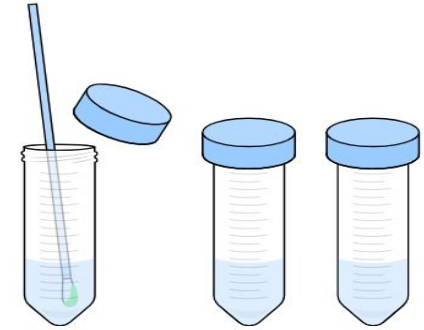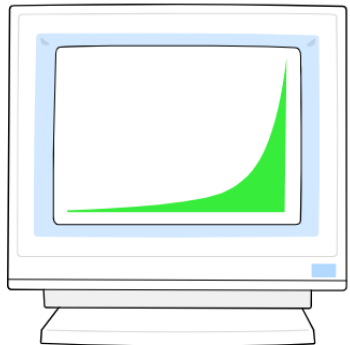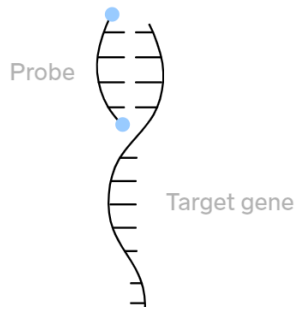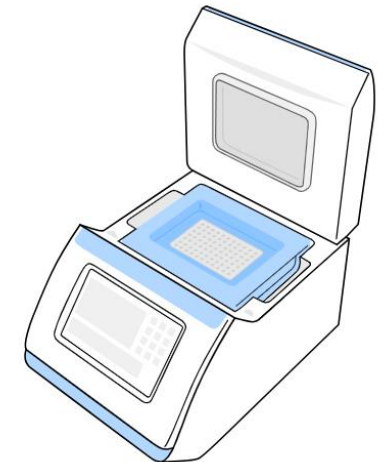In the name of God

COVID-19
CORONAVIRUS DISEASE 2019

Laboratory diagnosis for Covid-19 in Children

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Importance of Lab tests

- Epidemiological purpose
  - the main tools for the promotion, prevention, and control of infectious diseases
  - two main categories: immunological and molecular.

- Clinical purposes

Who should (and should not) be tested for SARS-CoV-2 infection?
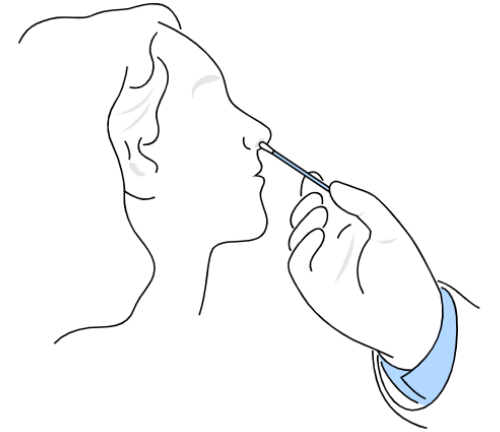
- **Patients with symptoms consistent with COVID-19**

- **Patients in close contact**
  - a distance of less than 6 feet for at least 15 minutes from a person with laboratory-con
  - Wearing a mask or cloth covering
    - They is presently not considered sufficient to alleviate the risk of transmission if close contact conditions are met. firmed or probable SARS-CoV-2 infection

- **Patients scheduled for an invasive medical procedure**
  - An elective surgery

Suggested priorities for SARS-CoV-2 (COVID-19) testing

Priority	IDSA guidance [1]
First/High priority	■ Critically ill patients receiving ICU-level care with unexplained viral pneumonia or respiratory failure (regardless of travel or exposure history) ■ Any individual (including health care workers) with fever or features of a lower respiratory tract illness **and** close contact with patients with laboratory-confirmed COVID-19 within 14 days of symptom onset (including all residents of long-term care facilities with a confirmed case) ■ Individuals with fever or features of a lower respiratory tract illness who are also immunosuppressed (including patients with HIV), older, or have underlying chronic health conditions ■ Individuals with fever or features of a lower respiratory tract illness who are critical to the pandemic response, including health care workers, public health officials, and other essential leaders
Second/Priority	■ Non-ICU hospitalized patients and long-term care residents with unexplained fever and features of a lower respiratory tract illness*¶

Suggested priorities for SARS-CoV-2 (COVID-19) testing

Third	■ Outpatients who meet criteria for influenza testing (eg, symptoms such as fever, cough, and other suggestive respiratory symptoms plus comorbid conditions, such as diabetes mellitus, chronic obstructive pulmonary disease, congestive heart failure, age >50 years, immunocompromising conditions); testing of outpatient pregnant women and symptomatic children with similar risk factors is also included in this priority level*
Fourth	■ Community surveillance as directed by public health and/or infectious diseases authorities

What is the outpatient testing criteria?

