

Laboratory Diagnosis of SARS-Cov-2

Dornsife School of Public Health, Drexel University
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Drexel University College of Medicine Disclosure Statement

Alan T. Evangelista, PhD, D(ABMM)

- **No financial or commercial relationships**
- **No conflicts of interest**

Objectives

- **Review the principles of PCR (polymerase chain reaction) testing.**
- **Describe the evaluation and limitations of nucleic acid testing: sensitivity, specificity, positive and negative predictive values.**
- **Review the diagnostic PCR tests for SARS-CoV-2 comparing viral targets, limit of detection, and specimen types. Describe rapid NAA and rapid Ag tests.**
- **Discuss availability and specificity of SARS-CoV-2 antibody tests and utility of IgM and IgG results.**

Requirements for PCR Test

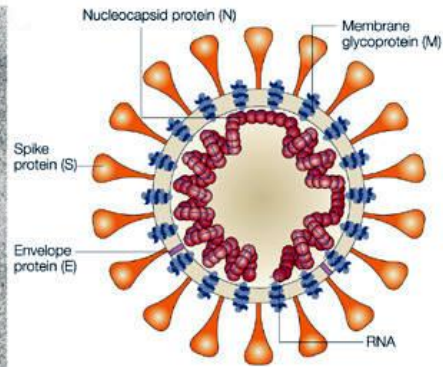
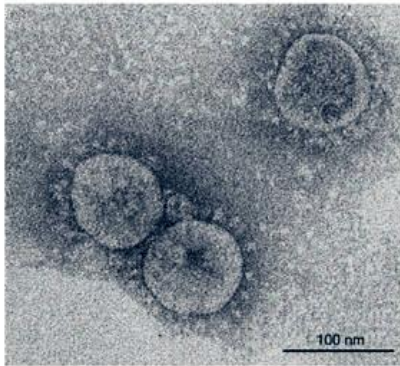
Extraction of specimen: protease +/- beads

Amplification

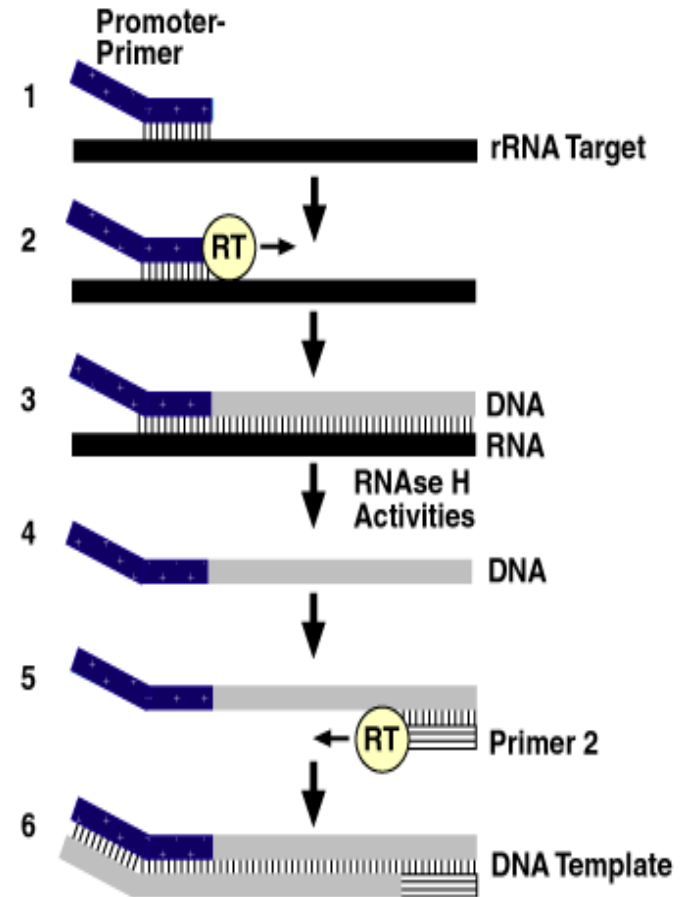
- Identify target sequences: usually 100-10,00 base pairs
- Reverse transcriptase step if RNA virus (make cDNA)
- PCR Primers: a pair of short oligonucleotides (20-30 bp) complementary to opposite strands of DNA flanking the sequence to be detected
- Deoxynucleotides of each base (dNTPs of A,T,C,G)
- Internal control in each sample: QC PCR inhibitors (hemn (hemin) (hemin, lipids)
- Heat stable DNA polymerase enzyme
- Thermal cycler: 95⁰C -> 55⁰C -> 72⁰C

