

Point-of-Care and Rapid Tests for COVID-19: One institution's experience

Virginia Point of Care Network Webinar Meeting
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DISCLOSURE

Speaker: Brad S. Karon

Relevant Financial Relationship(s)

None

Off Label/Investigational Usage

None

Objectives

- Understand types of rapid and point of care tests available for SARS-CoV-2 detection
- Define key issues to be addressed in implementing point of care testing for SARS-CoV-2
- Review study/validation data and observed performance for two rapid molecular tests for SARS-CoV-2 RNA
- Compare and contrast analytical sensitivity of lateral flow and fluorescence immunoassay rapid antigen tests for SARS-CoV-2

Rapid Testing Options for SARS-CoV-2

- Rapid nucleic acid amplification tests (NAAT)
 - Reverse transcription quantitative polymerase chain reaction (RT-qPCR)
 - Isothermal amplification, e.g. Loop-mediated isothermal amplification (LAMP)
- Rapid antigen diagnostic tests (Ag RDT)
 - Lateral flow immunoassays
 - Fluorescence immunoassays

Challenges in Evaluating/Implementing POCT NAAT Tests for SARS-CoV-2

- Emergency use authorization (EUA) studies
 - Very limited (30 positive and 30 negative) clinical data
- Single evaluation site for EUA claim
- Limited to no end user or “real world” testing
- Start-up companies produced limited numbers for EUA, ramp up production once approved
- Performance in hands of end user with commercial product?

Issues with Implementing POCT for SARS-CoV-2

- **Environmental Contamination**
 - Sample processing and testing outside lab by nurses, others
 - Environmental contamination producing false positives

- **Safety of Testing Personnel**
 - Aerosols created from mixing and opening specimens to dose devices

Approaches to Handling Contamination and Safety – Option 1

- Manipulate samples behind bench shield, end user wears mask and face shield (and universal PPE)
 - Controversial whether this is optimal protection for end user
 - Probably OK but currently against CDC guidelines

Approaches to Handling Contamination and Safety – Option 2

- Perform sample manipulation in biosafety cabinet or if not available chemical fume hood
 - Provides optimal protection for end user, may not be practical in POC setting
 - Solves both safety and contamination issues if regular cleaning BSC

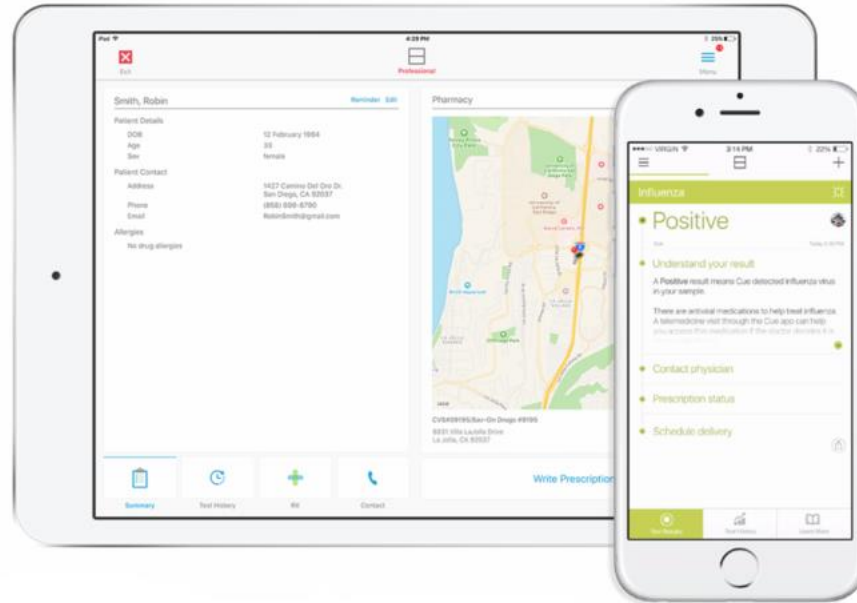
Approaches to Handling Contamination and Safety – Option 3

- Eliminate need for mixing of infectious media
 - Use device requiring no sample manipulation between collection and testing
 - Use device with viral inactivation buffer prior to instrument dosing
 - Direct dosing device probably solves both contamination and safety issues

