbiol., 2018

Conclusions of GAS study

- The Alere i™ had superior performance over the BD Veritor™
- More accurate results could assist in better utilization of antibiotics in real time
- Molecular platforms should be considered as viable alternative POCT devices for diagnosis of GAS pharyngitis

Berry et. al, J. Clin. Microbiol., 2018

Overall conclusions

- There are now user-friendly, CLIA-waived molecular testing platforms available for POCT.

- These new platforms are designed to accommodate almost any skill set and testing environment

- Molecular testing methodologies have the ability to drastically improve diagnostic turnaround times, increase overall testing accuracy, and drive more appropriate therapy choices for better patient outcomes

Thank you!

Questions?

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