- varies according to test availability and other resources

- for symptomatic patients (eg, fever, persistent cough) who are otherwise stable but have either:
  - Underlying conditions (eg, immune compromise, chronic cardiac or pulmonary disease)

  - Known in-person exposure to a laboratory-confirmed case of COVID-19 within the previous 14 days

An underlying condition that may increase the risk for severe disease; examples include

1) An immune-compromising condition
   - (eg, recipients of antineoplastic chemotherapy, recent hematopoietic cell transplantation, solid organ transplant recipients, primary immunodeficiency, HIV infection with CD4 count <15 percent)
2) Chronic cardiac disease
   - (eg, cardiomyopathy, unrepaired cyanotic congenital heart disease, single ventricle physiology)
3) Chronic pulmonary disease
   - (eg, requirement for supplemental oxygenation or noninvasive ventilation, severe persistent asthma)
4) Former preterm infants
5) Neuromuscular disease with impaired airway clearance
6) Poorly controlled type I diabetes mellitus
7) Severe obesity
   - (body mass index ≥120 percent of the 95th percentile values

When should patients be tested after starting symptoms consistent with COVID-19 ?

a) without delay

b) 2-4 days

c) 5-7 days

d) After one week

a

When should asymptomatic patients with close contact exposure to infected persons be tested?

a) without delay

b) 2-3 days after exposure

c) At least 4 days after exposure

d) At least 7 days after exposure

The probability of a false-negative result in an infected person decreases substantially over the first 4 days after exposure.
Because symptoms develop 2 to 14 days following exposure and most commonly between 5 and 6 days

c

What means a single negative test in a close contact?

- A single negative test does not mean you will remain negative at any time point after that test.

- Even if there is a negative test, a person with a close contact should still self-isolate for 14 days.

Testing is *not* generally recommended for ....? (more than 1 response)

a) Exposure to a close contact who is asymptomatic

b) Exposure to a close contact who is symptomatic

c) Exposure to a close contact who subsequently tests positive

d) Symptomatic close contact who have previously tested positive within the past 3 months.

e) Symptomatic patients with COVID-19 for return to school/work clearance

a, d, e

Which one is the "gold standard" for testing an individual child for acute SARS-CoV-2 infection?
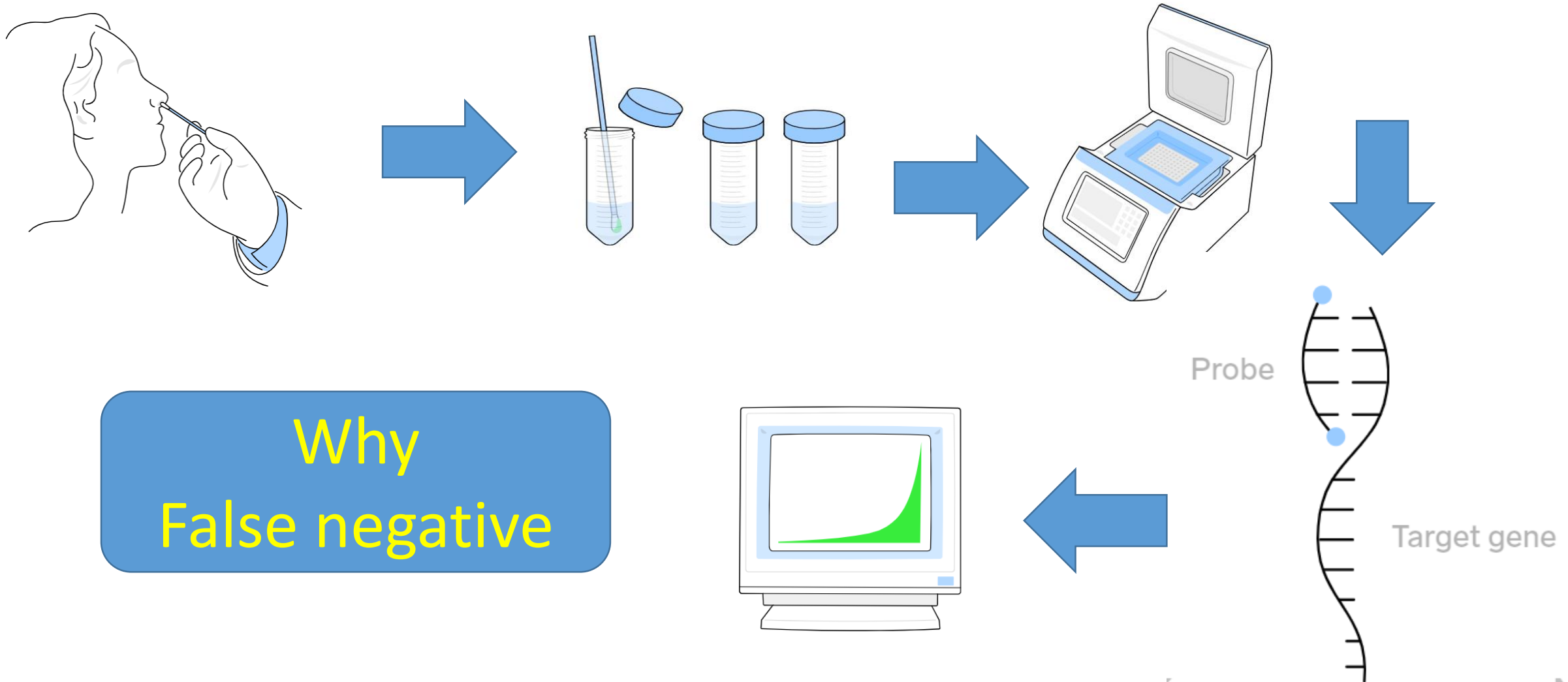
A. PCR tests

