您不能不知道的 COVID-19 檢測方式

花蓮慈濟醫院感染科

黃妙慧 醫師

核酸檢測
PCR
RT-PCR
Pooling PCR
到底 P 了什麼?

抗原

抗體

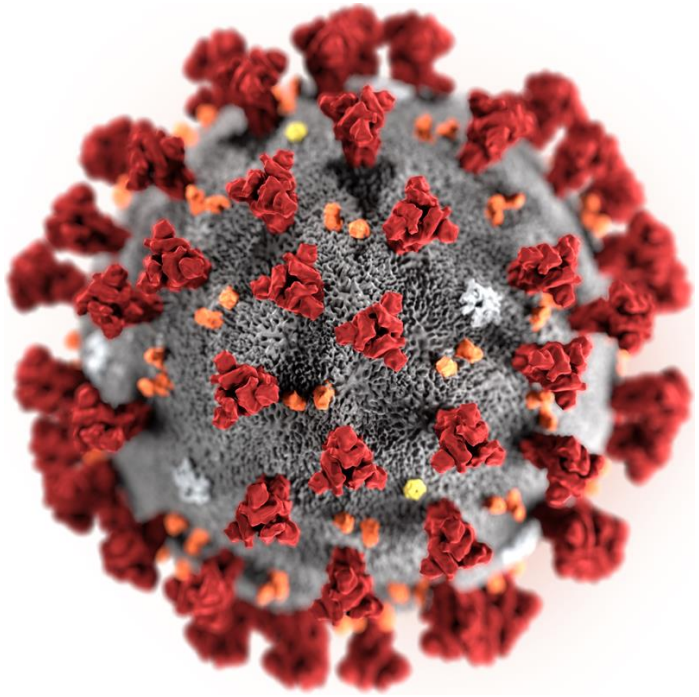
Ct 值??

“快篩”
核酸還是抗原??

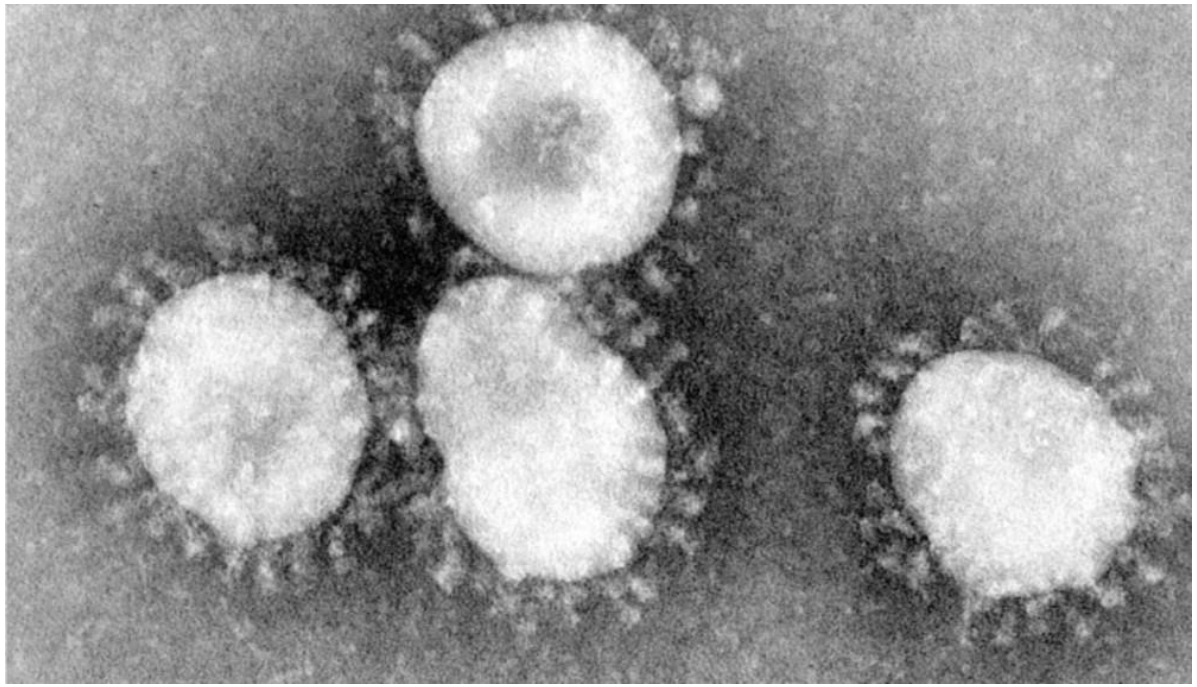
Coronavirus

From Latin, **“Corona”** means crown

Virion structure: spikes projecting from envelope



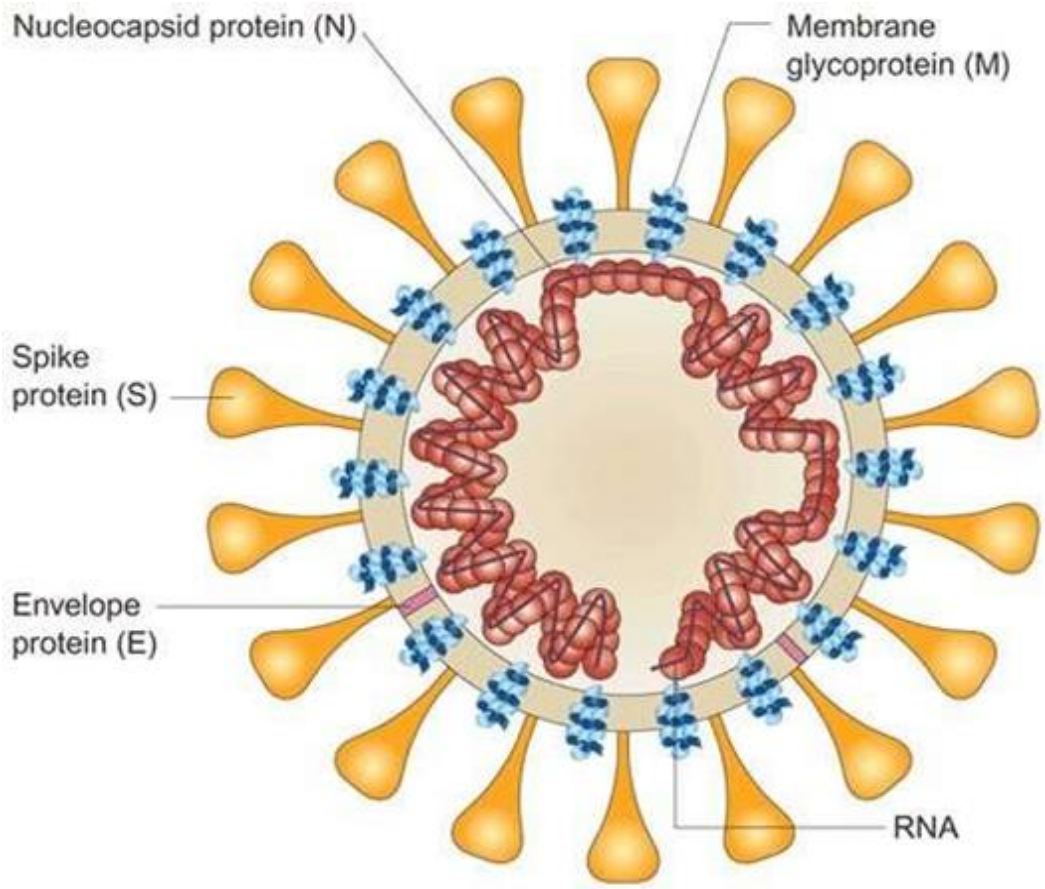
CDC/ Alissa Eckert, MSMI; Dan Higgins, MAMS