Cue Health Technology

- EUA approved isothermal amplification of SARS-CoV-2 at the point of care
 - EUA approval for lab and point of care use, CLIA high, moderate or waived complexity labs
- 25 minute sample to answer test
- Uses proprietary disposable cartridge, disposable nasal wand, and reader

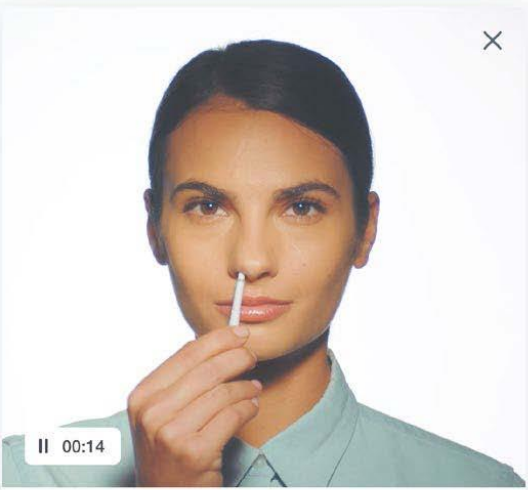
Cue Device and disposable



Cue collection and testing

No SIM 11:10 AM 100%

< Run a Test Cancel




00:14

Collect Sample Ready

- Use appropriate PPE. Unwrap the Sample Wand. Do not touch the Wand tip.
- Insert the tip of the Wand into one nostril about 1 inch up to the marker. If there is resistance, do not insert further.
- Keep gentle pressure on the outer wall of the nostril. Rotate the Wand against the wall 5 times.
- Repeat for the other nostril, again rotating 5 times.
- Insert the Sample Wand into the cartridge. The test will start automatically.

No SIM 9:45 AM 100%

< Run a Test Cancel



00:06

Collect Sample Ready

- Use appropriate PPE. Unwrap the Sample Wand. Do not touch the Wand tip.
- Insert the tip of the Wand into one nostril about 1 inch up to the marker. If there is resistance, do not insert further.
- Keep gentle pressure on the outer wall of the nostril. Rotate the Wand against the wall 5 times.
- Repeat for the other nostril, again rotating 5 times.
- Insert the Sample Wand into the cartridge. The test will start automatically.

Cue study

- 292 patients presenting for COVID-19 PCR at Mankato MCHS drive-thru swabbing site
- Cue Health nasal swab (5 rotations against outer nostril, both nostrils)
 - Nurses collected Cue nasal and reference NP swabs, lab techs performed Cue testing at drive-thru site
- NP swab collection for in-house PCR (Hologic with some Rochester LDT RT-PCR)
 - 206 reference Hologic TMA tests, 85 reference RT-PCR by LDT, 1 unknown

Cue study

	Number of samples with a reference result of:		
Number of samples with a Cue result of:	Positive	Negative	Total
Positive	22	4	26
Negative	2*	239	241
Positive percent agreement	91.7%*		
Negative percent agreement		98.4%	
Total	24	243	267

- *One discrepant positive reference sample did not have a tie-breaker method available, so positive percent agreement would be 22/23 (95.7%) excluding that sample.
- Overall concordance 97.8%
- Invalid/cancelled rate 8.6% (25 tests), with revised cut-off for detecting human DNA would have been 4.5% with revised cut-off

Cue at Mayo stat lab

- Went live Cue (VTM/dipping method) on Dec 9, 2020
- Detected, inconclusive or cancelled tests run on LIAT, result from LIAT released
- 6455 tests performed thru 4/27/21
 - 42 (0.7%) false positive (undetected by LIAT)
 - 361 (5.6%) invalid/cancelled
 - None reported false positive or inconclusive using VTM method
 - Since May 2022 invalid rate increased 10-15%

Cue at POC

- Went live direct dosing POC application in Feb 2021
- 534 tests thru April 2021 (20-30 per day)
- 1 false positive (patient negative by LIAT)
- 24 (4.5%) invalid and cancelled
- All positive, invalid or cancelled results patient gets re-swabbed and sent for LIAT rapid RT-PCR
 - Not patient satisfier, low volume but useful for same day procedures
 - Patient procedure designed around observed rates of invalid and false positive results
 - Recently discontinued POC application due to workflow issues handling invalid/cancelled and confirmation of positives

