The Application of molecular POCT for Influenza and Group A Strep Detection

Gregory J. Berry, Ph.D., D(ABMM)
Assistant Professor, Pathology and Laboratory Medicine
Zucker School of Medicine at Hofstra/Northwell
Director, Molecular Diagnostics/ Asst. Director, Infectious Disease Diagnostics
Northwell Health Laboratories

Northwell Health℠

Objectives

- Introduce Point-of-Care Testing (POCT) uses in diagnosis of infectious diseases
- Explain the difference between molecular POCT and traditional antigen-based assays
- Review different POCT methodologies and instruments for Influenza and group A strep
- Present data from molecular Influenza and group A strep studies done in the POCT arena

Point-of-care testing (POCT)

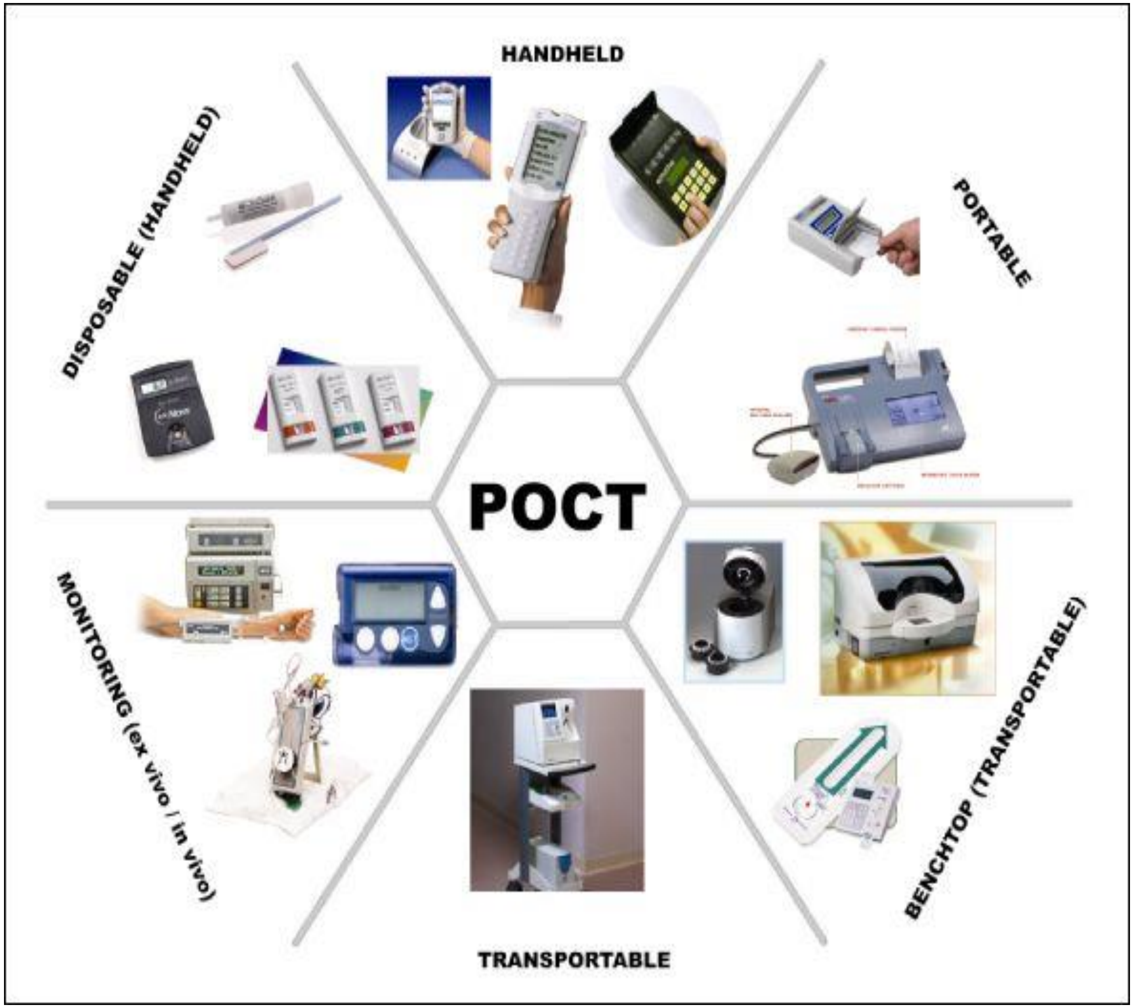
Testing performed while patient care is occurring

