COVID-19分子生物檢驗與抗原快篩

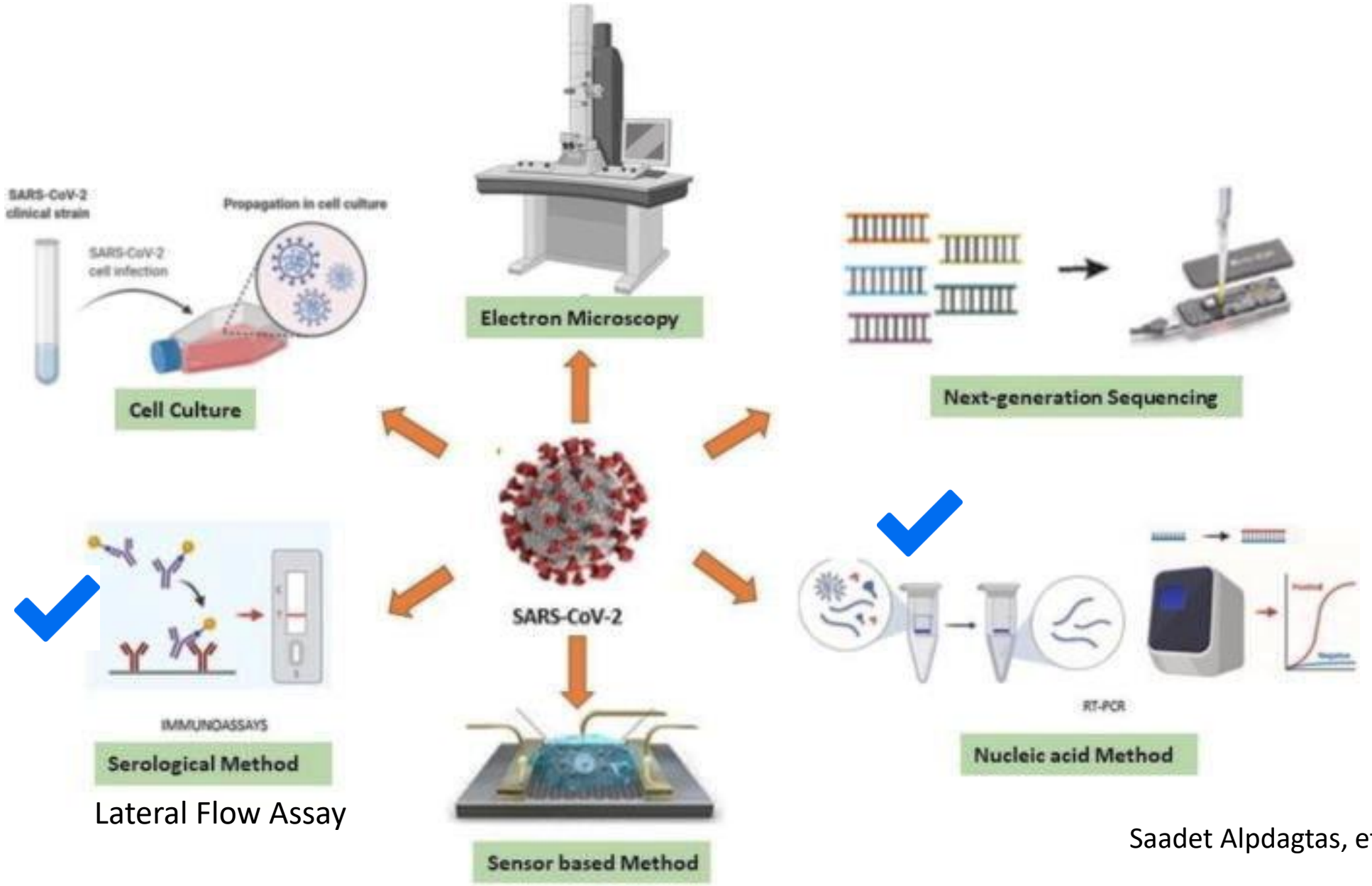
高雄醫學大學附設中和醫院感染科
檢驗醫學部微生物室
林尚儀

正確使用診斷工具遏制大流行

- **Case finding in symptomatic or asymptomatic individuals**
- **Households and other contacts of confirmed cases**
  - A meta-analysis of 54 studies (77 758 people infected with SARS-CoV-2) estimated an overall secondary attack rate of 16·6%
- **Screening of populations at increased risk of acquisition and transmission**
  - Health-care workers, and workers in residential care homes, essential frontline workers, and public transport and aviation transport operators
- **Testing as a public health tool to ensure safe environments and enable economic recovery**
- **Testing travelers to reduce the risk of importing COVID-19**

RW Peeling, et al. Lancet 2022
Madewell ZJ, et al. JAMA Netw Open 2020; 3: e2031756.

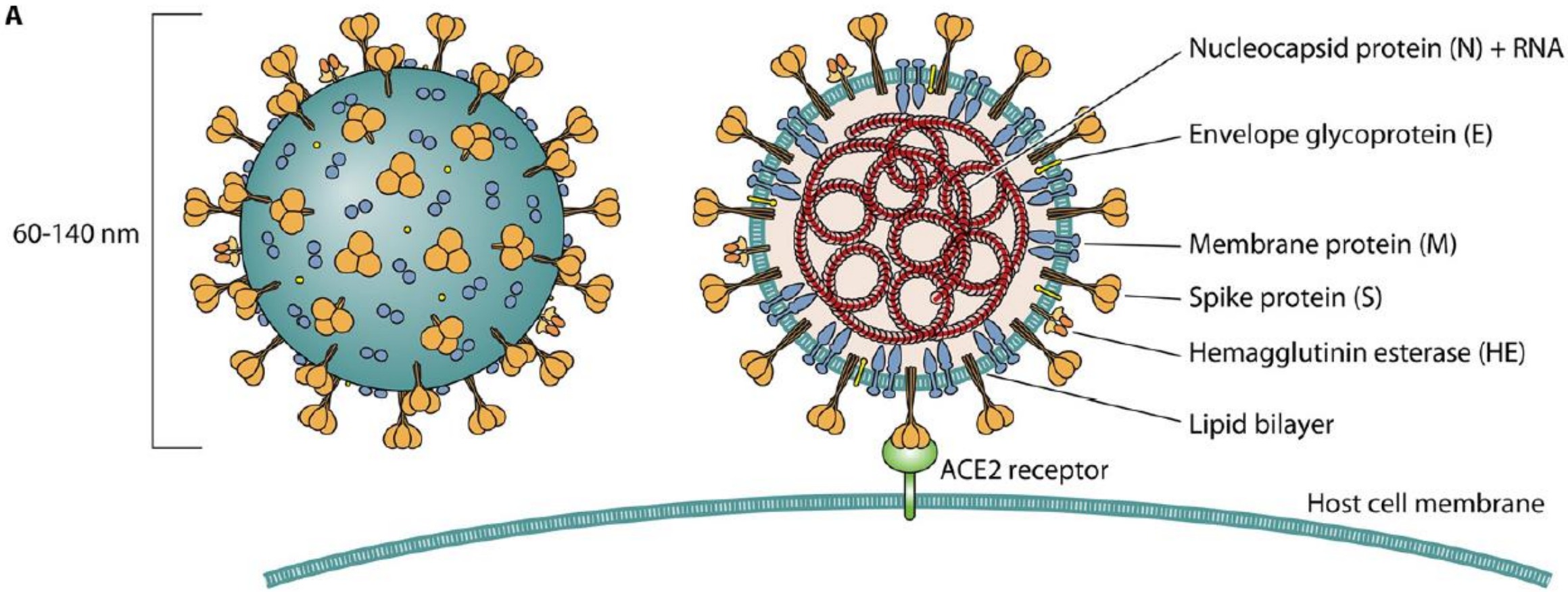
RNA 病毒的檢驗方法



Lateral Flow Assay

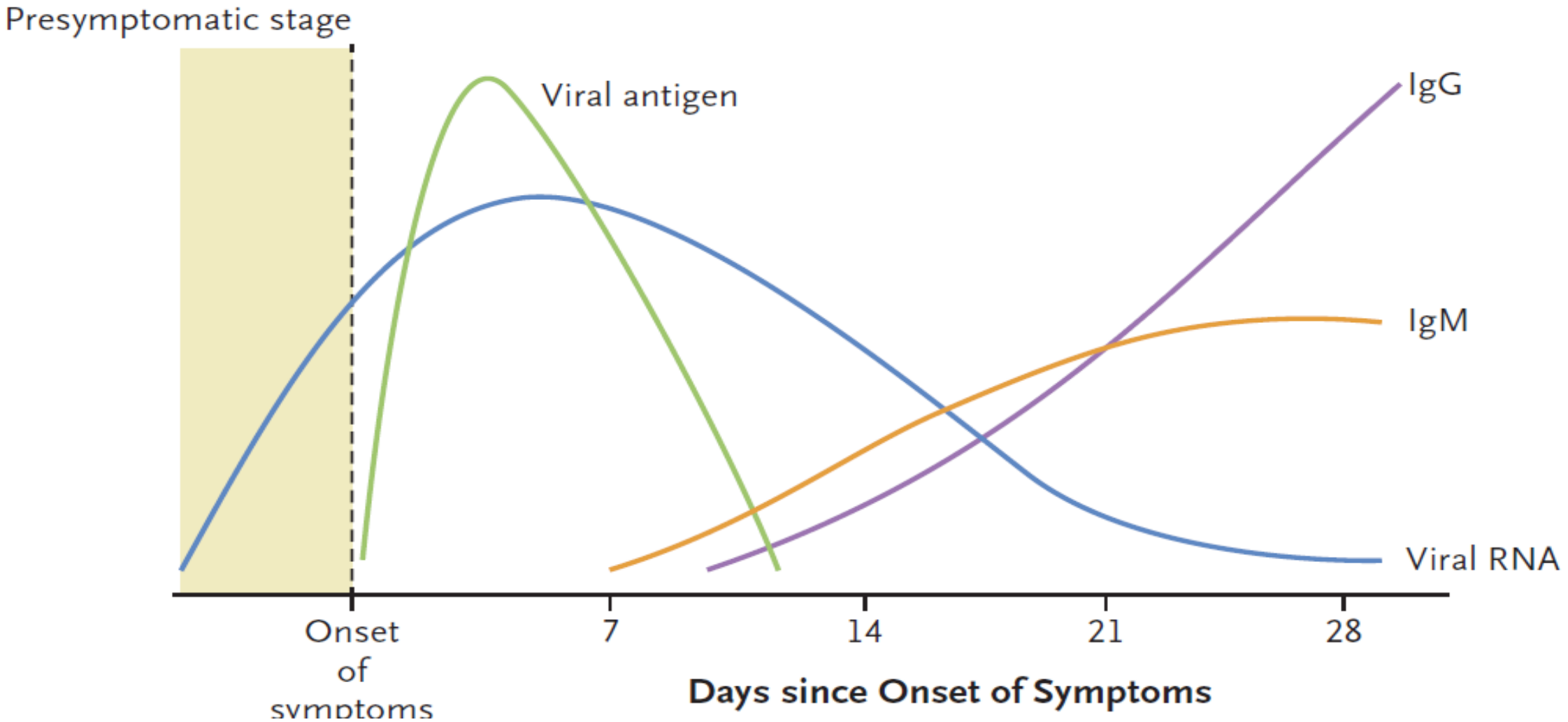
Saadet Alpdagtas, et al. APL Bioeng 2020

新型冠狀病毒的蛋白質及基因結構

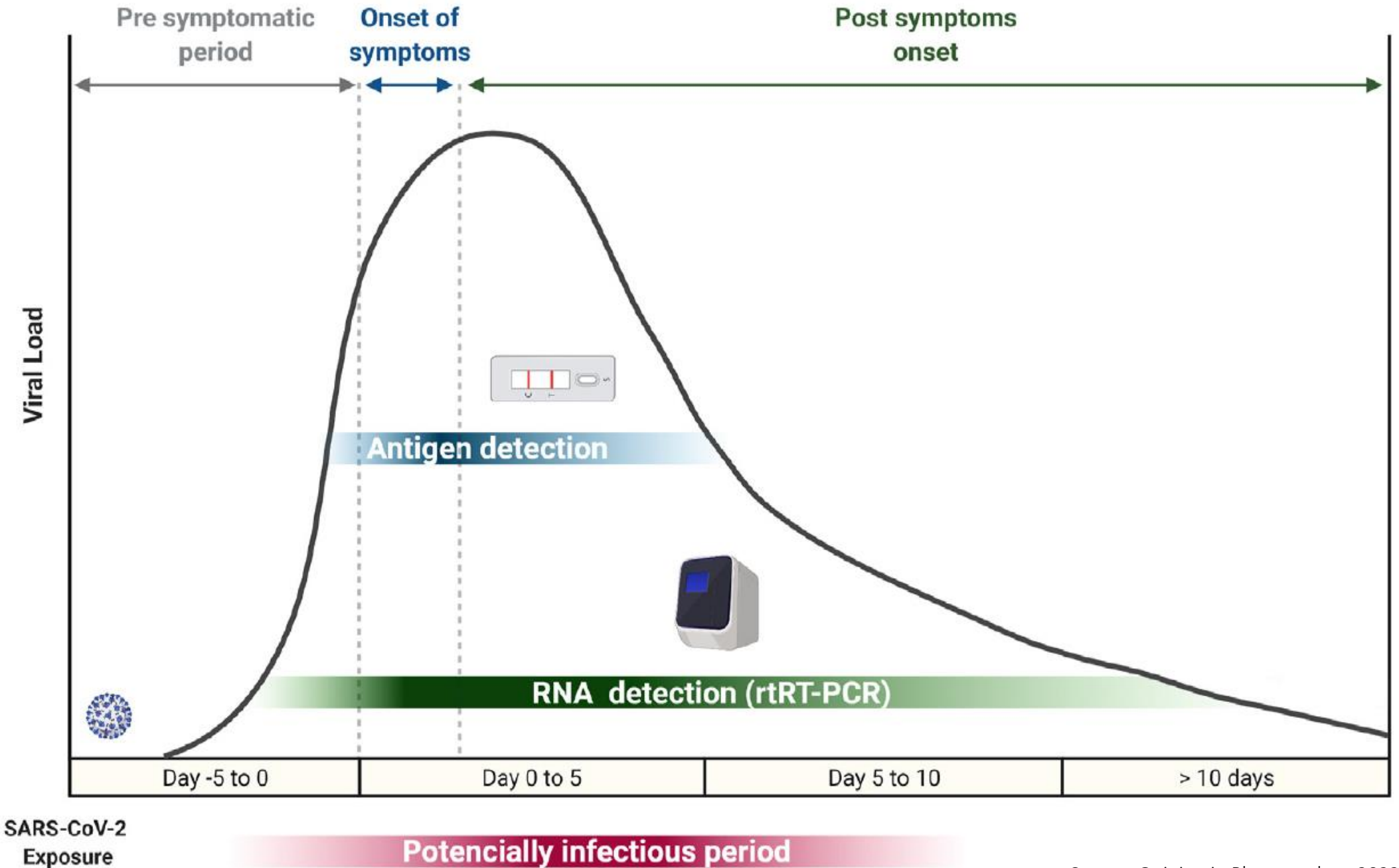


Safiabadi Tali SH, et al. Clin Microbiol Rev. 2021.