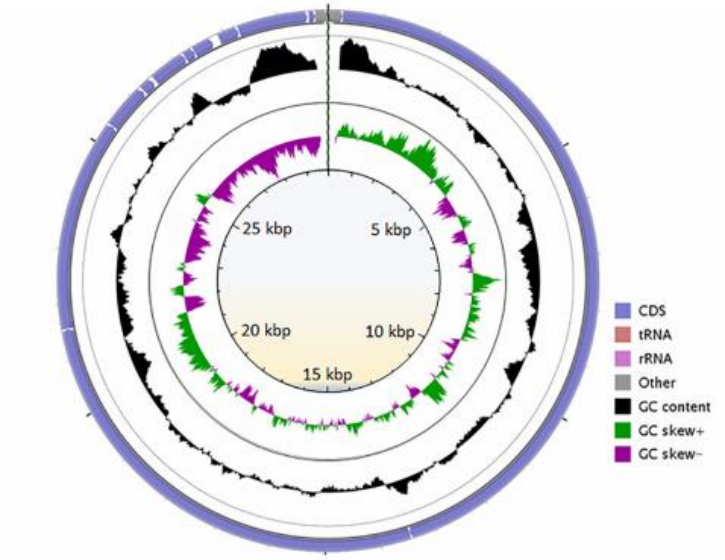
CDC, USA; Stanford University

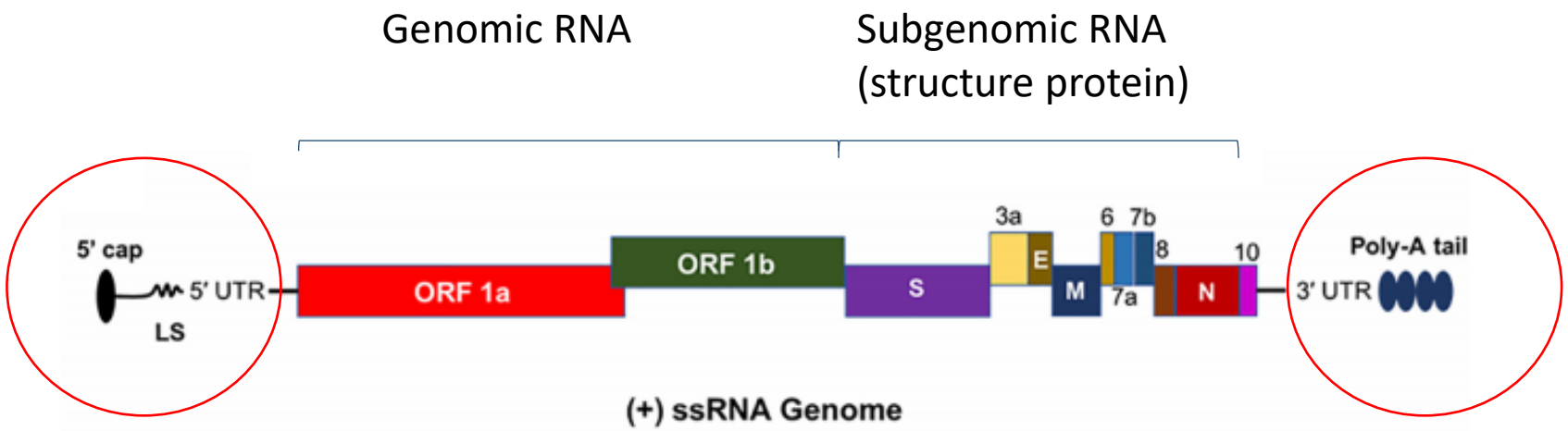
Virion structure

- **Lipid envelope**
  Lipid bilayer – Soap works to disrupt it
- **Spike protein (S)**
  - Attaches to ACE-2 receptor
- **Membrane protein (M)**
  - Provides shape
- **Envelope protein (E)**
  - Guides assembly and release
- **Nucleocapsid protein (N)**
  - Protects RNA
- **RNA viral genome**
  - ssRNA, ~30,000 nucleotides
  - Encodes 29 proteins



Genome structure





ORF= Open Reading Frames

Naqvi AAT, et al. Biochim Biophys Acta Mol Basis Dis. 2020;1866(10):165878.

COVID-19 Coronavirus Symptoms

World Health Organization

SERIOUS COVID-19 SYMPTOMS REQUIRING IMMEDIATE MEDICAL CARE

- If you develop any of these symptoms, call your healthcare provider or health facility and seek medical care immediately.
- This is not an exhaustive list. These are the most common symptoms of serious illness, but you could get very sick with other symptoms – if you have any questions, call for help immediately.


Shortness of breath/ Difficulty breathing


Loss of speech or mobility or confusion


Chest pain

MOST COMMON SYMPTOMS


Fever


Cough


Tiredness


Loss of taste or smell

LESS COMMON SYMPTOMS


Sore throat


Headache


Aches & pains


Diarrhea

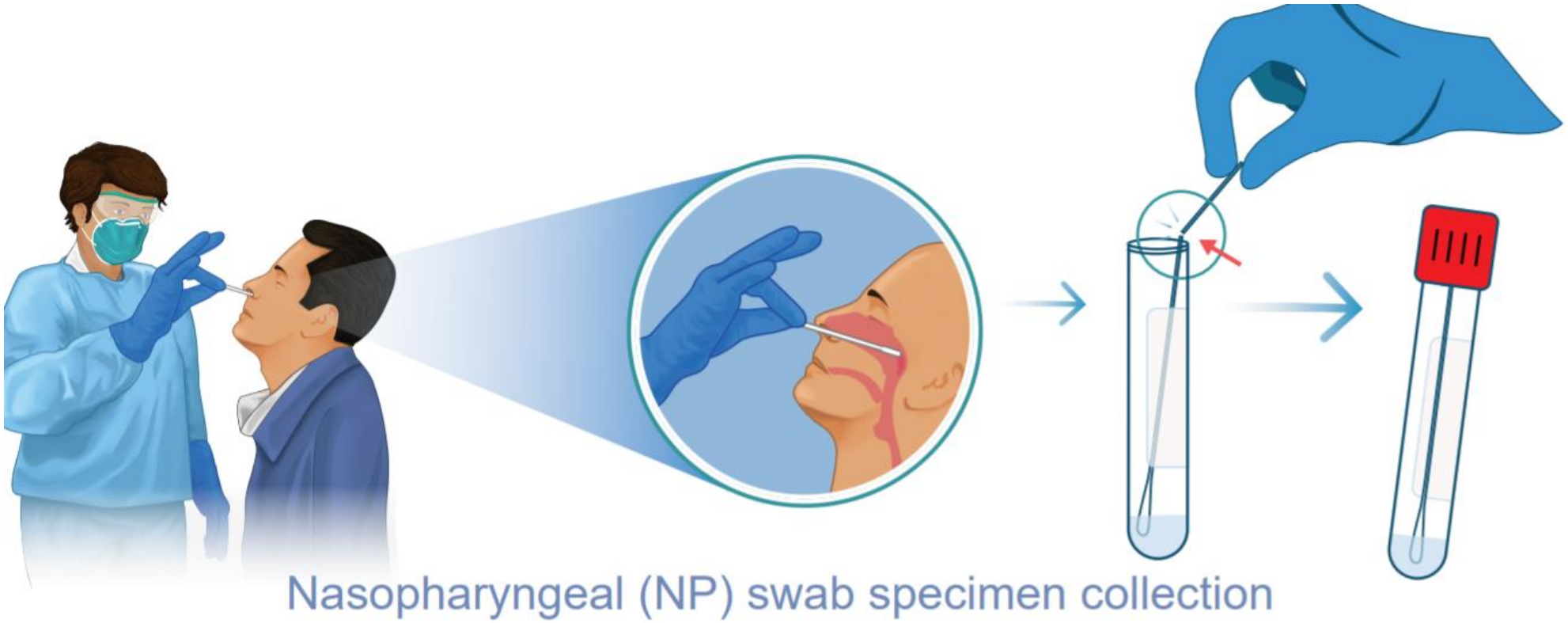

A rash on the skin or discolouration of fingers or toes


Red or irritated eyes

Whom to test

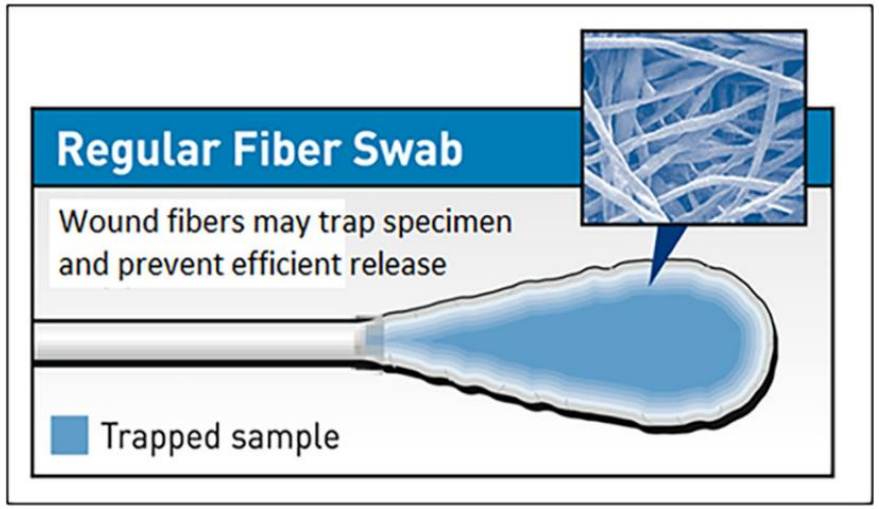
- **Symptomatic patients**
  - If possible, all symptomatic patients with suspected infection should undergo testing
- **Select asymptomatic individuals**
  - Following close contact with an individual with COVID-19
  - Early identification of infection in congregate living facilities that house individuals at risk for severe disease (eg, long-term care facilities, homeless shelters)
  - Prior to surgical procedures or aerosol-generating procedures.
  - Screening hospitalized patients at locations where prevalence is high (eg, ≥10% PCR positivity in the community).