Detection of amplicons: probes w fluorescent reporter

Reverse Transcription PCR (RT-PCR) for RNA viruses



Coronavirus



3 Steps of PCR Thermal Cycling



Denaturation @ 95°C – 10 sec

- Separate double-strained DNA

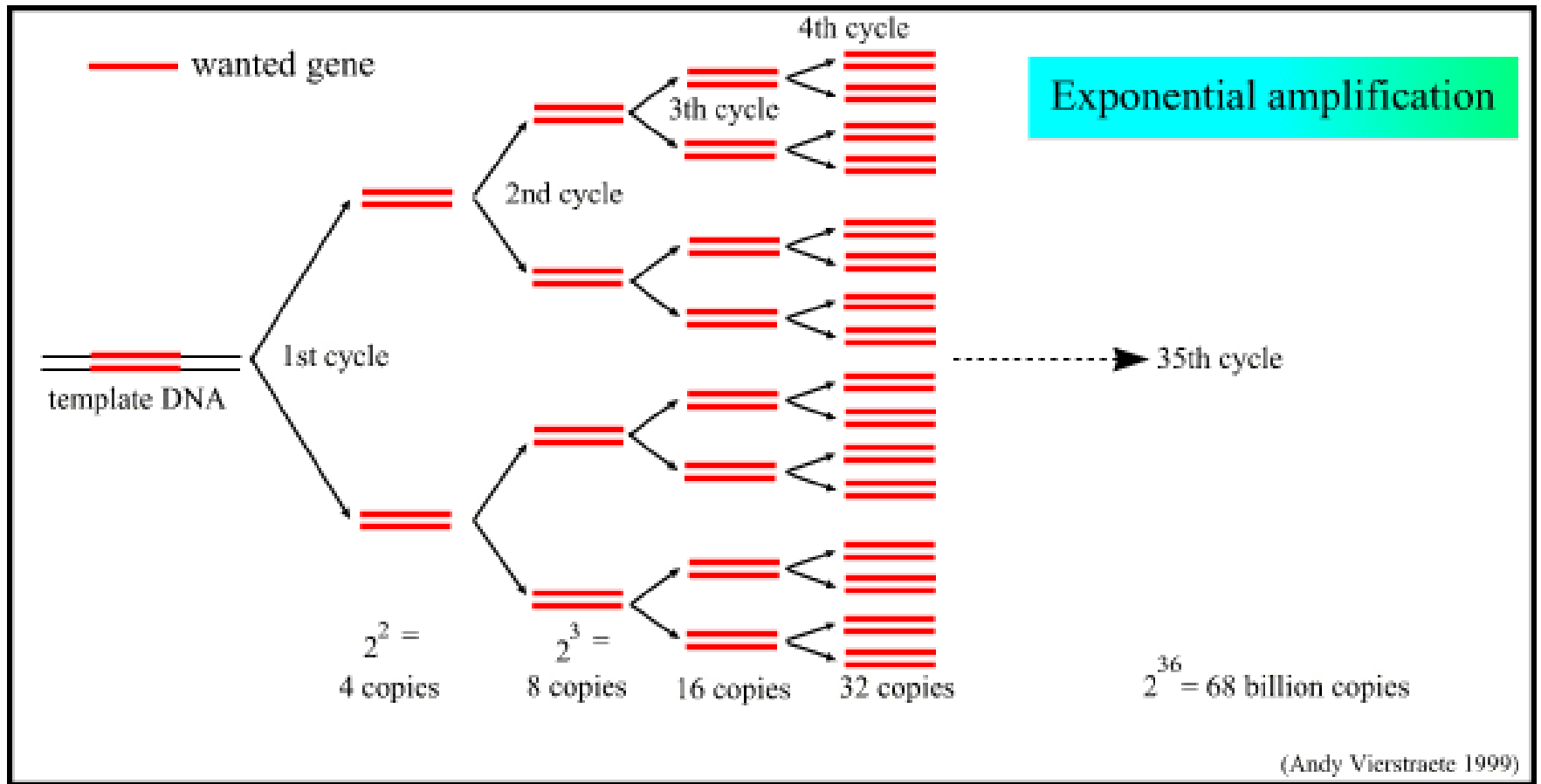
Annealing @ 50-60°C - 20 sec

- Bind PCR Primers to DNA

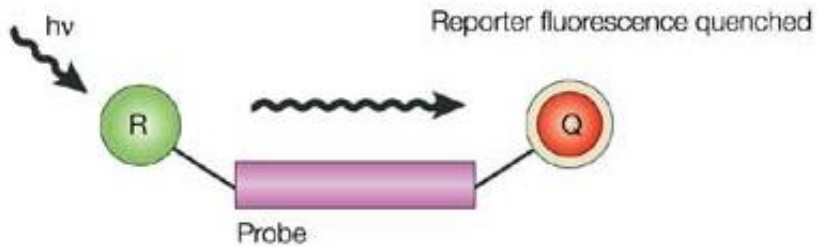
**Polymerization @ 72°C
(Extension Step) – 60 sec**

