Evaluation of Point-of-Care and Rapid Tests for COVID-19: The Mayo Clinic Experience

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DISCLOSURE

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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

Rapid Testing Options for SARS-CoV-2

- Some established in-vitro diagnostic companies developed emergency use authorization (EUA) tests
  - Roche, Abbott, BioFire, Cepheid
- Some start-up companies produced first diagnostic test as EUA for COVID-19
  - Cue Health, Visby, Lucira Health

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

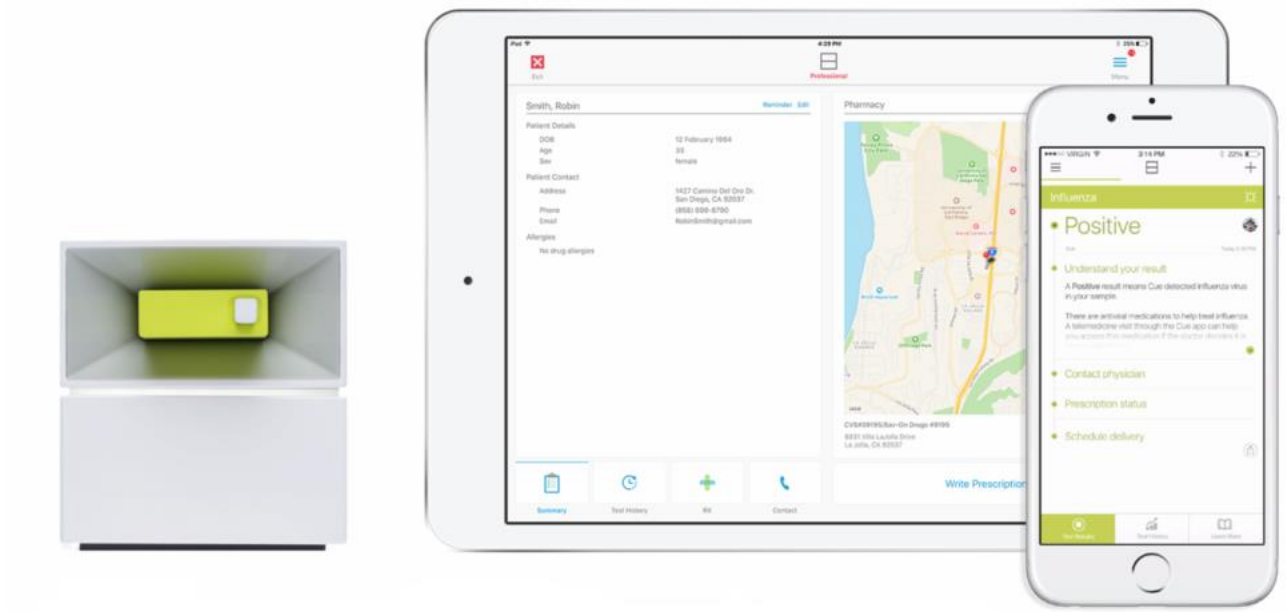
Approaches to Handling Contamination and Safety – Option 3