SARS-CoV-2 infection time course

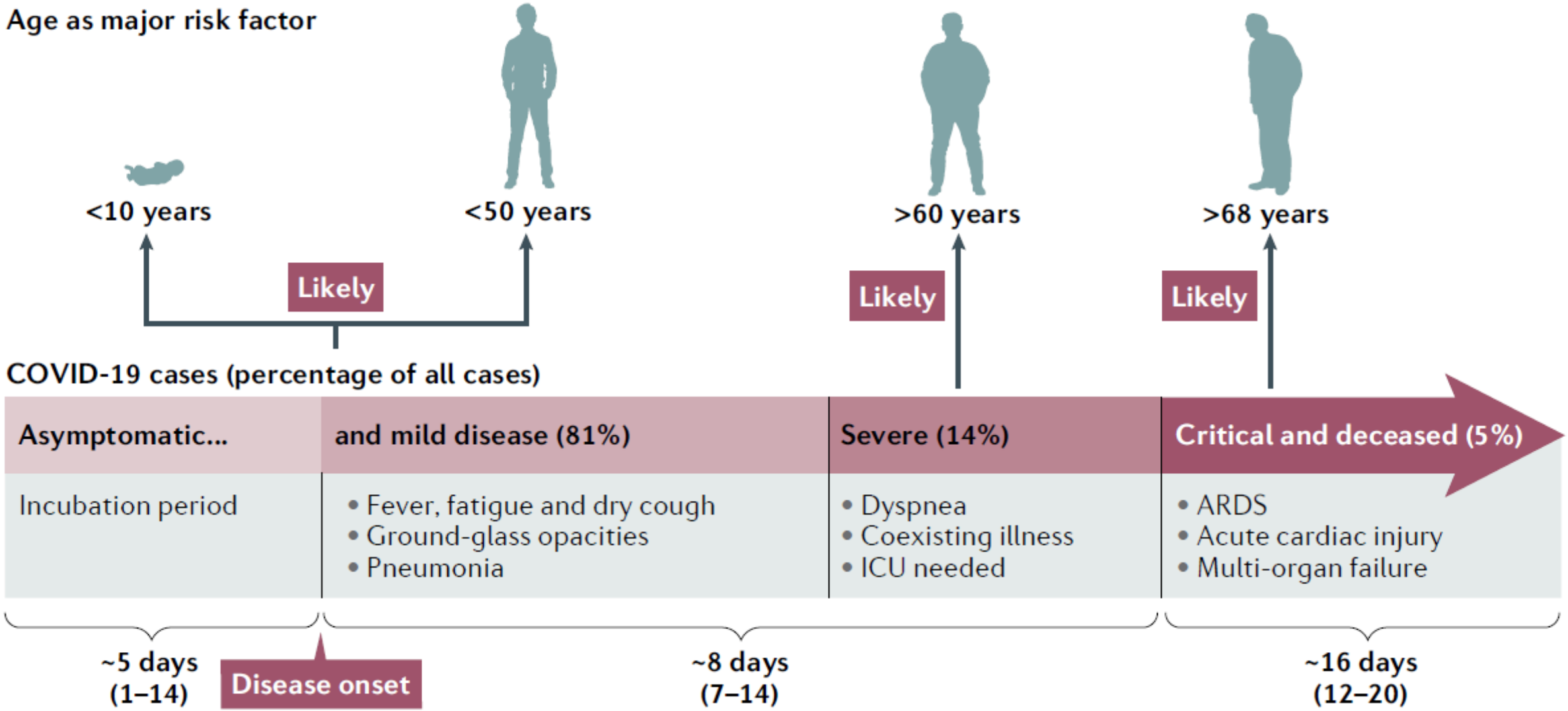


Paul K. Drain. NEJM 2022

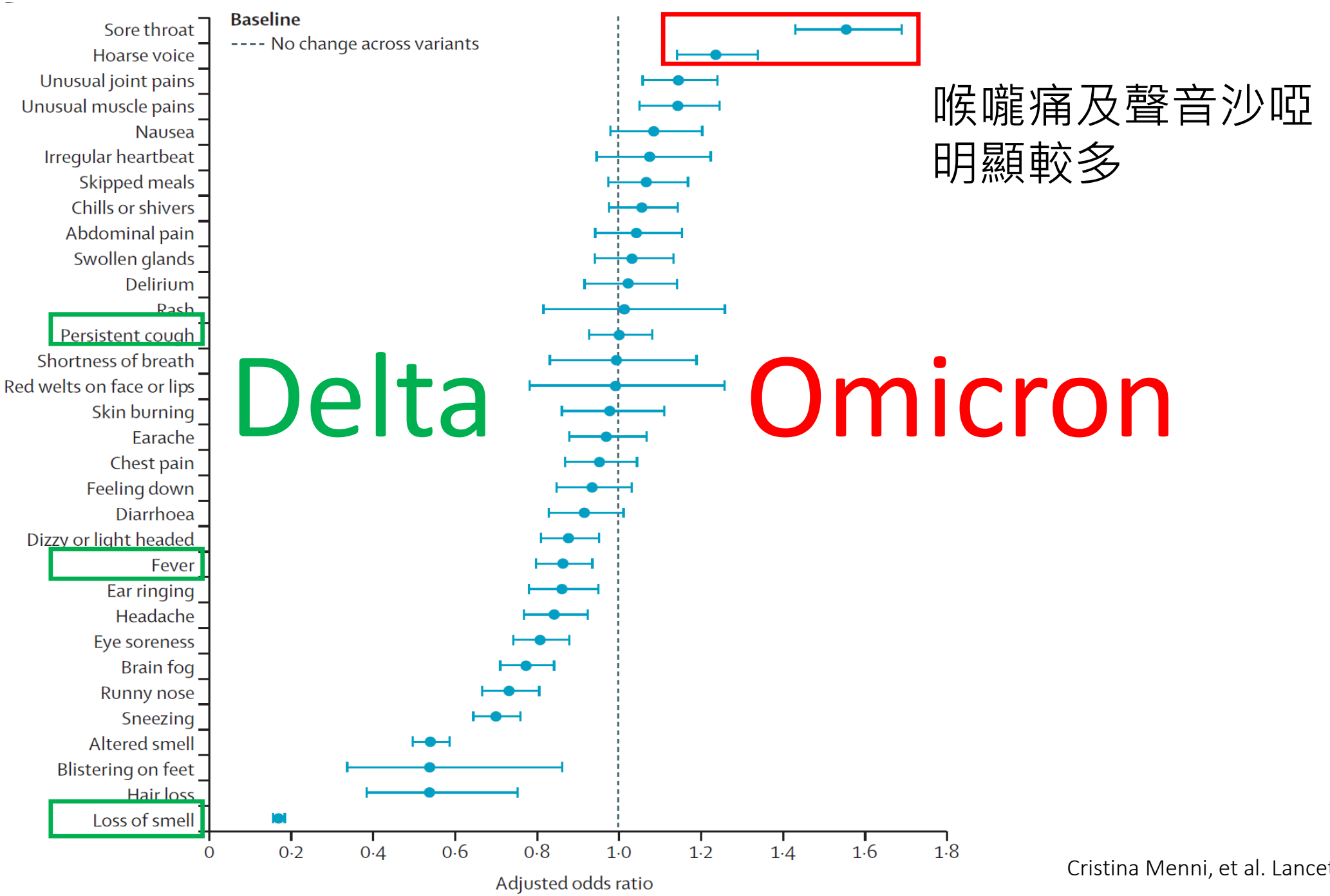


Current Opinion in Pharmacology 2022, 62:152–158

COVID-19的臨床表現



Ben Hu, et al Nat Rev Microbiol. 2021



喉嚨痛及聲音沙啞明顯較多

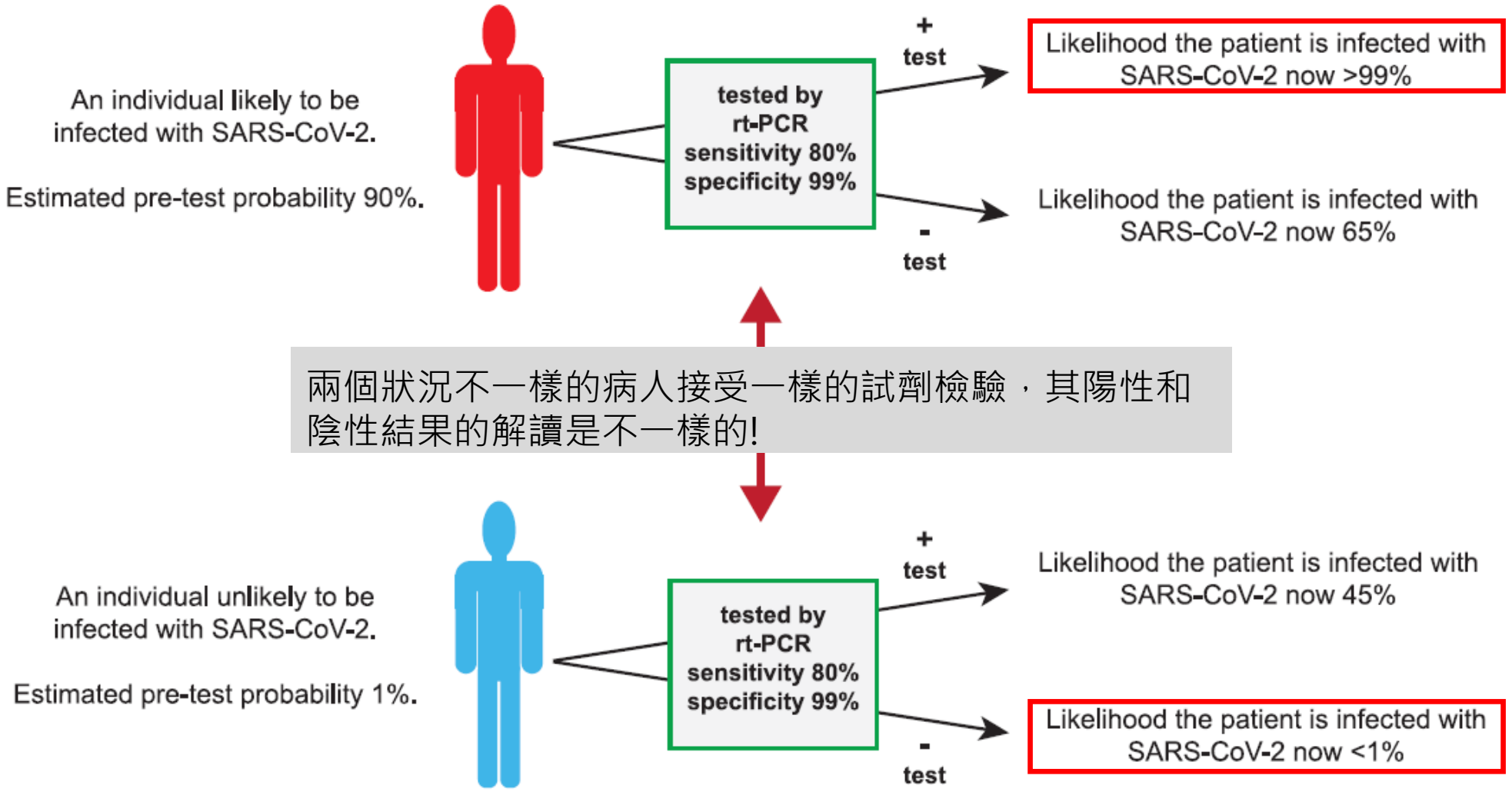
Cristina Menni, et al. Lancet 2022

無症狀的人員篩檢

- **Asymptomatic testing** for SARS-CoV-2 needs clear goals and protocols
- Select asymptomatic patients
  - Close contact with index case
  - individuals at risk for severe disease (eg, long-term care facilities, cancer patients…)
  - hospitalized patients at locations where prevalence is high
- Screening program
  - depends on population prevalence of infection, the different tests, different people's willingness to accept personal inconvenience…

1. Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19, updated December 23, 2020
2. CDC Guidance for Expanded Screening Testing to Reduce Silent Spread of SARS-CoV-2. https://www.cdc.gov/coronavirus/2019-ncov/php/testing/expanded-screening-testing.html
3. European Society for Blood and Marrow Transplantation. COVID-19 anbd BMT. https://www.ebmt.org/covid-19-and-bmt