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

Historical impediments to POCT

- 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 to POCT barriers

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

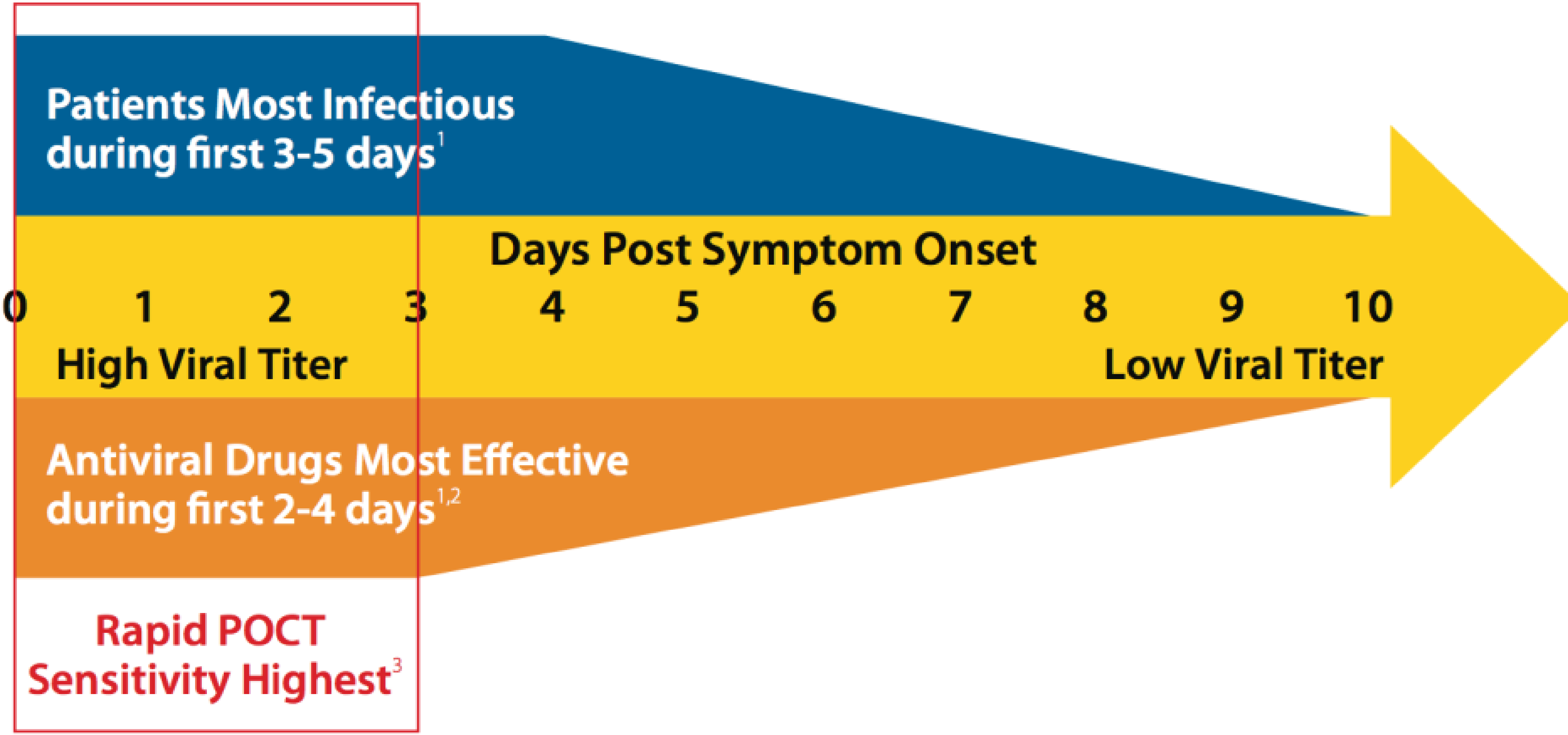
Solutions

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

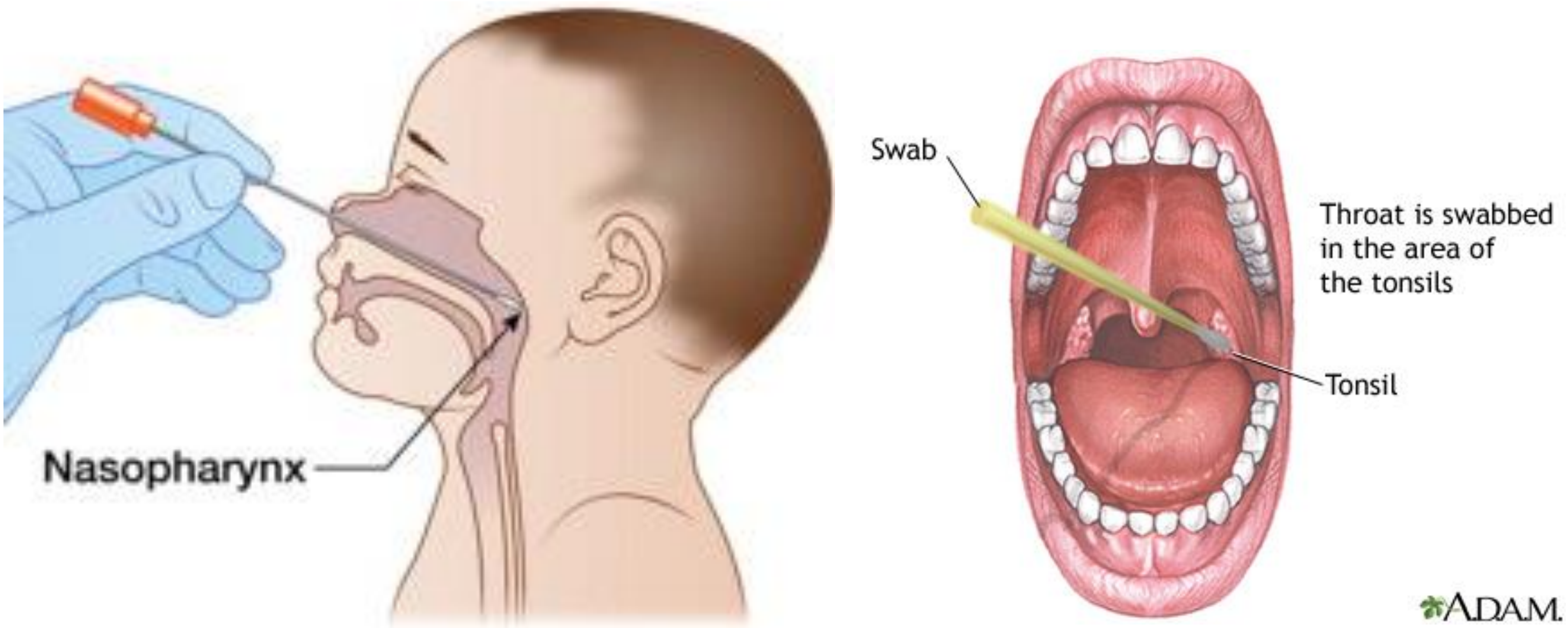
POCT in infectious disease diagnostics

- These are 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 proper specimen collection!



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

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 (NEW)

Rapid antigen detection tests

- Immunoassays— viral/bacterial antigens



- Qualitative resulting

- Vary greatly in their sensitivity
  - Negative strep a results need culture confirmation
  - **RIDTs reclassified to class II**


What changed with rapid influenza virus antigen detection tests (RIDTs)?

- **These tests were classified as Class I devices**
  - General controls were considered sufficient
- **FDA has re-classified them to Class II**
  - Both general and special controls must now be followed

FDA decision

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FEDERAL REGISTER

The Daily Journal of the United States Government

Rule

Microbiology Devices; Reclassification of Influenza Virus Antigen Detection Test Systems Intended for Use Directly With Clinical Specimens

A Rule by the Food and Drug Administration on 01/12/2017

PUBLISHED DOCUMENT

AGENCY:

Food and Drug Administration, HHS.

ACTION:

Final order.

SUMMARY:

The Food and Drug Administration (FDA) is reclassifying antigen based rapid influenza virus antigen detection test systems intended to detect influenza virus directly from clinical specimens that are currently regulated as influenza virus serological reagents from class I into class II with special controls and into a new device classification regulation.

DOCUMENT DETAILS

Printed version:
PDF

Publication Date:
01/12/2017

Agencies:
Food and Drug Administration

Dates:
This order is effective February 13, 2017. See further discussion in section IV, ``Implementation Strategy.''

Effective Date:
02/13/2017

Document Type:
Rule

Document Citation:

Site Feedback

Why the change with flu RIDTs?