- 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

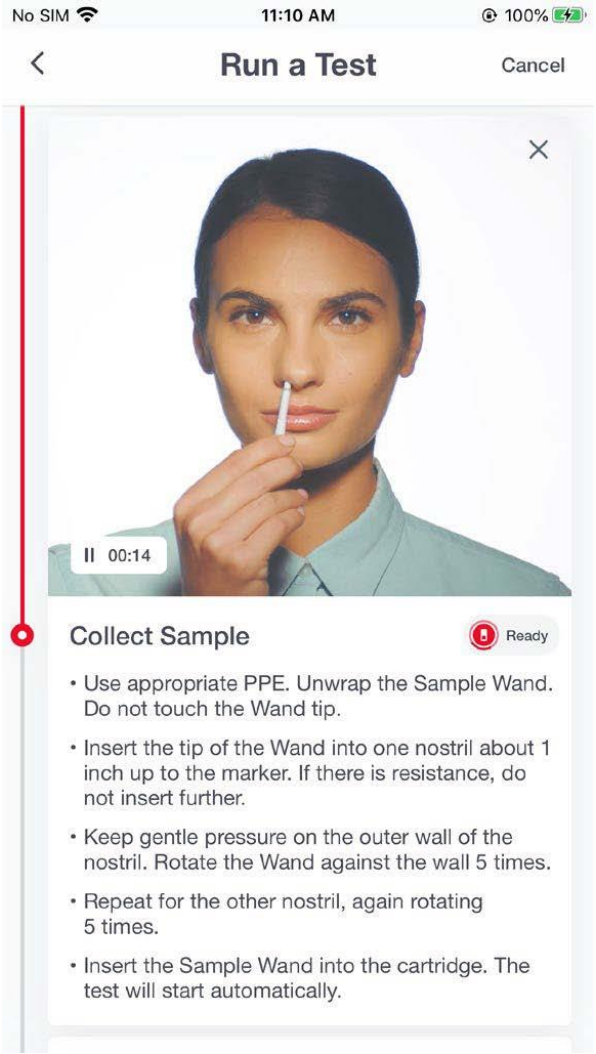
Cue Health Technology

- 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
- LAMP (loop-mediated isothermal amplification) with electrochemical detection
- 25 minute sample to answer test
- Uses proprietary disposable cartridge, disposable nasal wand, and reader

Cue Device and disposable



Cue collection and testing



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

Lab Cue study

- August 2020, Cue Health obtains modification of EUA to allow use with VTM (dipping method)
- 103 VTM specimens previously tested by Roche 8600 and LDT (53 pos and 50 neg)
- Cp values 14-37.8 (6 samples Cp ≥35)
- LoD experiment using BEI heat-inactivated virus diluted into VTM

Lab Cue study

	Number of samples with a reference result of:		
Number of samples with a Cue result of:	Positive	Negative	Total
Positive	45	4	49
Negative	8	46	54
Total	53	50	

- 45/53 (84.9%) positive percent agreement
- Excluding 6 samples with Cp ≥35, 93.6% PPA
- 46/50 (92.0%) negative percent agreement
- LoD studies
  - 8/12 positive at 5000 copies/mL
  - 6/6 positive at 10,000 copies/mL
  - LoD between 5000-10,000 copies/mL with dipping/VTM application method (2700 copies/mL direct application)

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
- Fewer invalid results with user experience, but more cancelled due to cartridge errors with some lots

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

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 has gone 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
  - Asymtpomatic 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

- Assumptions made about viral load of samples based upon Cp or Ct value
  - Crossing point (Cp) or cycle threshold (Ct) related to viral load
  - 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