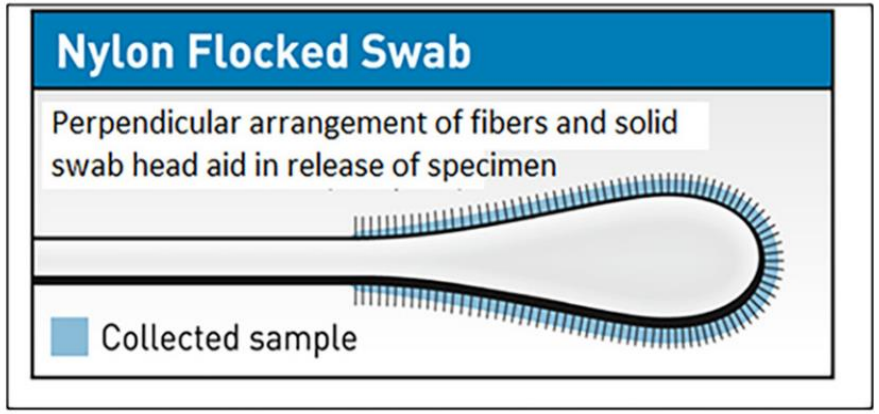
Nasopharyngeal specimen collection

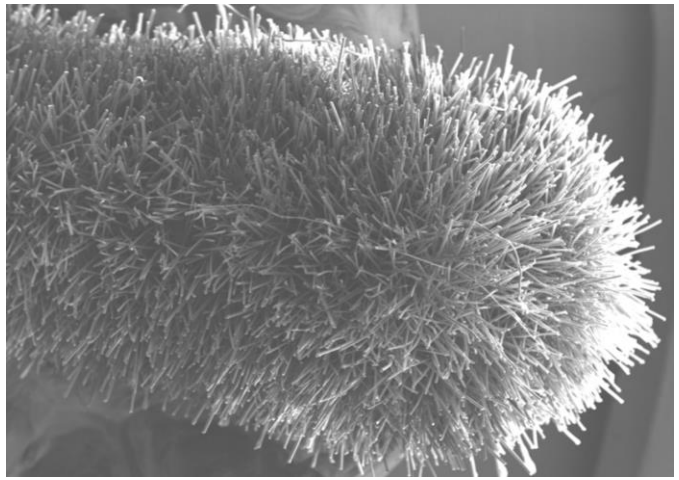


Source:https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

Optimal sample collection material





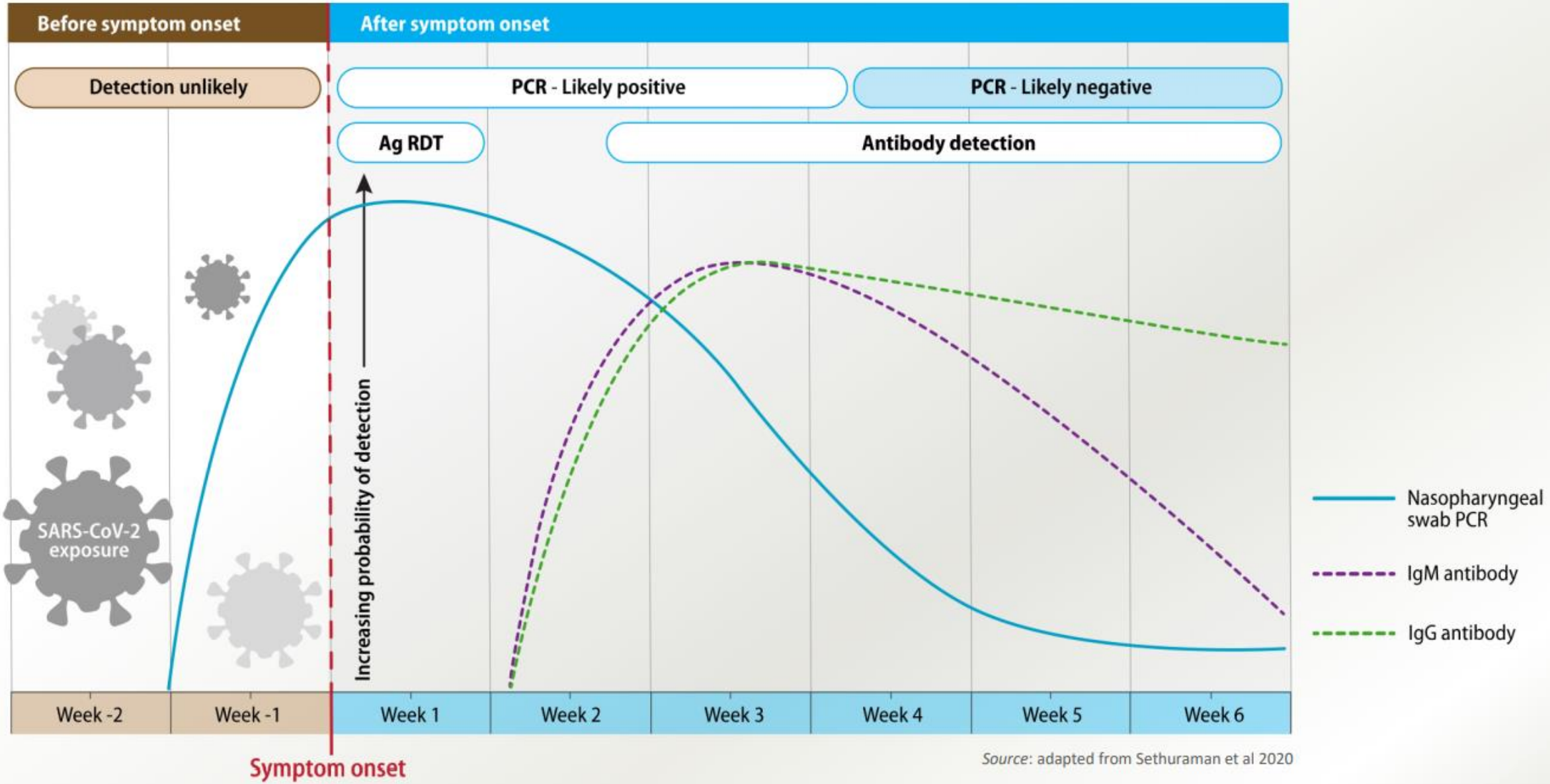


The flocked swab tip is coated with perpendicularly sprayed on Nylon fibers.

This coating creates a thin absorbent layer that allows for quick sample uptake and elution of more than 90% of the sample.

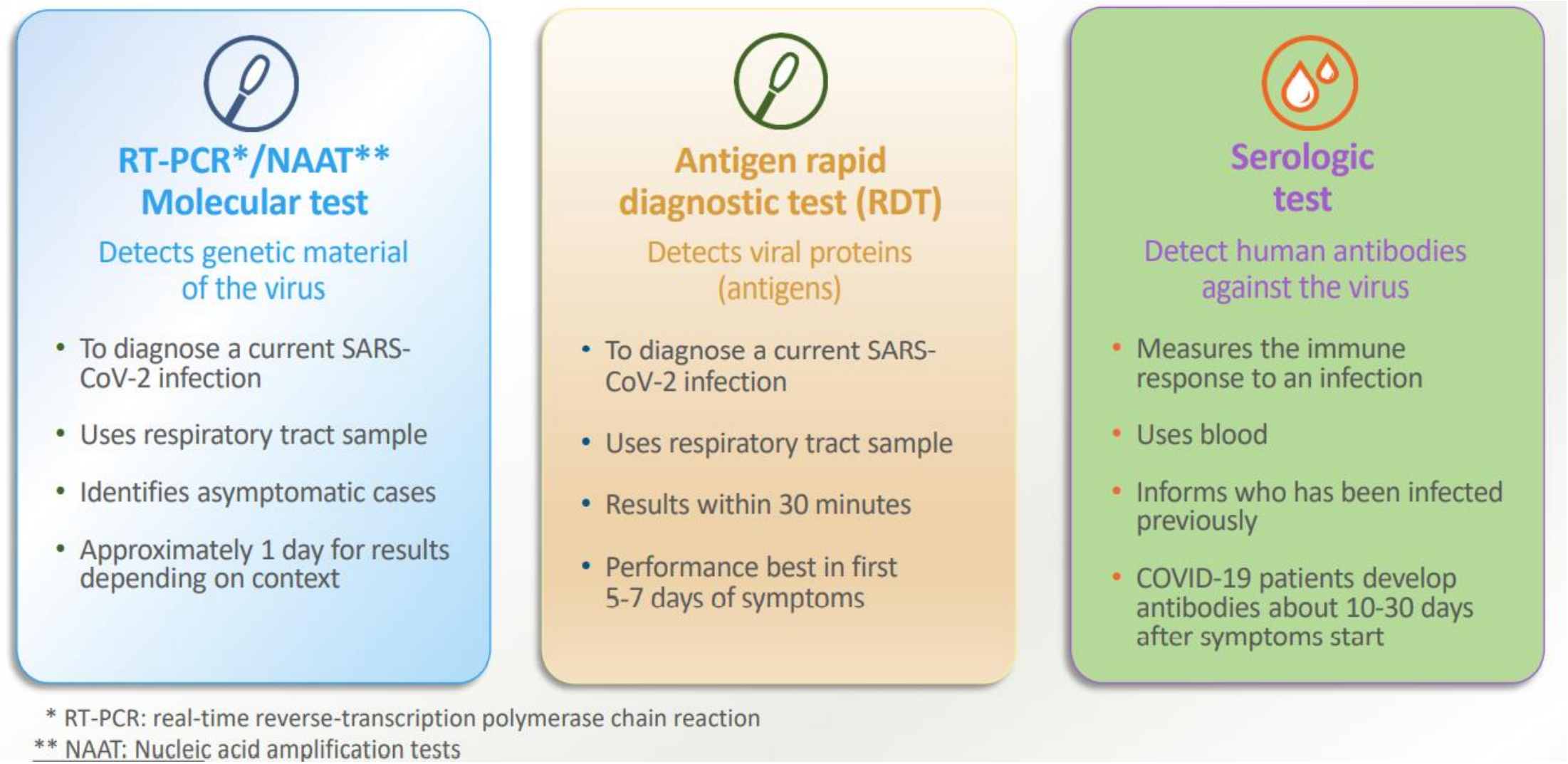
Detection of SARS-CoV-2 relative to symptom onset