- Duplicate copies made

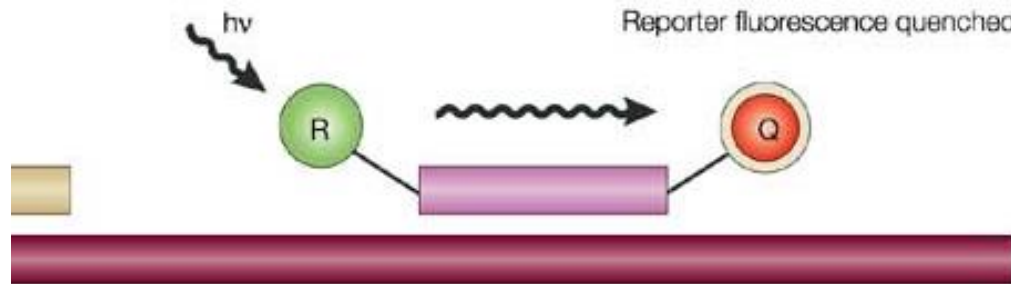
Polymerase Chain Reaction (PCR) Amplification



Fluorescence Detection: Taqman Probes

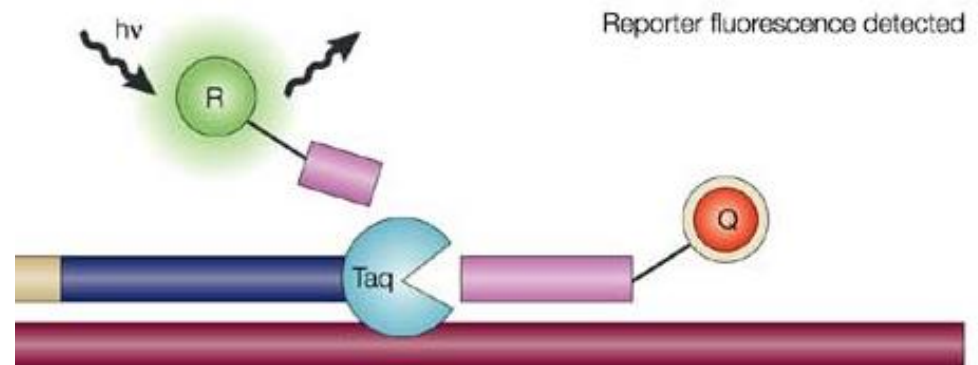


Denature

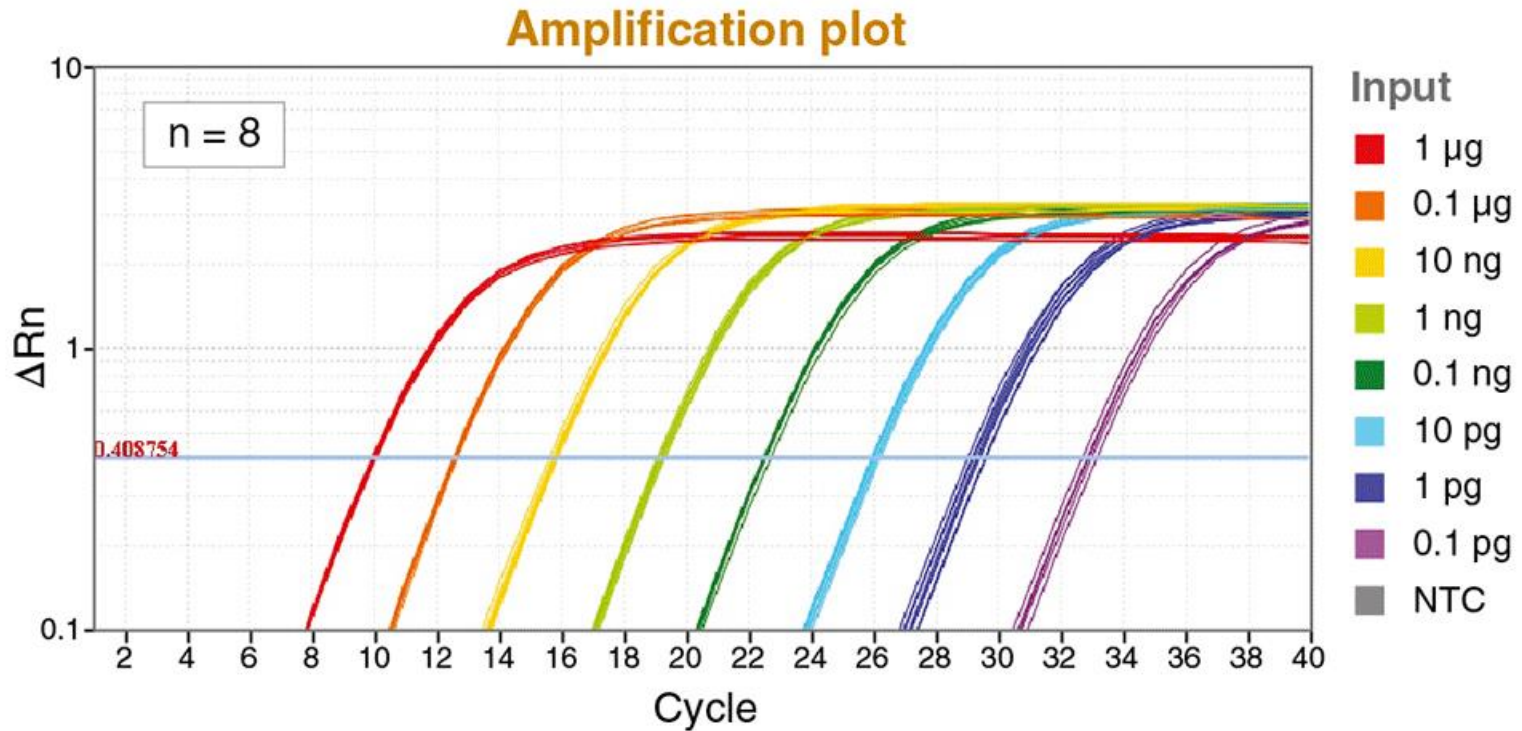


Anneal

Extend



PCR Cycle Threshold Values