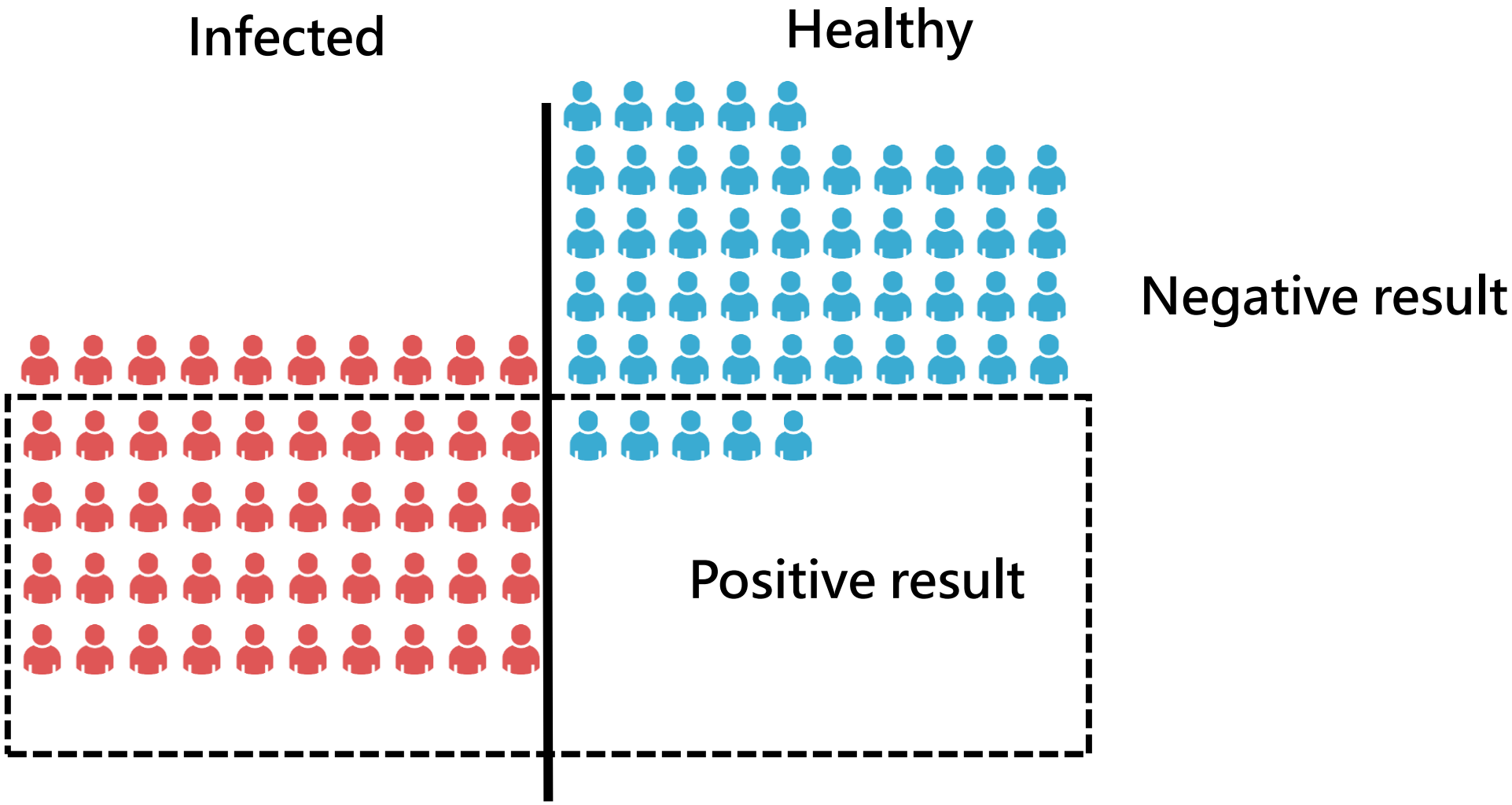
前測機率影響結果判讀



Edward C. Stites and Craig B. Wilen. Med (N Y) 2020

Disease prevalence= 50%

Diagnostic assay sensitivity 80%, specificity：90%



Disease prevalence= 50%

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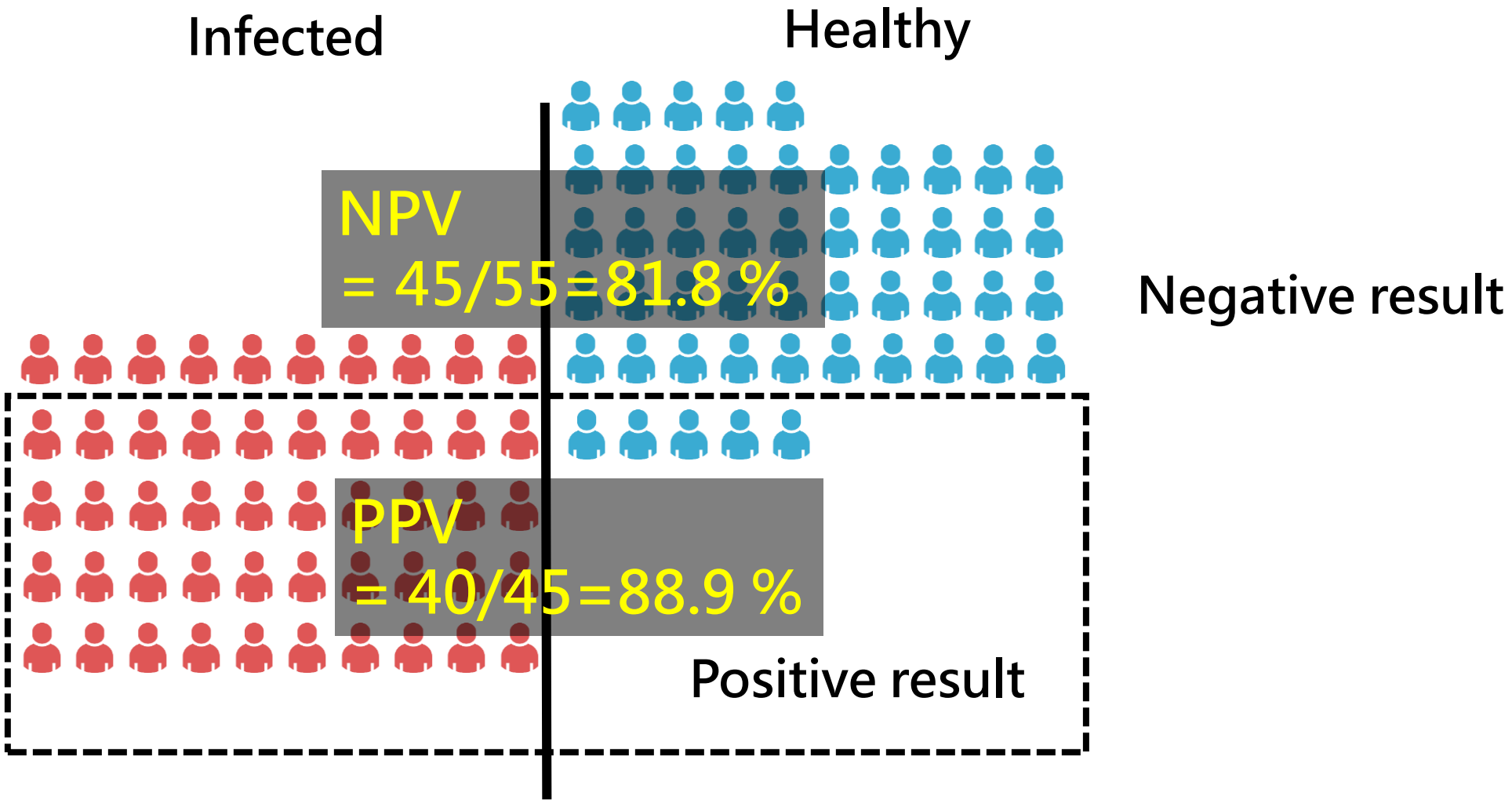
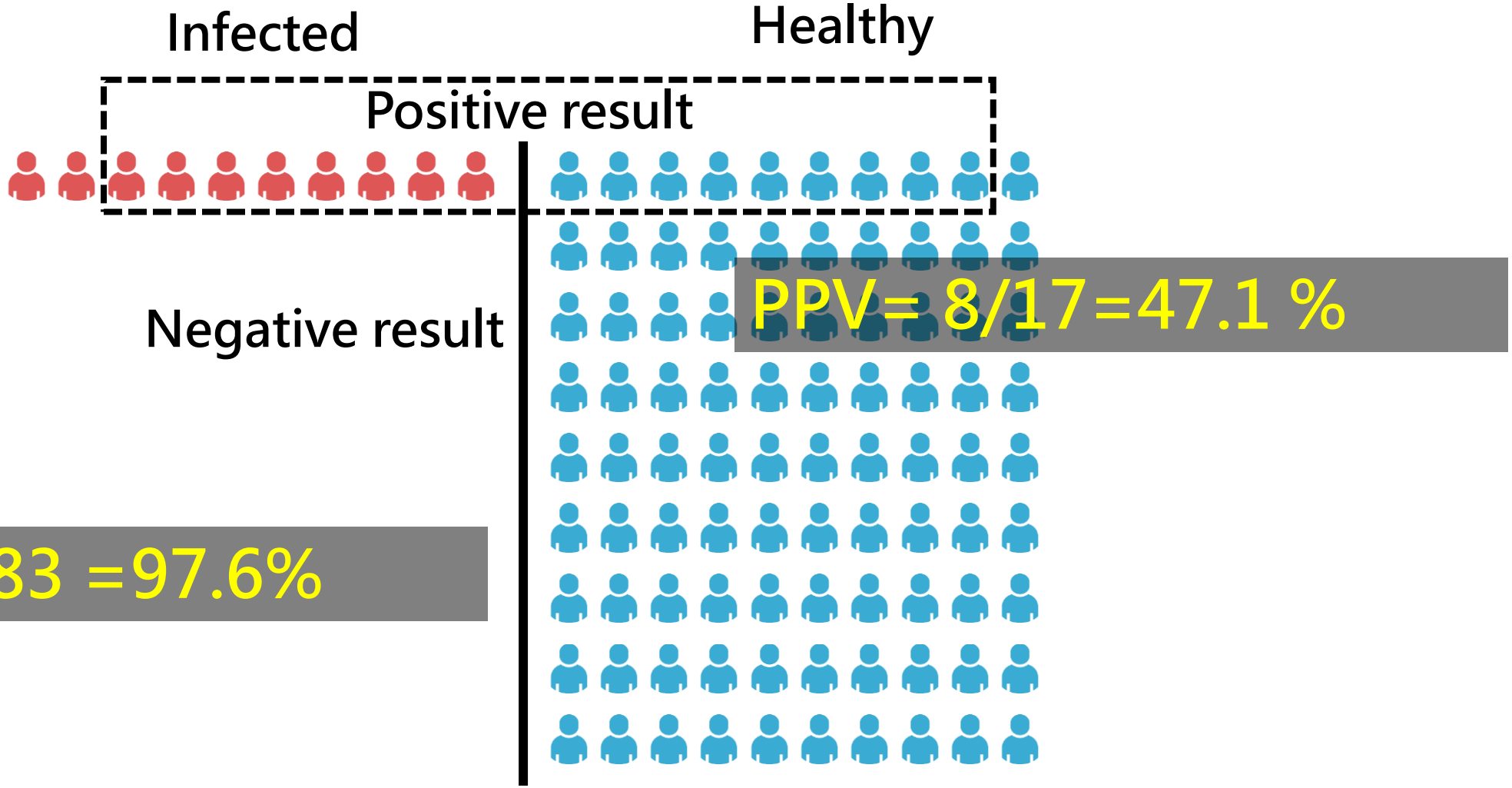
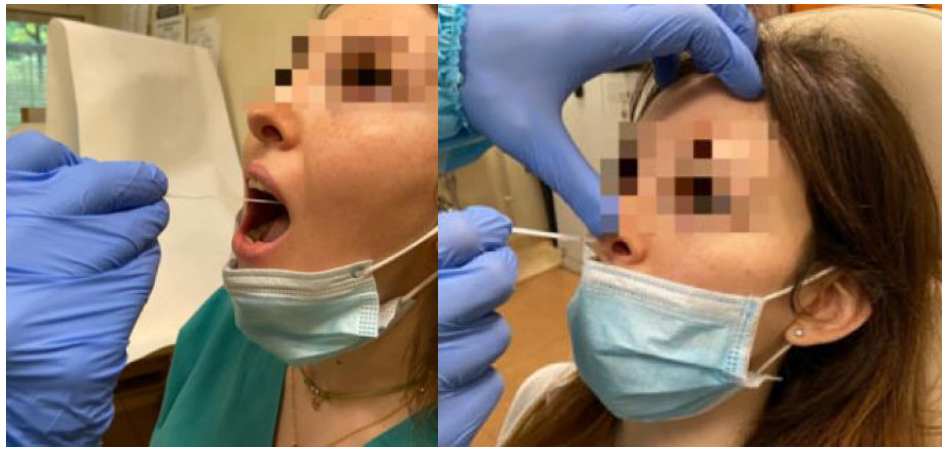


Disease prevalence= 10%

Diagnostic assay sensitivity 80%, specificity：90%



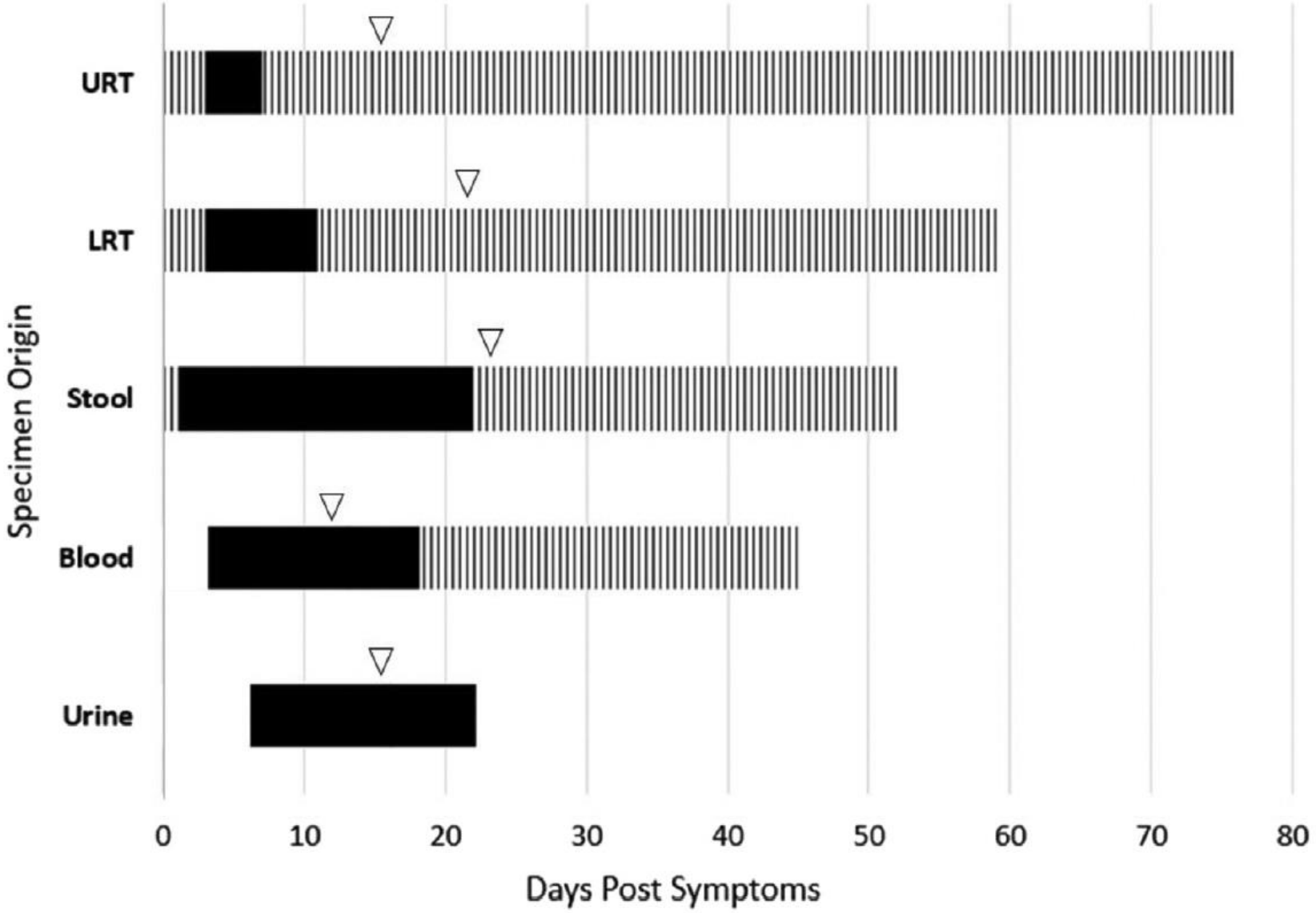
檢體別對檢驗結果的影響



- Oropharyngeal/nasopharyngeal swabs
  - the most frequently used biological samples
  - Spe ~100% and Sen 40-100%
- Salivary samples
  - reduce the need for PPE
  - Spe ~90% and Sen ~85%
  - Optimal method is unknown, and instructions on collection varied
- Sputum, bronchoalveolar lavage
  - higher viral loads and be more likely to yield positive tests
  - Difficulty collected
- Rectal swabs and stool samples
  - esp. in the advanced stages of infection
  - alternative diagnostic strategy