Figure. Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset



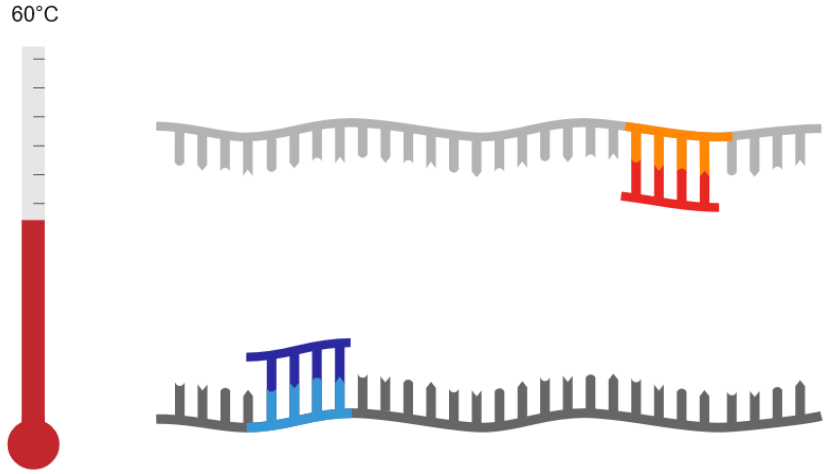
Source: adapted from Sethuraman et al 2020

Diagnostic tests for SARS-CoV-2

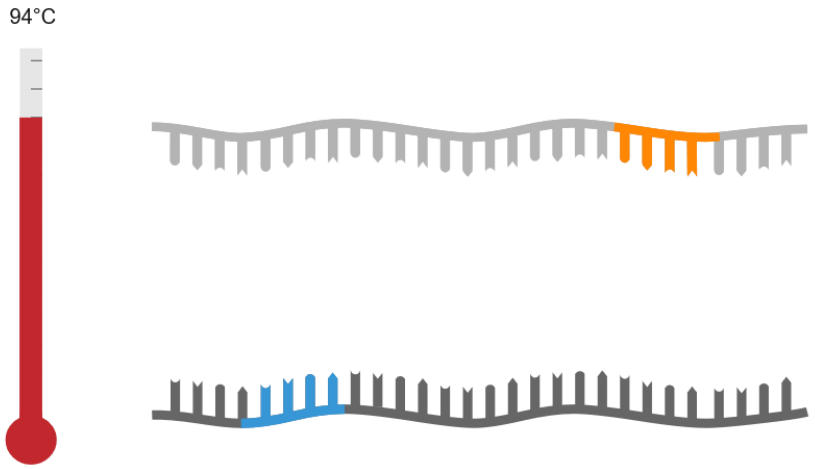


Polymerase Chain Reaction (PCR)

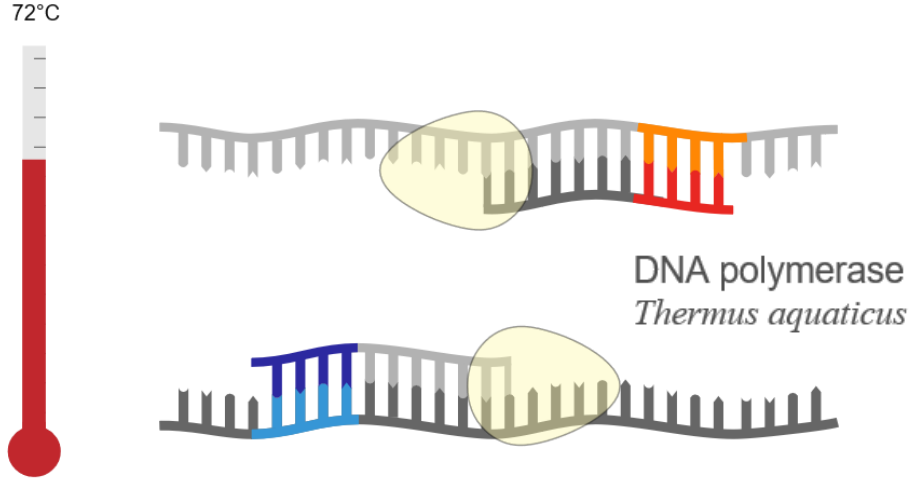
Anneal primers



Denature DNA



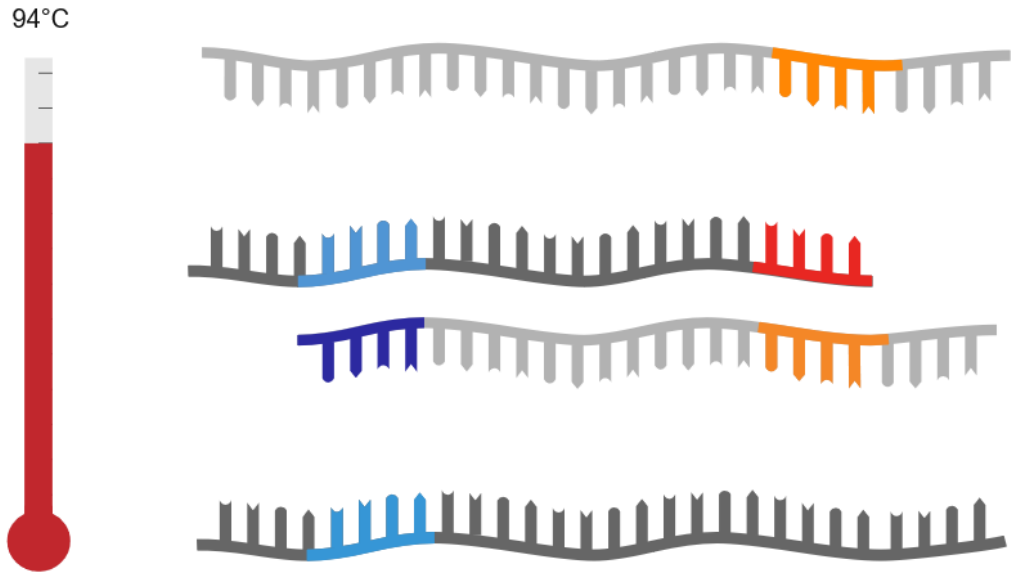
Extend primers



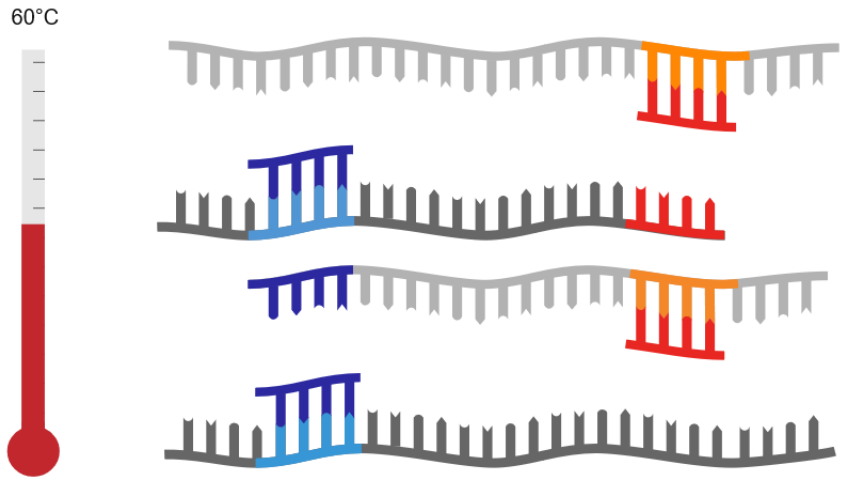
https://www.bio-rad.com/webroot/web/movies/lse/global/english/what-is-polymerase-chain-reaction/tutorial.html