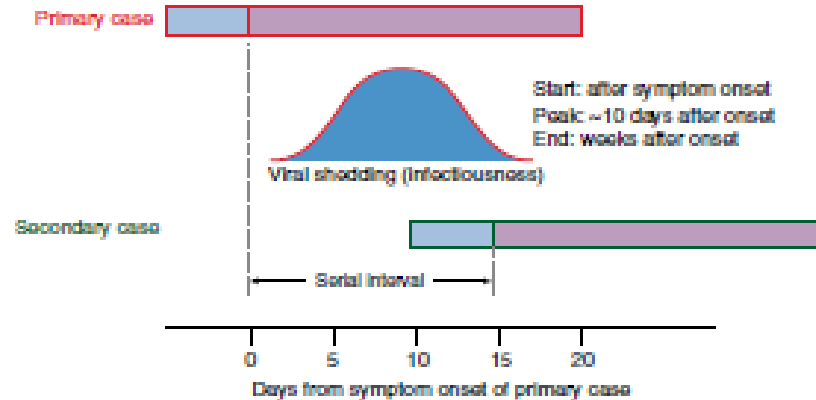


New England Biolabs. Luna Probe One-Step RT-qPCR
<https://www.neb.ca/index1.php>

Temporal Viral Shedding

SARS 2003

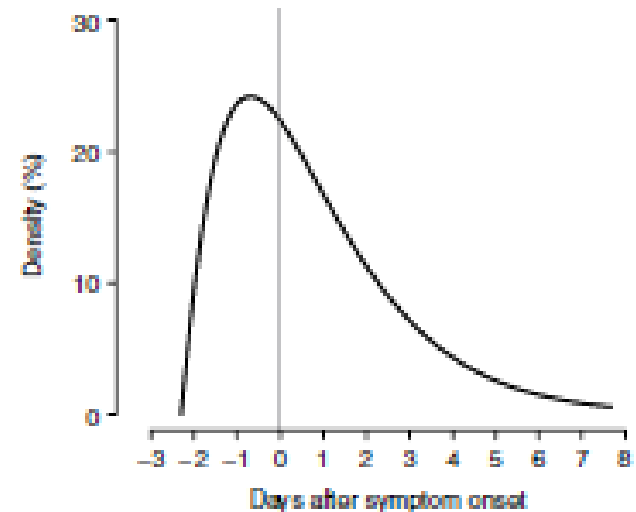
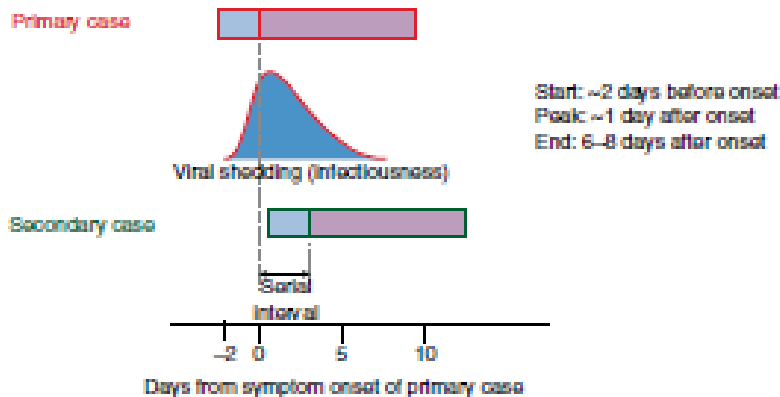
Estimated incubation period: 4–5 days
Estimated serial interval: 10–11 days