Visby Medical Disposable RT-PCR

- Single use, fully disposable RT-PCR test for SARS-CoV-2
 - No instrument required
- RT-PCR followed by horseradish peroxidase colorimetric detection
- < 30 min sample to answer
- Multi-step procedure
 - VTM → dilution buffer
 - Dilution buffer → device
- EUA POC February 2021 (USA)



Visby Disposable RT-PCR Data

- 100 sample comparison and LoD experiment
 - 70 negative and 30 positive comparison samples
- 69 of 70 (98.6%) NPA (specificity)
- 29 of 30 (96.7%) PPA (sensitivity)
 - One sample Cp 33.38 discrepant
 - Two samples Cp 35 detected
- LoD 3 of 3 detected at:
 - 1000 copies/mL (claim 1112 copies/mL) and
 - 500 copies/mL

Mayo Clinic Experience with POCT NAAT Testing

- Variable analytical sensitivity
 - RT-PCR > isothermal amplification
 - From as sensitive as central lab NAAT to considerably less sensitive
- All rapid methods more prone to analytical false positives compared to central lab NAAT
 - Cue > Visby > LIAT or Cepheid
 - Consider protocol to confirm positive rapid NAAT if lower specificity
- Learning curve with new methods
 - Invalid rate initially went down over time with Cue testing
 - Cancelled rate due to bumping reader down over time
 - Cancelled rate due to cartridge error trending up

SARS-CoV-2 antigen testing

- Laboratory NAAT testing widely available in US
 - Limitations of cost, instruments, time to result
- More emphasis on decentralized testing as vaccine distributed and infections rise in at-risk populations
- Role of antigen testing remains uncertain/controversial
- Clinical sensitivity
 - Asymptomatic 30-50%
 - Symptomatic 50-80%
- Is variability in analytical sensitivity of antigen tests one reason for differing findings on clinical sensitivity?

Challenges to assessing sensitivity/specificity of SARS-CoV-2 antigen tests

- No reference method for presence of SARS-CoV-2 antigen
 - Patients or samples that are RT-qPCR positive
 - We compared 4 POC antigen tests to a lab-developed, ultrasensitive mass spectrometric antigen test
 - Sample set RT-qPCR positive (RNA), digital droplet PCR (ddPCR) positive (RNA), antigen positive by one or more methods
- Impossible to use intended sample type (nasal swab direct to extraction buffer) to compare multiple antigen tests
 - Various dilution protocols used on residual VTM or PBS specimens
 - We validated dilution protocol with PBS samples on 4 POC tests

Challenges to assessing sensitivity/specificity of SARS-CoV-2 antigen tests

- Viral load and Cp or Ct value
 - Crossing point (Cp) or cycle threshold (Ct) related to viral load
 - In general higher Cp/Ct means less virus
 - Most tests Cp/Ct higher than 40 is negative
 - Relationship between Ct and viral load differs by method
 - Use of RNA standards to estimate viral load from Ct
 - PCR efficiency creates variability between Ct and viral load sample to sample, making estimation of viral load from Cp inexact
- ddPCR more sensitive, 4-20 fold more precise than RT-qPCR
 - We used ddPCR to measure viral load in each sample

