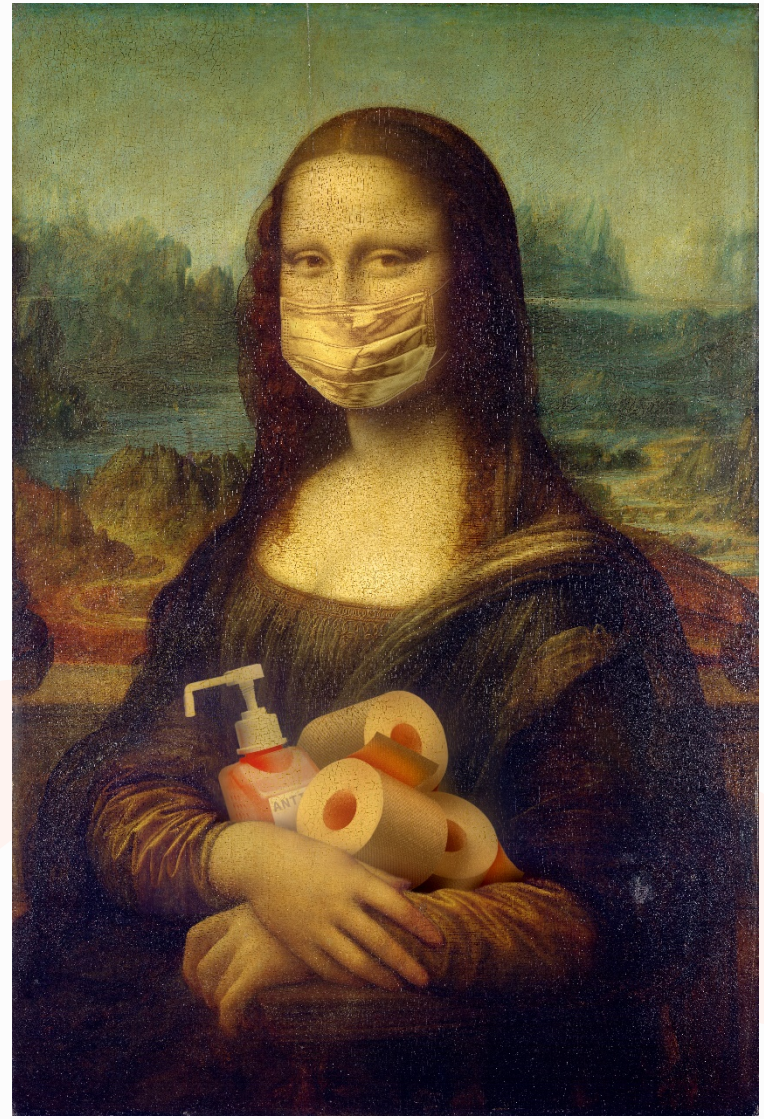
AETC AIDS Education & Training Center Program Pacific

Testing for COVID-19 and HIV:

It is what it is.



Bernard M Branson MD

March 24, 2021

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Use of Brand Names

- This presentation may refer to individual tests by brand name for the purposes of identification and clarity.

- No endorsement of any specific test is intended.

Session Objectives

- Understand the fundamentals of laboratory tests for SARS-CoV-2.
- Appreciate the role for serology tests in identifying exposure to SARS-CoV-2.
- Review new developments in HIV tests and their utility for a new diagnostic algorithm
- Describe how similar parameters affect the accuracy of tests for SARS-CoV-2 and HIV.

Poll Question 1: Which COVID-19 tests have received FDA-approval?

A. PCR tests
B. Antigen tests
C. Antibody tests
D. Home tests
E. All of the above
F. None of the above

FDA Regulation of Devices: a Quick Primer

- License: Biologics (tests for blood screening)
- Approval: high-risk devices (HIV Ag, Ab, RNA tests
  - Pre-market approval application
- Clearance: most diagnostic tests
  - 510(k) application
- Emergency Use Authorization: **not** approval

A few more regulations apply…

- CLIA: Clinical Laboratory Improvement Amendments (of 1988)
  - Part of CMS *(Center for Medicare and Medicaid Services)* classifies devices and certifies laboratories to perform tests based on complexity:
    - High
    - Moderate
    - Waived *(Home tests are automatically waived)*
- LDTs : Laboratory-developed tests *(FDA requirements suspended)*
  - For use only by laboratory that developed the assay
  - Can be for novel assay (SARS-CoV-2) or one for which assays exist (Vitamin D)

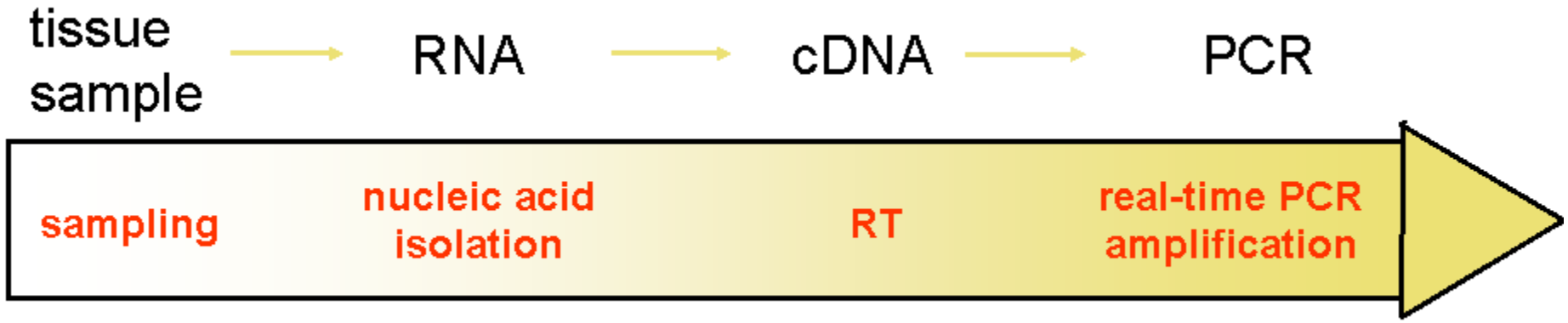
How Confident in Results? It Depends.

		No. of Specimens	No. of Specimens	Sensitivity/ PPA (95% CI)	Specificity/ NPA (95% CI)
		Positive	Negative		
FDA-Approved					
APTIMA HIV	RT-PCR	1041	3515	100 (99.6-100)	99.8 (99.6-100)
Bio-Rad Ag/Ab	EIA	1300	7000	100 (99.7-100)	99.8 (99.7-99.9)
FDA-Cleared					
COBAS Flu A	RT-PCR	175	1175	98.3 (95.1-99.4)	96.0 (94.7-97.0)
COBAS Flu B	RT-PCR	42	1308	95.2 (84.2-98.7)	99.4 (98.8-99.7)
Binax NOW Flu A	Rapid Ag	69	266	80 (71-87)	93 (89-95)
BinaxNOW Flu B	Rapid Ag	50	288	65 (54-75)	97 (95-99)
Emergency Use Authorization					
Abbott SARS-CoV-2	RT-PCR	84	122	100 (88-100)	100 (93.5-100)
Binax NOW SARS CoV-2	Rapid Ag	35	67	97 (85-99)	98.5 (92-100)

Current Status, SARS-CoV-2 Tests

- 336 Emergency Use Authorizations
  - RT-PCR: >200
    - 18 for saliva specimens
    - 29 home swab collection systems
    - 15 COVIC-19/Influenza A-B multiplex
  - Antigen: 16
    - 8 High or moderate complexity lab assays
    - 7 rapid, waived complexity
    - 1 rapid home test, instrument read
  - Antibody: 65

Initial Steps: RT-PCR for SARS CoV-2 RNA



205 patients, 1070 specimens

- BAL 14/15 (93%)
- NP swab 5/8 (63%)
- Throat swab 126/398 (32%)
- Sputum 75/104 (72%)
- Saliva
- Feces 44/153 (29%)