SARS-CoV-2: virus culture
(infectiousness profile)
PCR pos up to 14 days after symp
(some residual RNA up to 21 days)

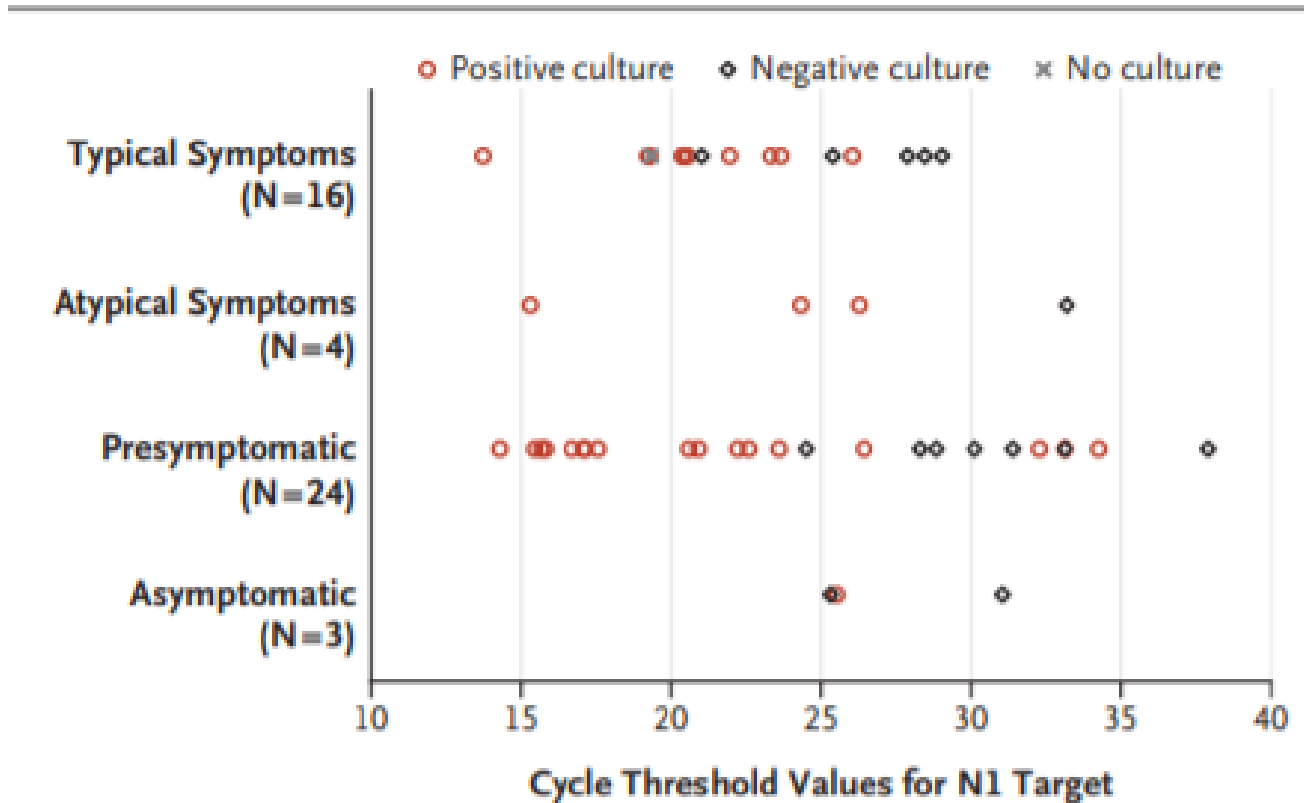
Seasonal influenza

Estimated incubation period: 2 days
Estimated serial interval: 2–4 days



He, X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 15 Apr 2020. <https://doi.org/10.1038/s41591-020-0869-5>

SARS-CoV-2 Viral Culture vs PCR Cycle Threshold Values



**Presymptomatic:
Median Ct: 24
(high viral load)**

**Neg viral culture
if viral load was
< 10⁶ copies/mL**

Figure 2. Cycle Threshold Values and Results of Viral Culture for Residents with Positive SARS-CoV-2 Tests According to Their Symptom Status.