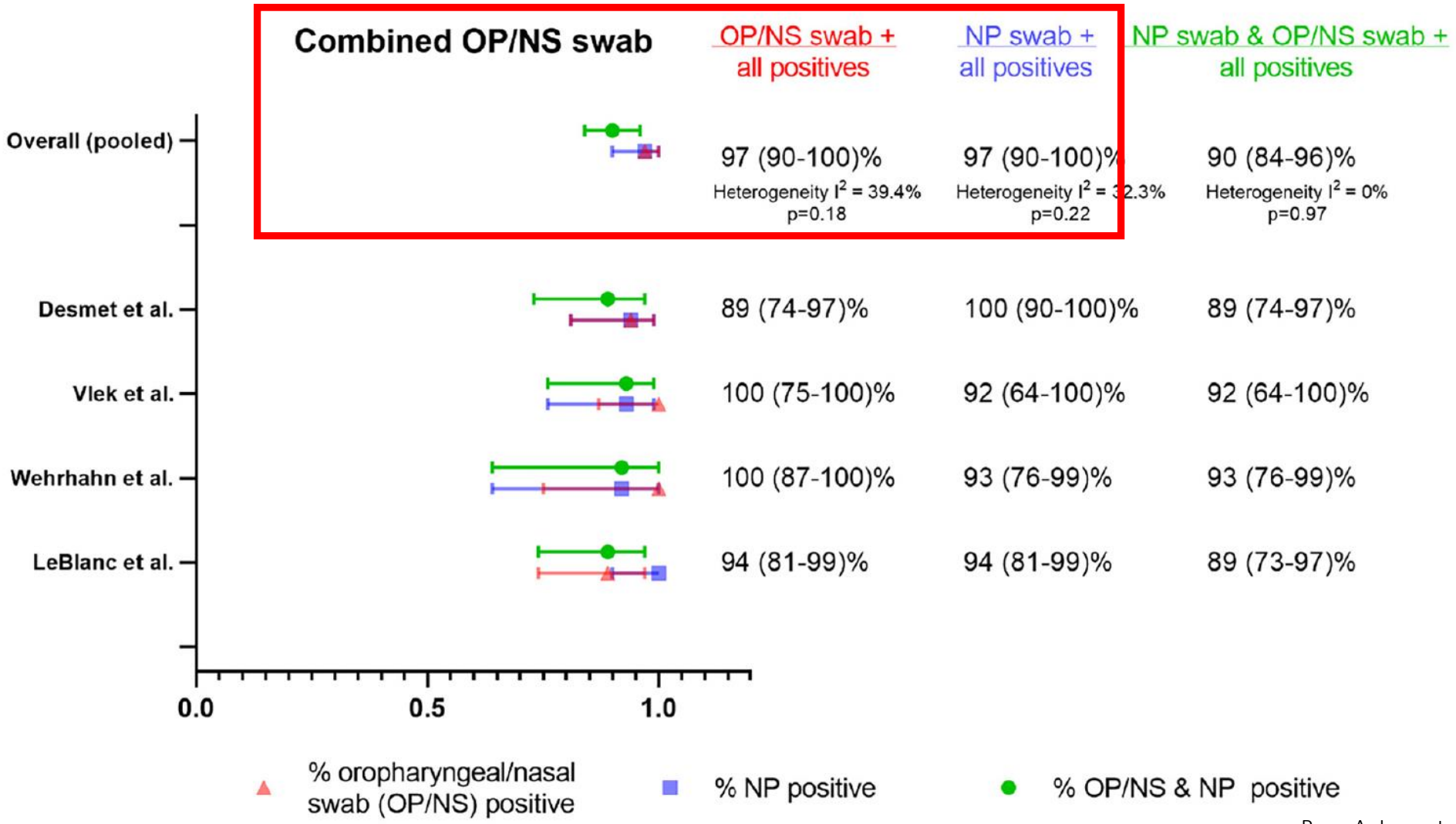
Wang W,et al. JAMA. 2020; Wo¨ lfel R, et al. Nature 2020; 6. Yang Y, et al. BMJ 2020; Sara Torretta, et al. Ear, Nose & Throat Journal 2021
Butler-Laporte G, et al. JAMA Intern Med 2021; Yu F, et al. Clin Infect Dis 2020; Wang H, et al. Eur J Clin Microbiol Infect Dis. 2020

不同檢體別可偵測到SARS-CoV-2 RNA的時序



Kukull, et al. Clin Lab Med 2022

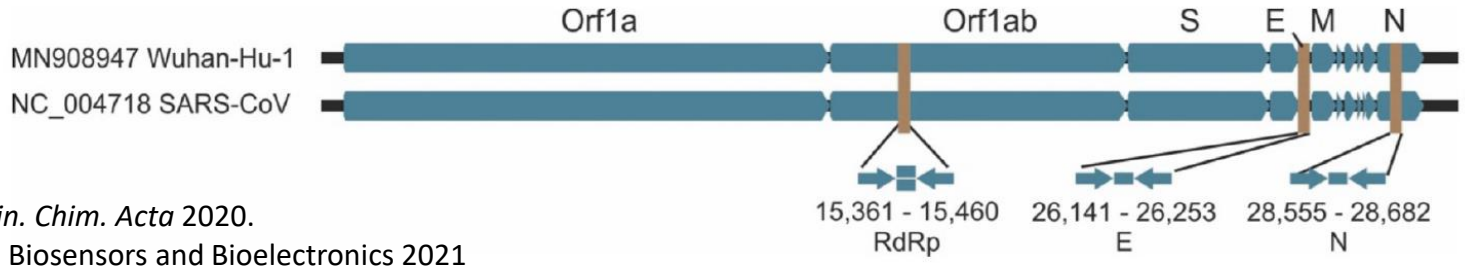
口咽合併鼻腔檢驗的效果與鼻咽檢驗相當



Rose A. Lee, et al. Journal of Clin Microbiol 2021

Nucleic acid amplification testing (NAAT)

- Gold standard method with a RT-PCR assay
  - the standard for population-scale testing
  - Based on measuring the amplification of *RdRP*, *E*, *N* or *S* gene fragments
  - Sensitivity 80-90% and specificity ~99%
- Disadvantage
  - typically conducted in large, centralized laboratories, efficient sample collection is critical to minimize reporting delays
  - false-negative rate across different specimens and time periods
  - Occasional false positive results may occur due to technical errors and reagent contamination



1. Corman, V. M. et al. *Eur. Surveill.* 2020.
2. Liu, R. et al..*Clin. Chim. Acta* 2020.
3. Wikramaratna, P., et al. *Euro Surveill.* 2020
4. M. Yüce, et al. Biosensors and Bioelectronics 2021

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Flow Chart for COVID-19 Diagnostic through RT-PCR

Symptoms:

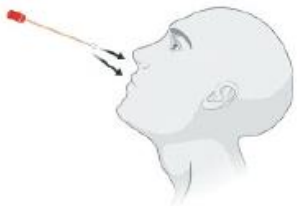
Cough

Fever

Difficulty breathing

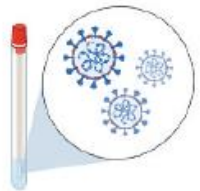
Pneumonia (severe cases)

1 Nasopharyngeal or Oropharyngeal swab



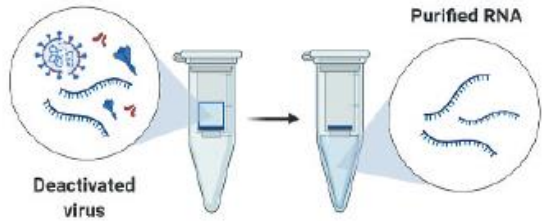
2 Sample Handling

The collected sample should be immediatedly inactivated and proceed to RNA extraction.



3 RNA extraction and purification

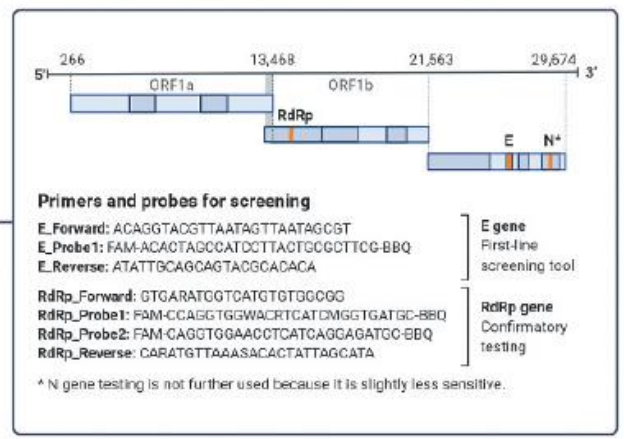
Purified RNA is extracted from deactivated virus.



4 RT-qPCR

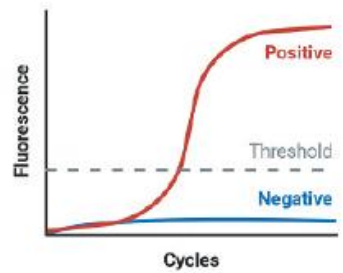
Purified RNA is reverse transcribed to cDNA and amplified by qPCR.

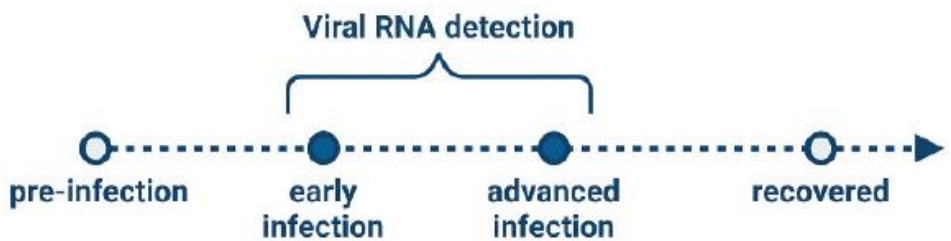
Retro transcription



5 Test results real-time

Positive SARS-CoV-2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).





L. BENEVIDES LIMA, et al. EXPERT REVIEW OF MOLECULAR DIAGNOSTICS 2021

高通量自動化以提升檢測量能

全自動化cobas® 6800 系統

將檢體簡易處理後即可上機，在儀器內自動完成核酸萃取及PCR

- 每批可處理94支檢體
- 檢驗效率：第一批檢體(自上機到結束)需時3小時，第二批以後後每1.5小時可完成檢測

- 24小時可檢測約1400支檢體

高通量自動化以提升檢測量能

Panther® system

- 第一批可上機120支檢體，之後可隨到隨上機
- 檢驗效率：第一批檢體上機3.5小時後，開始每5分鐘發出5份報告

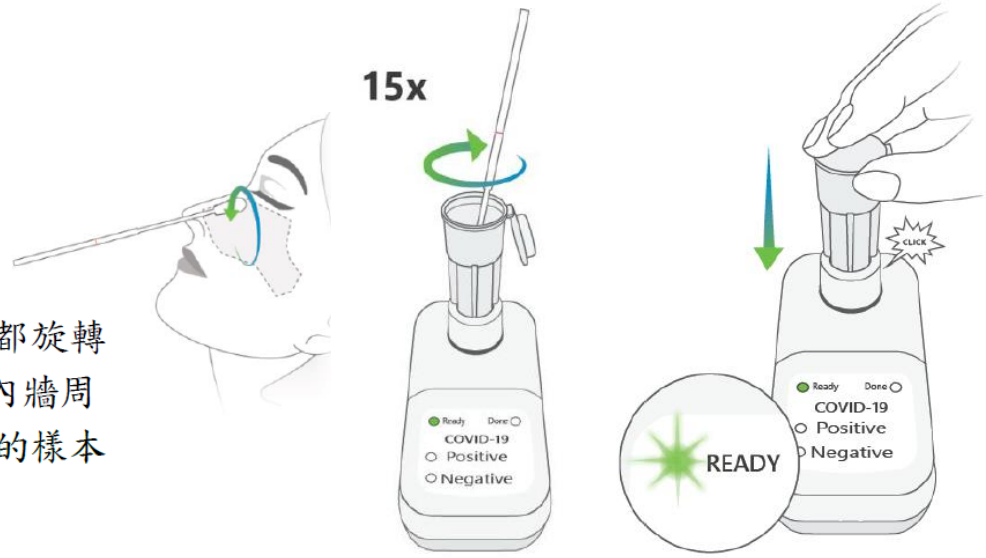
- 24小時可檢測約1000支檢體

快速核酸檢測工具

檢測工具	操作時間	一次可檢測的檢體量	說明
Xpert Xpress SARS-CoV-2 (Cepheid)	1 小時	1-4 tests	• 已有相當多的實證顯示檢驗效能與標準做法相當 • 很貴
ID NOW COVID-19 assay (Abbott)	15 分鐘	1 test	• 速度非常快 • 無法提供Ct值 • 效能略低於標準做法，但仍算是相對可靠的診斷工具
Liat System (Roche)	20-30 分鐘	1 test	• 速度快 • 能提供Ct值 但不易比較 • 可同時檢測流行性感冒病毒