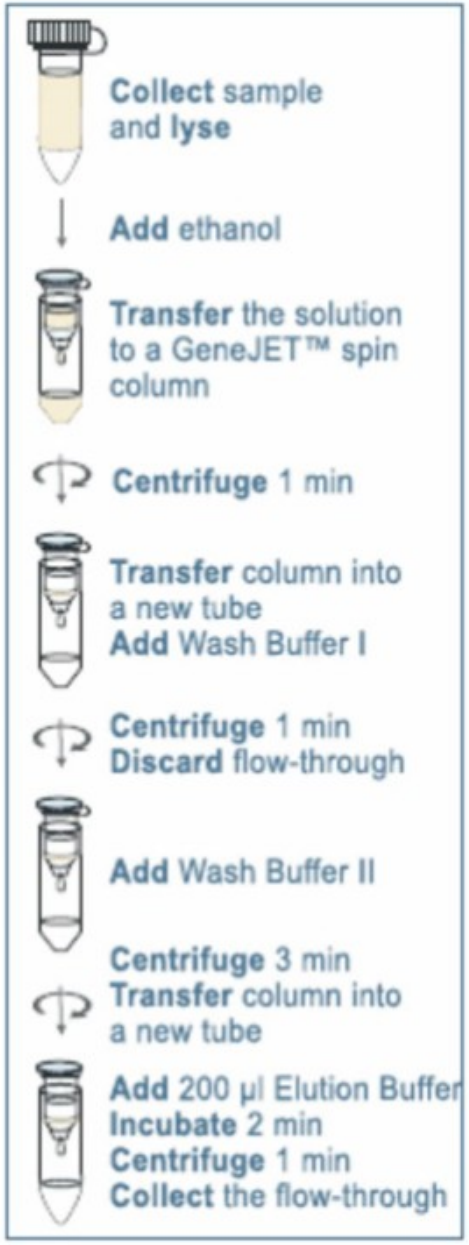
Wong et al, JAMA March 2020

Extract RNA from cellular debris, eliminate DNA

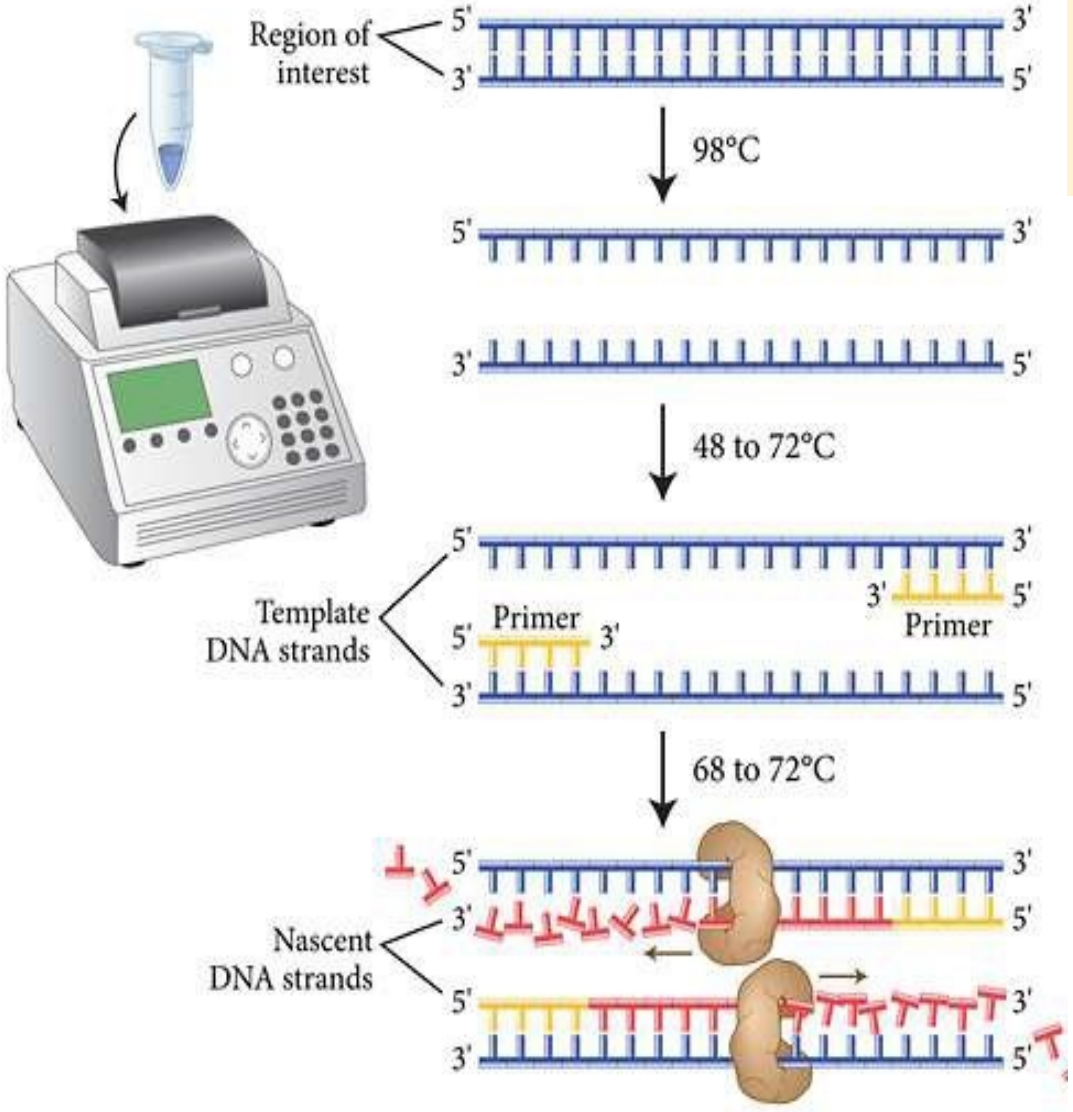
Use reverse transcriptase to form complemenatry DNA

Real time DNA PCR

RNA extraction



RT-PCR procedure



Reverse Transcriptase makes cDNA from viral RNA

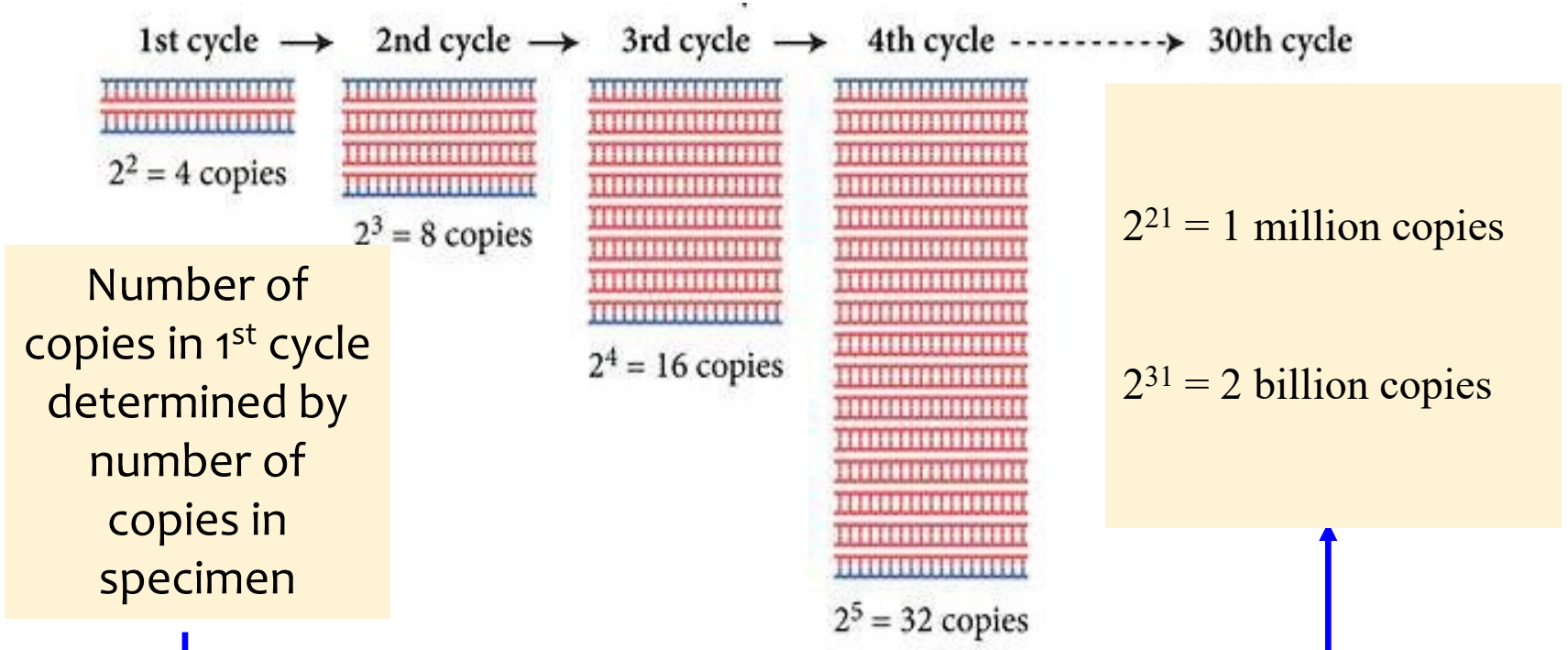
Heat to denature cDNA to create two single DNA strands

Lower temp, primers bind to 3' and 5' ends of single-stranded DNA

Add DNA polymerase to create 2 new cDNA molecules

Repeat "thermocycle"

Chain Reaction: Exponential Amplification



Comparative Analytical Sensitivity

FDA Performance Panel

Product LOD (NDU/mL*)	Developer	Test
180	Perkin Elmer	P.E. New Coronovirus Nucleic Acid Detection Kit
600	Hologic, Inc.	Panther Fusion/Aptima SARS-CoV-2 Assay *(2.4 hours)*
1,800	Roche Molecular	cobas SARS-CoV-2
1,800	Becton Dickenson	BD SARS-CoV-2 Reagents for BD MAX System
1,800	Quest Diagnostics	Quest SARS-CoV-2 rRT-PCR
5,400	Abbott Molecular	Abbott RealTime SARS-CoV-2 assay *(2.4 hours)*
5,400	CDC	Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay
5,400	Cepheid	Xpert Xpress SARS-CoV-2 *(45 minutes)*
18,000	CDC	CDC2019-nCoV Real-Time RT-PCR Dx Panel
300,000	Abbott Diagnostics	ID NOW COVID-19 *(13 minutes)*
540,000	Quidel Corporation	Lyra Direct SARS-CoV-2 Assay *(70 minutes)*

*NAAT-detectable units/mL

- FDA.gov/medical-devices as of 9/15/2020

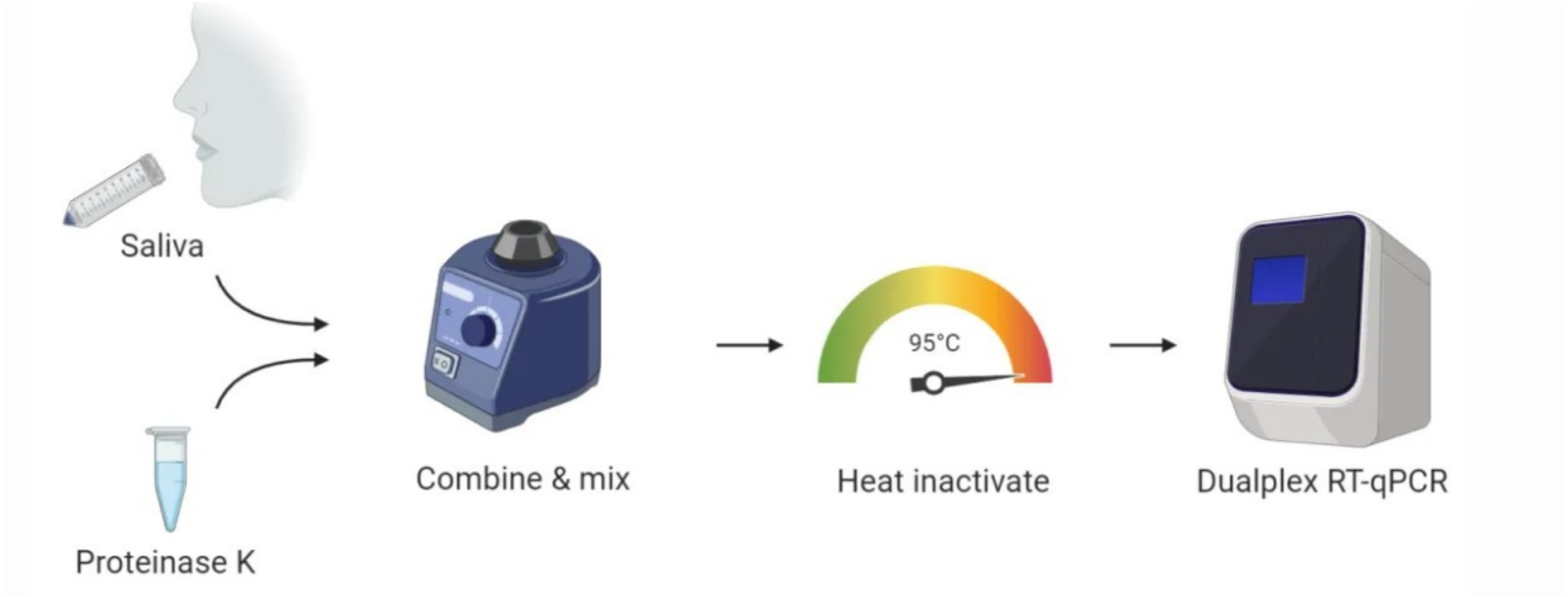
Poll Question 2:

Compare saliva specimens with nasal swabs.