Shown are N1 target cycle threshold values and viral culture results for 47

Temporal SARS-CoV-2 Ct Values from Symptomatic Patients

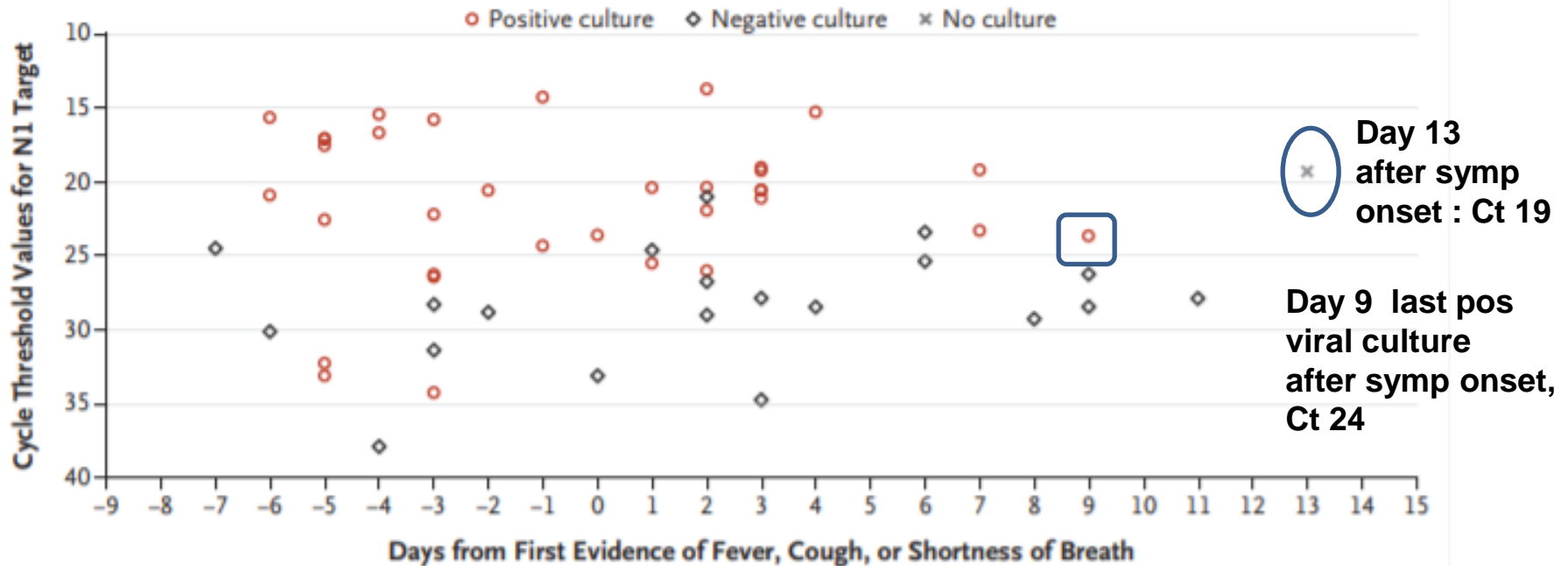


Figure 3. Cycle Threshold Values Relative to First Evidence of Fever, Cough, or Shortness of Breath.

Shown are N1 target cycle threshold values and viral culture results for each resident's positive tests for SARS-CoV-2

Arons, M, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. NEJM 24 Apr 2020. doi: 10.1056/NEJMMoa2008457

Test Sensitivity and Specificity

- **Sensitivity:**

- Ability of the test to correctly identify those patients with the disease
- A test with 90% sensitivity: 10% with disease go undetected (10% false negatives)
- Analytic sensitivity of PCR: depends of limit of detection
- Clinical sensitivity of PCR: depends on specimen collection and time of collection

- **Specificity:**

- Ability of the test to correctly identify those patients without the disease
- A test with 90% specificity: 10% without the disease are incorrectly identified as test positive (10% false positives)

Disease Prevalence of 5%

- **Test Sensitivity and Specificity of 95%**
 - **Negative predictive value: 99.7%**
 - **False neg rate: 0.3%**
 - **Positive predictive value: 50%**
 - **False pos rate 50%**
- **Test Sensitivity and Specificity of 99%**
 - **Negative predictive value: 99.9%**
 - **False neg rate: 0.1%**
 - **Positive predictive value: 84%**
 - **False pos rate 16%**