FDA grants first emergency use ok for at-home COVID-19 test

兩個鼻孔取樣都旋轉5圈，確保在內牆周圍滾動收集好的樣本



陽性結果	陰性結果	不正確結果
陽性燈號亮	陰性燈號亮	所有燈號閃爍

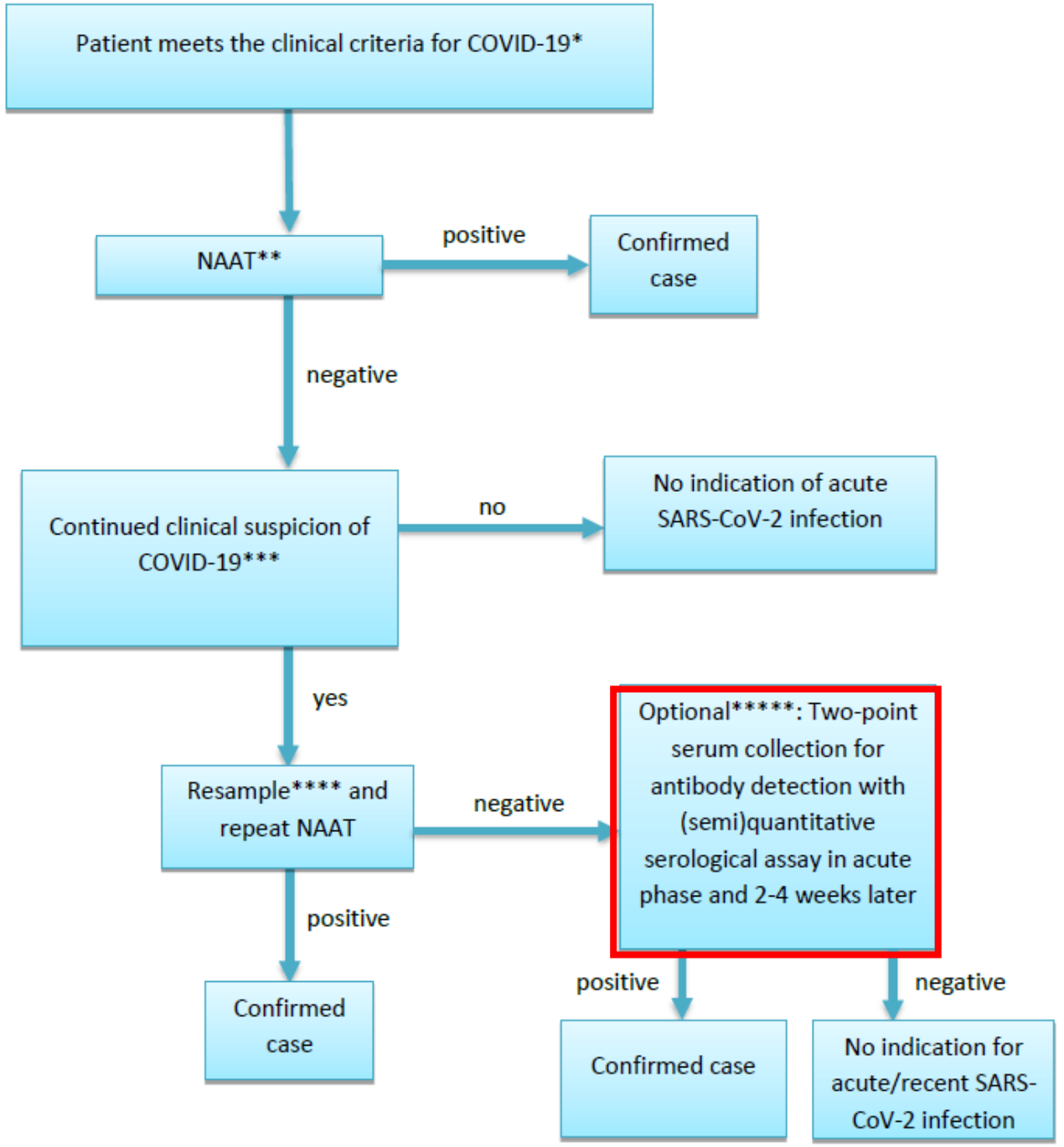
Diagnostic testing for SARS-CoV-2

Interim guidance

11 September 2020

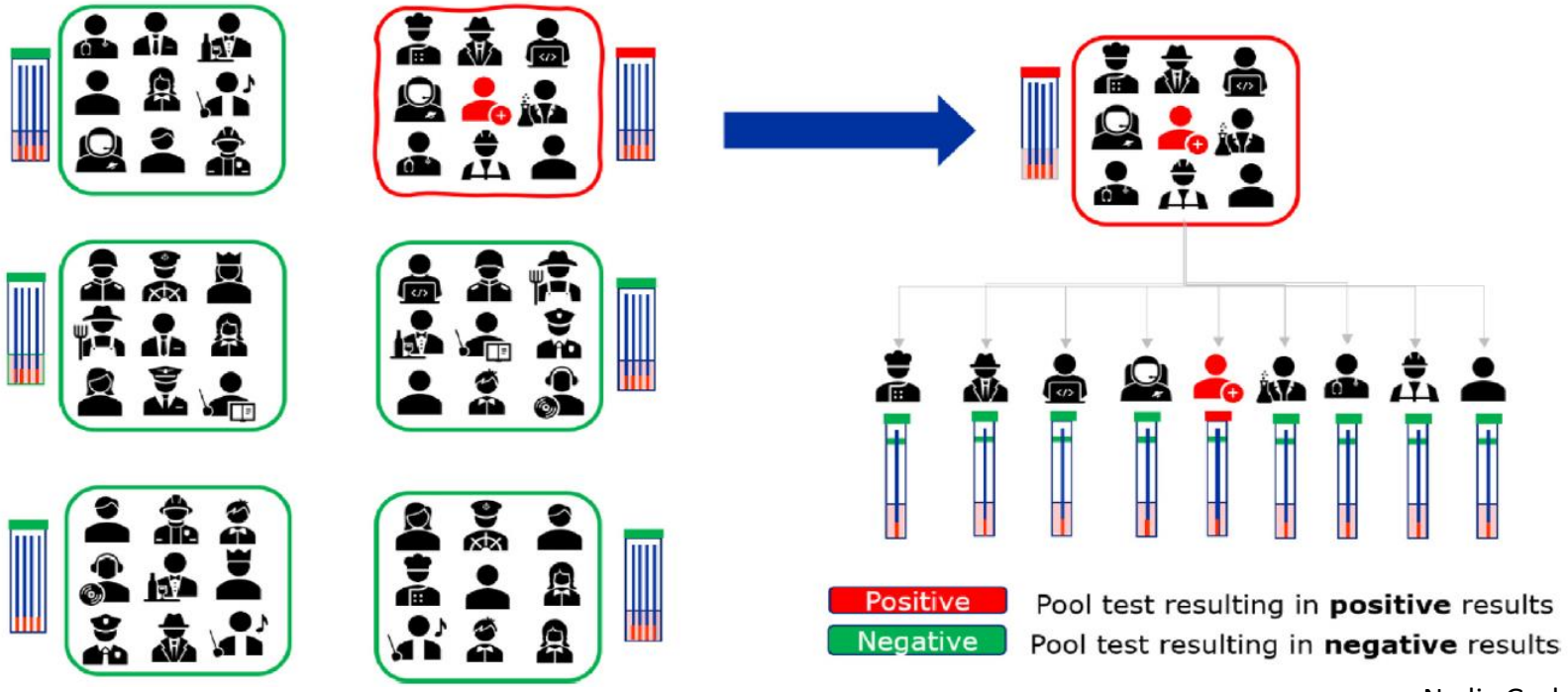
World Health Organization

Diagnostic flow diagram for the detection of acute SARS-CoV-2 infection in individuals with clinical suspicion for COVID-19



池化PCR的策略

- **優點:** 增加檢驗量能、降低檢驗費用、減少試劑耗用、適用於大規模的篩檢及流病的調查
- **缺點:** 池化的數量越大越容易發生偽陰性的風險



Nadja Grobe, et al. Clin Microbiol Infect 2021
Daniel EA, et al. Diagn Microbiol Infect Dis. 2021

嚴重特殊傳染性肺炎核酸檢測池化(pooling)方式操作步驟

110/6/28

操作步驟

1. 各待測檢體先依常規流程完成前處理(例如有些實驗室會先移液分管)。
2. 將各待測檢體以每五支為一組，取等體積混合成一管(一個 pool)。每個 pool 中各檢體須取的體積量請依各檢測平台所投入之檢體總體積計算。例如單一檢體投入體積為 300 ul，則將每個 pool 視為單一檢體時，亦須投入 300 ul 進行檢驗，因此各 pool 所含的 5 支檢體每支須取 60 ul (使總和為 300 ul)；若再考慮分注時的誤差，建議可多取 10 %的體積(例如每支取 66 ul)，5 支混合後每個 pool 含 330 ul，再取 300 ul 進行檢驗。

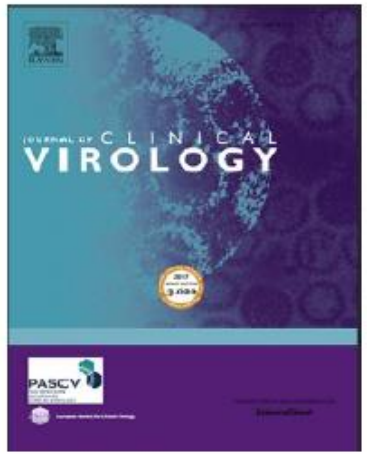
備註：若各單位所使用的檢測平台原廠有提供 pooling 之操作步驟，亦可自行參考，依該步驟執行，惟每個 pool 所含的檢體上限為 5 支。

2022/4/1 改為**1:10**

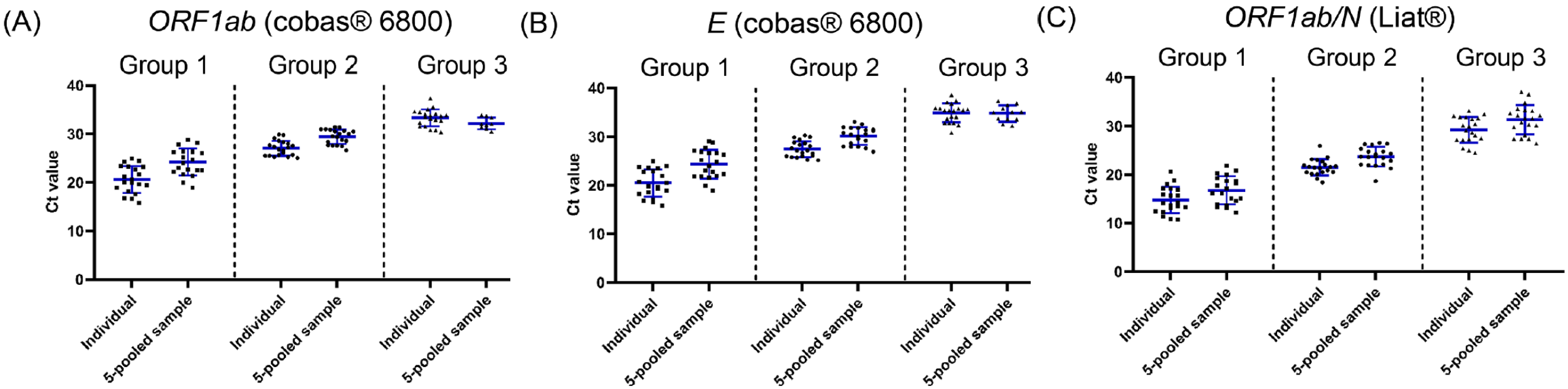
結果判讀

1. 若單一 pool 的檢驗結果呈現陰性，則該 pool 內的 5 支檢體均核發陰性報告。
2. 若單一 pool 的檢驗結果呈現陽性，則該 pool 內的 5 支檢體均須個別重新檢測，再依各檢體的檢驗結果核發報告。

台北榮總池化PCR經驗

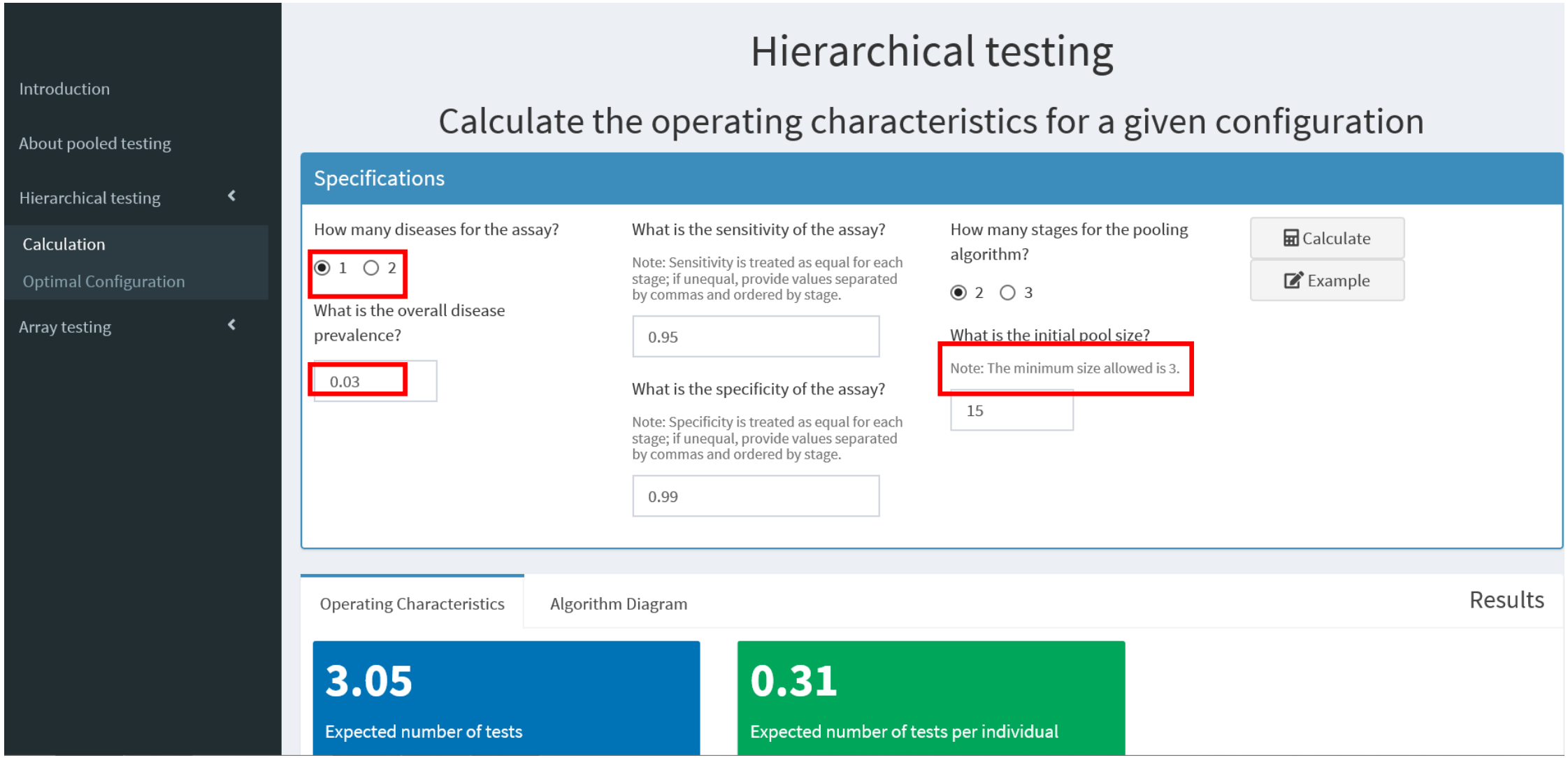


- Cobas 6800及Liat 1:5池化比較:
- 7,606 鼻咽檢體在36小時內檢驗完畢



Journal of Clinical Virology 149 (2022) 105133

池化檢驗的數量預估



https://bilder.shinyapps.io/PooledTesting/

Hou P, et al. Biostatistics 2020

CT value(cycle threshold)的迷思

- WHO: Ct values as a surrogate for the level of viral load

The Depiction of Viral load and infectivity basis on the RT PCR Ct (Cycle threshold) values.