Which statement is incorrect:

A. Saliva is less accurate than nasal swabs.
B. Saliva is easier to collect than nasal swabs.
C. Virus is detected longer in saliva than in nasal swabs.
D. Saliva specimens require special preservatives for RT-PCR.
E. RT-PCR is more expensive with saliva specimens because of special handling and extraction procedures.

Saliva Test: EUA for SalivaDirect Protocol



- EUA issued to Yale School of Public Health August 15, 2020
- Uses unprocessed saliva, collected into sterile urine cup or test tube
- No extraction step required

SalivaDirect Protocol for SARS-CoV-2 RT-PCR

- Eliminates need to collect NP swab, so no swab or transport medium
- Eliminates need for RNA extraction kit (supply issues)
- Validated for use with reagents, primers and instruments from several different diagnostics manufacturers – and counting
- Distributed free to individual laboratories
- Commercial laboratories must obtain a license (free) that negotiates retail price they can charge

The NEW ENGLAND JOURNAL of MEDICINE

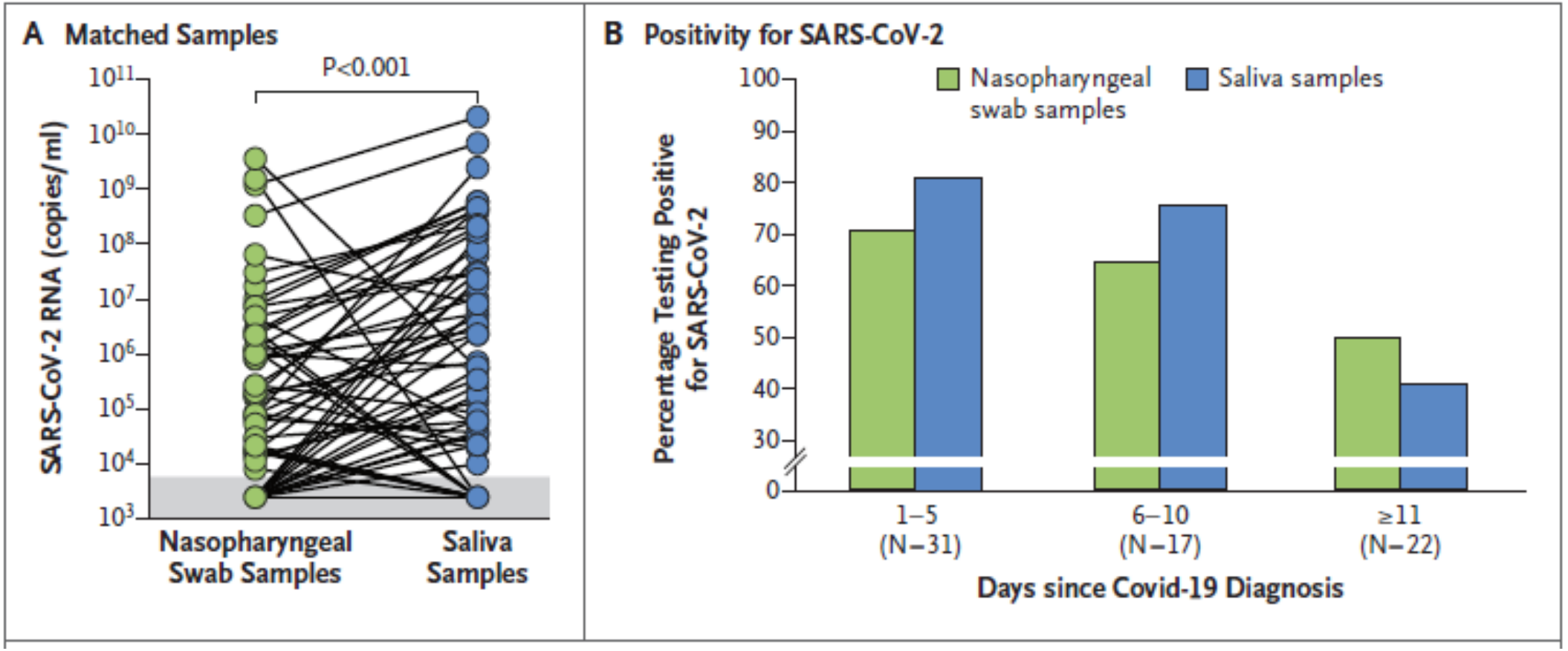
CORRESPONDENCE

Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2

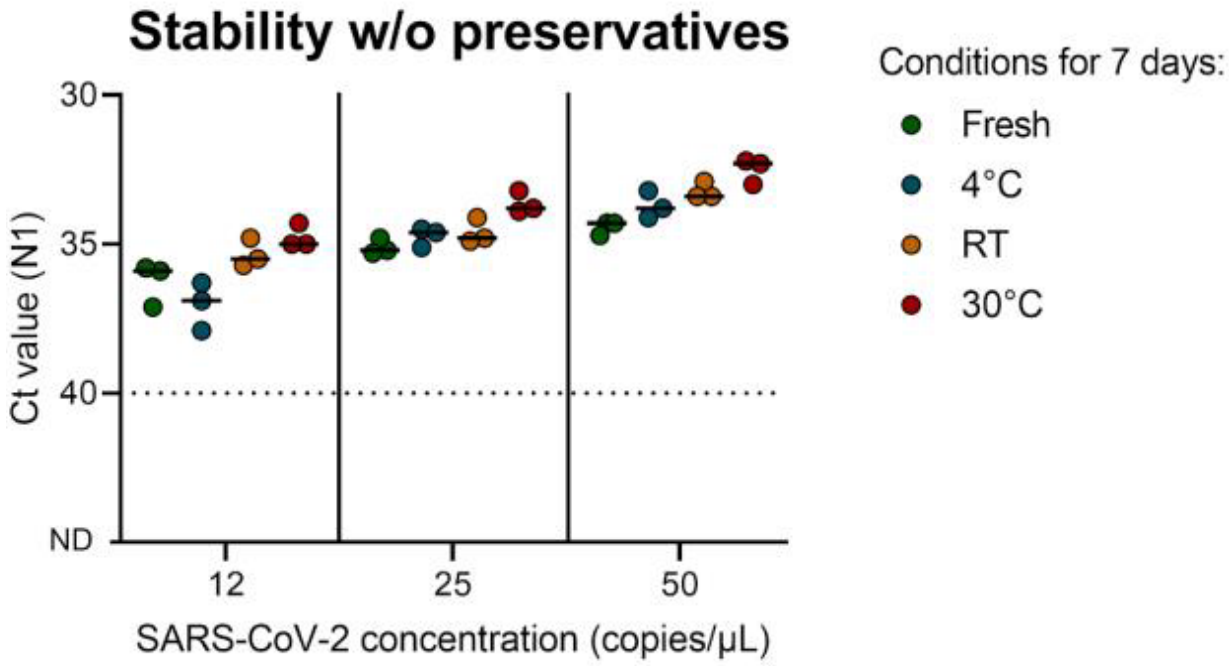
September 7, 2020

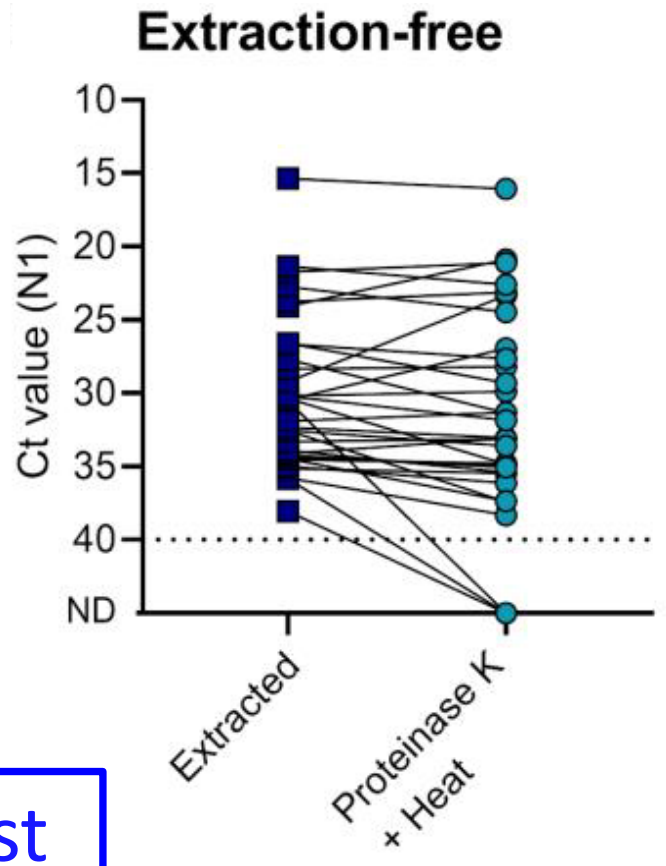
Paired specimens from 70 hospitalized patients with NP swabs positive for SARS-CoV-2 on hospital admission