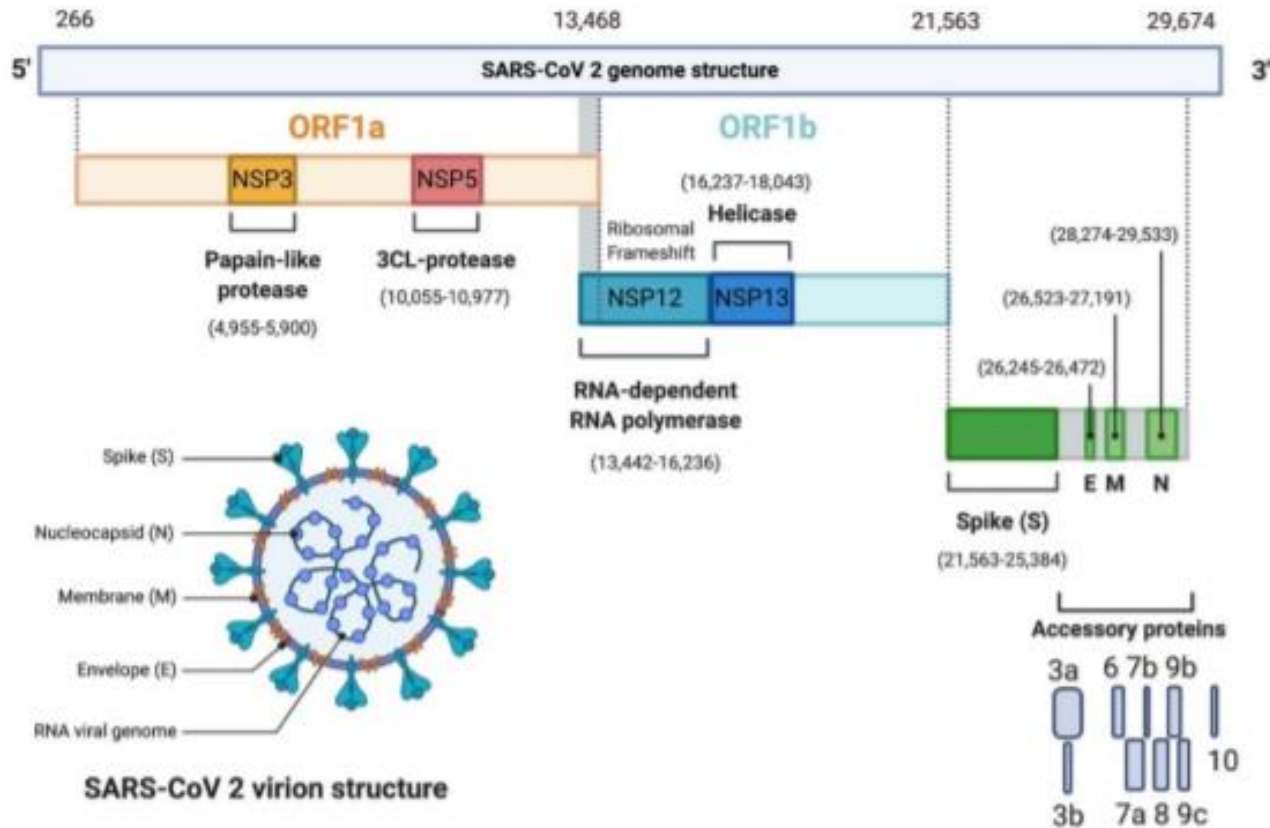
Cairns, E. COVID -19 antibody tests face a very specific problem.
Evaluate Vantage COVID-19 Report.16 Apr 2020. www.evaluate.com

Specimen Source Sensitivity for SARS-CoV-2 PCR Assays (Symptomatic Patients)

Specimen	Sensitivity	Collection	Swab Type
Nasopharyngeal (NP)	97%	HCW	Flocked, Synthetic
Nasal (mid-turbinate)	100%	HCW Self-collection	Flocked, foam, synthetic
Saliva	85%	Self-collection	Wide tube, Urine cup
Nasal (anterior nares)	76%	HCW Self-collection	Flocked, foam, synthetic
Oral (throat)	56%	HCW Self-collection	Synthetic

Hanson, et al. IDSA Guidelines Diagnosis of COVID-19. 6 May 2020
[.www.idsociety.org/COVID19guidelines/dx](http://www.idsociety.org/COVID19guidelines/dx)

SARS-CoV-2 RT-PCR Targets



Alanagreh, et al. Human coronavirus disease COVID-19. Pathogens 9:331 MDPI 29 Apr 2020. doi:10.3390/pathogens9050331

RT-PCR Assays for SARS-CoV-2 (96 well, high throughput)

Company	Instrument Name	PCR Kit Sample size	Targets	Limit of Detection	Assay Time
CDC	ABI 7500 Fast DX	TaqPath COVID-19 (110 μ L sample) (5 μ L eluate extract)	N1 N2	150- 780 copies/mL	4-5 hr
Roche	Cobas 6800	Cobas SARS-CoV-2 (400 μ L sample)	Orf 1ab E	150 copies/ mL	3-4 hr
Abbott	m2000	Alinity m SARS-CoV-2 (100 μ l sam, 5 μ l ext)	N RdRp	150 copies/mL	4-5 hr
Thermo Fisher	ABI 7500 Fast DX	TaqPath CovID-19 (400 μ l sample) (5 μ l extract)	N S Orf 1ab	250 copies/mL	4 hr
LabCorp	ABI Quant Studio Flex	TaqPath COVID-19 (200 μ L s)	N1 N2	150 copies/mL	3-4 hr
Quest	ABI 7500 Fast DX	TaqPath COVID-19 (250 μ L s) (10 μ L ext)	N1 N3	150 copies/mL	4 hr