Second cycle

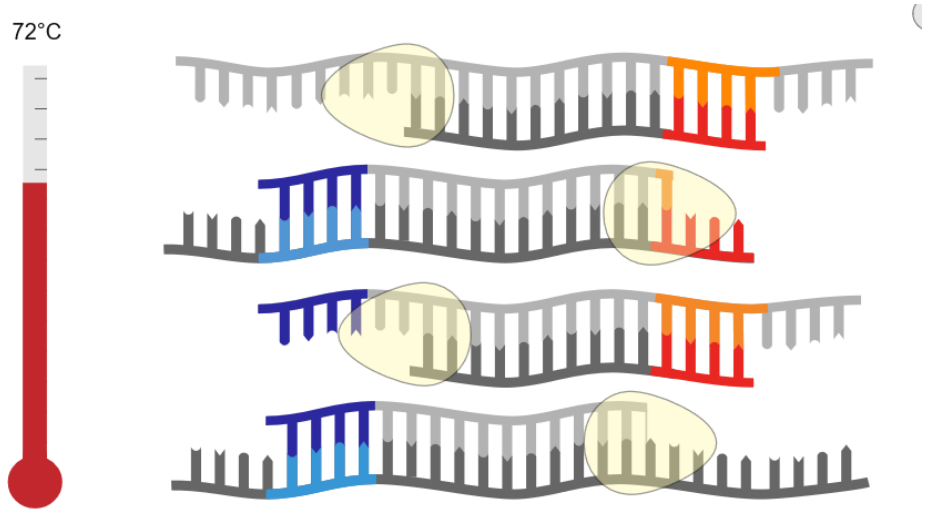
Denature DNA



Anneal primers



Extend primers



$x2^n$

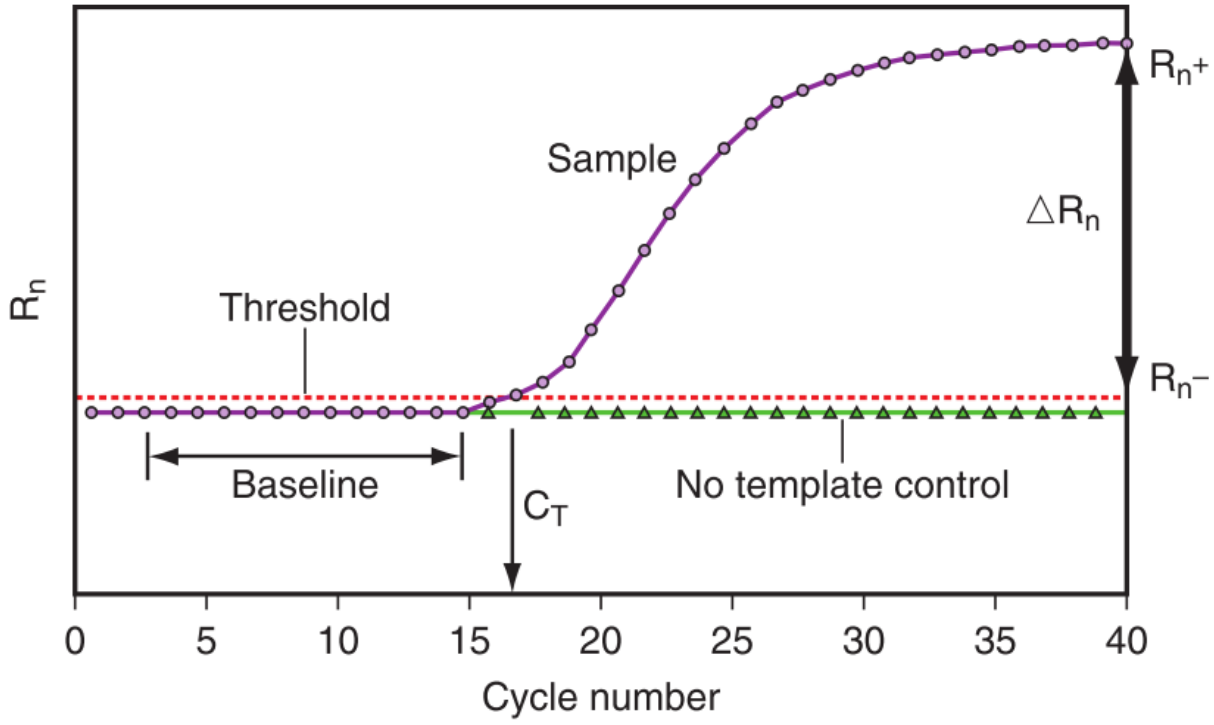
Real-Time PCR

The number of cycles required to pass the detection threshold depends the amount of starting template (viral RNA level)

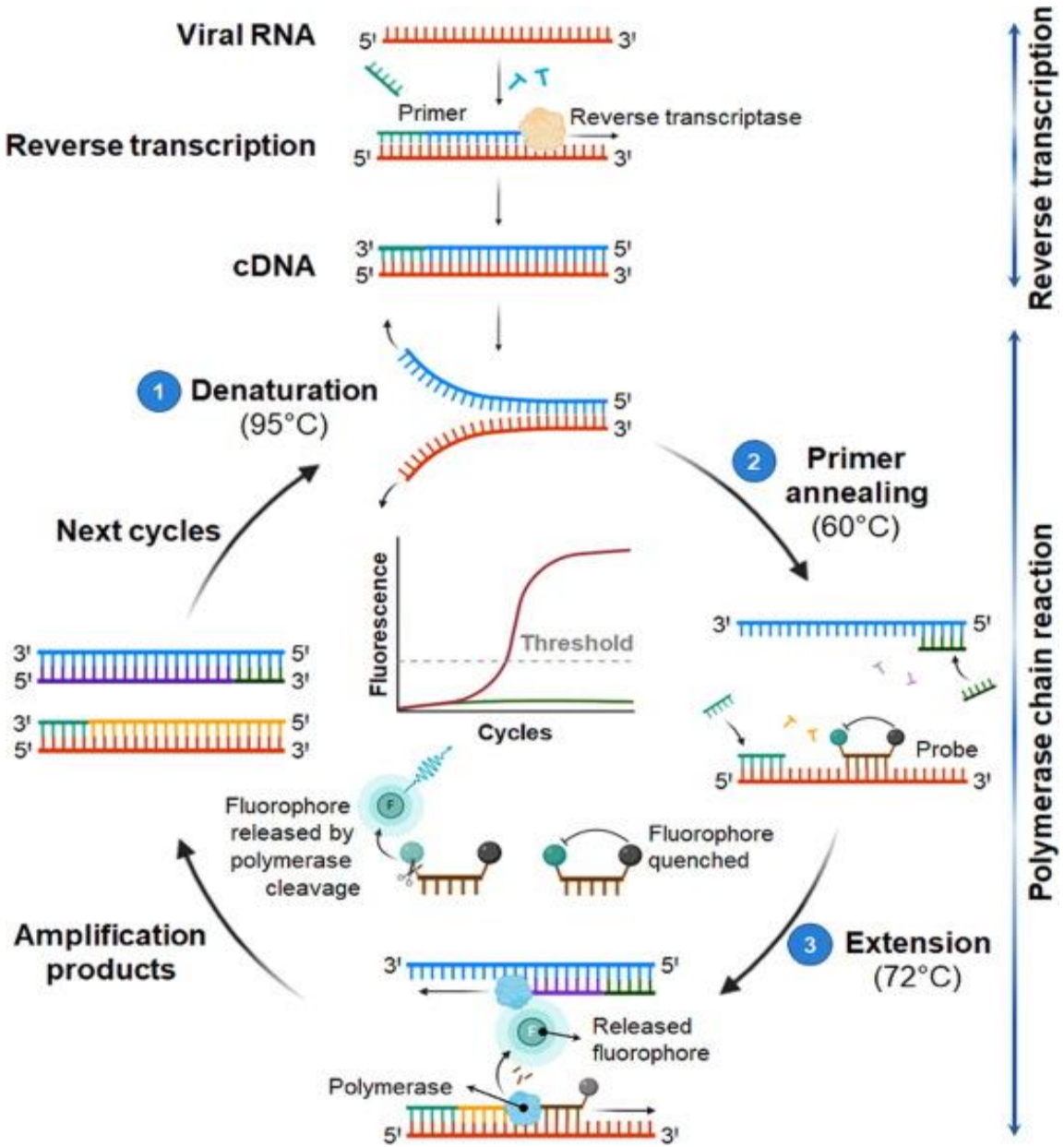
In that way, real-time PCR can be quantitative **("qPCR")**

Fluorescence detection:

- Requires no gel electrophoresis
- Higher throughput
- More sensitive, faster than PCR

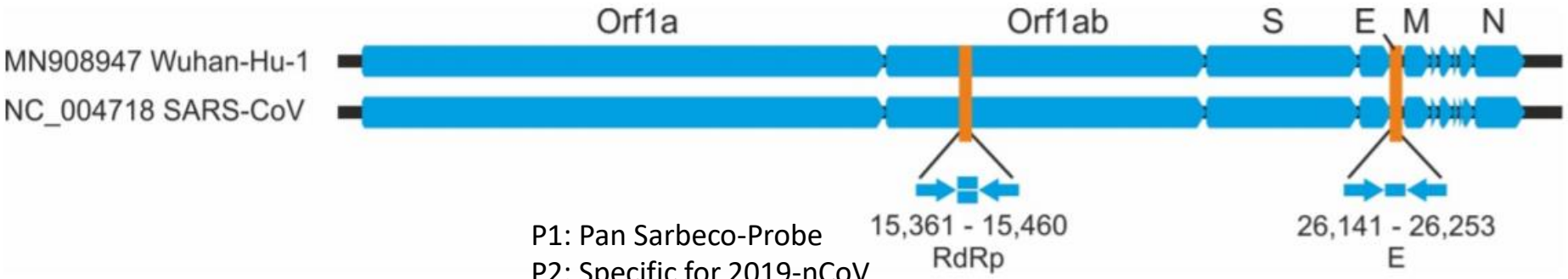


Reverse Transcription qPCR (RT-qPCR)



Afzal A. J Adv Res. 2020;26:149-159

WHO protocol v2



P1: Pan Sarbeco-Probe
P2: Specific for 2019-nCoV

Figure 1 relative positions of amplicon targets on SARS-CoV an 2019-nCoV genome. ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay

Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs.

World Health Organization

Berlin, Jan 17th, 2020

Table 1. Primers and probes

Optimized concentrations are mol per liter of final reaction mix.