-Wyllie et al, Yale University

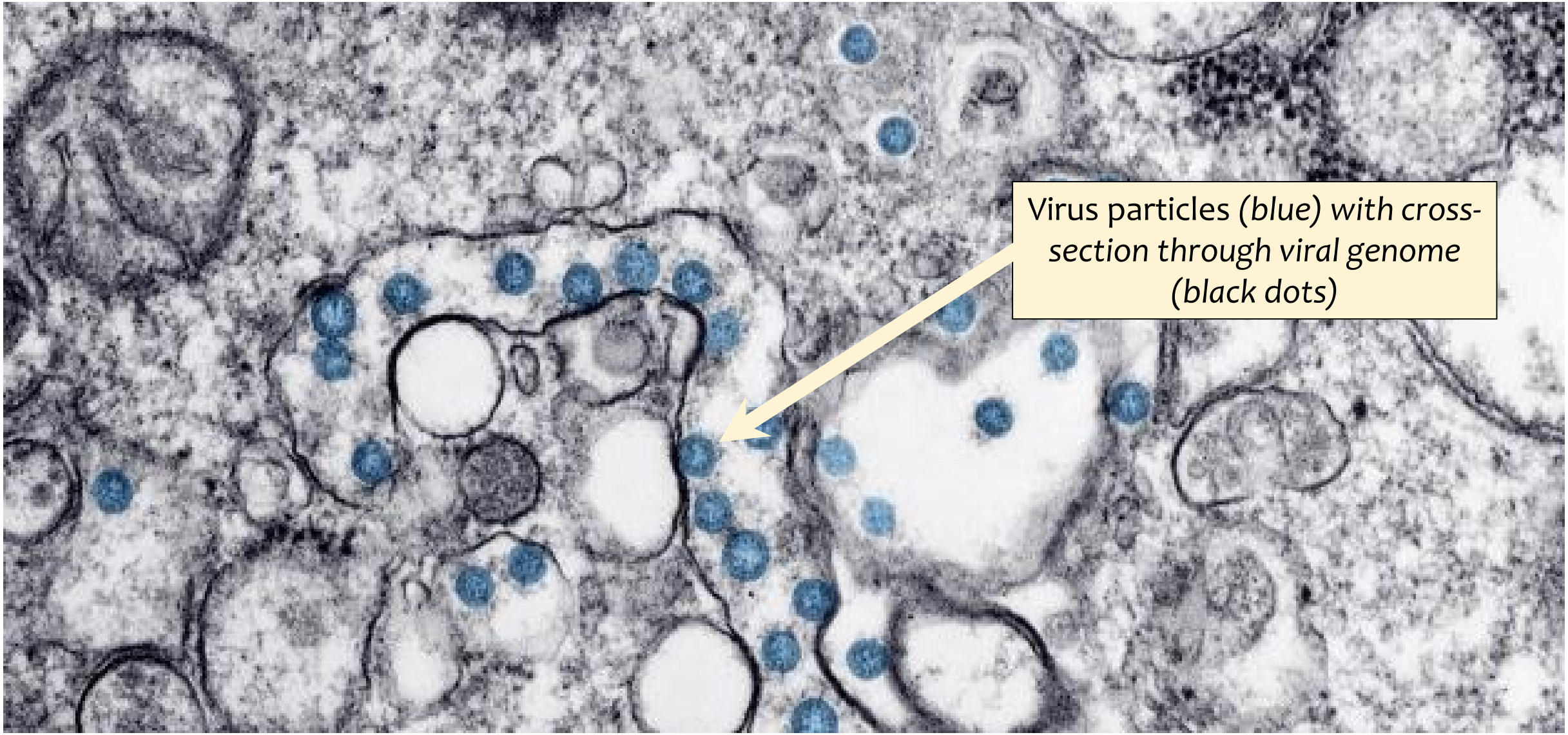


Simplified, sensitive, economical SalivaDirect method



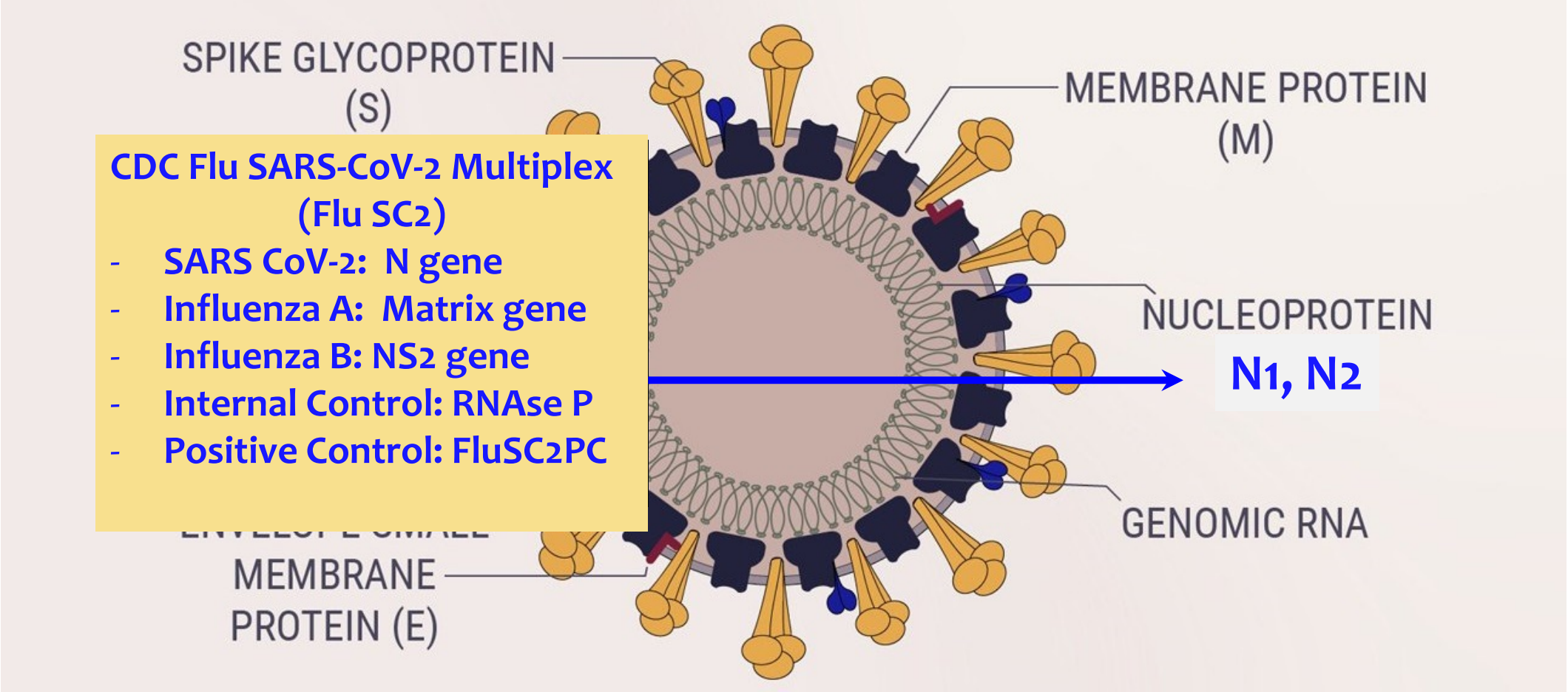


Total minimum reagent cost per sample: $1.29 - $4.37



From CDC: Transmission electron micrograph of isolate from first U.S. case of COVID-19

SARS-CoV-2 viral antigens and RNA



CDC Flu SARS-CoV-2 Multiplex (Flu SC2)

- **SARS CoV-2: N gene**
- **Influenza A: Matrix gene**
- **Influenza B: NS2 gene**
- **Internal Control: RNAse P**
- **Positive Control: FluSC2PC**

Testing strategies

- Symptomatic persons only (*"revised" CDC guidelines)*
- Test symptomatic persons and known contacts

- Test anyone on demand

- Test everyone twice a week *(Saliva: University of Illinois)*
- Focused testing after sentinel testing of sewage from specific locations *(e.g., dorms – U of Arizona)*