- During the H1N1 influenza pandemic of 2009, questions were raised about the sensitivity of RIDTs
  - Lower sensitivity than package insert
- Concerns raised about the overall quality of influenza testing
- **Overall goal:** lower the number of misdiagnosed influenza infections by increasing the number of devices that can reliably detect the influenza virus

https://www.federalregister.gov/d/2017-00199/p-19

Minimum acceptance criteria

<u>Sensitivity</u>

Flu A Point estimate of 90% with 80% lower bound of the 95% confidence interval
Flu B Point estimate of 80% with 70% lower bound of the 95% confidence interval

<u>Specificity</u>

All influenza detection devices should demonstrate specificity with a lower bound of the 95% confidence interval exceeding 90% for both, Flu A and Flu B.

b. When compared to a molecular comparator method:

<u>Sensitivity</u>

Flu A Point estimate of 80% with 70% lower bound of the 95% confidence interval
Flu B Point estimate of 80% with 70% lower bound of the 95% confidence interval

<u>Specificity</u>

All influenza detection devices should demonstrate a specificity estimate with a lower bound of the 95% confidence interval exceeding 90% for both, influenza A and influenza B.

Molecular POCT

Molecular POCT tests for infectious diseases

- Traditionally designated by CLIA as moderate/high complexity and have been performed in the clinical laboratories
  - Only rapid antigen testing was available as CLIA waived

- CLIA waived tests have recently become available

CLIA waived molecular tests for infectious diseases

- **January 8th, 2015:** First CLIA waived test for influenza A and B (Alere i Influenza A&B)
- Followed by the Roche cobas Influenza A/B
- Both of these tests are classified as class II, so they are already compliant

Group A Strep and RSV are also now available on both platforms

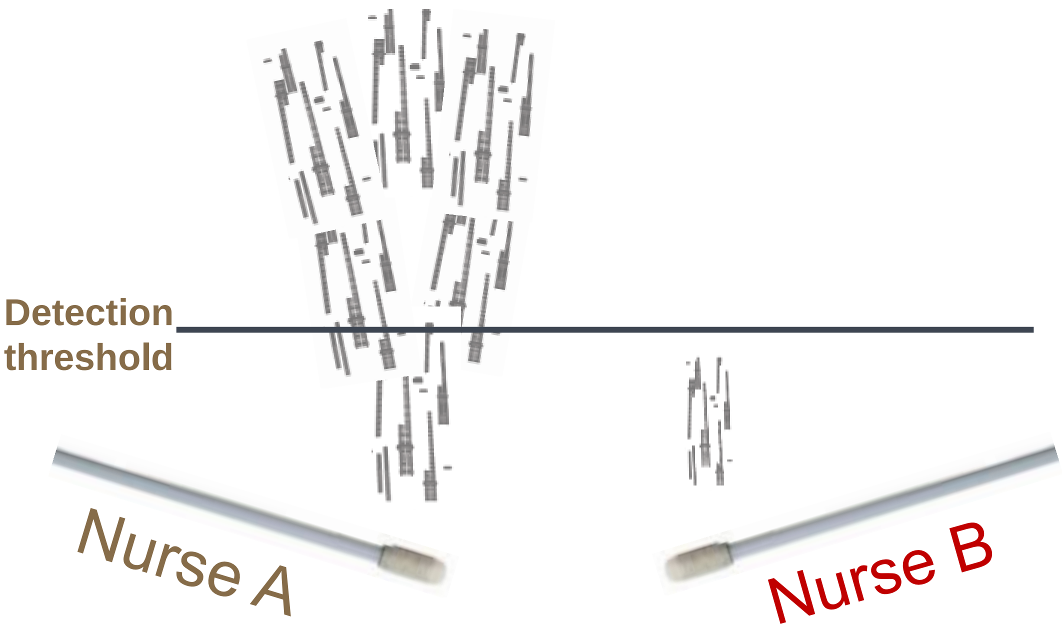
Molecular testing pros and cons

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

Technology comparison

	IMMUNOASSAY		MOLECULAR	
	RAPIDS	LAT FLOW READERS	PCR	Rapid
FAST	✓	✓		✓
CONVENIENT	✓	✓		✓
POC-FRIENDLY	✓	✓		✓
ACTIONABLE RESULTS	✓	✓		✓
REMOVES SUBJECTIVITY		✓	✓	✓
CONNECTED		✓	✓	✓
EXCELLENT PERFORMANCE			✓	✓

The power of sample amplification



Amplified
Flu+ Sample

Not Amplified
Flu+ Sample

Molecular tests on the market

PCR – Polymerase Chain Reaction

- Rely on the ability to amplify due to temperature cycling
- Many traditional molecular companies
- Alere q - Competitive Reporter Amplification
- Cepheid – GeneExpert
- POCT → Roche LIAT – Lab in a tube