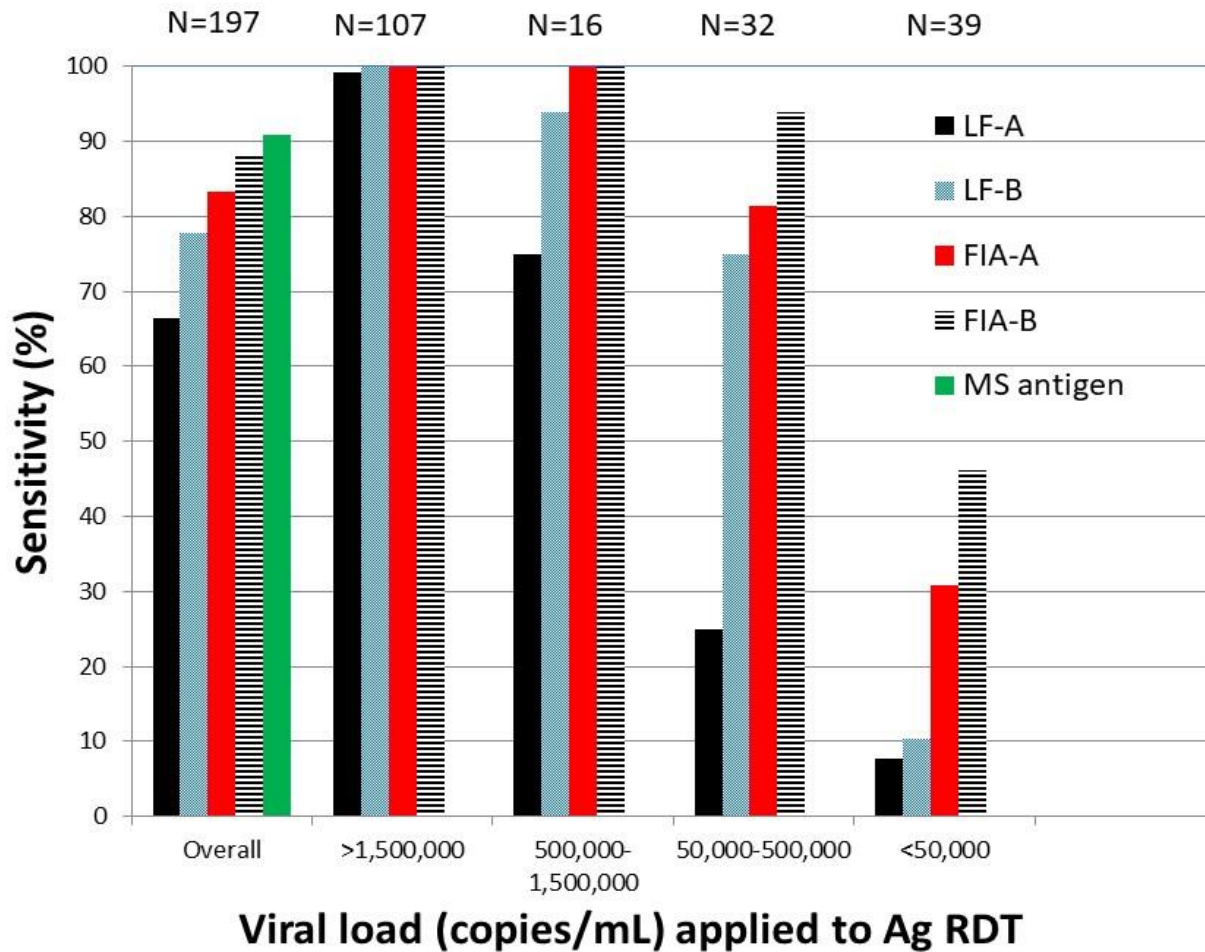
“4 way” POC antigen study

- Four POC antigen tests compared
- Digital reader lateral flow (LF), visual LF, two fluorescence immunoassay (FIA)
- Four measurement technologies used
 - POC antigen tests (4 methods)
 - RT-PCR reference test
 - ddPCR to obtain viral load for each sample
 - MS antigen test
- 350 PBS samples tested by LDT
 - 150 RT-PCR negative samples
 - 200 RT-PCR positive samples (targeted Cp ranges)