Inversely Proportional relationship of Ct values and Viral load.

Lower the Ct values = Higher the viral load

Higher the Ct values = Lower the viral load

Score	Viral load
17-24	High Viral load
24-35	Moderate Viral load
≥ 36	Non-diagnostic result

Ali A. Rabaan, et al. Diagnostics 2021
Rao, S.N, et al. Infect. Dis. Ther. 2020

CT value(cycle threshold)的迷思

- CT值跟嚴重度有關嗎?
  - 低CT值與嚴重的臨床表現相關
  - ex, CT value of 14 vs 35 ?
- CT值能預測感染力嗎?
  - 一般而言，高CT值表示病毒量較低(ex, 大於35)
  - ex, CT value of 35 比較不會傳染嗎?
  - 病人最常問: CT值還驗得到，真的不會傳染給其他人...
  - 不必遵守防疫措施，因為我CT值很高...

Rao SN, et al. Infectious diseases and therapy 2020
https://www.idsociety.org/globalassets/idsa/public-health/covid-19/idsa-amp-statement.pdf

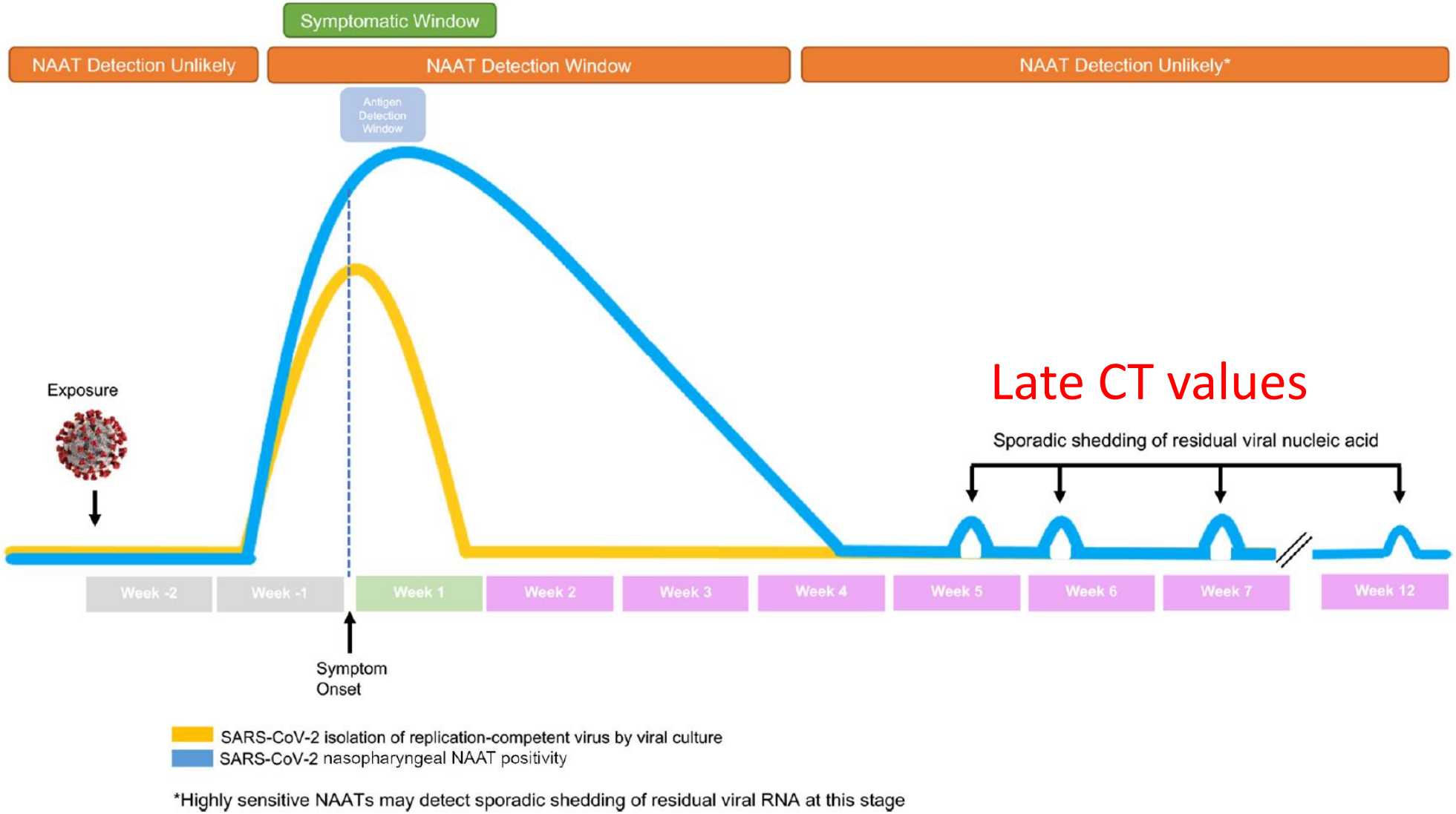
影響CT值的因子

IDSA Infectious Diseases Society of America

AMP Association for Molecular Pathology

Patient factors	Specimen factors	Test factors
Presence or absence of symptoms	Adequacy of specimen collection	Volume of sample subjected to testing
Severity of symptoms	Reproducibility of the specimen collection method	Gene target
Time from symptom onset	Specimen type (e.g., nasal swab, saliva, BAL)	PCR primer and probe design, which may be variably affected by emerging viral variants
Immune status	Dilution of the sample in transport medium or other liquid	Nucleic acid extraction efficiency (note, not all tests include a nucleic acid purification and extraction step to remove potential PCR inhibitors in the specimen)
Age	Specimen transport and storage conditions	Gene target amplification efficiency
		PCR instrument parameters and settings

SARC-CoV-2 viral load kinetics and nucleic acid detection

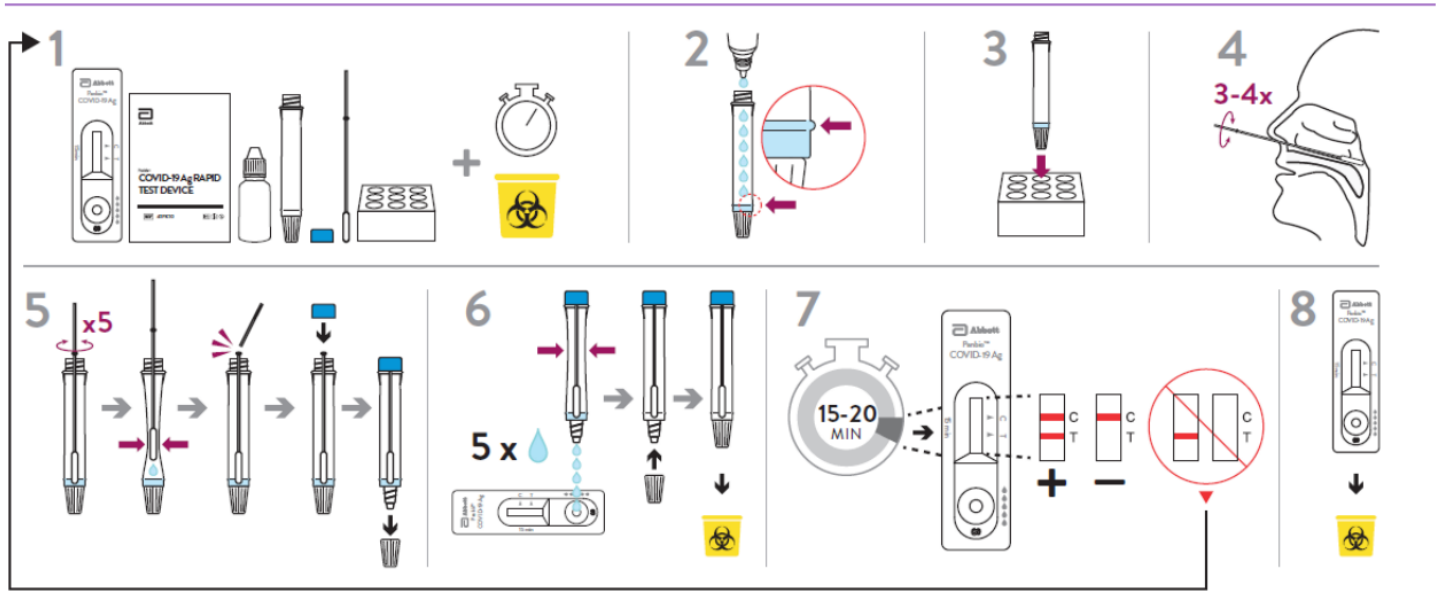


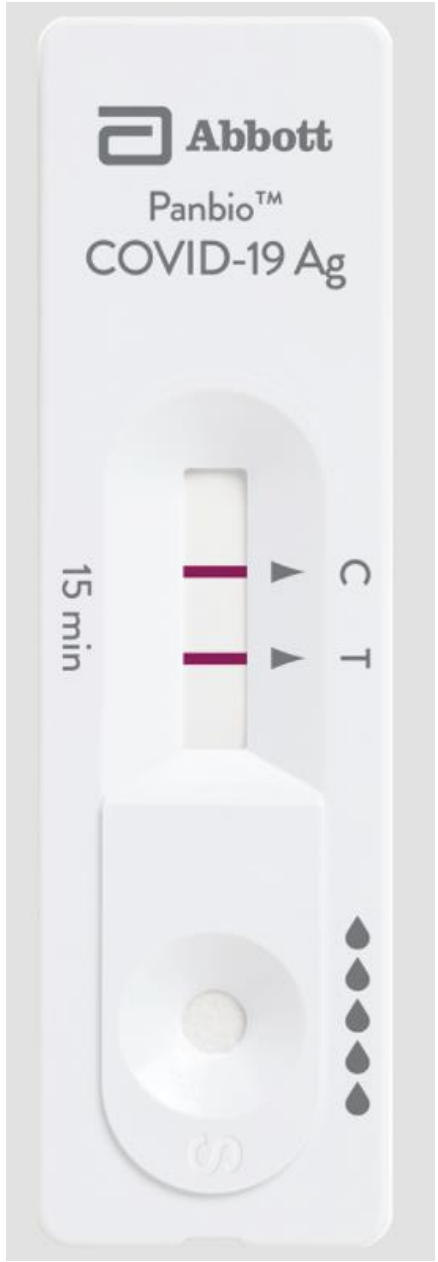
Stephanie L. Mitchell, Michael J. Loeffelholz, Journal of Clin Microbiol 2022 Jun

快速抗原檢測工具

對於疑似患者的快速篩檢工具

- 操作簡易(有居家試劑)、快速
- 需要時建議進一步以核酸檢驗確認





症狀發生後篩檢 抗原快篩的表現都不錯!

	Sample type	Time of sample collection*	Result reading	Sensitivity, specificity†	Comments
Abbott BinaxNOW, USA	Nasal swab	0–7 days	Visual, 15 min	97%, 99%	WHO Emergency Use Listing; US FDA Emergency Use Authorization; app for results; influenza A and B tests available
Abbott Panbio, USA	Nasal swab, nasopharyngeal swab	0–7 days	Visual, 15–20min	93%, 99%	WHO Emergency Use Listing; US FDA Emergency Use Authorization pending
Access Bio CareStart, USA	Nasal swab, nasopharyngeal swab	0–5 days	Visual, 15–20min	88%, 100%	US FDA Emergency Use Authorization
BD Veritor, USA	Nasal swab	0–5 days	Instrument, 30 min	84%, 100%	US FDA Emergency Use Authorization
LumiraDx, UK	Nasal swab	0–12 days	Instrument, 12 min	98%, 97%	US FDA Emergency Use Authorization
Quidel Sofia SARS Antigen Fluorescent Immunoassay, USA	Nasal swab, nasopharyngeal swab	0–5 days	Instrument, 20 min	97%, 100%	US FDA Emergency Use Authorization; does not differentiate between SARS-CoV and SARS-CoV-2
Quidel Sofia Flu and SARS Antigen Fluorescent Immunoassay, USA	Nasal swab, nasopharyngeal swab	0–5 days	Instrument, 20 min	95%, 100%	US FDA Emergency Use Authorization
SD Biosensor, South Korea	Nasal swab, nasopharyngeal swab	Not stated	Visual, 15–30min	97%, 100%	WHO Emergency Use Listing

Data from the Foundation for Innovative New Diagnostics.[2] SARS-CoV=severe acute respiratory syndrome coronavirus. FDA=Food and Drug Administration. *Days after symptom onset. †Data from manufacturers.