B. Antigen tests

C. Antibody tests

A

PCR test

Probe

Target gene

Why
False negative

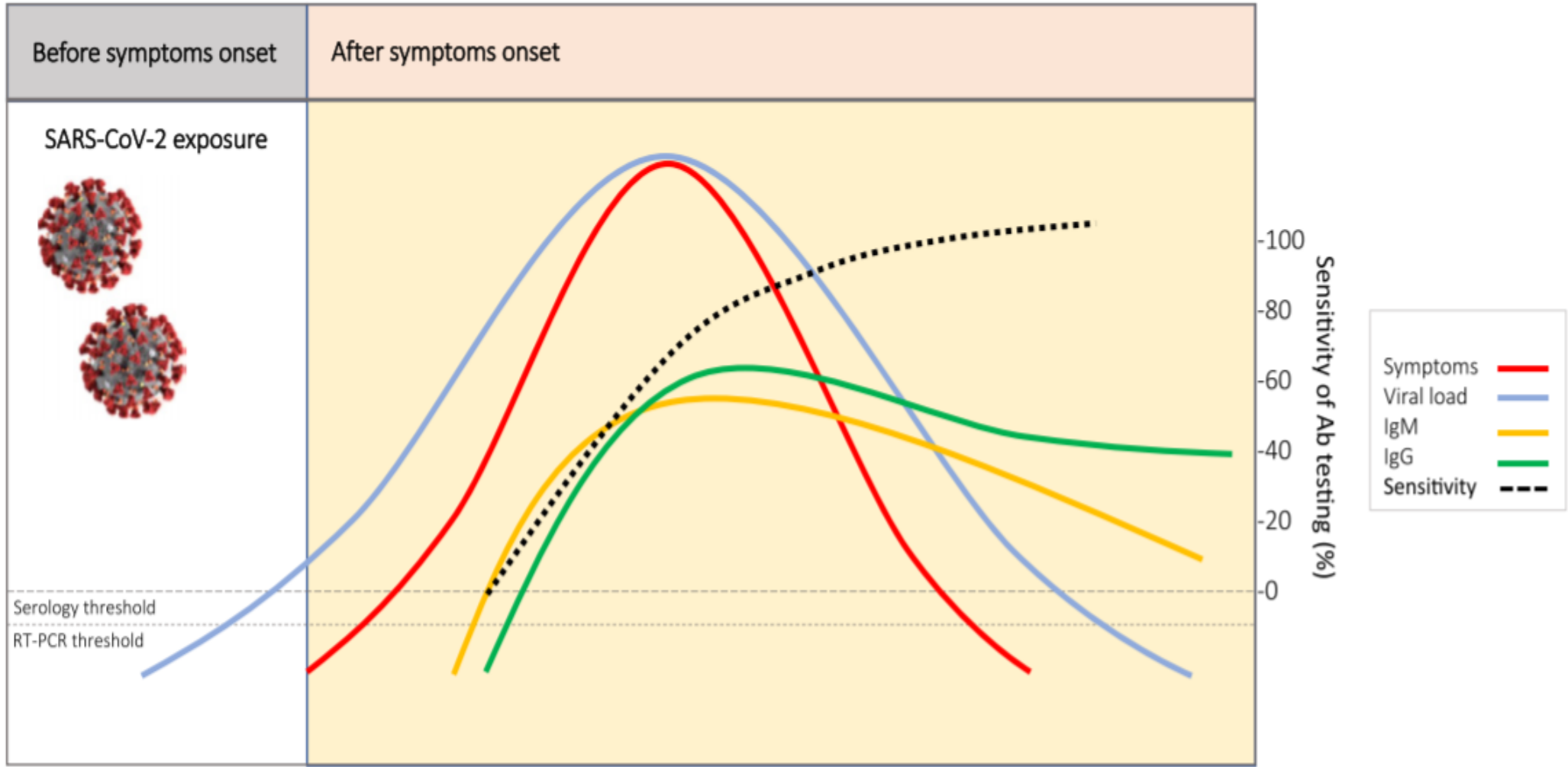Which one is the most often respiratory samples that PCR tests perform on it?