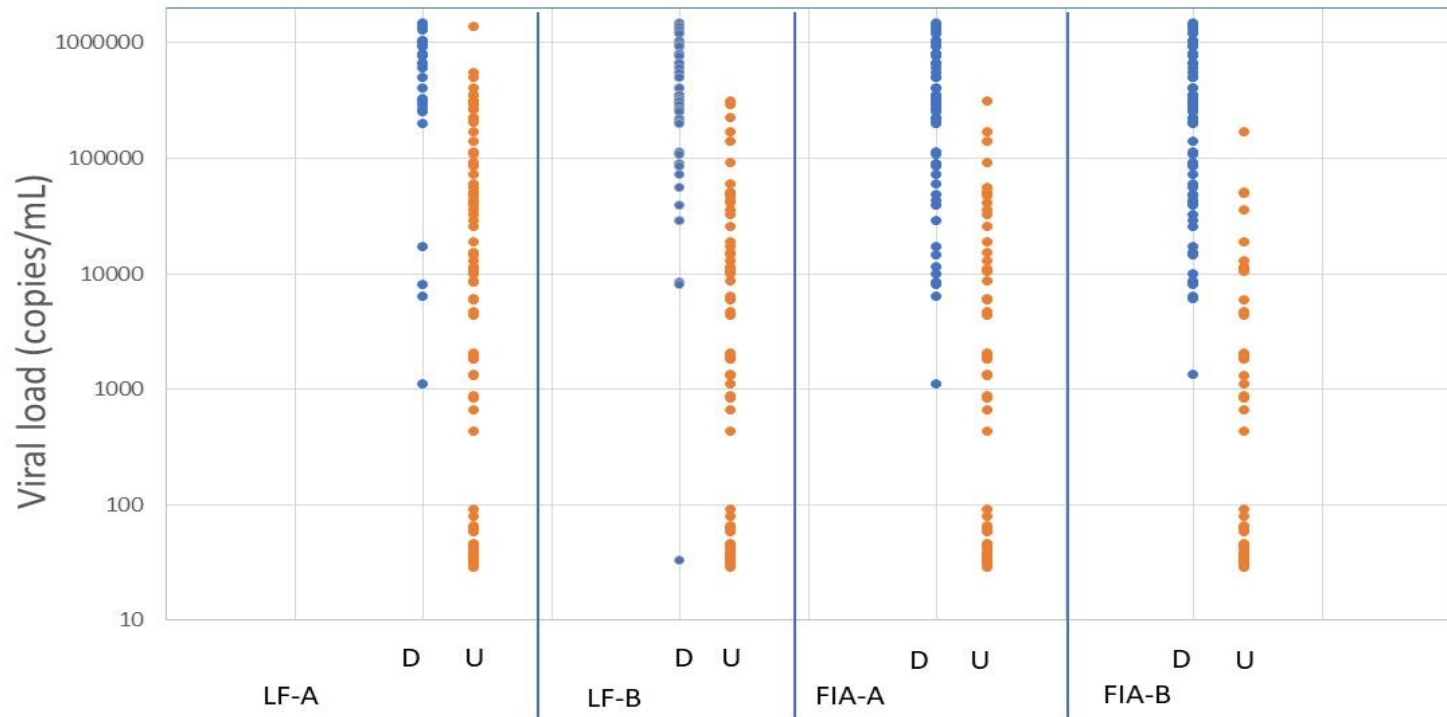
POC antigen study results

- Specificity
 - 150 PBS samples, diluted 10x into extraction buffer
 - Two lab techs performed all testing, third tech for visual tests
 - LF-A, FIA-A, FIA-B 100% specificity
 - LF-B (visual) 97.3% specificity (146/150)

POC antigen study results--sensitivity



POC antigen results—viral load detected vs undetected antigen



Summary of antigen testing study

- SARS-CoV-2 antigen tests differ in analytical sensitivity, mainly for samples with viral load <500,000 copies/mL
- Lateral flow (LF) antigen tests are significantly less sensitive than fluorescence immunoassay (FIA), though differences were found between two LF assays
- When antigen is present, more sensitive antigen tests can detect it in most samples with viral load <50,000 copies/mL
- Less sensitive tests will fail to detect antigen in many samples with <500,000 copies/mL
- The relationship between Cp (Ct) and viral load measured by ddPCR is highly variable, limiting use of Cp (Ct) to predict viral load
- Current project re-evaluate antigen sensitivity 2 tests with Omicron variant

Conclusions

- Must address safety and environmental contamination if using point of care SARS-CoV-2 assay
- Rapid and point of care molecular tests for SARS-CoV-2 RNA vary from less to equally sensitive to central lab RT-qPCR tests
 - tendency towards more false positives
- Role of antigen testing remains uncertain, variable analytical sensitivity at viral loads 50,000-500,000 copies/mL

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Thank You

Karon et al, Analytical sensitivity and specificity of four point of care rapid antigen diagnostic tests for SARS-CoV-2 using real-time quantitative PCR, quantitative droplet digital PCR, and a mass spectrometric antigen assay as comparator methods, Clin Chem, <https://doi.org/10.1093/clinchem/hvab138>