Isothermal

- Rely on the ability to do the reaction at a single temperature
- Meridian's LAMP (loop mediated isothermal amplification)
- Quidel Solana – HDA (Helicase dependent amplification)
- POCT → Alere i – NEAR / RPA (Nicking enzyme amplification rxn/ Recombinase polymerase amplification)

Alere™ i

8-13 minutes to result for Flu/RSV

4-8 minutes to result for Strep A

< 2 minutes hands on time

Small footprint (8.15” W x 5.71” H x 7.64” D)

Weight= 1.4 lbs / 3 kg

FDA-cleared for use with both nasal swabs (direct) and NP or nasal swabs in VTM
CLIA-waived for use with nasal swabs (direct) only

LIAT - Lab In a Tube

- 20 minutes to results Flu/ RSV
- 15 minutes to results Strep A
- Footprint 4.5 x 9.5 x 7.5
- Weight 8.3 lbs

CLIA-waived by FDA for use with nasopharyngeal swabs only

INFLUENZA A/B STUDY

FOCUSED REPORT

Comparison of the Alere i and BD Veritor Assays for the Rapid Detection of Influenza A and B Viruses

Gregory J. Berry,[1,2] Olajumoke Oladipo,[1] Debbie Wittnebert,[3] Michael J. Loeffelholz,[1] and John R. Petersen[1]*

Background: The use of point-of care testing (POCT) in patient management decisions is becoming increasingly common. Our goal was to evaluate the diagnostic performance of 2 commercially available rapid POCT devices for influenza viruses A and B: the Alere™ i Instrument (Alere, Scarborough) and the BD Veritor™ System (BD Diagnostics).

Methods: Paired nasopharyngeal swabs were collected from patients (18–71 years) presenting with influenza-like symptoms at 3 outpatient clinics. A total of 65 samples were obtained. The Alere i and BD Veritor were performed according to the manufacturers' instructions. Discordant results were resolved using real-time reverse transcription PCR (RT-PCR).

Results: In a head-to-head comparison involving symptomatic adult patients visiting outpatient clinics during the 2014–2015 and 2015–2016 influenza seasons, the Alere i and BD Veritor had 90.63% agreement in the detection of influenza A virus and a statistically significant observed κ coefficient of 0.754 (P <0.0001). Discordant results between the Alere i and BD Veritor were further investigated using RT-PCR, showing that the BD Veritor missed 5 positive influenza A virus results (false negatives) and detected 1 false positive, while the Alere i results agreed with all RT-PCR results. There were no discordant results between the Alere i and BD Veritor in the detection of influenza B virus.

Conclusions: Our data suggest that the Alere i has higher sensitivity and specificity than the BD Veritor in the detection of influenza A virus. Both assays showed equal performance in the detection of influenza B virus.

Berry et. al, JALM. May 2017

Goal

- Evaluate the diagnostic performance of 2 commercially available rapid POCT devices for influenza viruses A and B:

BD Veritor™

RIDT with reader

Alere™ i

Isothermal amplification

Study design

- Paired nasopharyngeal swabs were collected from patients (18–71 years) presenting with influenza-like symptoms at 3 outpatient clinics
  - A total of 65 samples were obtained

- The Alere i and BD Veritor were performed according to the manufacturers' instructions

- Discordant results were resolved using real-time reverse transcription PCR (RT-PCR)

Table 1. Comparison of the Alere I and BD Veritor in the detection of Influenza A and B viruses.

	Alere i			
	Influenza A		Influenza B	
	Positive	Negative	Positive	Negative
BD Veritor				
Positive	13	1	7	0
Negative	5	45	0	57
Agreement %	90.63		100	
Observed k, linear weighting	0.754, 95% CI 0.569-0.938		1.00	
p	<0.0001		0.00	

Berry et. al, JALM. May 2017

Results

- Influenza A:
  - RT-PCR was done on discordants
    - BD Veritor missed 5 positive results (false negatives); detected 1 false positive result
    - Alere i agreed with all RT-PCR results
- Influenza B:
  - No discordant results

One Alere i invalid was also excluded from analysis, but was positive by the BD Veritor and confirmed by RT-PCR.

