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Interpreting diagnostic tests for Sars-CoV-2

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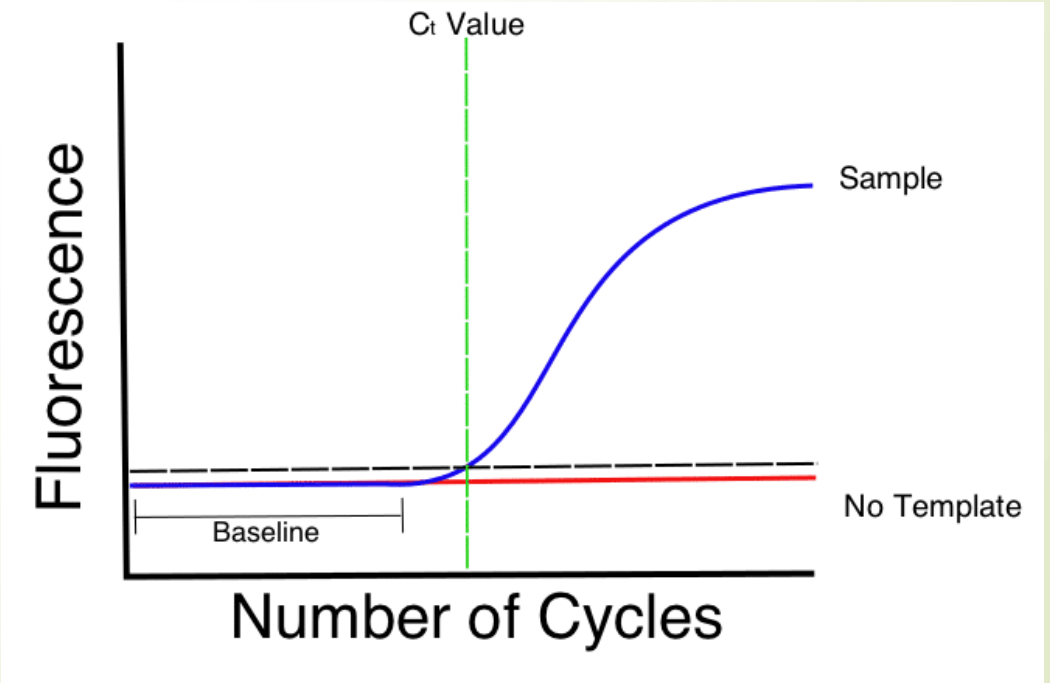
Detection of viral RNA by RT-PCR

- RT-PCR test 目前是最常見被使用來診斷COVID-19。可以從鼻咽、Throat、Saliva等等組織。
- 定義：
- PCR: polymerase chain reaction (from DNA)
- RT-PCR: reverse transcriptase PCR (from RNA)
- Real time PCR (or quantitative PCR, qPCR): a PCR assay with relative values
- Real time RT-PCR