Commercial RT-PCR Assays for SARS-CoV-2

Company	Instrument Name	Format	Targets	Limit of Detection	Assay Time
Cepheid	GeneXpert Xpress (300 µL sample)	Single cartridge 12,16,24/ins	N2 E	200 copies/mL	45 min
DiaSorin Molecular	Liaison MDX (50 µL sample)	8 test disc	S Orf 1ab	40 ^a -150 copies/mL	80 min
GenMark	ePlex (200 µL sample)	Single cartridge 3-24/instr	N	1,000 copies/mL ^a	3-4 hr
Biofire	Torch Film Array (300 µL sample)	Single pouch (22 targets) 8-12/instr	S M	160 copies/mL	45 min
Luminex	Aries (200 µL sample)	Single cassette 6-12/instr	N Orf 1ab	75,000 copies/mL	2 hr

a: Zhen, et al. Comparison of 4 molecular in vitro assays for SARS-Cov-2.
Medrxiv <https://doi.org.1101/2020.04.17.20069864>

Commercial RT-PCR Assays for SARS-CoV-2

Company	Instrument Name	Format	Targets	Limit of Detection	Assay Time
Becton Dickinson	BD Max (750 μ L sample)	Single cartridge 12,16,24/instr	N1 N2	200 copies/mL	2 hr
Hologic	Panther Fusion (500 μ L sample)	10 test cartridge 120/instr	Orf 1a Orf 1b	83-625 copies/mL ^a	3 hr

a: Zhen, et al. Comparison of 4 molecular in vitro assays for SARS-Cov-2. Medrxiv <https://doi.org.1101/2020.04.17.20069864>

Commercial NAA Assays for SARS-CoV-2

Company	Instrument Name	Format	Targets	Limit of Detection	Assay Time
Abbott	ID NOW Direct-dry	Single cartridge	RpRp (template)	20,000 copies/mL ^a	15 min
	ID NOW in VTM	Single cartridge	RpRp	15-30% false neg ^a	15 min

Rapid Commercial Ag Assays for SARS-CoV-2

Company	Instrument Name	Format	Targets	Limit of Detection	Assay Time
Quidel	Sofia: lateral Flow FIA (120 µl sample)	Single test cassette	N (acute infection)	850 copies/mL (confirm neg with molec test) IFU 80% sensitivity	15 min

a: Basu, et al. Performance of NAA by Abbott ID NOW COVID-19. Medrxiv <https://doi.org.1101/2020.05.11.089896> (posted 12 May 2020)

Commercial Ab Assays for SARS-CoV-2

Company – Test	Instrument Name	Format	Targets	Sensitivity Specificity	hCoV -X PPV at 5% prev	Assay Time
DiaSorin IgG	Liaison XL (Chemilum)	12/rack 120/run	S1 S2	97.6% 99.3%	No 88%	30 min
	Pos Percent Agreement	≤ 5 days 6-14 days ≥ 15 days	25% 90% 97.6%			
Abbott IgG	Architect (Chemilum)	5/rack 50/run	N	100% 99.0%	Yes 92.9%	30 min
Eurolmmun IgG	ELISA: (man/auto)	96 well	S1	90% 99.1%	No 88%	2-3 hr
Ortho Clinical Diag IgG & Total	Vitros (Chemilum)	10/rack 100/run	S1	87.5% 99.1%	No 88%	1 hr

<https://www.fda.gov/medical-devices/emergency-situations>

Summary of SARS-CoV-2 Diagnostic Tests

I. Acute Infection

- Molecular testing: RT-PCR, NAA less sensitive

II. Recent Infection

- IgM: 1-7 days after symp, 85% of patients (Guo, CID 2020)
 - IgM useful for suspected COVID patients w neg molec PCR
 - IgM is not a viral neutralizing Ab
- IgA: 1-3 days after symp onset
 - IgA most abundant Ig in mucosal surfaces
 - IgA has viral neutralizing activity

III. Recent or Remote Past Infection

- IgG: 6-14 days after symp onset, 90% of patients
 - IgG has viral neutralizing activity
 - IgG is a long lasting Ab
 - Low level false pos rate due to autoantibodies

Utility of SARS-CoV-2 IgG Tests

I. Screening of Recovered COVID-19 Patients

- Convalescent plasma: treat acutely ill patients
- Donor plasma with Ab titer of 1:160

II. SARS-CoV-2 Seroprevalence studies

- Prevalence of total disease in community
 - Asymptomatic prevalence 50%, mild infection 30%
- Screen HCW for immune status & patient exposure
- Guidance for return to work status (PCR + Ab)

III. Monitor Immune Responses for Vaccine Candidates

- Prescreen individuals prior to vaccine clinical trial
- Monitor temporal immune response
- Determine if serologic assay is able to detect neutralizing Ab
- Determine duration of protective immunity