COVID-19 Screening Strategies

ScienceAdvances

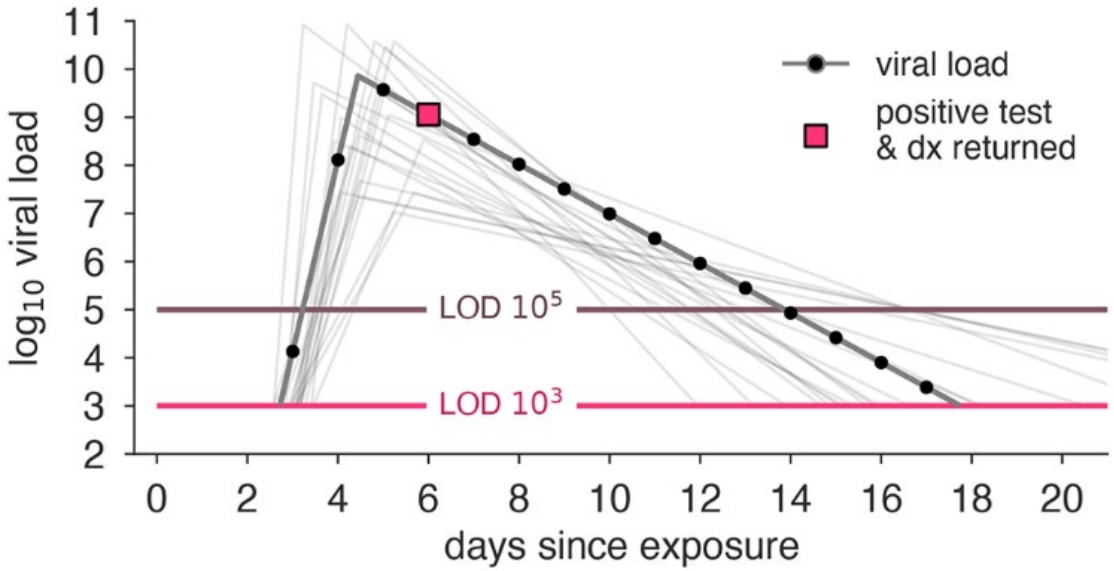
RESEARCH ARTICLES

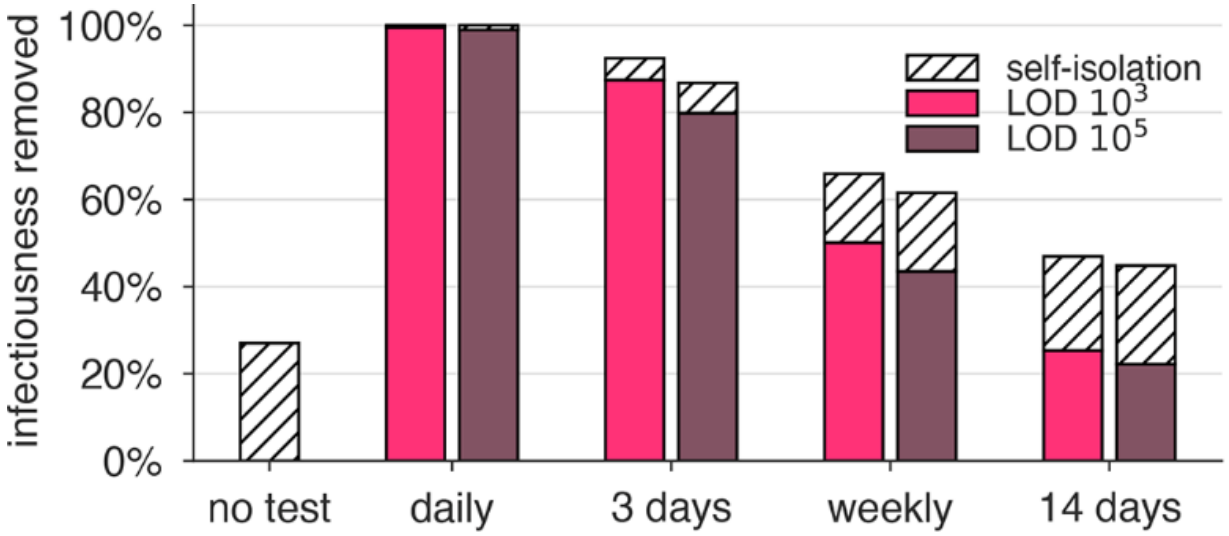
Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening

Daniel B. Larremore,[1,2*] Bryan Wilder,[3] Evan Lester,[6,5] Soraya Shehata,[4,5] James M. Burke,[6] James A. Hay,[7,8] Milind Tambe,[3] Michael J. Mina[7,8,9*†] and Roy Parker[4,6,10,2*†]

First release: 20 November 2020

Serial Testing Strategy – Congregate setting





The number of infections identified and quarantined **depends more on the frequency of the test than the test's sensitivity.**

RT-PCR vs Antigen tests

- RT-PCR is exquisitely sensitive for presence of viral material.
- Most tests report **sensitivity** compared with RT-PCR
- RT-PCR also detects viral transcripts (fragments) that cells produce in excess compared with infectious virions
- Infectious virus is rare at RT-PCR RNA values $<10^6$ copies/ml

- Cevik et al, Lancet Microbe 2021

Antigen vs RT-PCR tests

- Antigen tests are visually read, antigen-antibody interactions.

- Antigen "Sensitivity" = Positive Percent Agreement *with RT-PCR*
  - 1098 Paired swabs from WI college students (5.2% overall prevalence)[1]
    - **80%** among 227 (21%) participants with one or more symptoms
    - **41%** among 877 (77%) asymptomatic participants
  - UK Oxford study
    - **79%** when performed by lab professionals
    - **73%** when performed by trained health care workers
    - **57%** when performed by self-trained public using a protocol

[1]*MMWR Jan 29, 2021*

[2]*Public Health England Nov 8, 2020*

RT-PCR vs Antigen tests Limits

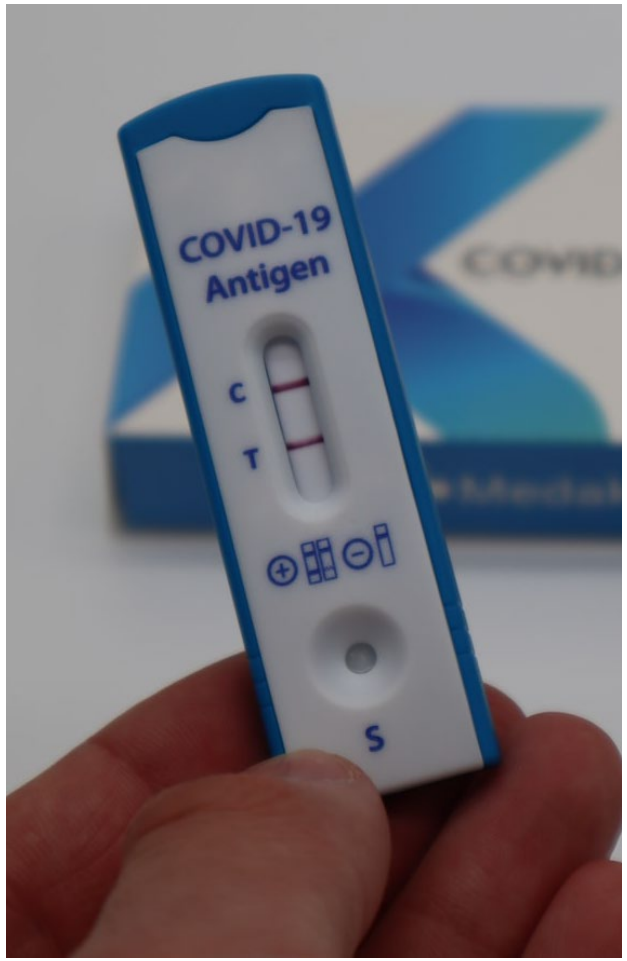
- No study identified infectious virus >9 days after onset of symptoms despite weeks of persistently positive RT-PCR

- RT-PCR have limits of detection of 10^3-10^4 copies/ml or lower, but Infectious virus is rare at RT-PCR RNA values <10^6 copies/ml, a level that Antigen tests can achieve

- Genetic variants might lead to false-negative RT-PCR if the mutation involves the part of the genome detected by the test.

- Cevik et al, Lancet Microbe 2021

The role for Antigen tests



- Antigen tests take 15-20 minutes, RT-PCR takes 4^{+} hours.
- Antigen tests are less expensive and suitable for POC.
- Antigens likely detect persons who are infectious, but might be false-negative in some cases.
- RT-PCR remains positive long after infectious virus is gone.

The role for Antigen tests

- Antigen tests make it possible to conduct repeated serial testing
  - often enough
  - deliver results fast enough
  - at costs low enough

to make surveillance testing (dormitories, schools, nursing homes, job sites…) feasible.

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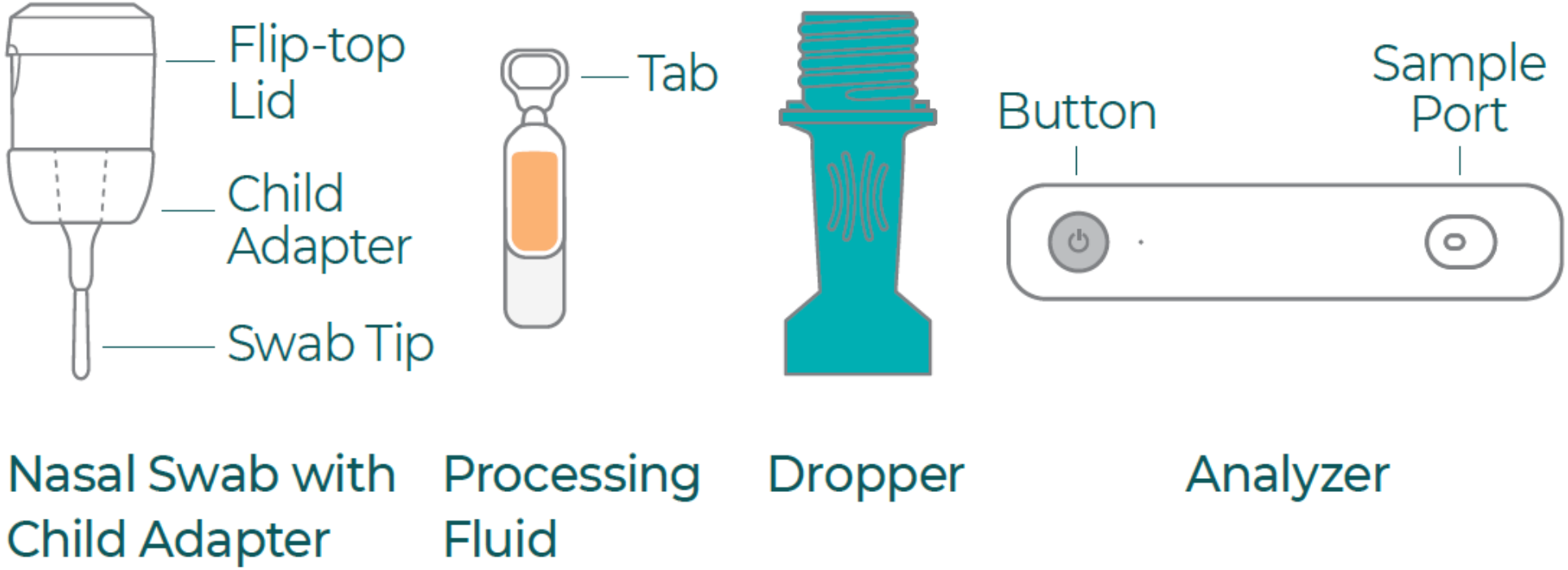
First release: 20 November 2020

Ellume Home test for SARS-CoV-2 Antigen



Detects nucleocapsid antigen from nasal swabs

Ellume COVID-19 Home Test: FDA EUA December 15, 2020

- 15-minute fluorescent antigen test
- Testing app for iOS, Android
- Video and step-by-step instructions on app
- Battery operated analyzer connects with phone via Bluetooth
- Result appears on phone after 15 minutes
- Test result also reported to health department
- Email result optional

IMMEDIATE RELEASE

DOD Awards $231.8 Million Contract to Ellume USA LLC to Increase Domestic Production Capacity and Deliver COVID-19 Home Tests

FEB. 1, 2021

Poll question 3: Which statement is true about antibody tests?