A. Throat swab

B. nasopharyngeal swab

C. Nasal swab

D. Sputum

B

RT-PCR performance by time since symptom onset or exposure

- The estimated rates of false-negative

  a) 100 % on the day of exposure

  b) 38 % on day 5 (estimated as the first day of symptoms)

  c) 20 % at day 8

  d) 66 % at day 21

Antigens tests

1) Tests that detect SARS-CoV-2 antigen
2) more accessible with a faster time to results than some molecular studies

- WHO advise:
a) A minimum sensitivity ≥80 percent and specificity ≥97 percent,
b) within the first five to seven days of symptoms

Which is <u>not correct</u> about a**ntigen tests** ?

a) They generally performed on nasal or oral swab specimens

b) They are the point-of-care test.

c) They generally have lower sensitivity than molecular tests.

d) The positive test results are generally reliable.

e) A negative test result will often suffice for return to school.
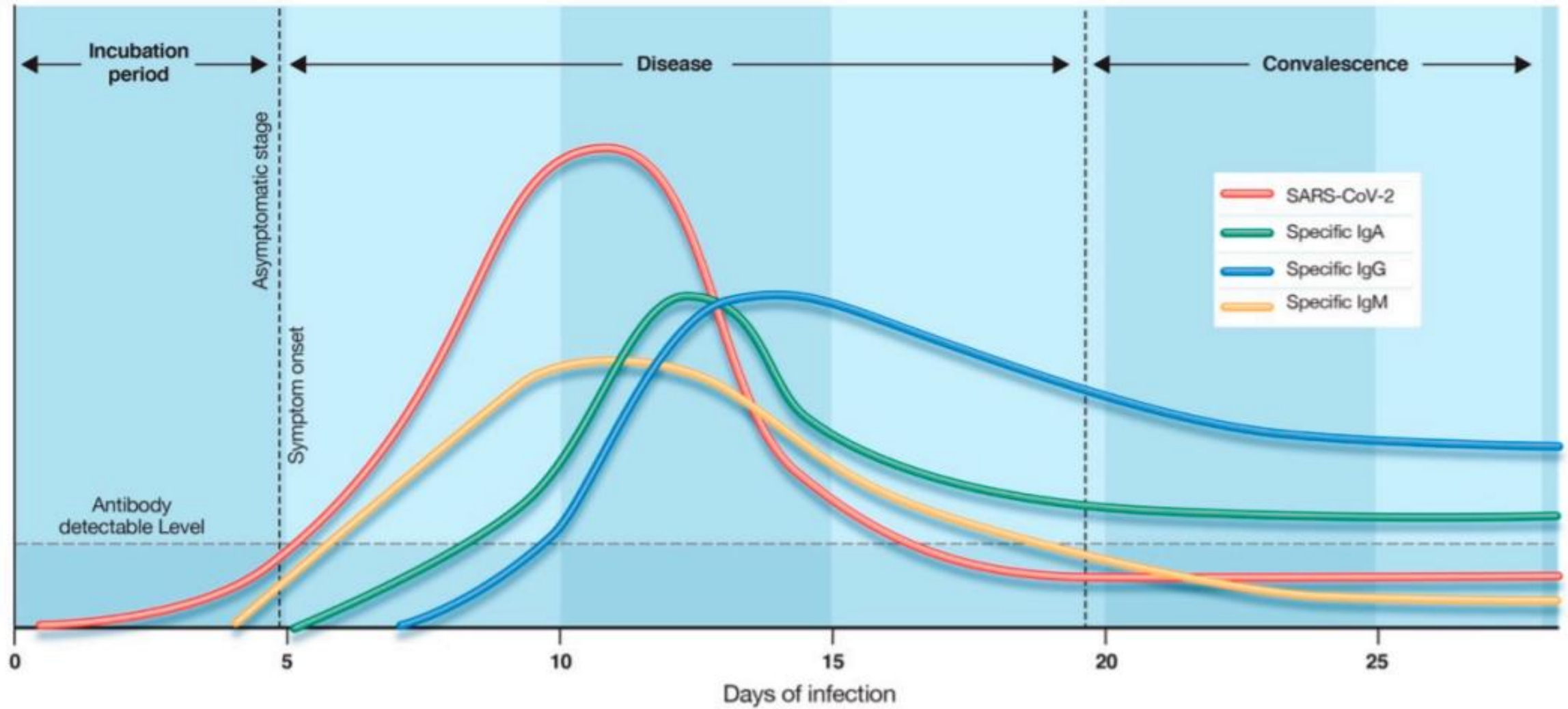
e