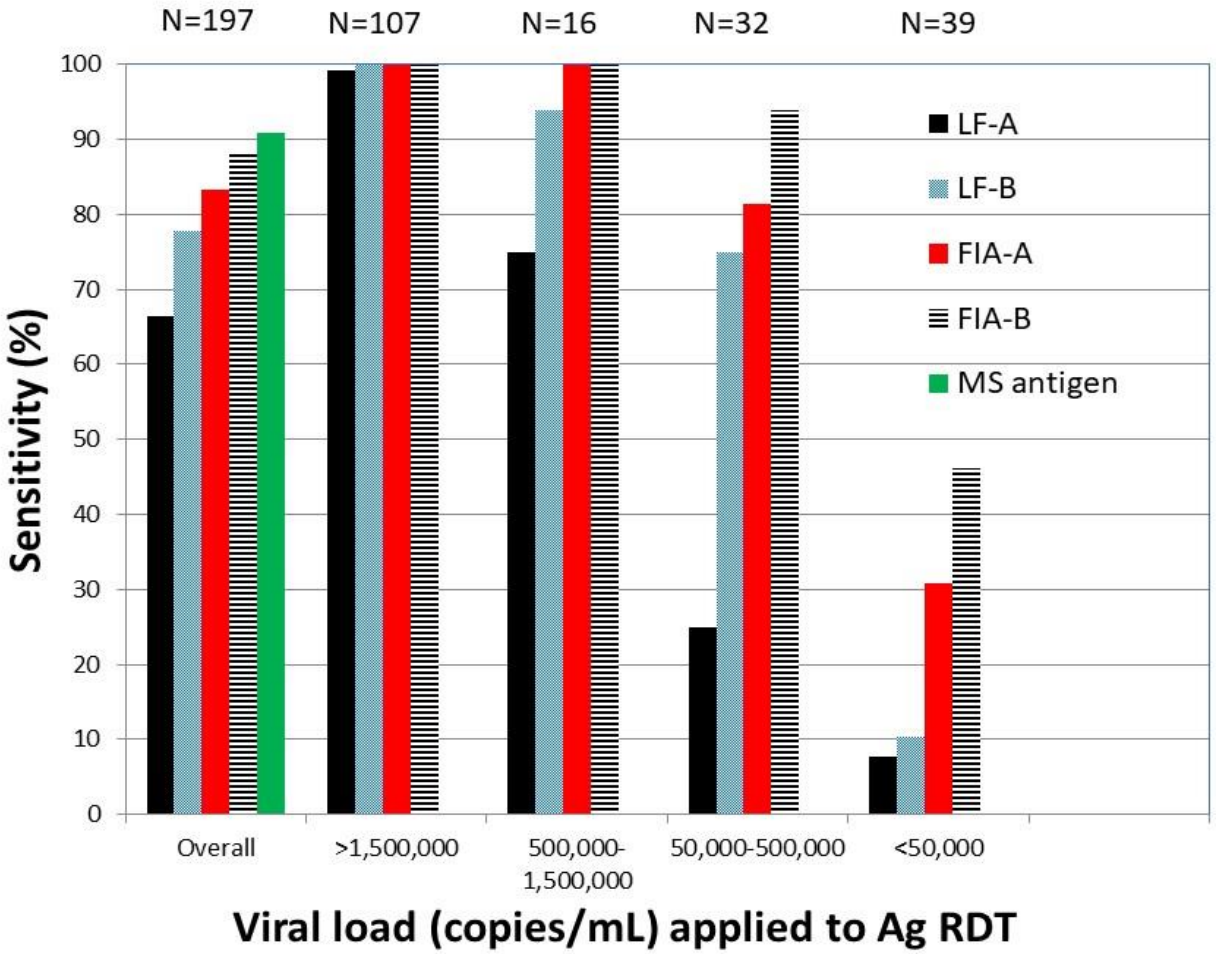
“4 way” POC antigen study

- 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)