A. A positive antibody test means a person has COVID-19.
B. A positive antibody test means a person is immune.
C. A positive antibody test provides insight into the prevalence of COVID-19 in a population.
D. Antibody tests help identify new mutant viral variants.

PCR vs Antibody tests

- PCR tests detect the presence of viral material, but cannot determine whether a person has been infected previously and recovered.

- Serology tests determine whether a person has been previously exposed to a pathogen
- Serology tests provide insight into the prevalence of a disease in the population by identifying those with specific antibodies

FDA Regulation: Lessons from HIV testing

- Antibody tests came first.
  - Intended for screening the blood supply so…
  - "High risk" designation by FDA (= PMA); CBER vs CDRH
  - First quantitative HIV-1 viral load monitoring test approved in 1996
  - First HIV-1 RNA diagnostic test (qualitative) in 2006
- FDA (CBER & CDRH) proposed "down-classification" of HIV and HCV tests to class II (recommended by Advisory Committee in March 2018) – public comment spring 2020

Lessons from HIV testing?

- FDA required COVID-19 LDTs to obtain Emergency Use Authorizations, then
- FDA granted blanket EUAs, first to LDT PCRs
- FDA first granted waiver from EUAs for antibody tests
  - CLIA waiver also OK'd
- FDA issued EUAs for several antibody tests, then later rescinded them and required EUAs for the others
- FDA has rescinded 254 EUAs for COVID-19 tests – so far.

Types of SARS CoV-2 antibody tests

- Lateral flow assays (LFAs)

Rapid, point of care

- ELISA

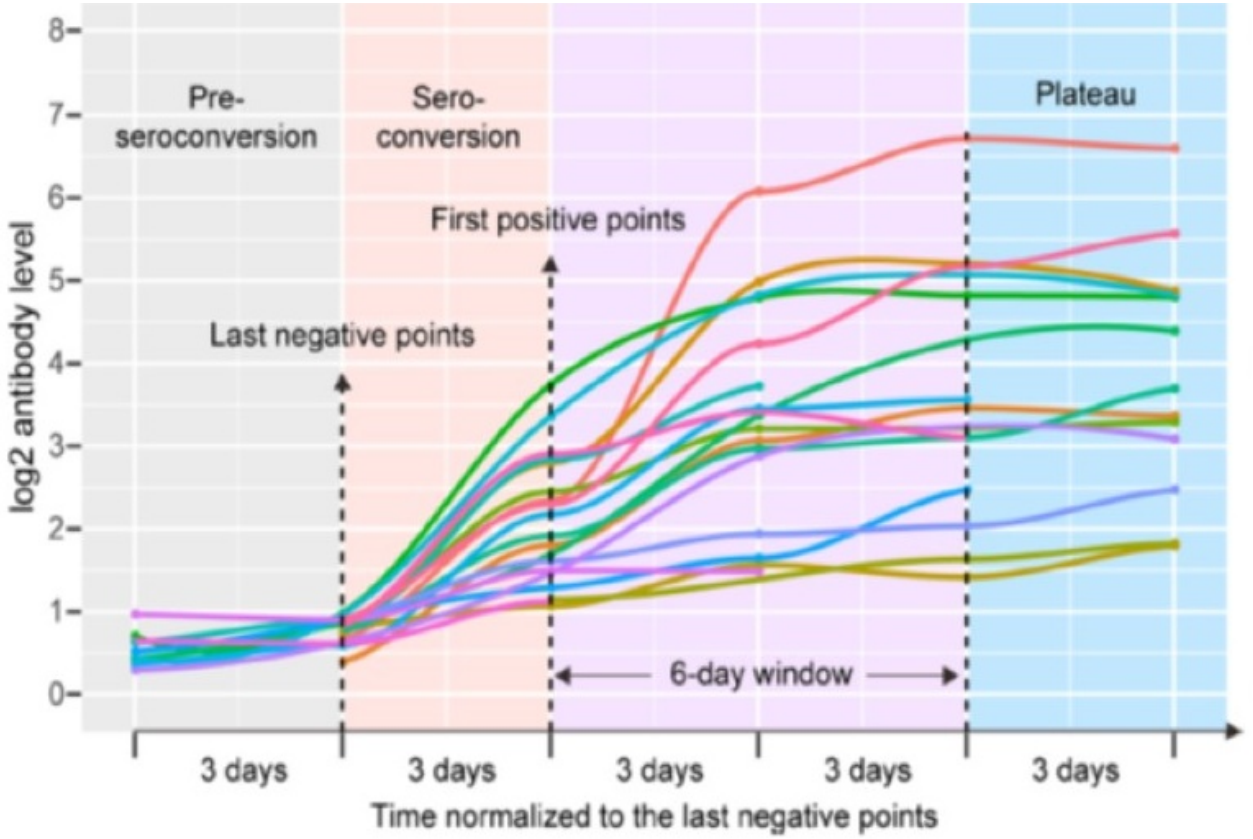
Chemiluminescent, electrochemiluminescent
Potential for high throughput

- Neutralizing antibody assays

Serial dilutions of serum added to viral cultures to look for areas of inhibition

COVID-19: Dynamic Changes in IgG Levels

- 285 patients with COVID-19:
  - 100% tested positive within 19 days after symptom onset
  - Seroconversion for IgG and IgM occurred simultaneously or sequentially



- Long et al, Nature Medicine 2020

Antibody tests: HIV versus COVID-19

- Positive HIV antibody tests indicate active HIV infection

- Positive COVID-19 antibody tests:
  - Single test has low positive predictive value in most populations
  - Indicative of past exposure, not necessarily active infection
  - Unknown whether antibodies indicate immune protection
  - If antibodies confer immunity, not certain how long it will persist

Interpreting screening test results

For a laboratory test:

Sensitivity: Probability test=positive if patient=positive

Specificity: Probability test=negative if patient=negative

For COVID tests:

Positive Percent Agreement: Probability test2=positive if test1=positive

Negative Percent Agreement: Probability test2=negative if test1=negative

Predictive value:

Probability patient=positive if test=positive

Probability patient=negative if test=negative

Specificity of SARS CoV-2 antibody tests

Specificity in 108 blood donor plasma specimens collected before July 2018												
	IgM				IgG				IgM or IgG			
Assay	Total N	positive	%	95% CI	Total N	positive	%	95% CI	Total N	positive	%	95% CI
Immunochromatographic Lateral Flow Assays												
Biomedomics	107	13	**87.9**	80.1 - 93.4	107	4	**96.3**	90.7 - 99.0	107	14	**86.9**	79.0 - 92.7
Bioperfectus	104	3	**97.1**	91.8 - 99.4	104	2	**98.1**	93.2 - 99.8	104	5	**95.2**	89.1 - 98.4
DecomBio	107	10	**90.7**	83.5 - 95.4	107	9	**91.6**	84.6 - 96.1	107	11	**89.7**	82.3 - 94.8
DeepBlue	108	17	**84.3**	76.0 - 90.6	108	1	**99.1**	94.9 - 100.0	108	17	**84.3**	76.0 - 90.6
Innovita	108	4	**96.3**	90.8 - 99.0	108	0	**100.0**	96.6 - 100.0	108	4	**96.3**	90.8 - 99.0
Premier	108	2	**98.1**	93.5 - 99.8	108	1	**99.1**	94.9 - 100.0	108	3	**97.2**	92.1 - 99.4
Sure	108	0	**100.0**	96.6 - 100.0	108	0	**100.0**	96.6 - 100.0	108	0	**100.0**	96.6 - 100.0
UCP	107	2	**98.1**	93.4 - 99.8	107	2	**98.1**	93.4 - 99.8	107	2	**98.1**	93.4 - 99.8
VivaChek	99	5	**94.9**	88.6 - 98.3	99	4	**96.0**	90.0 - 98.9	99	5	**94.9**	88.6 - 98.3
WondFo									106	1	**99.1**	94.9 - 100.0
									Mean		**94.23**	
ELISAs												
Epitope	108	3	**97.2**	92.1 - 99.4	108	10	**90.7**	83.6 - 95.5	108	11	**89.8**	82.5 - 94.8
In-House									108	1	**99.1**	94.9 - 100.0

Poll question: What happens with results of tests when the prevalence of disease is low?

A. The number of false-positive tests is higher.
B. There is a higher likelihood that a positive result is a false-positive.
C. Chance of false-positive results are much reduced.
D. Nothing. False-positive results are unaffected by prevalence.