Antibody tests

Figure 4. Time kinetics of antibody response in coronavirus disease 2019 (COVID-19). The illustration

Antibody rests: Methods

- Enzyme-linked immunosorbent assay (ELISA)

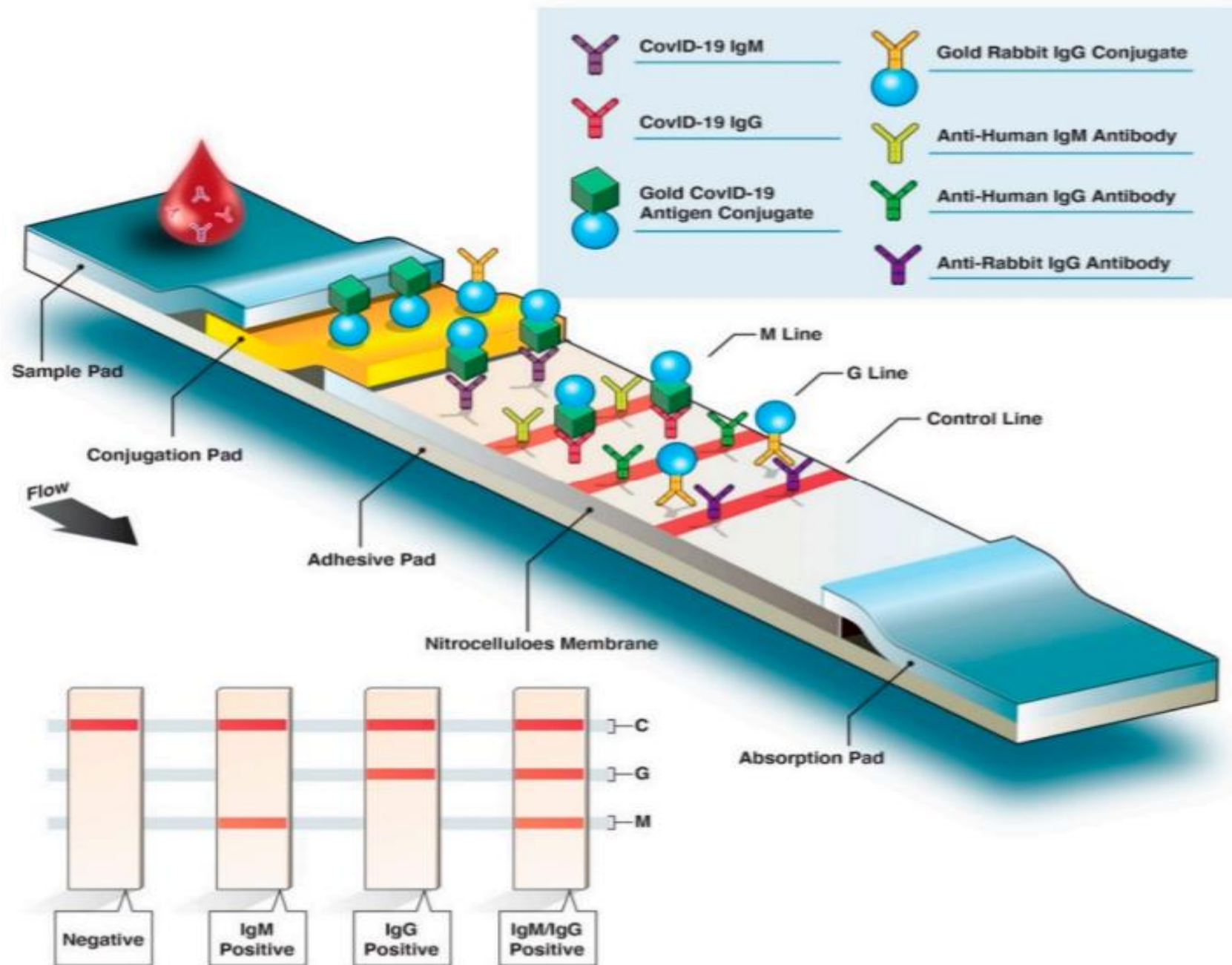- Lateral flow immunochromatography (LFI)