Structure of SARS-CoV 2 and the targeting primers

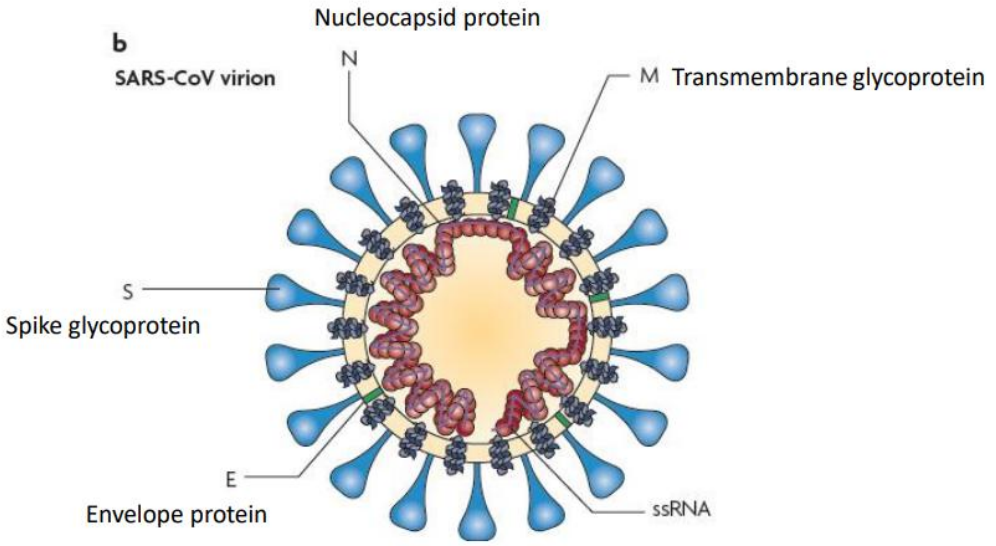


TABLE 19.2 Group II coronavirus proteins and functions

Protein	Functions
nsp1, 2, 7-10, 11	Unknown functions in replication; nsp1 may mediate cell-cycle arrest during overexpression in culture.
nsp3	One or two papain-like proteinase domains (PLP1, PLP2) responsible for cleavage of nsp1, nsp2, and nsp3; zinc ribbon motifs with predicted transcription factors; transmembrane sequences with membrane integration.
nsp4, nsp 6	Membrane-spanning proteins, may localize replication complexes to membranes.
nsp5	Picornavirus 3C-like proteinase (3CLpro or Mpro) responsible for cleavage of nsp4 through nsp16.
nsp12	Predicted RNA-dependent RNA polymerase responsible for genome replication and transcription.
nsp13	RNA helicase, nucleoside triphosphatase activity *in vitro*. Likely involved in genome unwinding, separation, and packaging; may be virulence factor.
nsp14, 15, 16	Predicted RNA modifying enzymes: 3′ to 5′ exonuclease (14), endoribonuclease (15), and O-methyl transferase (16).

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2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes

Division of Viral Diseases

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

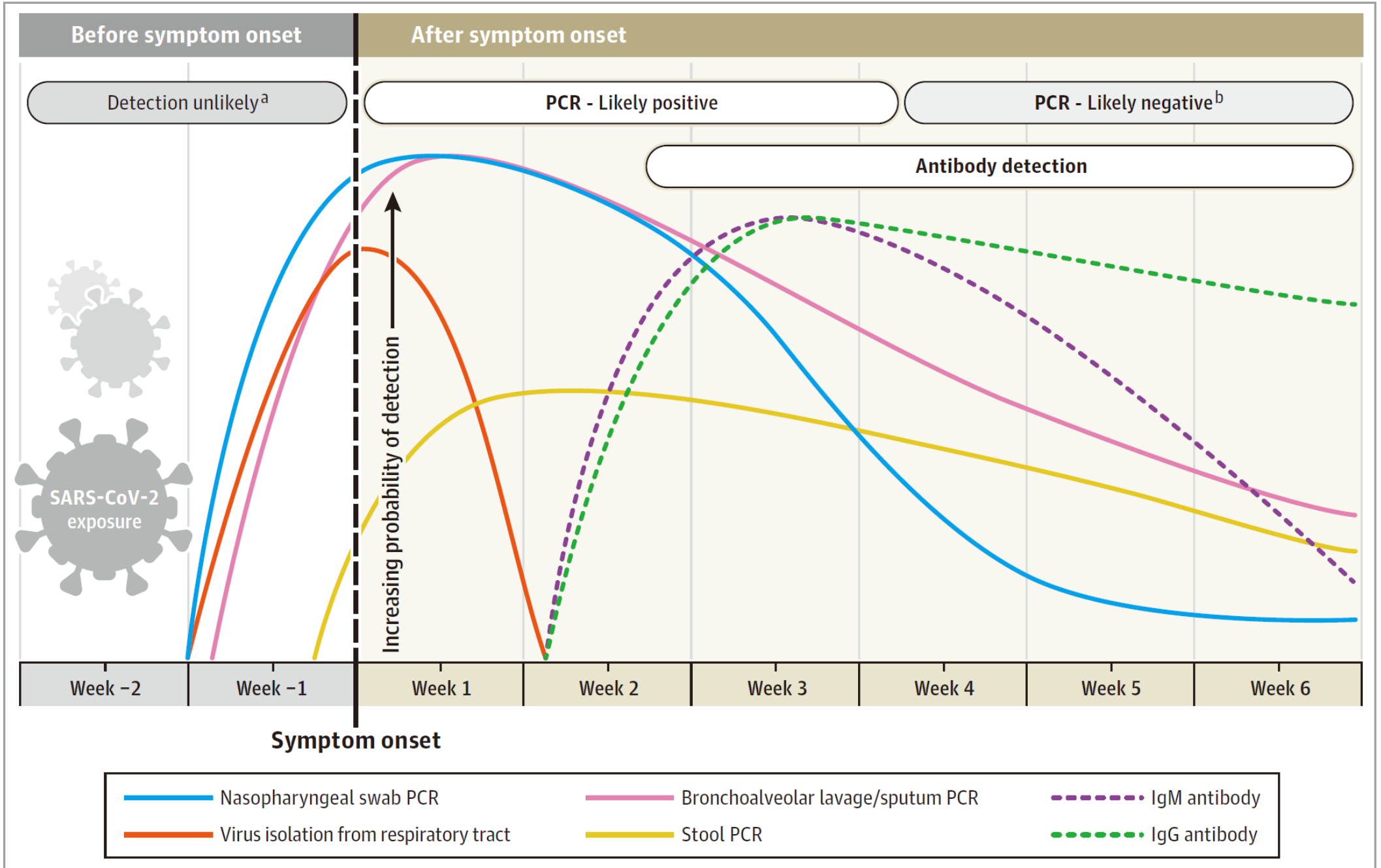
[1]TaqMan® probes are labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher 1 (BHQ-1) (Biosearch Technologies, Inc., Novato, CA) at the 3'-end.