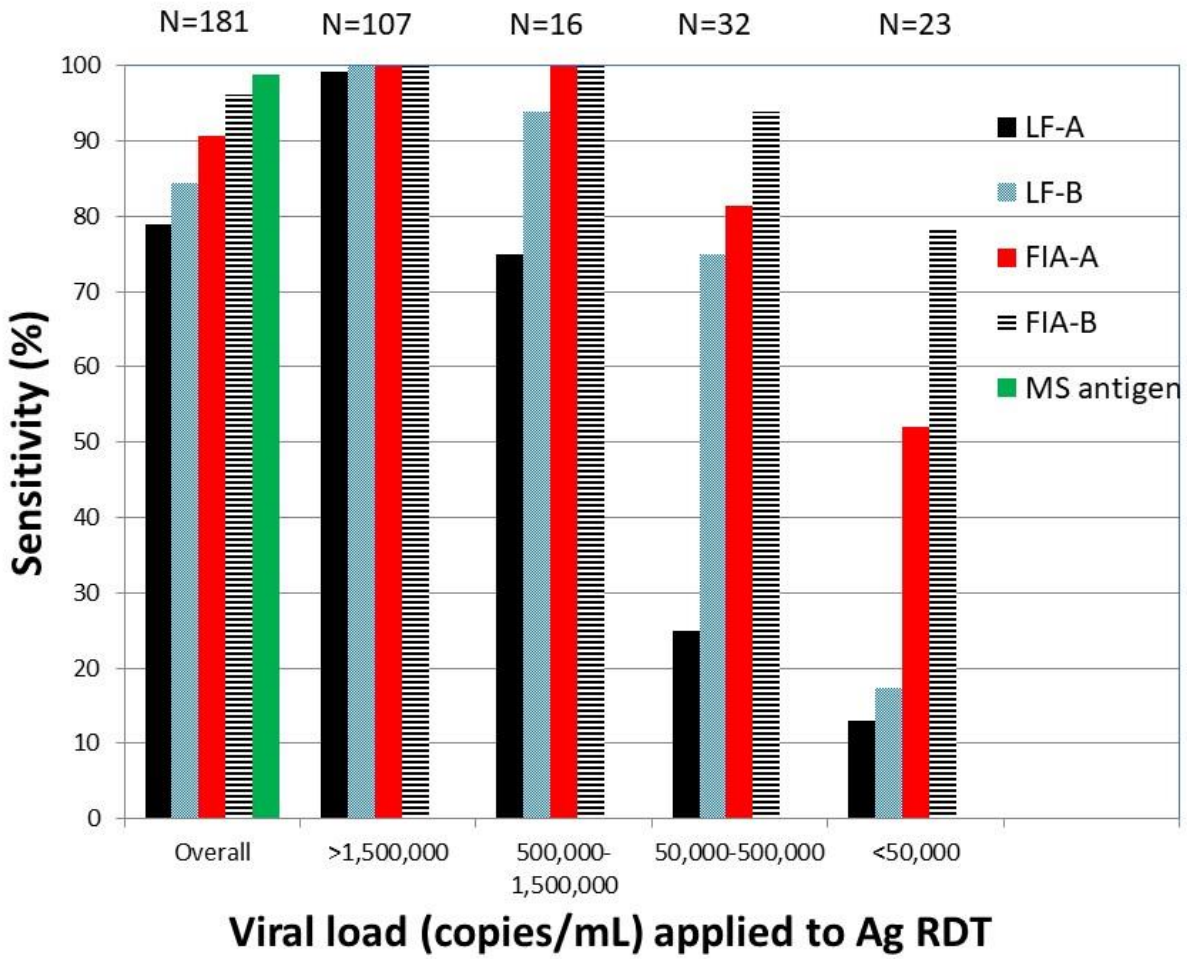
POC antigen study results

- 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)