Figure 2. Overview of rapid diagnostic serological test. Rapid diagnostic tests (RDTs) are typically

Antibodies tests

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

- Sensitivity of IgM :
- by one week: 23 percent
- by two weeks: 58 percent
- by three weeks: 75 percent

Sensitivity of IgG :
by one week: 30 percent
by two weeks: 66 percent
by three weeks: 88 percent

- Other studies have suggested that the rate of positive IgG approaches 100 percent by 16 to 20 days

Antibody tests: specificity

- IgM and IgG antibodies detected on ELISA have more than 95% specificity in the diagnosis of COVID-19

- High titers of IgG antibodies detected by ELISA demonstrate a positive correlation with neutralizing antibodies.

Antibody tests

- FDA has not authorized using antibody tests to diagnose SARS-CoV-2 infection

- CDC does not currently recommend using antibody testing as the sole basis for diagnosis of acute infection.

In certain situations, serologic assays may be used to support clinical assessment

1. Patient present late in their illnesses when used in conjunction with viral detection tests

2. If a person is suspected to have a post-infectious syndrome caused by SARS-CoV-2 infection

The duration of detectable antibodies is uncertain.

- Detectable IgG at **8 weeks following infection :** Negative
  - **40** percent of asymptomatic patients
  - **13** percent of symptomatic patients

- Another study from Iceland:
  - Total Ig antibody tests were reactive in 90 percent
  - with titers increasing over the first two months after diagnosis
  - remaining steady for another two months

What type of personal protective equipment (PPE) do I need to use when I conduct testing?

- At a minimum, the use of gloves, face mask, and eye protection is necessary for all specimen handling and collection.

- For patients for whom crying, gagging, or coughing is likely; or for patients who are otherwise deemed as higher risk, such as high suspicion of SARS-CoV-2 infection based on household contacts, the addition of **gowns and N95 masks** is encouraged.

Should I additionally test for influenza during flu season?

- The range of symptoms for influenza and COVID-19 are similar
  - It is not possible to differentiate these two viral syndromes on the basis of symptoms alone

- Coinfection is entirely possible.

Coinfection was detected in 5.6 percent

- Detection of other respiratory pathogens (does **not** exclude COVID-19

- In a systematic review of COVID-19 in 1183 children from 26 countries
  - a) *M. pneumoniae* was most common (58 percent of coinfections)
  - b) Influenza (11 percent)
  - c) RSV (9.7 percent)

Other **Laboratory findings**

- The complete blood count
  - normal in most children
  - 17 %: Leukopenia
  - 13 %: neutropenia or lymphocytopenia

- C-reactive protein (CRP, >5 mg/L) and Procalcitonin (defined as >0.5 ng/mL)
  - Elevated in one-third

-

- Elevated inflammatory markers and lymphocytopenia may indicate multisystem inflammatory syndrome in children (MIS-C)

- Serum aminotransferases : elevated in 12 %

- Creatine kinase (CPK): elevated in 15 %

فاکتورهای پیش بینی کننده آزمایشگاهی پیشرفت بیماری در فرد بستری

یافته های آزمایشگاهی
• افت پیشرونده تعداد لنفوسیتها نسبت به تست پایه با و یا بدون انمی و ترومبوسیتوپنی. • افزیش ۵۰ درصدی در LDH و یا CRP نسبت به تست پایه. • کاهش GFR به زیر ۵۰ ml/min
※ موارد زیر هم پیش بینی کننده پیشرفت بیماری است و در صورت درخواست مورد توجه قرار گیرد: • Troponin • PT, PTT, INR • High ferritin (Ferritin > 500 ug/L) • D-dimer(>1000 ng/ml[1])

Initial labs

- CBC with differential
- CRP; ESR
- D dimer
- PT / INR, PTT
- Fibrinogen

- LDH
- Troponin
- Procalcitonin *
- Ferritin
- VBG
- Serum Lactate

<div dir="rtl">

جدول ۲- آزمایشات اولیه

</div>

Predictors	Predictive factor	Cut off	Score
Lab Tests	Lymphopenia	According to COVID-19 algorithm in children*	2
	LDH**	>500	2
	or	245-500	1
	CRP	>70mg/L	2
		40-70mg/L	1

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*لنفوپنی عبارتست از میزان لنفوسیت کمتر از ۳۰۰۰ برای سنین ۱-۱۲ ماه ، کمتر از ۲۰۰۰ برای سنین ۱ تا پنج سال و کمتر از ۱۱۰۰ برای سنین بالاتر از پنج سال.