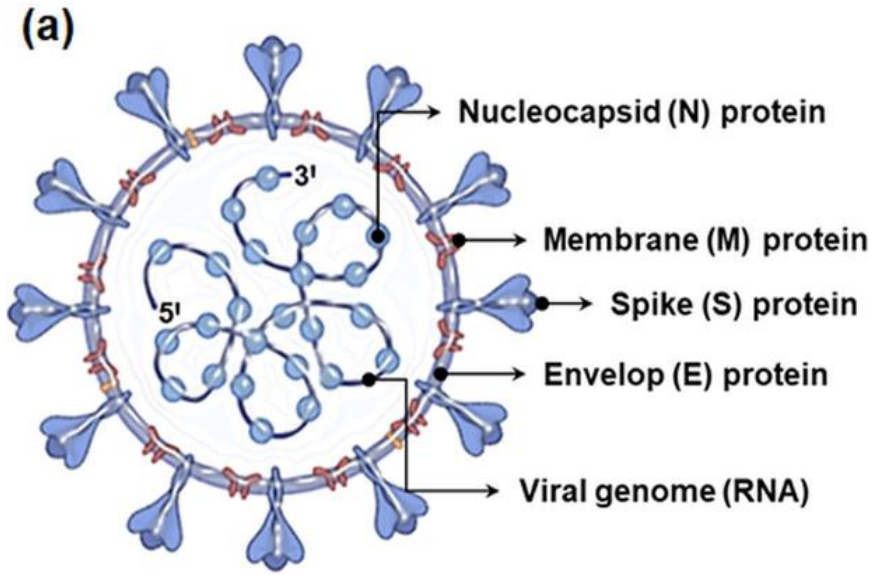
(e.g., 1.5 microliters of a 10 micromolar (uM) primer stock solution per 25 microliter (ul) total reaction volume yields a final concentration of 600 nanomol per liter (nM) as indicated in the table)

-note that standard, non-optimized reaction conditions as indicated by suppliers of one-step RT-PCR kits will generally yield sufficient sensitivity-

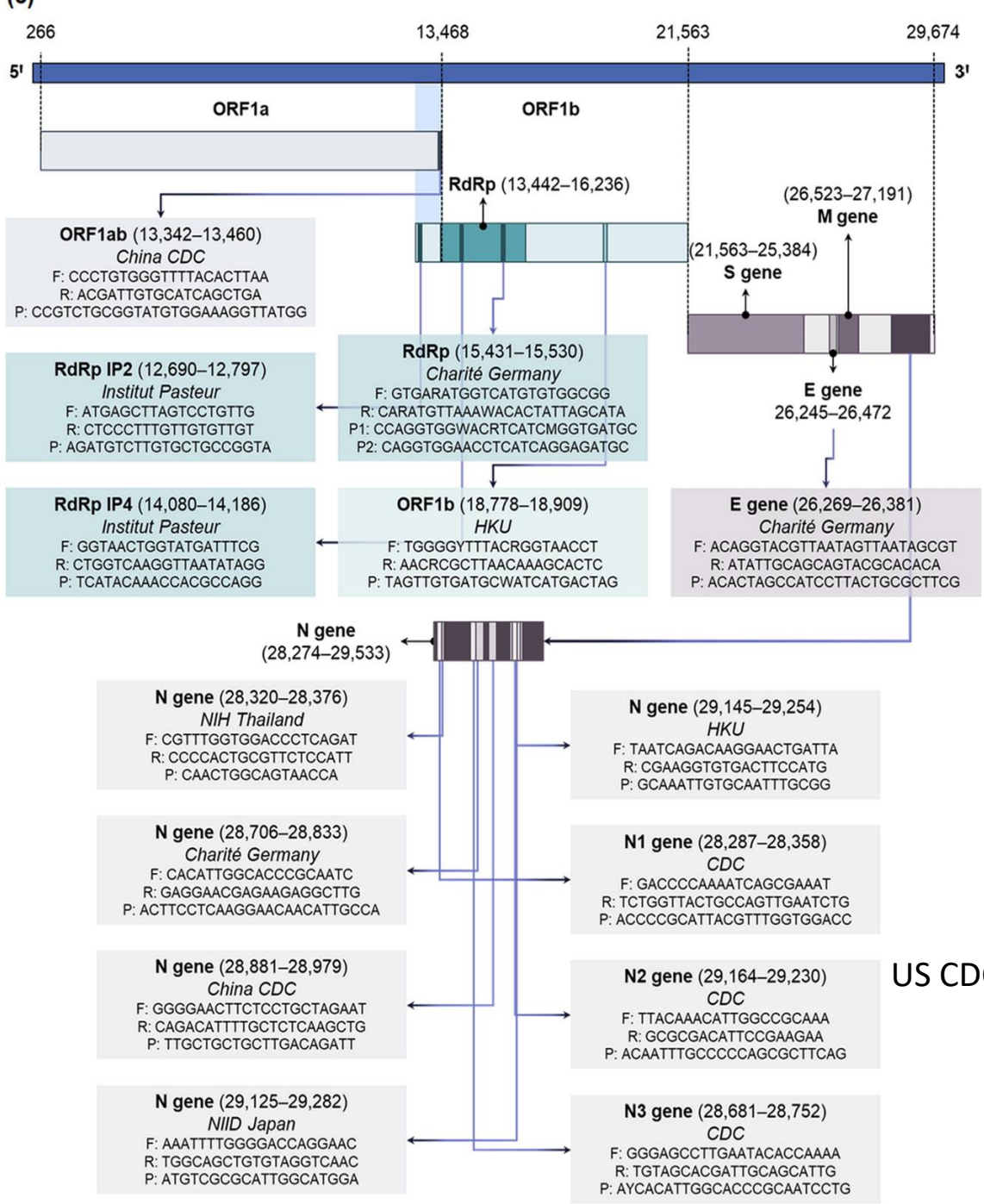
Assay/ Use	Oligonucleotide ID	Sequence (5'–3')	Comment
RdRP gene	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	use 600 nM per reaction
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA	use 800 nM per reaction
	RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe, will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs use 100 nM per reaction and mix with P2
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	use 400 nM per reaction
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	use 200 nM per reaction

W is A/T; R is G/A; M is A/C ; FAM, 6-carboxyfluorescein; BBQ, blackberry quencher

Target gene of SARS-CoV-2



Nucleocapsid (**N**), envelope (**E**), and spike (**S**) genes, and regions in the first open reading frame (**ORF1a/b**), RNA-dependent RNA polymerase (**RdRp**) gene



US CDC

US CDC

2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes				
Name	**Description**	**Oligonucleotide Sequence (5'>3')**	**Label[1]**	**Working Conc.**
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	20 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None	20 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	20 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'	None	20 µM
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'	None	20 µM
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'	FAM, BHQ-1	5 µM
RP-F	RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	20 µM
RP-R	RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	20 µM
RP-P	RNAse P Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'	FAM, BHQ-1	5 µM

[1]TaqMan® probes are labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher 1 (BHQ-1) (Biosearch Technologies, Inc., Novato, CA) at the 3'-end.

2019-nCoV rRT-PCR Diagnostic Panel Results Interpretation

2019 nCoV_N1	2019 nCoV_N2	2019 nCoV_N3	RP	Result Interpretation[a]
+	+	+	±	2019-nCoV detected
If only one, or two, of three targets is positive			±	Inconclusive Result
-	-	-	+	2019-nCoV not detected
-	-	-	-	Invalid Result

RNase P (human)

SARS-CoV-2 Reference Panel of US FDA

Table 2. Sensitivity Mean Estimates of the EUA authorized SARS-CoV-2 molecular diagnostic tests using the FDA SARS CoV-2 Reference Panel.

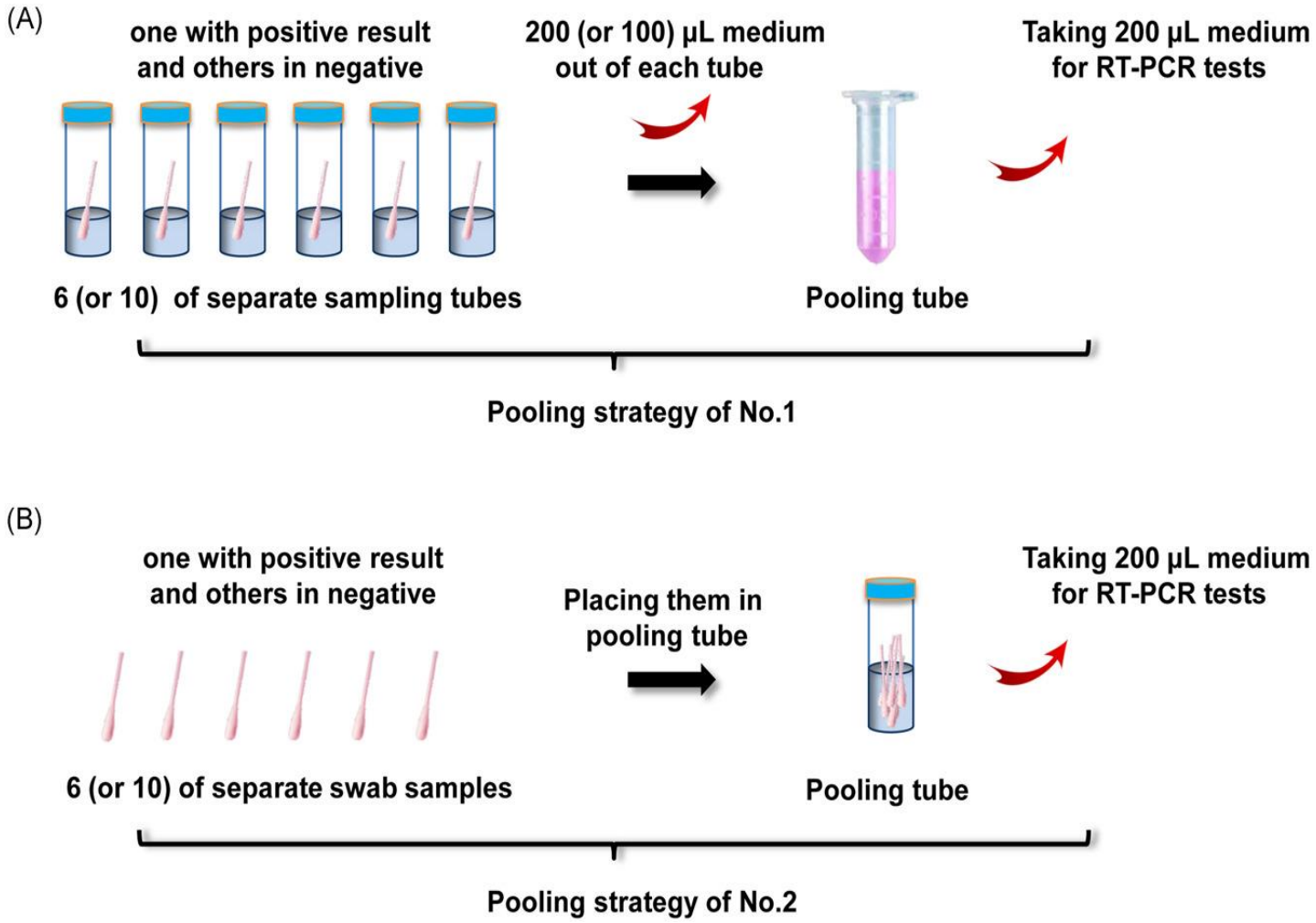
NDU/mL = NAAT Detectable Units/mL

Product LoD (NDU/mL)	**Developer**	**Test**	**Target gene**
5400	Roche Molecular Systems, Inc.	cobas SARS-CoV-2 & Influenza A/B Nucleic Acid Test for use on the cobas Liat System	ORF1ab, N
5400	Becton, Dickinson & Company	BD SARS-CoV-2 Reagents for BD MAX System	N1, N2, RNase P (human)
5400	Cepheid	Xpert Xpress SARS-CoV-2 test	E, N2
6000	BioFire Diagnostics, LLC	BioFire Respiratory Panel 2.1	S, M