Conclusions

- The Alere i has higher sensitivity and specificity than the BD Veritor in the detection of influenza A virus

- Both assays showed equal performance in the detection of influenza B virus

GROUP A STREPTOCOCCUS STUDY

Group A Strep study goal:

- Compare the BD Veritor, Alere i, and culture for detection of Group A Streptococcus
- Evaluate the hypothetical impact of results on antibiotic utilization

BD Veritor™

RIDT with reader

Alere™ i

Isothermal amplification

Culture

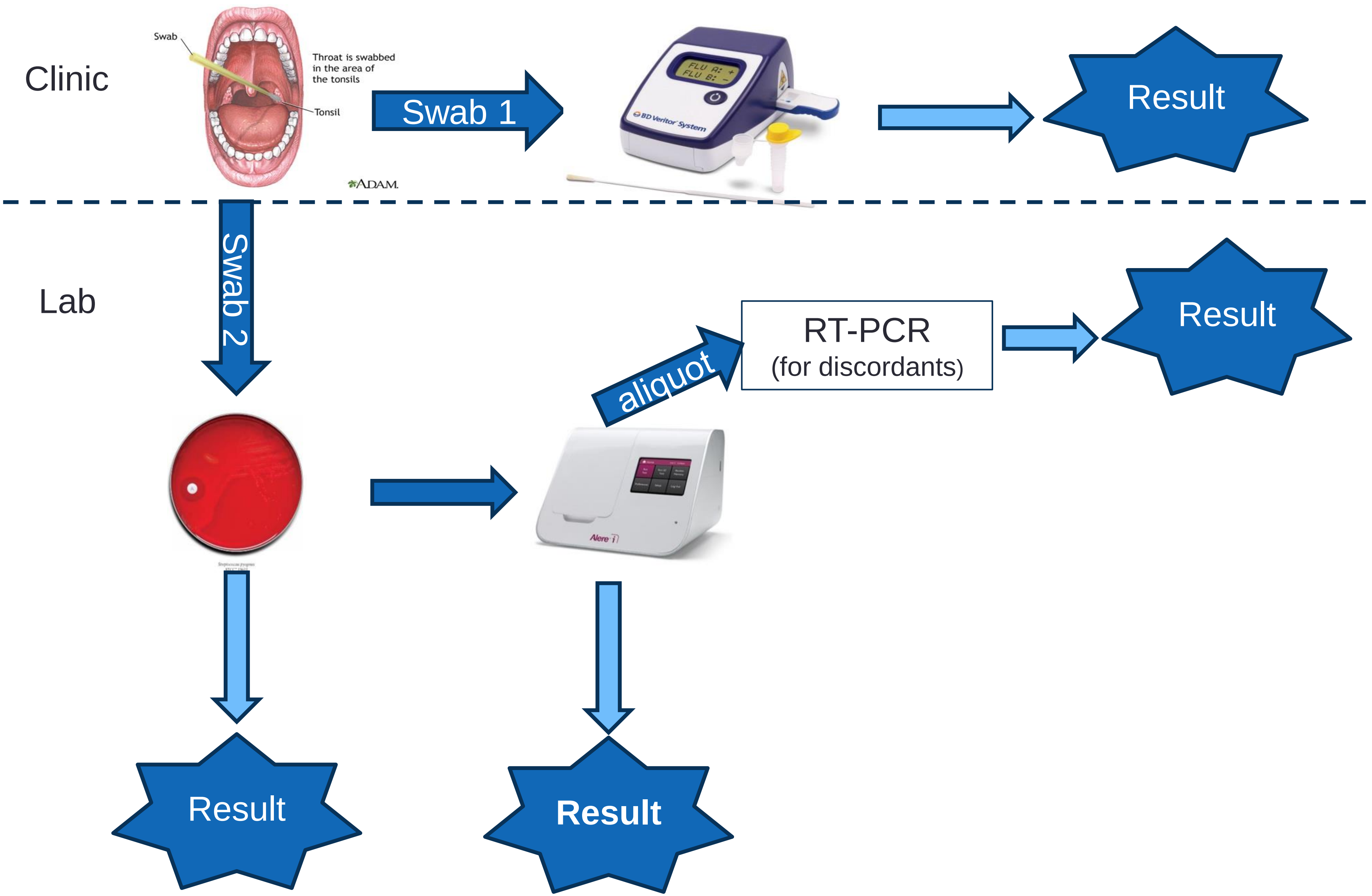
Berry et. al, *J. Clin. Microbiol. JCM.01310-17; Accepted manuscript posted online 5 January 2018*

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 **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. JCM.01310-17; Accepted manuscript posted online 5 January 2018*

Study Design



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Distribution of positive results