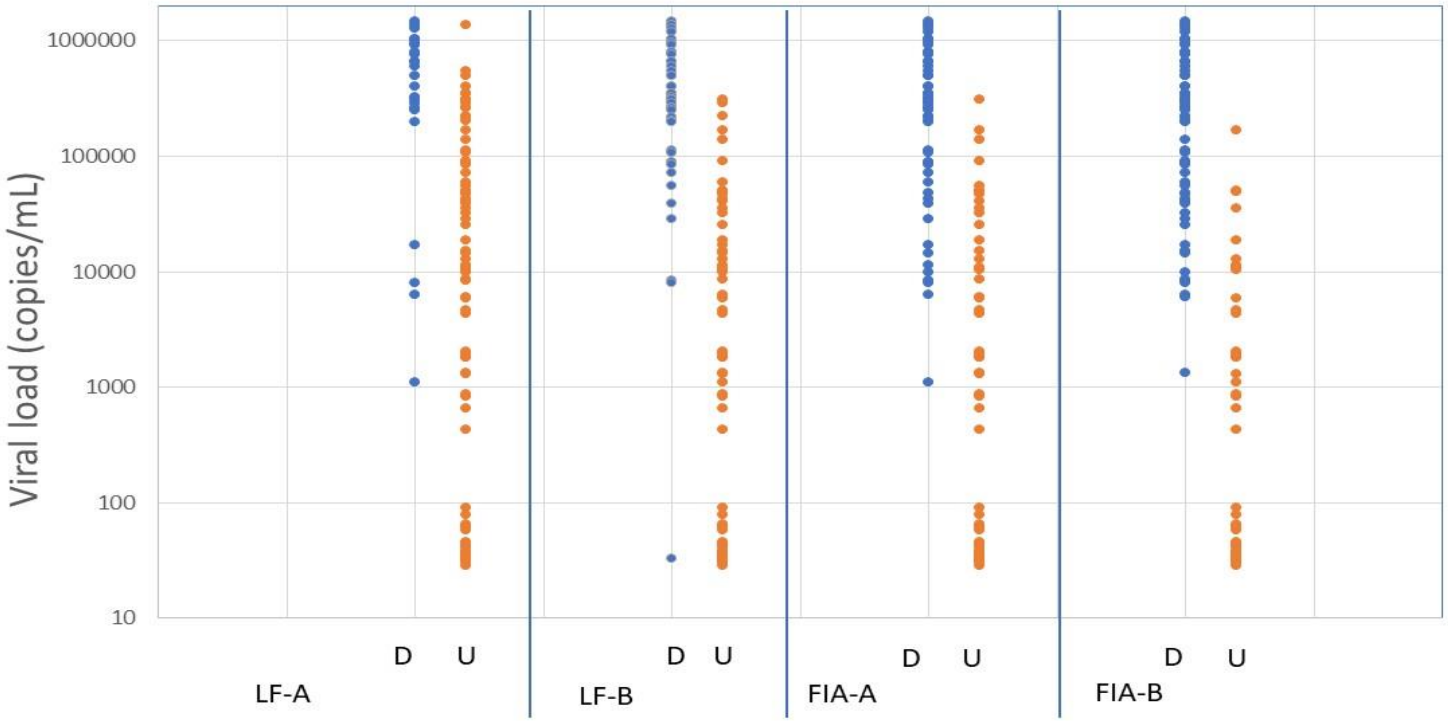
POC antigen study results--sensitivity



POC antigen study results—sensitivity, RT-qPCR and antigen positive samples



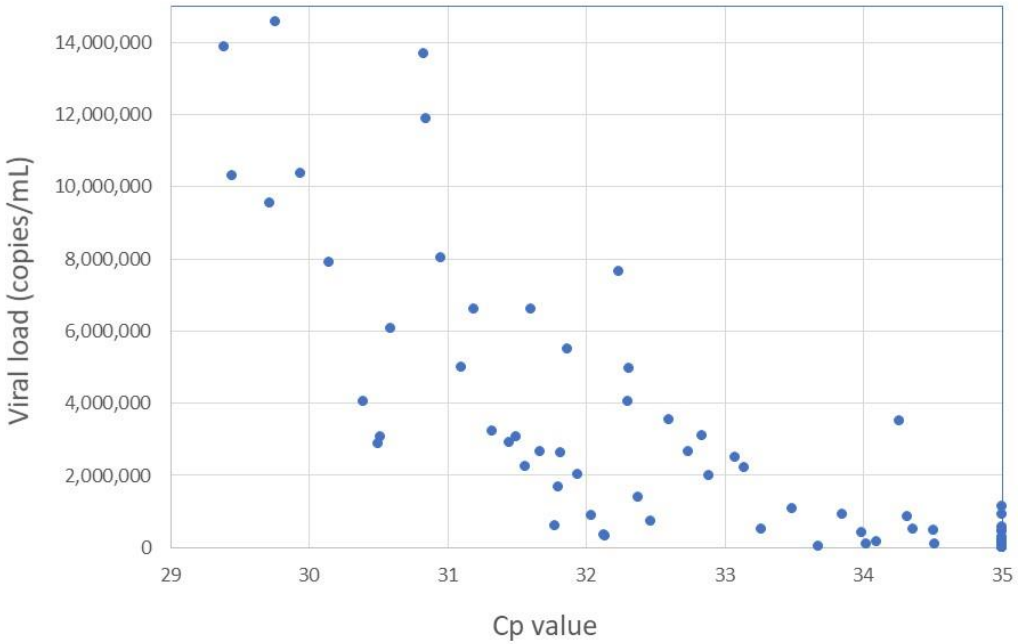
POC antigen results—viral load detected vs undetected antigen



POC antigen results--Cp (Ct) vs viral load by ddPRC

Viral load Cp 35 samples

Ranged 378 to 1,119,259 copies/mL



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

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

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