林口長庚醫院


花蓮慈濟醫院

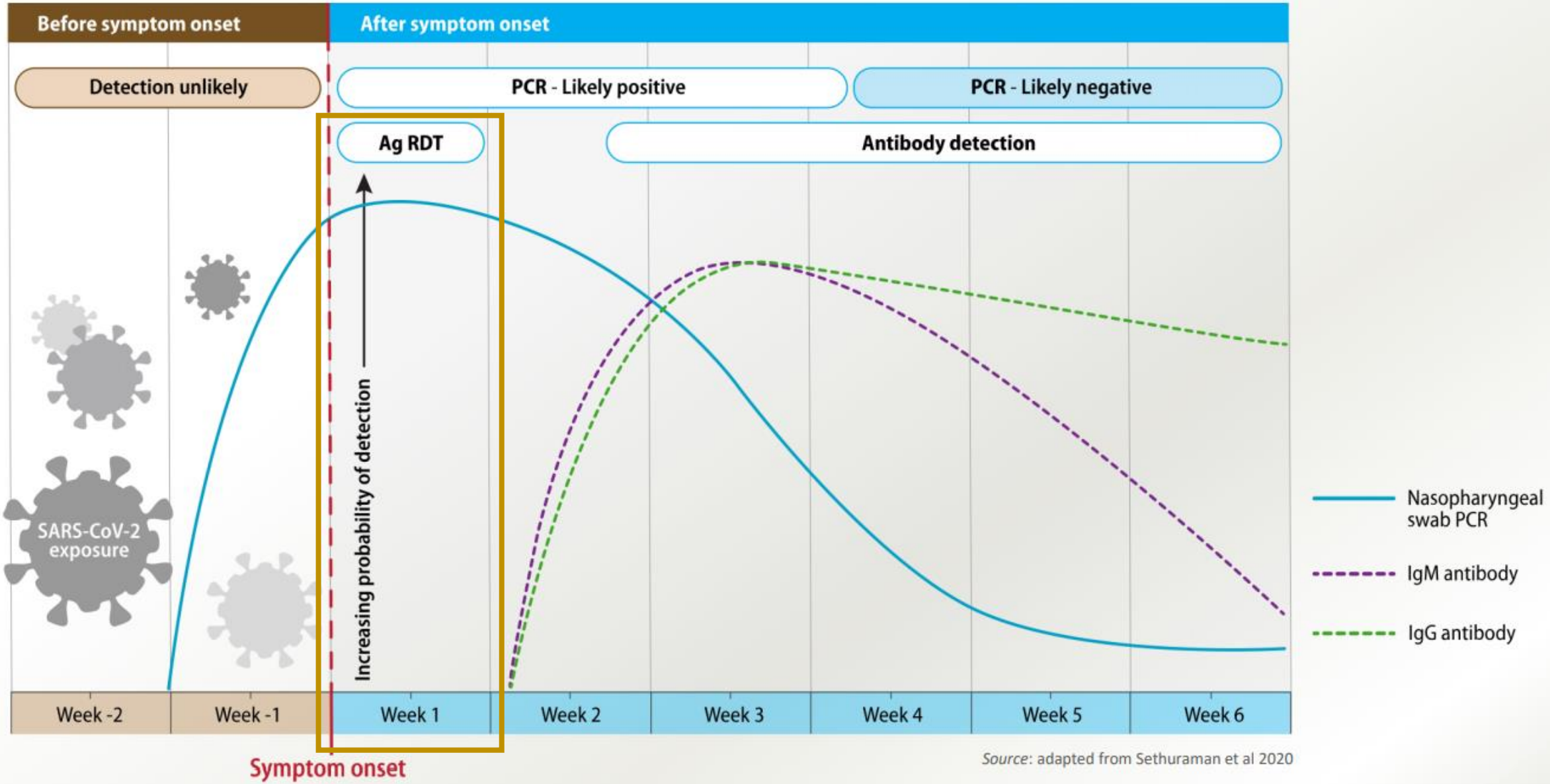
Screening Testing Using a Pooling Strategy



Chen F, et al. J Med Virol. 2021;93:2805-2809

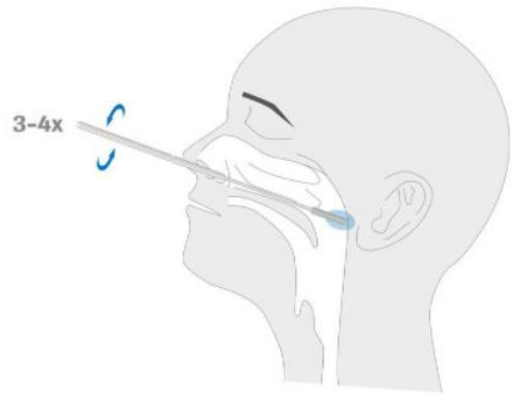
Detection of SARS-CoV-2 relative to symptom onset

Figure. Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset

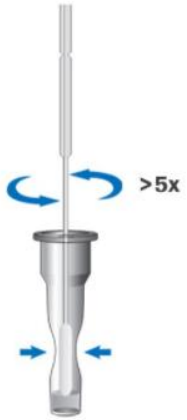


Source: adapted from Sethuraman et al 2020

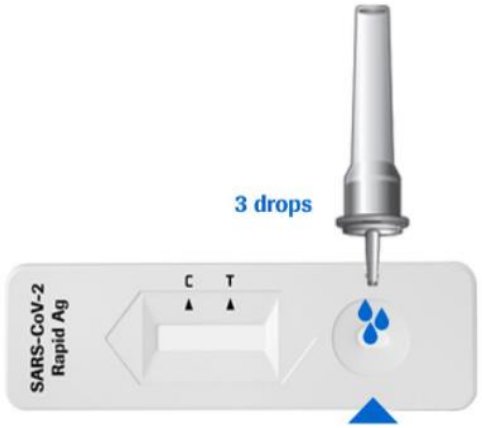
SARS-CoV-2 Rapid Antigen Test



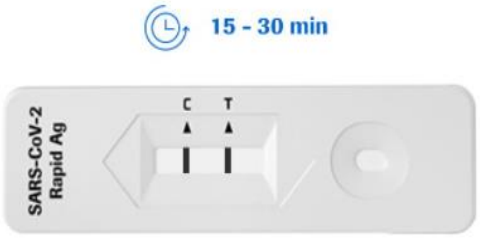
1. Collecting a sample (nasypharyngeal swab)*



2a. Preparing a sample



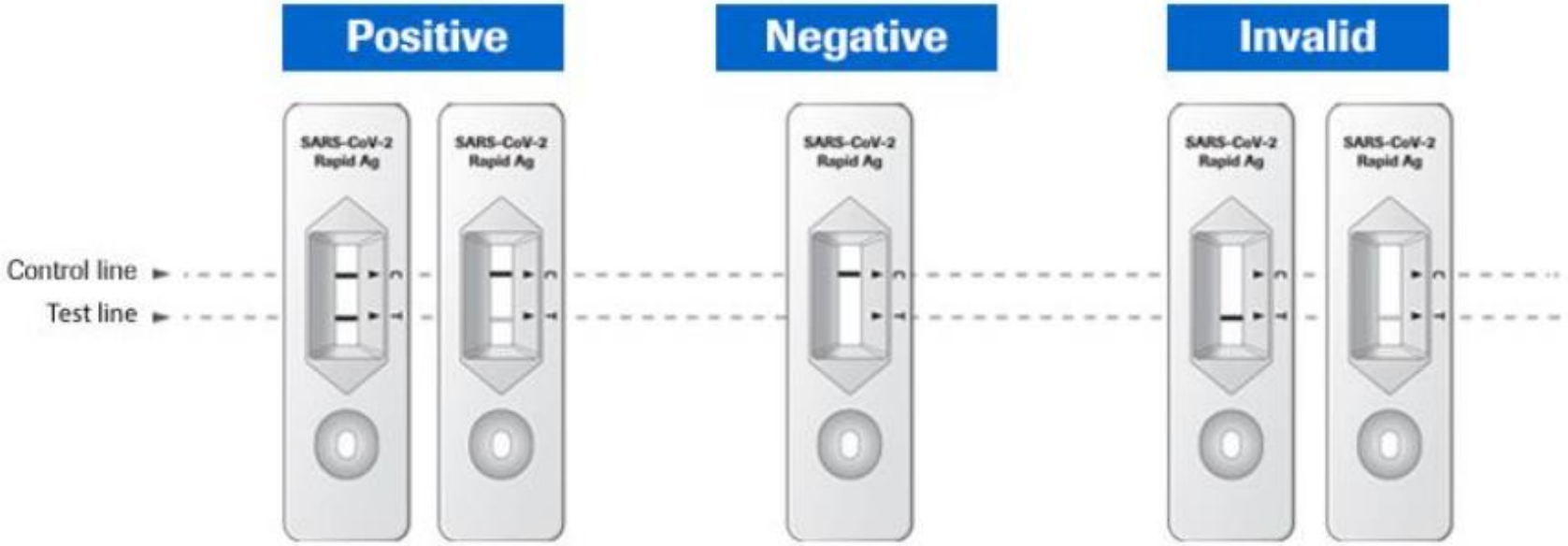
3a. Performing a test



3b. Performing a test

Source: https://diagnostics.roche.com/global/en/products/params/sars-cov-2-rapid-antigen-test.html