Example: Test 10,000 persons

Test Specificity = 94.2% *(58/1000)*

SARS-CoV-2 prevalence = 10.5%

True positive: 1050 False positive: 580

Positive predictive value: 1050/1630 = 65%

Example Continued: Test 10,000 persons

Test Specificity = 94.2% *(58/1000)*

SARS-CoV-2 positivity = 10%

True positive: 1050 False positive: 580

Positive predictive value: 1050/1630 = 65%

SARS-C0V-2 positivity = 1.3%

True positive: 130 ⇩ False positive: 580

Positive predictive value: 130/710 = 18%

Continued Example: Test 10,000 persons

Test Specificity = 94.2% *(58/1000)*

SARS-CoV-2 positivity = 1.3%

True positive: 130 False positive: 580

Positive predictive value: 130/710 = 18%

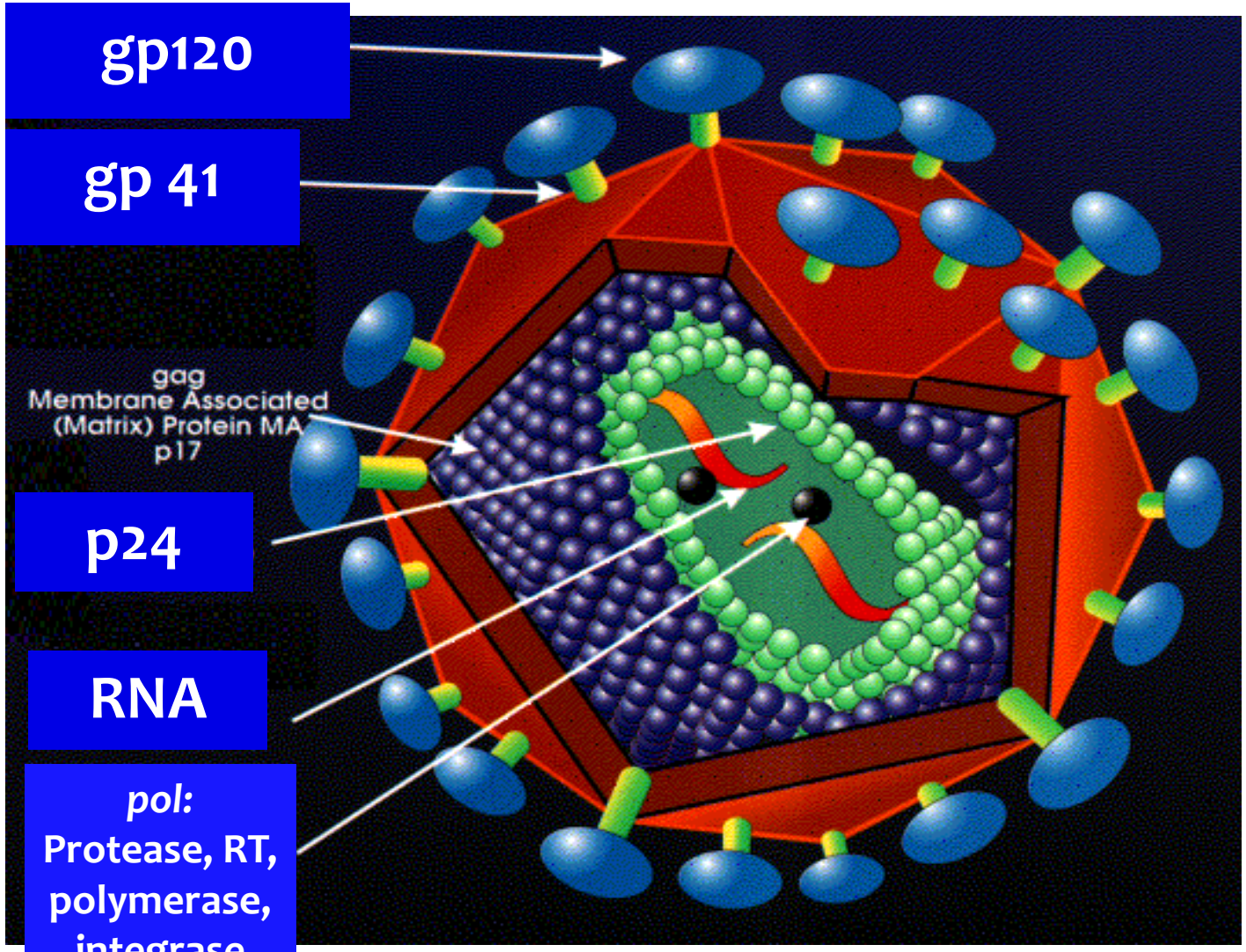
Test Specificity = 98.5% *(15/1000)*

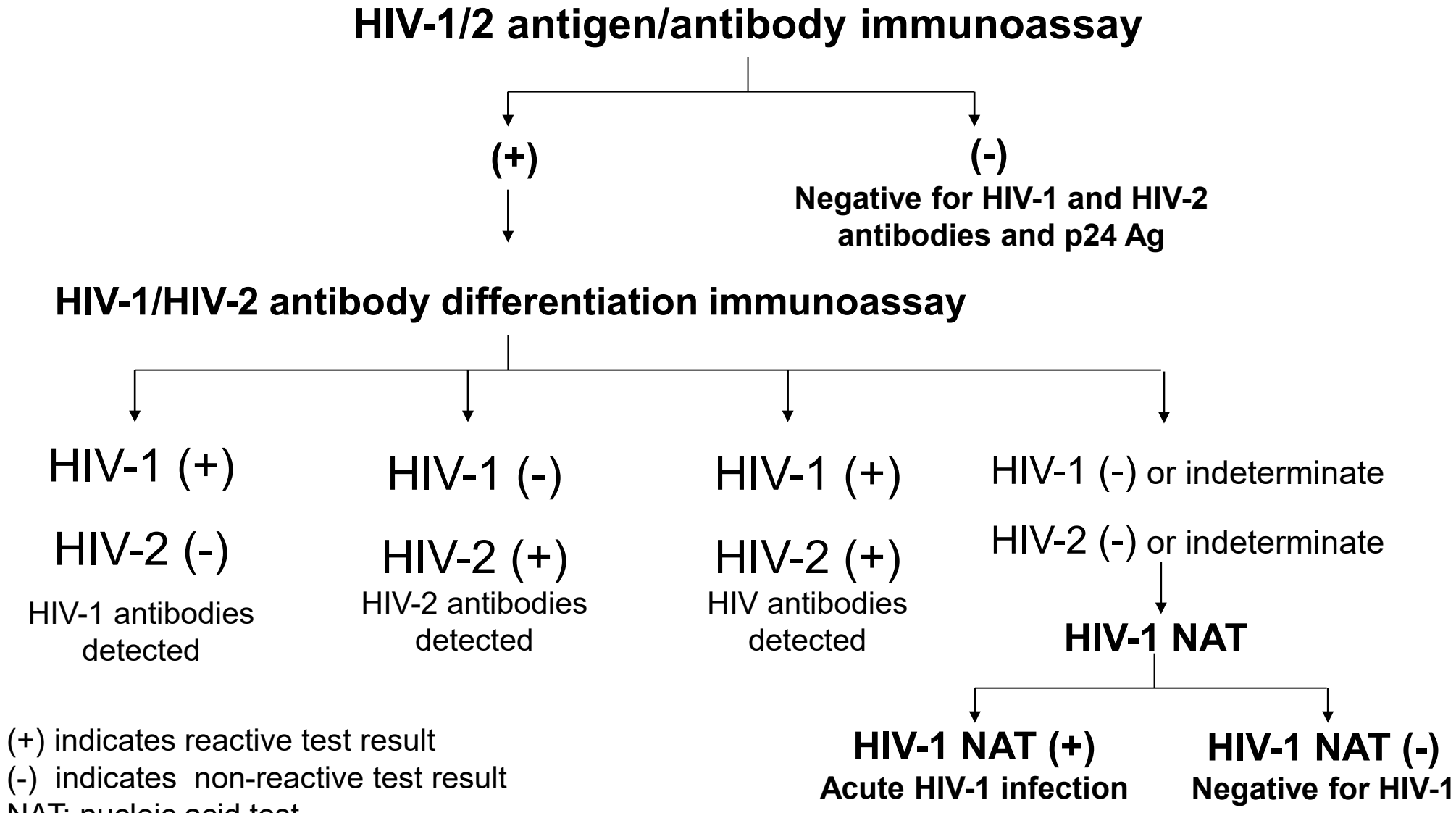
SARSCoV-2 positivity = 1.3%

True positive: 130 False positive: 150

Positive predictive value: 130/280 = 46%

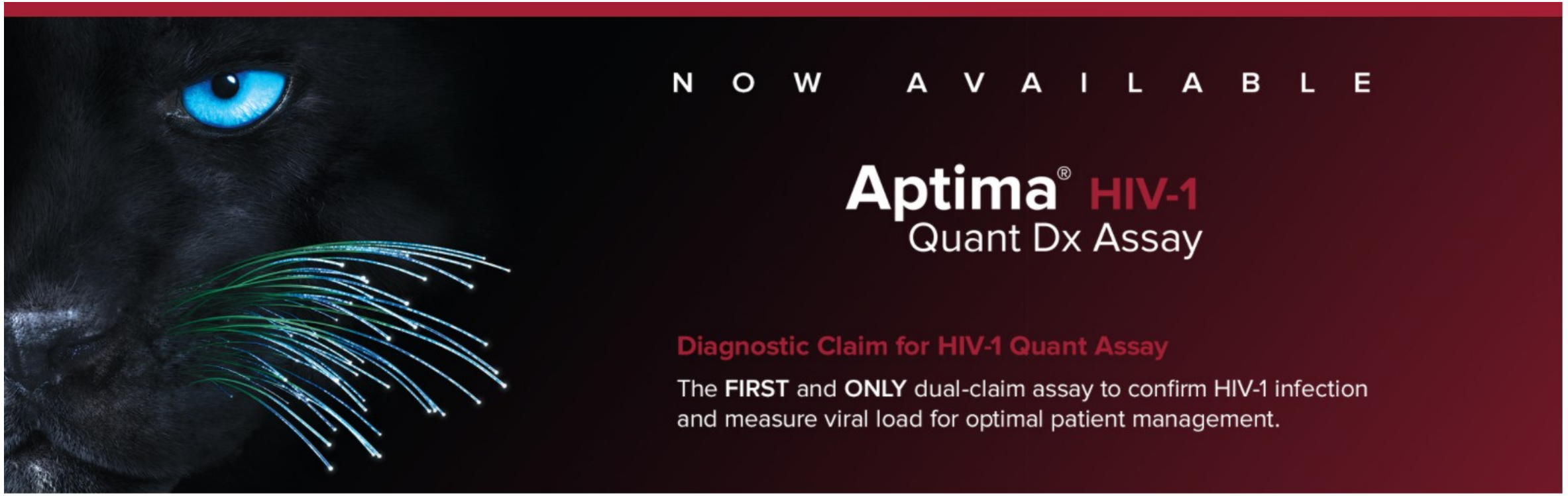
HIV-1: viral antigens and RNA





(+) indicates reactive test result
(-) indicates non-reactive test result
NAT: nucleic acid test