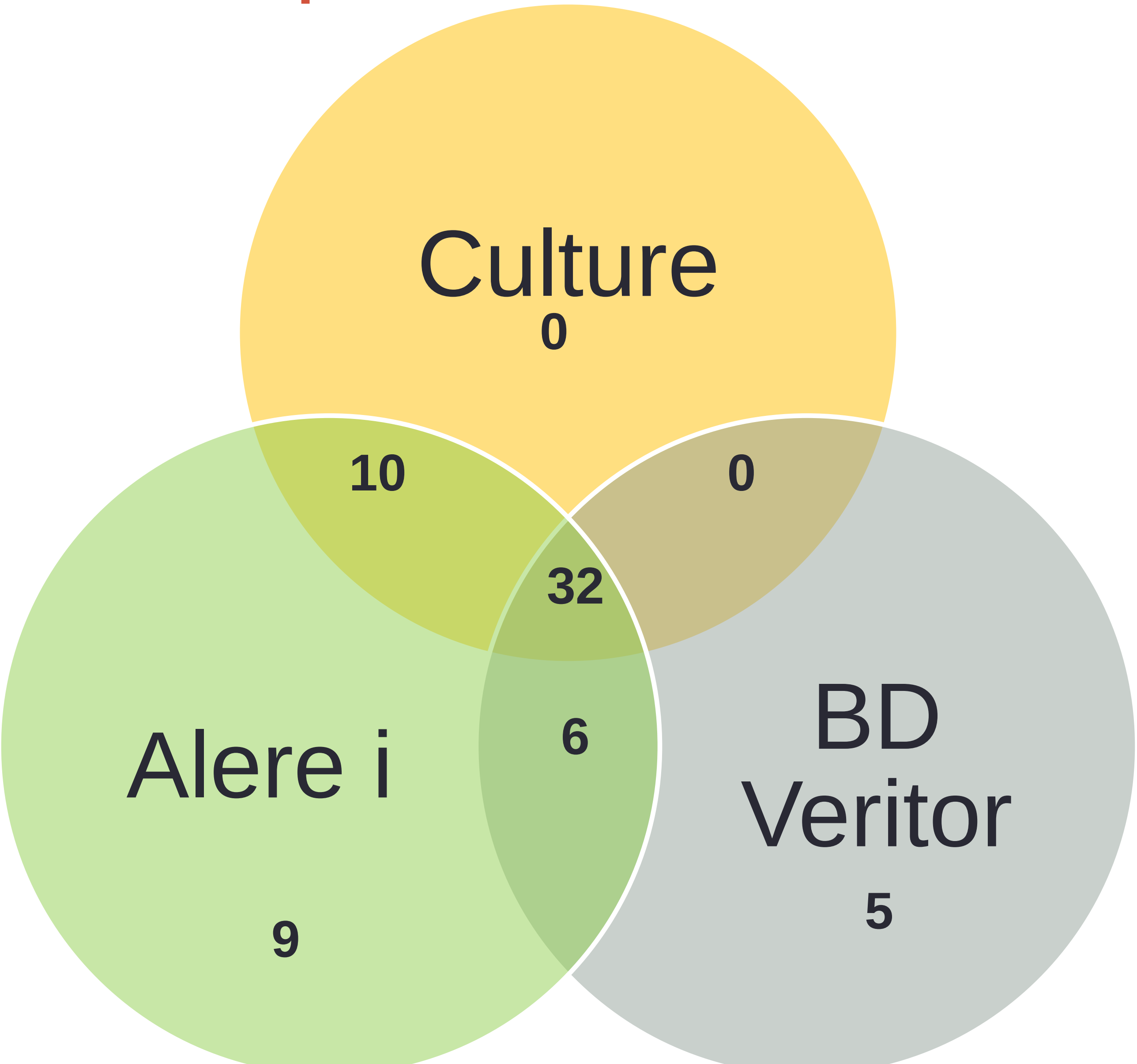

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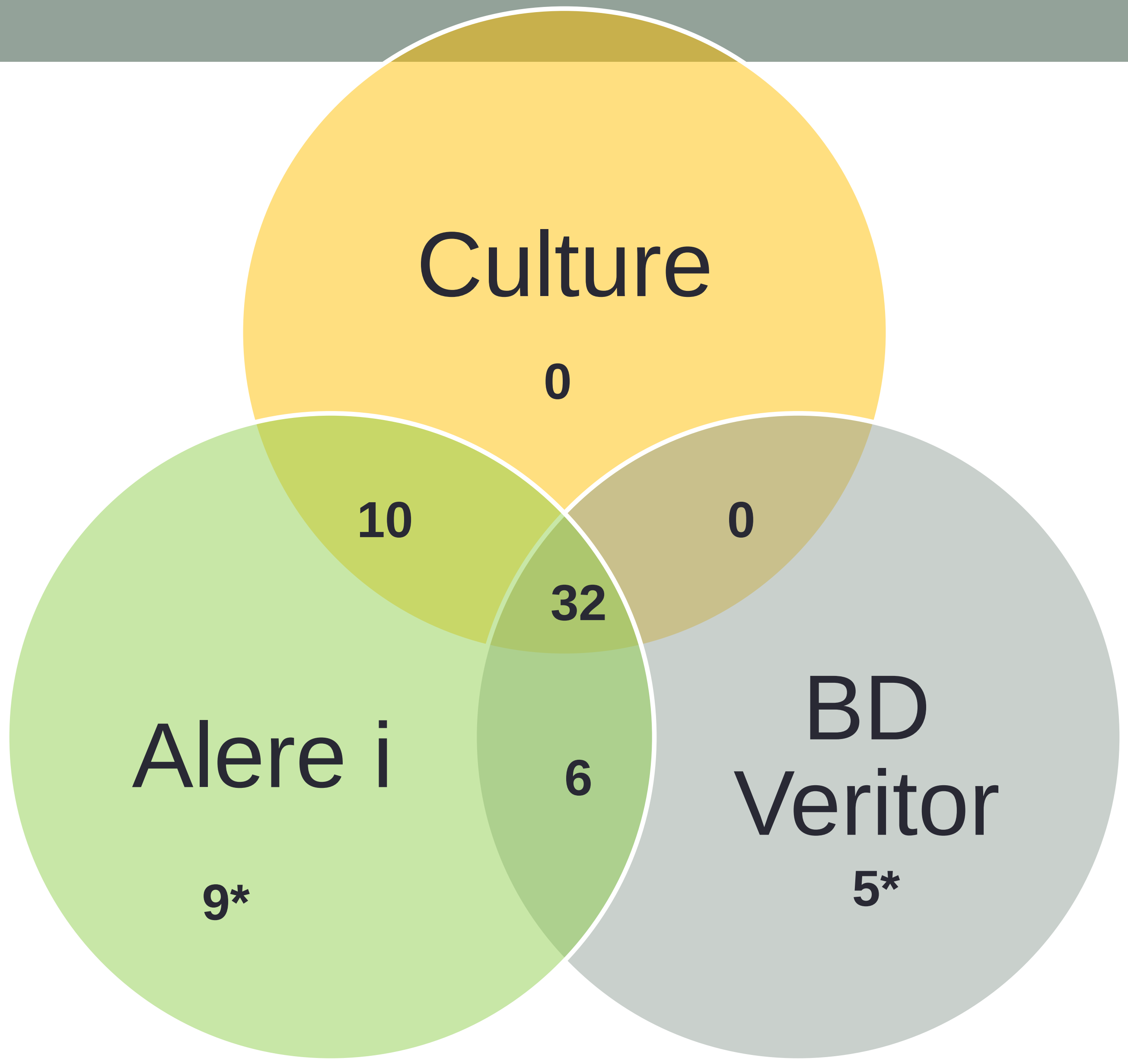
Table 2: Agreement between the Alere i and BD Veritor

		Veritor		
Test	**Result**	**Pos**	**Neg**	**Total**
Alere	Pos	38	19	57
	Neg	5	153	158
	Total	43	172	215
	Agreement	.	.	0.888 (95% CI 0.838-0.927)
	Kappa Index	.	.	0.689 (95% CI 0.575-0.803)
	P-value	.	.	<.0001

Berry et. al, *J. Clin. Microbiol. JCM.01310-17; Accepted manuscript posted online 5 January 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. JCM.01310-17; Accepted manuscript posted online 5 January 2018*



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

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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

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. JCM.01310-17; Accepted manuscript posted online 5 January 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. JCM.01310-17; Accepted manuscript posted online 5 January 2018*

Summary- 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 and the cobas Liat would have led to 4/6 (67%) of these patients being treated.

Berry et. al, *J. Clin. Microbiol. JCM.01310-17; Accepted manuscript posted online 5 January 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

Overall conclusions

- Infectious disease testing will continue to enter the POCT
- Molecular POCT is as sensitive/specific as most lab tests, but has the huge advantage of a rapid answer
- These tests have the ability to drive more appropriate therapy choices for better patient outcomes

Acknowledgements

utmb Health

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THANK YOU!

Questions?