SARS-CoV-2 Rapid Antigen Test



Source: https://diagnostics.roche.com/global/en/products/params/sars-cov-2-rapid-antigen-test.html

民眾使用COVID-19家用快篩試劑流程 110年6月30日修訂

請注意:若出現嚴重特殊傳染性肺炎相關症狀，不宜使用家用快篩試劑自行在家快篩，應佩戴醫用口罩，儘速前往醫療院所就醫，且前往就醫時勿搭乘大眾運輸工具。

可至醫療器材販賣業者(如藥粧店、醫療器材行、便利商店等)或藥局購買家用快篩試劑

↓

依使用說明書進行採檢及操作

↓

快篩結果

陰性

1. 請遵循CDC防疫規範，做好個人防護，持續自我健康管理。
2. 採檢完之家用快篩試劑及試劑棒用塑膠袋密封包好，以一般垃圾處理。

◆ 僅能代表採檢當下所收集之檢體，沒有偵測到病毒，不能代表安全無虞。

◆ 受限於產品性能其結果可能會出現偽陽性或偽陰性，故不可取代PCR檢驗作為診斷COVID-19感染之依據。

◆ 在非疫情熱區使用快篩，其結果可能會出現較高機率的偽陽性。

陽性

↓

居家隔離或居家檢疫者:

請立即與當地衛生局聯繫，或撥1922，依指示方式處理。

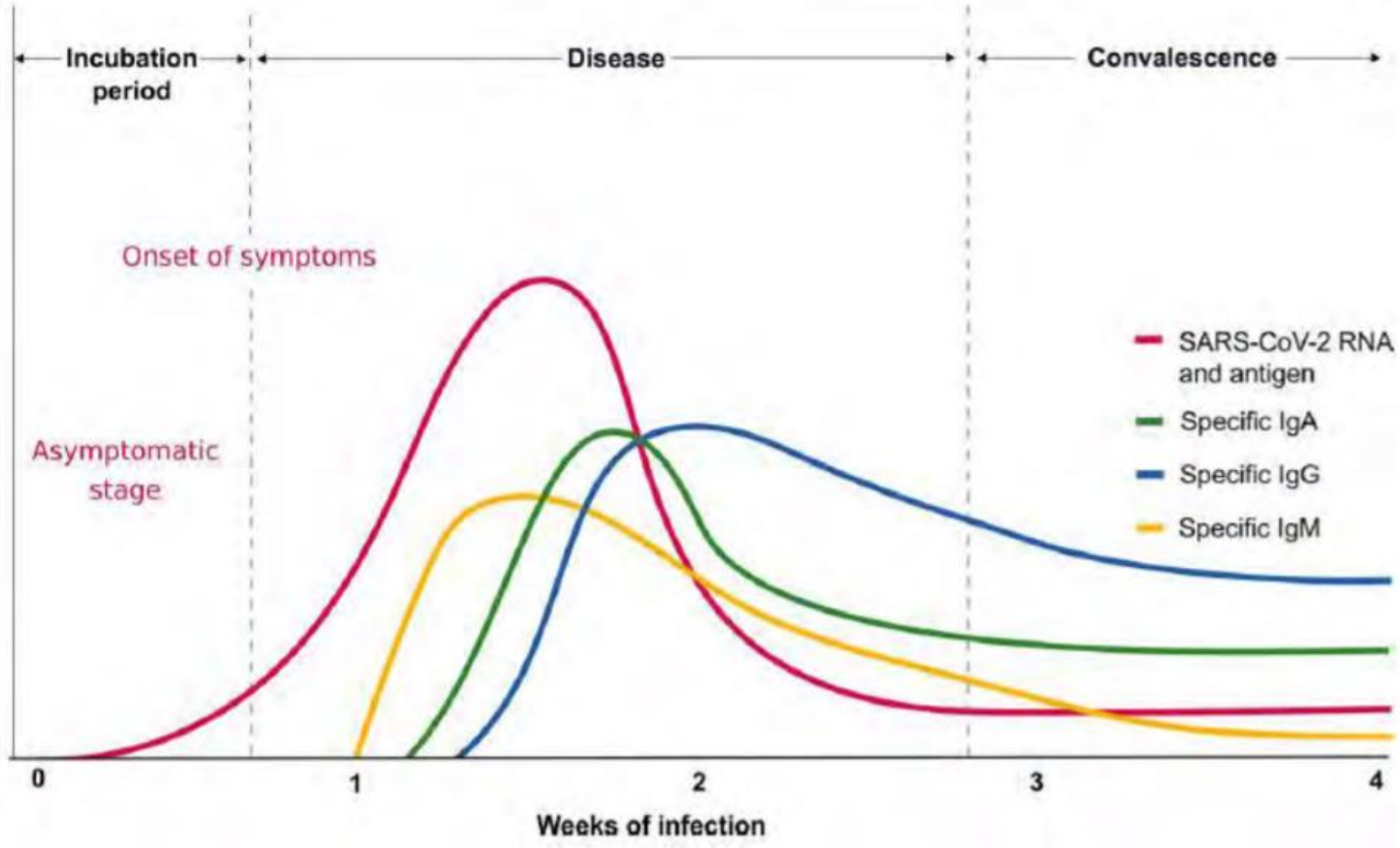
非居家隔離且非居家檢疫者:

1. 儘速至鄰近的社區採檢院所*進一步檢測。
2. 請戴好口罩、勿搭乘大眾運輸工具，另使用過之採檢器材，請用塑膠袋密封包好，攜帶至社區採檢院所，交予院所人員。
3. **後續處置請依防疫人員指示。**

SARS-CoV-2 and antibody response

Serologic tests detect antibodies to SARS-CoV-2 in the blood

Identify previously had SARS-CoV-2 infection as well as patients with current infection who have had symptoms for 3 to 4 weeks.



No.	床號	年齡	入院日	O2 condition	Day1	Ct1 E gene	Ct1 N2 gene	Serum date	Serum Ab
1	2002>2552	25	2021/5/24	room air SpO2: 97	2021/5/24		27.8	2021/5/24	neg.
2	2010>2005>2553	80	2021/5/24	cannula 3L/min	2021/5/24		23	2021/5/25	neg.
3	2001 > 2560	76	2021/5/26	High flow N/C	2021/5/26	15.4	17.3	2021/5/26	neg.
4	2011	68	2021/5/26	ETT (5/27)	2021/5/26	26.2	27.3	2021/5/26	neg.
5	2015>2555-2	33	2021/5/27	cannula 2-3L/min SpO2: 95-96	2021/5/26	23	24.5	2021/5/28	neg.
6	2013>2558	3	2021/5/27	room air SpO2: 96	2021/5/26	26.2	27.7	2021/5/28	neg.
7	2006 > 2552	50	2021/5/28	High flow NC 20 L/min + awake prone	2021/5/28	20.8	22.5	2021/5/28	neg.

Diagnostic tests for COVID-19

Test category	Clinical use	Specimen type	Performance characteristics	Comments
RT-PCR	Diagnosis of current infection	Nasopharyngeal swab (NPS)	High analytic sensitivity and specificity in ideal settings. Clinical performance depends on the type and quality of the specimen and the duration of illness at the time of testing. Reported false-negative rate ranges from <10%.	Turnaround time (TAT) is ranges from 30 minutes to 8 hours.
Antigen rapid test	Diagnosis of current infection	Nasopharyngeal swab (NPS) or nasal swab	Antigen tests are generally **less sensitive** than nucleic acid tests. Sensitivity is highest in symptomatic individuals within 5 to 7 days of symptom onset.	TAT <1 hour.

Cheng MP, et al. Ann Intern Med. 2020;172(11):726-734

Diagnostic tests for COVID-19

Test category	Clinical use	Specimen type	Performance characteristics	Comments
Serology (antibody detection)	Diagnosis of prior infection (or infection of at least 3 to 4 weeks' duration)	Blood (Serum)	Sensitivity and specificity are highly variable. Detectable antibodies generally take several days to weeks to develop; IgG usually develops by 14 days after onset of symptoms. Serologic tests that have high specificity still have a low positive predictive value.	TAT ranges from 30 minutes to 2 hours. It remains uncertain whether a positive antibody test indicates immunity against future infection.

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Thanks for your listening!

CREDITS: 部分投影片資料來自花蓮慈濟醫院檢驗醫學科辜明慧副主任