Rosanna W Peeling,et al. Lancet Infect Dis 2021

抗原快篩與PCR的比較
- 三總經驗

- 2,096 samples
- Sensitivity and specificity:
  - 76.39% [95%CI, 64.91–85.60%] and 99.26% (95% CI 98.78–99.58%)

Sensitivity of the Coronavirus Disease 2019 (COVID-19) Rapid Antigen Test Kit (ART) by reverse transcription polymerase chain reaction (RT-PCR) cycle threshold (Ct) intervals.

		n	ART positive	Sensitivity % (95% CI)	ART negative	False-negative rate (%)
RT-PCR Ct value	≤20	39	39	100 (88.8–100)	0	0
	20–≤ 25	19	12	63.15 (38.63–82.77)	7	36.84
	25–<30	14	4	28.57 (9.58–57.99)	10	71.43

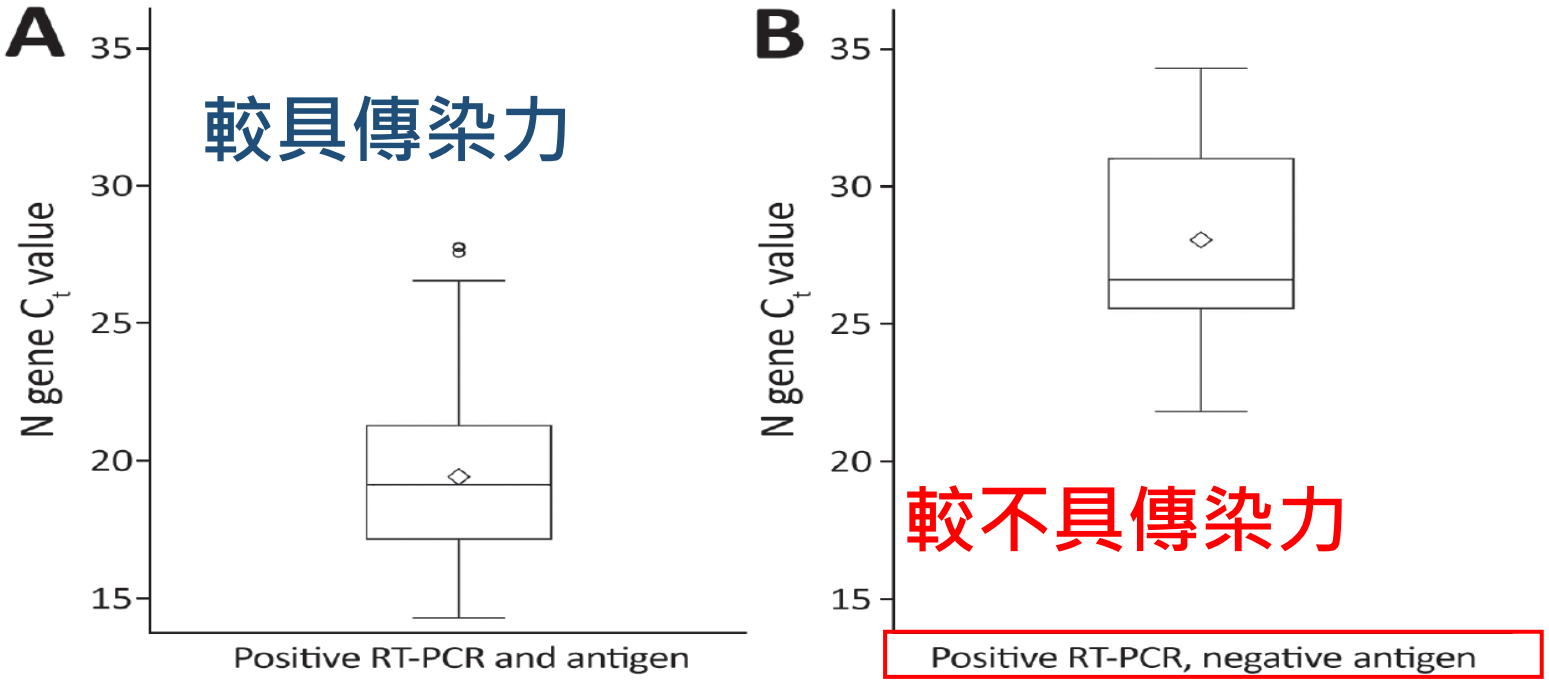
Jian MJ, et al. International Journal of Infectious Diseases 2022

抗原快篩、PCR及病毒培養的關係

Table 1. Positive predictive value of the BinaxNOW COVID-19 Antigen Card Test and RT-PCR relative to viral culture, Winnebago County, Wisconsin, USA, November–December 2020*

SARS-CoV-2 diagnostic test result	No. culture positive	No. culture negative	Total	Positive predictive value, %
BinaxNOW positive	191	78	269	71.0
RT-PCR positive	200	134	334	59.9

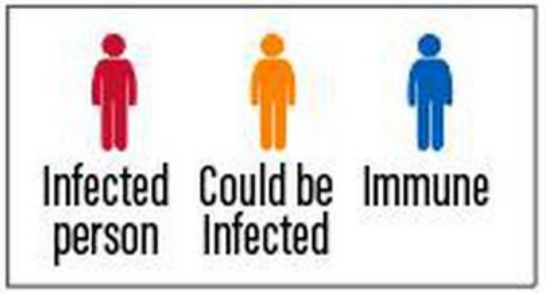
*BinaxNOW, https://www.abbott.com. RT-PCR, reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

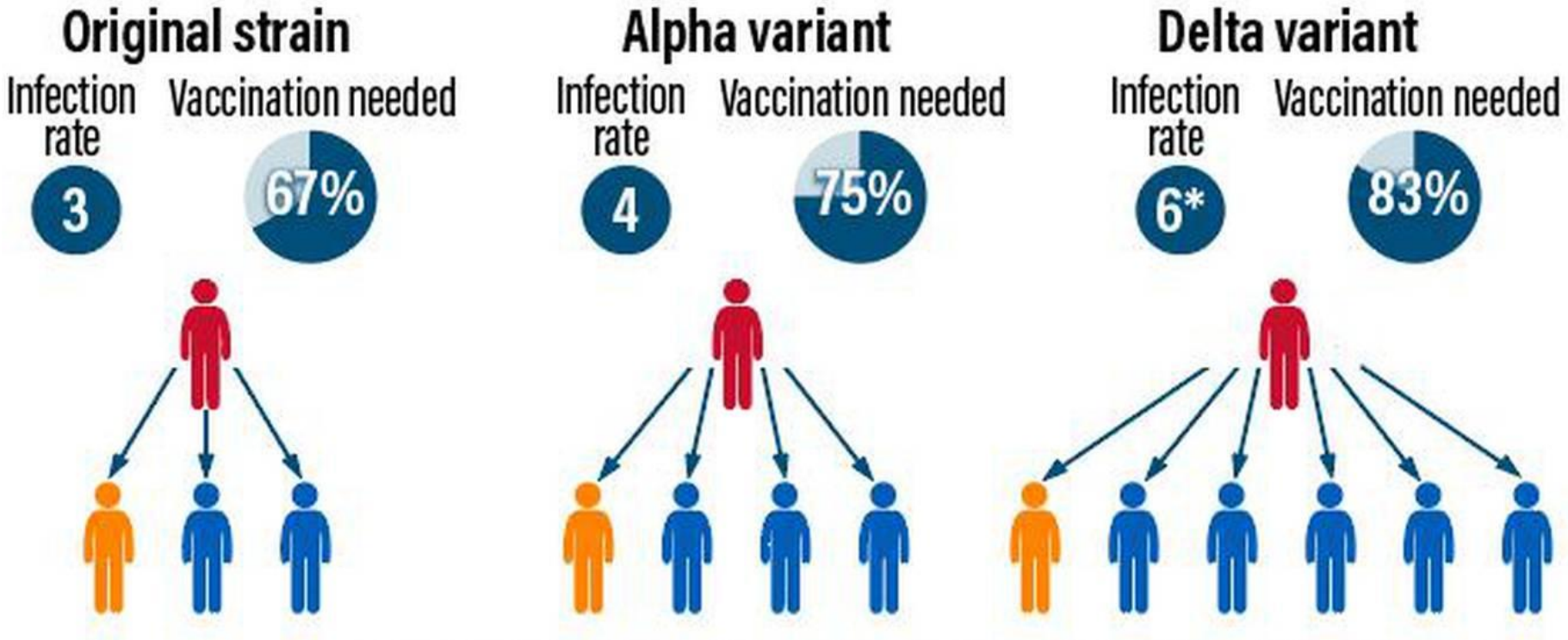


Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 28, No. 3, March 2022

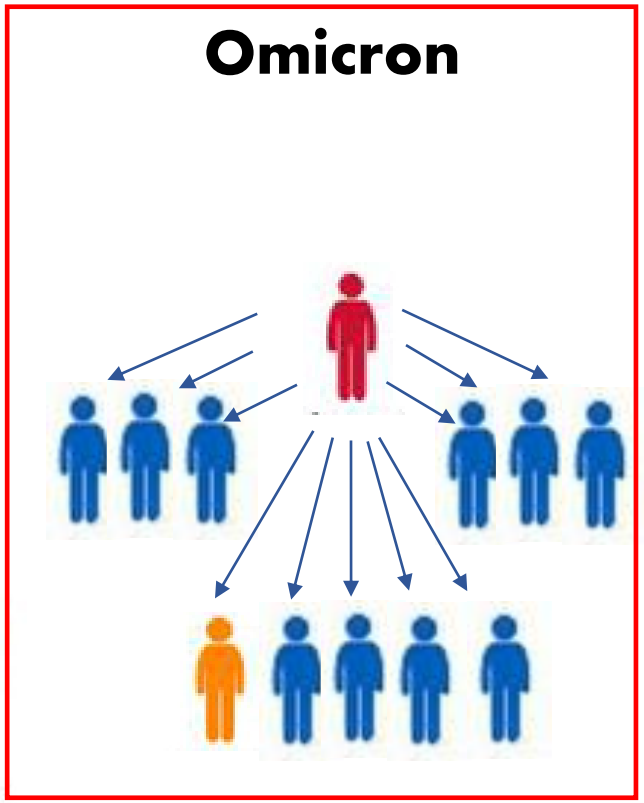
HOW DELTA VARIANT AFFECTS HERD IMMUNITY

The Delta variant is more highly infectious than the original strain of Covid-19 or the Alpha variant. This means that the goal of herd immunity is more difficult, as more people must be vaccinated to ensure the virus cannot spread further





*According to latest estimates, and assuming no lockdown or social distancing measures are in place



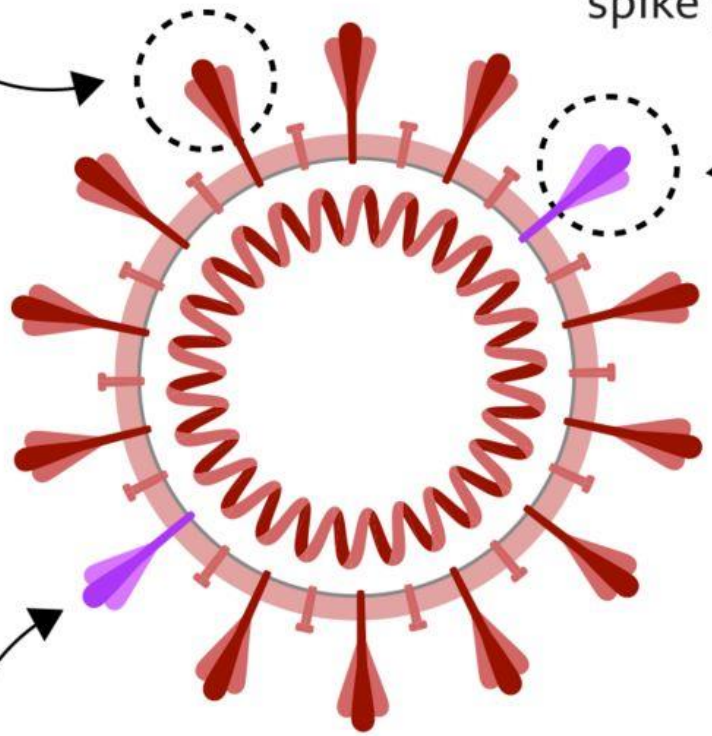
Talha Khan Burki , Lancet Respir Med 2021

The new Covid-19 variant: B.1.1.529

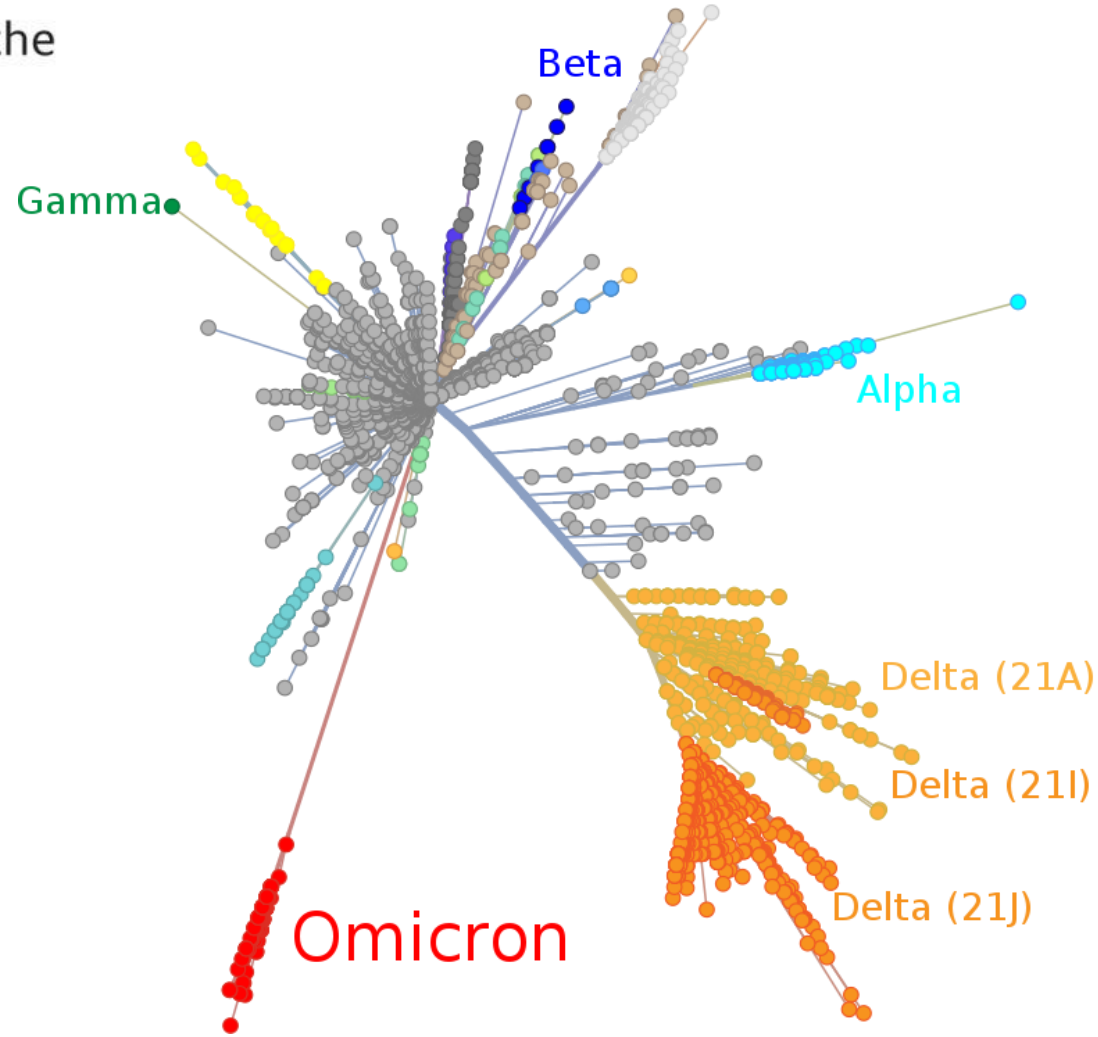
More mutations may make it spread faster