Note: Oligonucleotide sequences are subject to future changes as the 2019-Novel Coronavirus evolves.

鼻咽檢體

- 通常有症狀的COVID-19病人，鼻咽大概第一天就可以檢出，大概到7天最高。
- Ct value less than 40 is viewed as PCR positive clinically (reference: Ct value for house keeping genes of GAPDH or b-actin is usually 26-29).
- 一般3週降到測不到。
- 嚴重的案例可能比輕微的案例的Ct值低，可能會超過三週都還可測得到。
- 重要的是，測得到表示有病毒的RNA存在，但不表示有活的病毒存在。
- 有些案例6週還是測得到，有些案例連續2天測不到後，第3天復陽。
  - Testing errors, reinfection, or reactivation (?)

Figure. Estimated Variation Over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection Relative to Symptom Onset



醫療人員被感染後多久可上班?

- the Centers for Disease Control and Prevention (CDC) indicates that health care workers can return to work, if "at least **3 days** (72 hours) have passed since recovery defined as resolution of fever without the use of fever-reducing medications and improvement in respiratory symptoms (e.g., cough, shortness of breath);
- and, at least **10 days** have passed since symptoms first appeared.

其他部位的RT-PCR

- Sputum的病毒量降低較慢。
- Stool在57%的病人中陽性，且在鼻咽測不到4-11天後仍然陽性(n=96)
- 一研究顯示陽性率：bronchoalveolar lavage specimens (93%), followed by sputum (72%), nasal swab (63%), and pharyngeal swab (32%) (n=205)

Accuracy of RT-PCR

- False-negative results mainly occurred due to inappropriate timing of sample collection in relation to illness onset and deficiency in sampling technique, especially of nasopharyngeal swabs.
- Specificity of most of the RT-PCR tests is 100% because the primer design is specific to the genome sequence of SARS-CoV-2.
- Occasional false positive results may occur due to technical errors and reagent contamination.

Detection of antibodies against SARS-CoV-2

- 人體產生對抗SARS-CoV-2的抗體，表示曾感染過它。
- 對輕微症狀的人特別重要。
- An important tool to understand the extent of COVID-19 in the community and to identify individuals who are immune and potentially "protected" from becoming infected.
- The most sensitive and earliest serological marker is **total antibodies**, levels of which begin to increase from the **second week** of symptom onset.
- Although IgM and IgG ELISA have been found to be positive even as early as the fourth day after symptom onset, higher levels occur in the second and third week of illness.

IgG and IgM seroconverstions

- IgM and IgG **seroconversion** occurred in all patients between **the third and fourth week** of clinical illness onset as measured in 23 patients by To et al and 85 patients by Xiang et al.
- Thereafter IgM begins to decline and reaches lower levels by **week 5** and almost disappears by week 7 whereas IgG persists beyond **7 weeks.**
- During the first **5.5 days**, quantitative PCR had a higher positivity rate than IgM, whereas IgM ELISA had a higher positivity rate after day 5.5 of illness.

- ELISA-based IgM and IgG antibody tests have greater than 95%specificity for diagnosis of COVID-19. Testing of paired serum samples with the initial PCR and the second 2 weeks later can further increase diagnostic accuracy.
- 一般來講，抗體最多是對抗最常見的抗原，也就是NC，因此，對抗NC抗原的抗體一般是比較敏感的。
- 但是，Receptor-binding domain of S (RBD-S)是host attachment protein，因此抗體對抗RBD-S的是比較有專一性及中和性。
- Therefore, using one or both antigens for detecting IgG and IgM would result in high sensitivity.
- Antibodies may, however, have cross-reactivity with SARS-CoV and possibly other coronaviruses.

ELISA抗體測試品質不一

- 有很多公司的抗體assay已經在市場上，但品質不一。
- 有很多司公司甚至沒有把antigen講出來，這些測驗無法定量，只能定性。
- 中和性抗體只能使用Plaque reduction neutralization assay。
- However, high titers of IgG antibodies detected by ELISA have been shown to positively correlate with neutralizing antibodies.
- The long-term persistence and duration of protection conferred by the neutralizing antibodies remains unknown.

Answers to be addressed

- Most of the available data are for adult populations who are not immunocompromised.
- The time course of PCR positivity and seroconversion may vary in children and other groups, including the large population of asymptomatic individuals who go undiagnosed without active surveillance.
- Many questions remain, particularly how long potential immunity lasts in individuals, both asymptomatic and symptomatic, who are infected with SARS-CoV-2.