**مقادیر LDH که در جدول آمده است برای سنین بعد از دوره نوزادی قابل قبول است.

</div>

جدول ۳- آزمایشات بیماران بستری در بیمارستان

	آزمایش های روتین
روز اول بستری (درخواست این آزمایشات و تکرار آن می تواند بر اساس نیاز فرد و به صلاحدید پزشک و متناسب با بیماری زمینه ای فرد نیز باشد): CBC-diff, ESR, Quantitative CRP or LDH, SGOT, CPK, SGPT, BUN/Cr, ALP, B, Ca, Mg, Na, K, P, U/A	
	آزمایشات زیر بر اساس شرایط بالینی بیمار ممکن است درخواست شود:
PT, PTT, INR D-dimer, LDH, Ferritin, ABG, Fibrinogen, IL6, NT-proBNP B/C (در صورت شک به عفونت باکتریال) Procalcitonin (شک به عفونت ثانویه باکتریال) Urine Prot/Cr	

Age-related lymphopenia defines as below:

- Between2-6 years less than 1800 (microlit), and
- Over six years less than 1,500 (109/lit) ;

رادیوگرافی قفسه سینه

- حساسیت عکس قفسه سینه در مراحل اولیه بیماری و یا در بیماری خفیف کم است.

- با پیشرفت بیماری تا ۷۰ درصد بیماران در بدو بستری و تا ۸۰ درصد در حین بستری غیر طبیعی می شوند.

- یافته های شایع :

- airspace opacities
   - ✔ به شکل Consolidationیا
   - ✔Opacity Ground Glass (GGO)

- عمدتا دو طرفه، محیطی و در قسمت های تحتانی ریه

- پیشرفت بیماری: White Lung
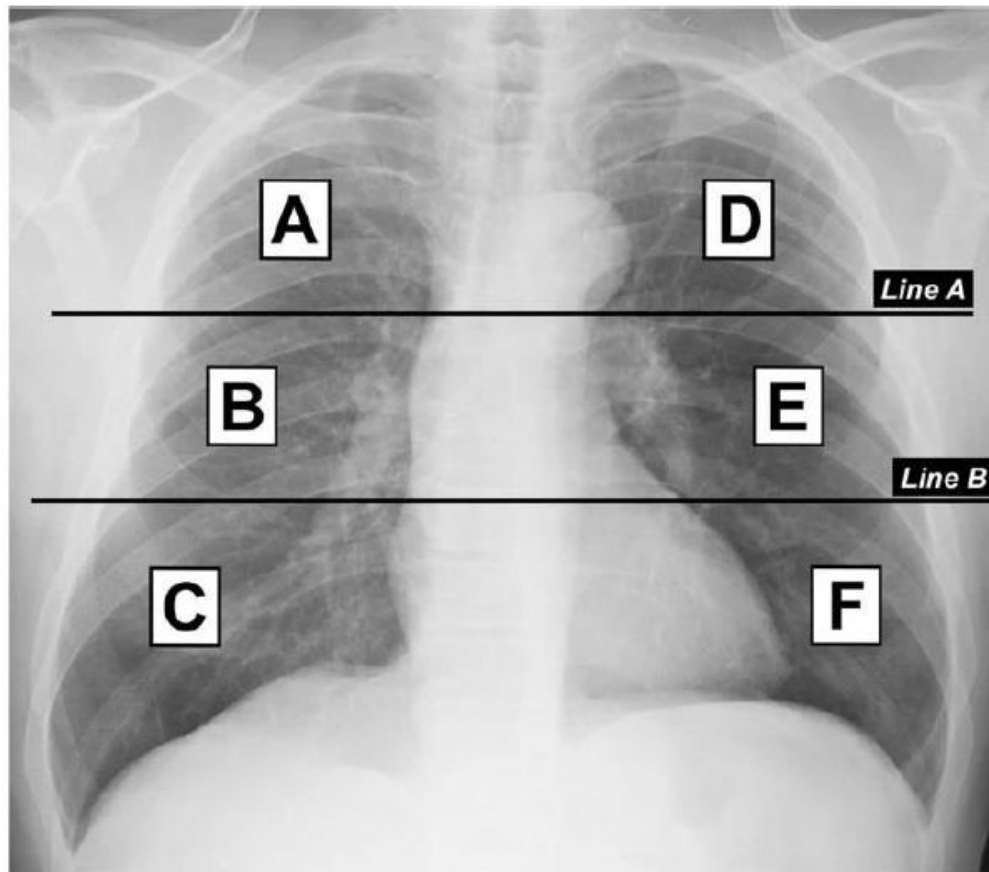
CXR ریه به ٦ منطقه تقَسیم می شود. خط A در سطح دیواره تحتَانی قوس آئورت و خط B به موازات سطح تحتَانی ناف ریه چپ رسم می شود. A و D منطقه فوقانی، B و E منطقه میانی، C و F منطقه تحتَانی.