Spike protein helps the virus enter human cells

New variant has 32 mutations on the spike protein



New variant has 10 mutations on the 'receptor binding domain' - which gains entry to cells

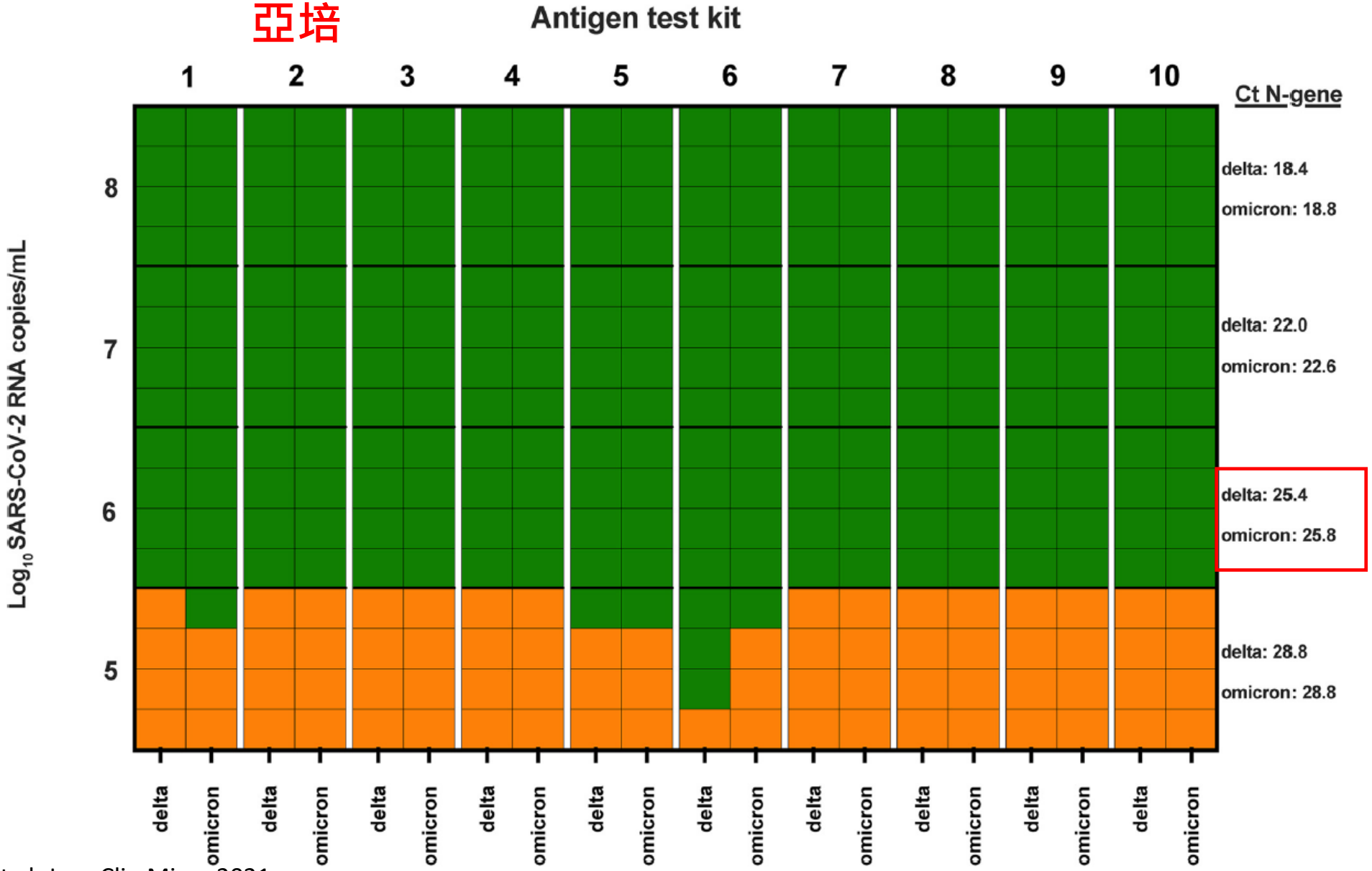


Source: South Africa Centre for Epidemic Response and Innovation

BBC

目前市面上抗原快篩的表現

亞培



Joshua Deerain, et al. Jour Clin Micro 2021

Omicron大流行- 快篩仍準確

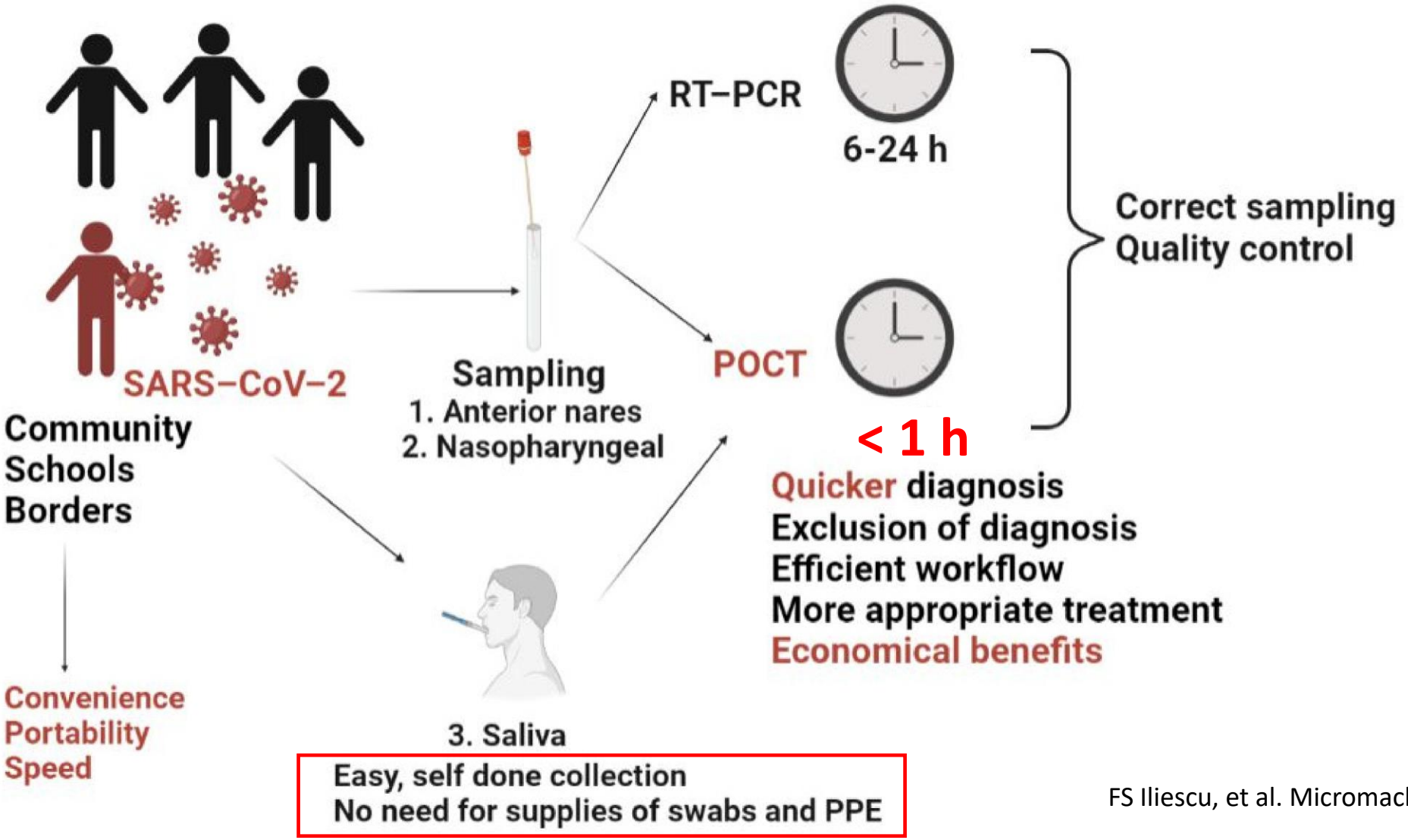
- 西班牙觀察性研究 (n=244):
- specificity and sensitivity was 100% and 81.8%, respectively.

Overall sensitivity of the Panbio™ COVID-19 Ag rapid test device according to the SARS-CoV-2 RNA load in nasopharyngeal specimens.

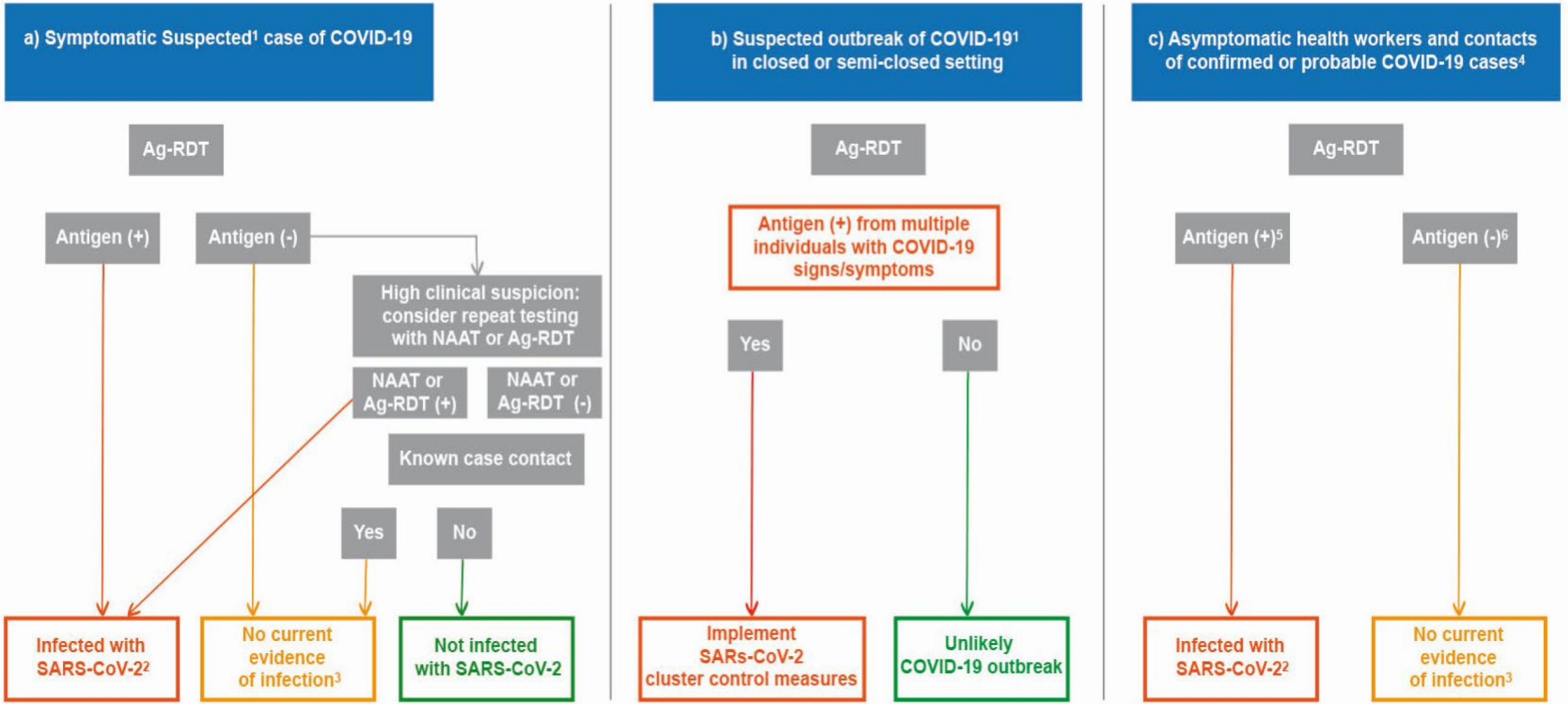
RT-PCR cycle threshold value	SARS-CoV-2 RNA load (log_{10} copies/ml)	Sensitivity (95% CI)
≤ 20	≥ 7.5	95.6 (89.2–98.3)
≤ 25	≥ 5.8	92.6 (86.6–96.1)
≤ 30	≥ 4.3	87.2 (80.7–91.8)
≤ 35	≥ 2.7	81.8 (75–87.1)

P. de Michelena, I. Torres, Journal of Infection 84 (2022) e64–e66

Point-of-Care Testing advantages for the diagnosis and surveillance of SARS-CoV-2 infection



FS Iliescu, et al. Micromachines 2021



* The results of Ag-RDTs will be most reliable in areas where there is ongoing community transmission (≥5% test positivity rate)

Ag = antigen, NAAT = nucleic acid amplification test.

WHO: Antigen-detection in the diagnosis of SARS-CoV-2 infection: Interim guidance

核酸及抗原檢測的比較 (症狀發生兩星期內)

	原理	優點	缺點
核酸檢測	偵測病毒的核酸片段 (黃金準則)	• 高敏感性及特異性的檢驗方法	• 技術門檻高 • 需要特定的設備及實驗室要求 • 檢驗時間較久
抗原檢測	偵測病毒抗原	• 快速提供結果 • 便宜 • 不一定需要專門的技術人員執行	• 敏感性較核酸檢驗差 • 檢驗品質不易掌握 (尤其居家快篩)

結語

- 沒有完美的檢測，除了檢驗試劑本身的表現，我們必須考量檢測的時序性、檢測方式的可近性及負擔能力等等
  - 綜合檢驗前評估與檢驗後結果才能做出接近事實的預測
- 檢驗工具
  - PCR和抗原快篩都可用來做急性新冠感染的檢測工具
  - PCR:
    - 準確性高，可透過池化策略提升檢驗量能
    - 謹慎的判讀CT值結果
  - 抗原快篩
    - 對於高度懷疑的患者能提供快速且準確的檢驗結果