FDA Approved November 20, 2020



Aptima HIV-1 Quantitative Dual Claim

- Diagnosis:
  - Serum or plasma
- Monitoring:
  - Plasma only

Plasma/Serum Comparative Performance Aptima HIV-1 RNA QT Assay

Lab ID	Plasma	Serum	BioCollections Plasma			Aptima QL (plasma)	Aptima QL (serum)
11563	<1.47	<1.47		<1.47	**Copies**	reactive (7.61 s/co)	reactive (5.42 s/co)
11564	1.48	<1.47		<1.47		reactive (6.04 s/co)	nonreactive
11565	1.71	<1.47		<1.47	**32**	nonreactive	nonreactive
11566	1.51	TND		<1.47		nonreactive	nonreactive
11567	<1.47	TND		<1.47		nonreactive	nonreactive
11568	2.87	2.78		2.86		reactive	reactive
11569	2.89	2.71		2.98		reactive	reactive
11570	2.41	2.63		2.72	**300**	reactive	reactive
11571	2.92	2.44		2.97		reactive	reactive
11572	2.44	2.26		2.24		reactive	reactive

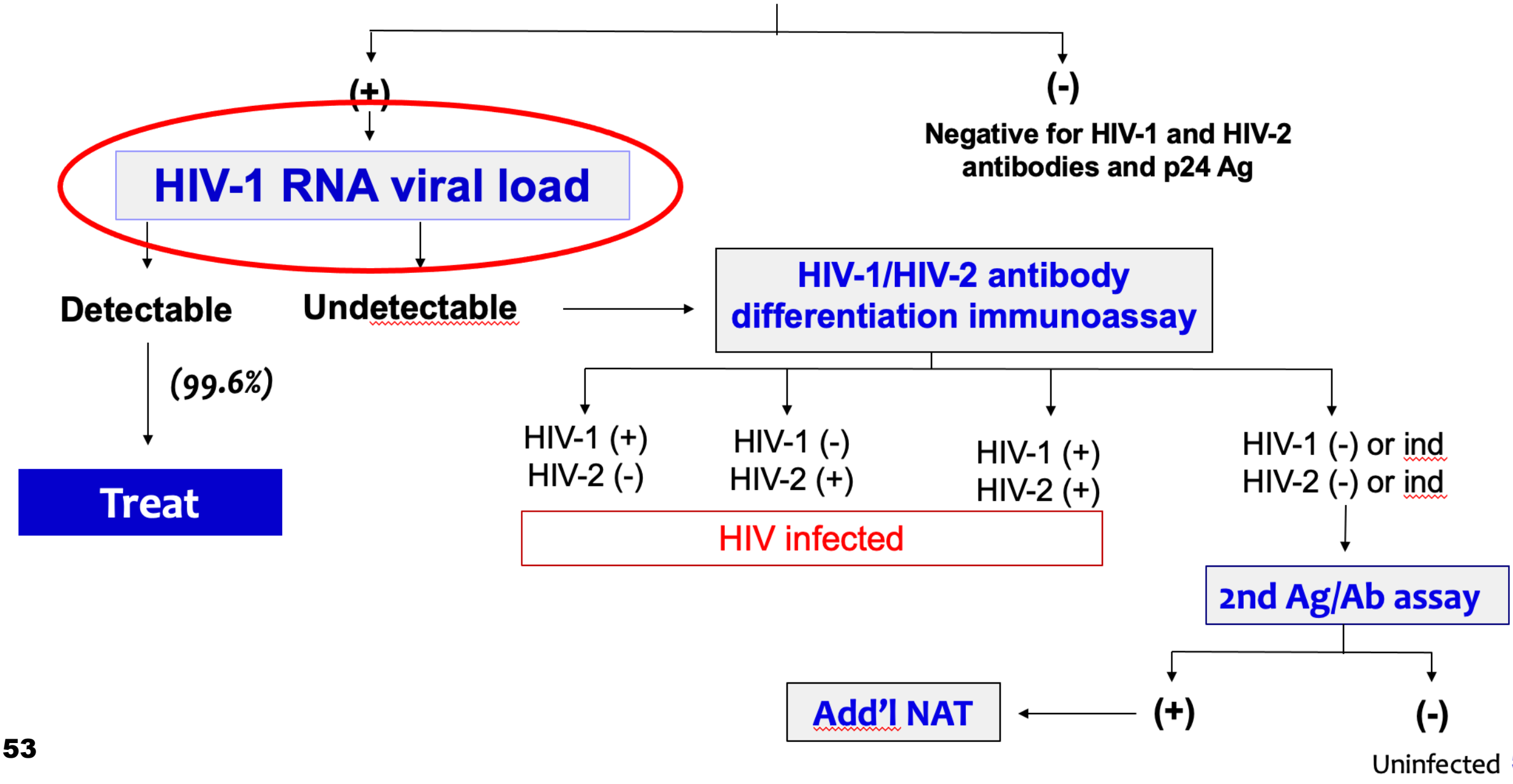
Source: FL Public Health Lab validation (with permission)

Plasma/Serum Comparative Performance Aptima HIV-1 RNA QT Assay Continued

Lab ID	Plasma	Serum	BioCollections Plasma		Aptima QL (plasma)	Aptima QL (serum)
11573	4.94	5.17	4.93	**100,000**	reactive	reactive
11574	4.18	4.05	4.17		reactive	reactive
11575	4.89	4.81	4.95		reactive	reactive
11576	4.91	5	4.86		reactive	reactive
11577	4.28	4.31	4.27		reactive	reactive
11578	5.6	5.11	5.66		reactive	reactive
11579	5.66	5.27	5.61		reactive	reactive
11580	5.84	5.54	5.98		reactive	reactive
11581	5.64	5.28	5.66	**500,000**	reactive	reactive
11582	5.18	4.95	5.2		reactive	reactive

Source: FL Public Health Lab validation (with permission)

HIV-1/2 antigen/antibody immunoassay (review)



“Point-of-Care” Nucleic Acid Tests

GeneXpert

- Xpert HIV-1 viral load
  - 1 ml plasma
  - Results in 90 minutes
  - LOD 32 copies/mL
  - CE-marked December 2014

Not yet available in U.S.

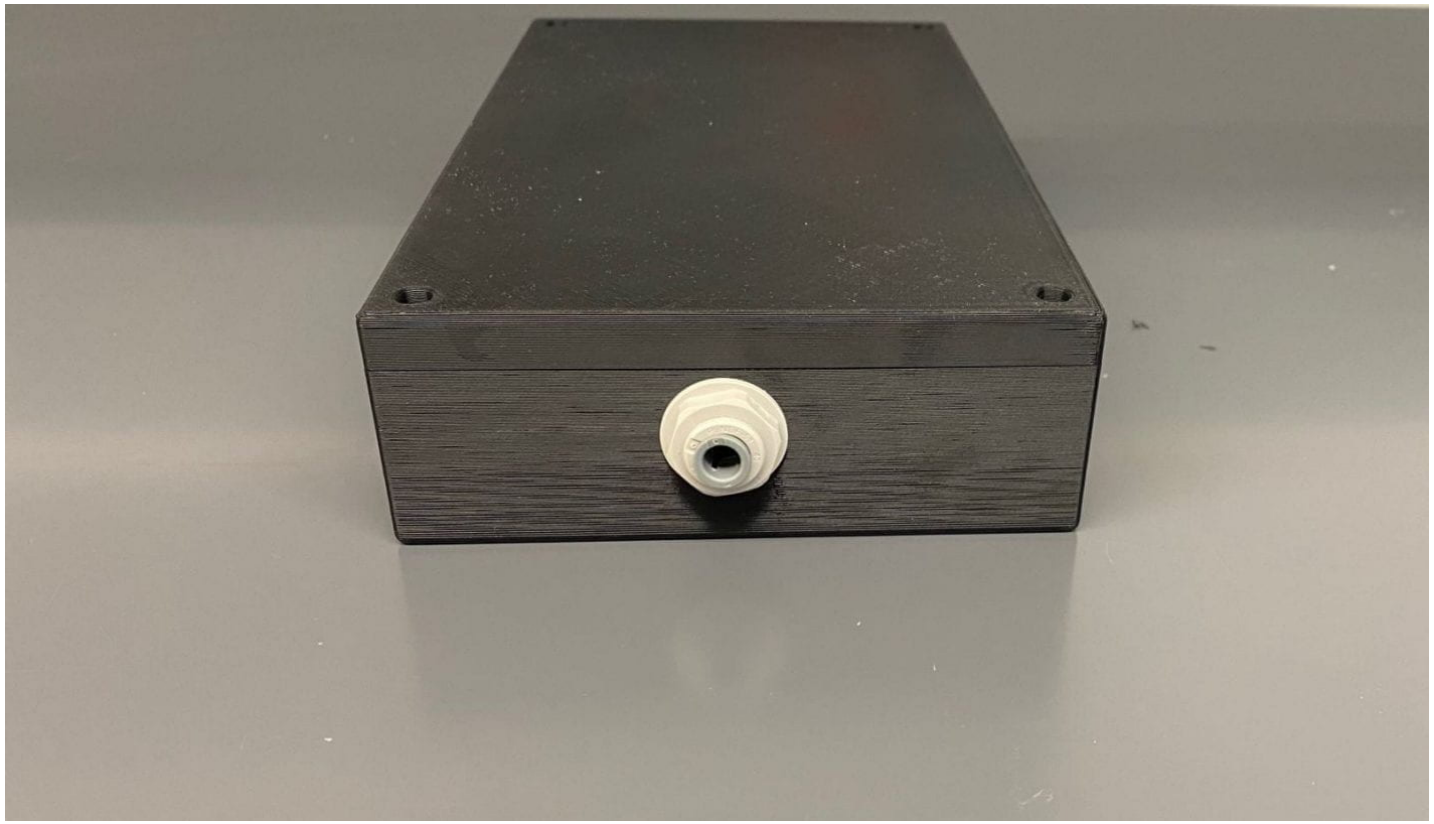
Summary

- Regulation of COVID-19 tests is substantially different than that for HIV tests – and it shows!
- RNA & viral load will play an increasingly important role in HIV diagnosis

- Most covid-19 diagnoses depend on PCR testing of NP swabs, but saliva specimens offer an attractive alternative
- Antibody testing will help to identify persons who have already been infected.
- Serial antigen testing might play a useful role in limiting COVID-19 transmission

OHIO STATE'S COVID-19 BREATHALYZER TEST STILL AWAITING FDA APPROVAL

January 25, 2021



Questions?