https://link.springer.com/article/10.1007/s11547-020-01200-3

امتیاز صفر= طبیعی (No lung abnormalities)

امتیاز ۱= اینفیلتراسیون بینابینی (Interstitial infiltrates)

امتیاز ۲= اینفیلتراسیون بینابینی و آلوئولار (با ارجحیت بینابینی) Interstitial and alveolar infiltrates (interstitial predominance)

امتیاز ۳= اینفیلتراسیون بینابینی و آلوئولار (با ارجحیت آلوئولار) Interstitial and alveolar infiltrates (alveolar predominance)

سی تی اسکن ریه

- **-حساسیت بالا**

- GGO (Ground Glass Opacity) **مولتی فوکال** **یک طرفه یا دوطرفه**

- **موقعیت پریفرال، ساب پلورال و یا پریوسکولار**

- **به شکل گرد یا با الگوی** Crazy paving

- **وجود** Halo Sign**و یا** Reverse Halo Sign

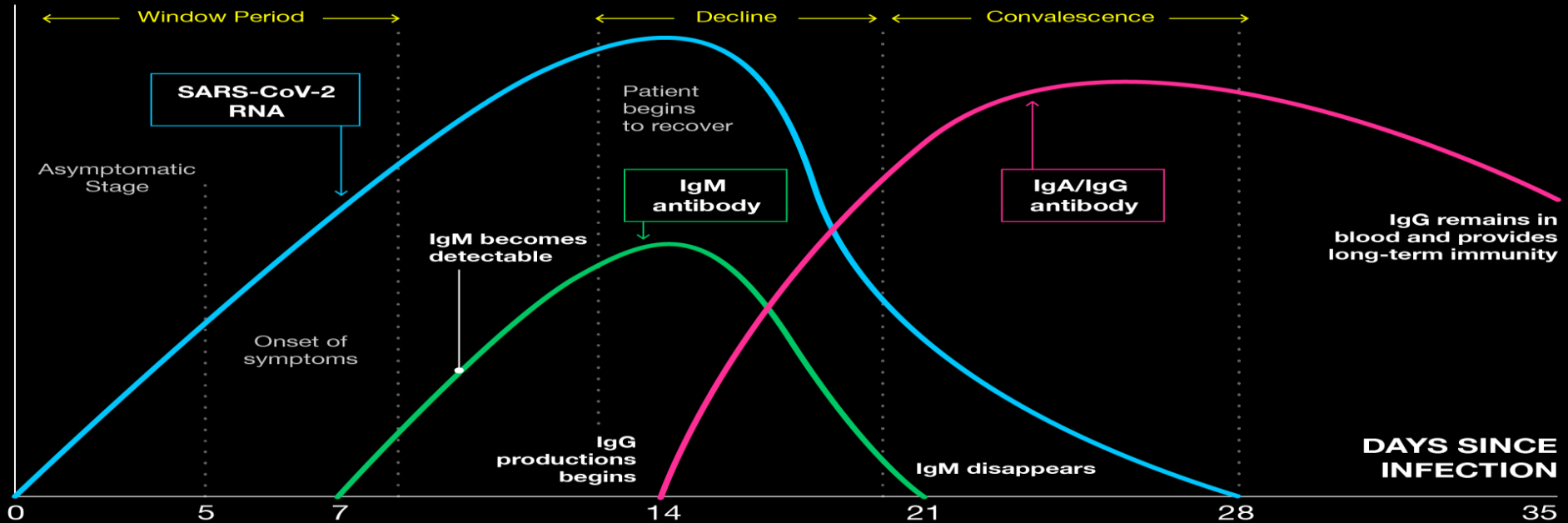# Crazy paving
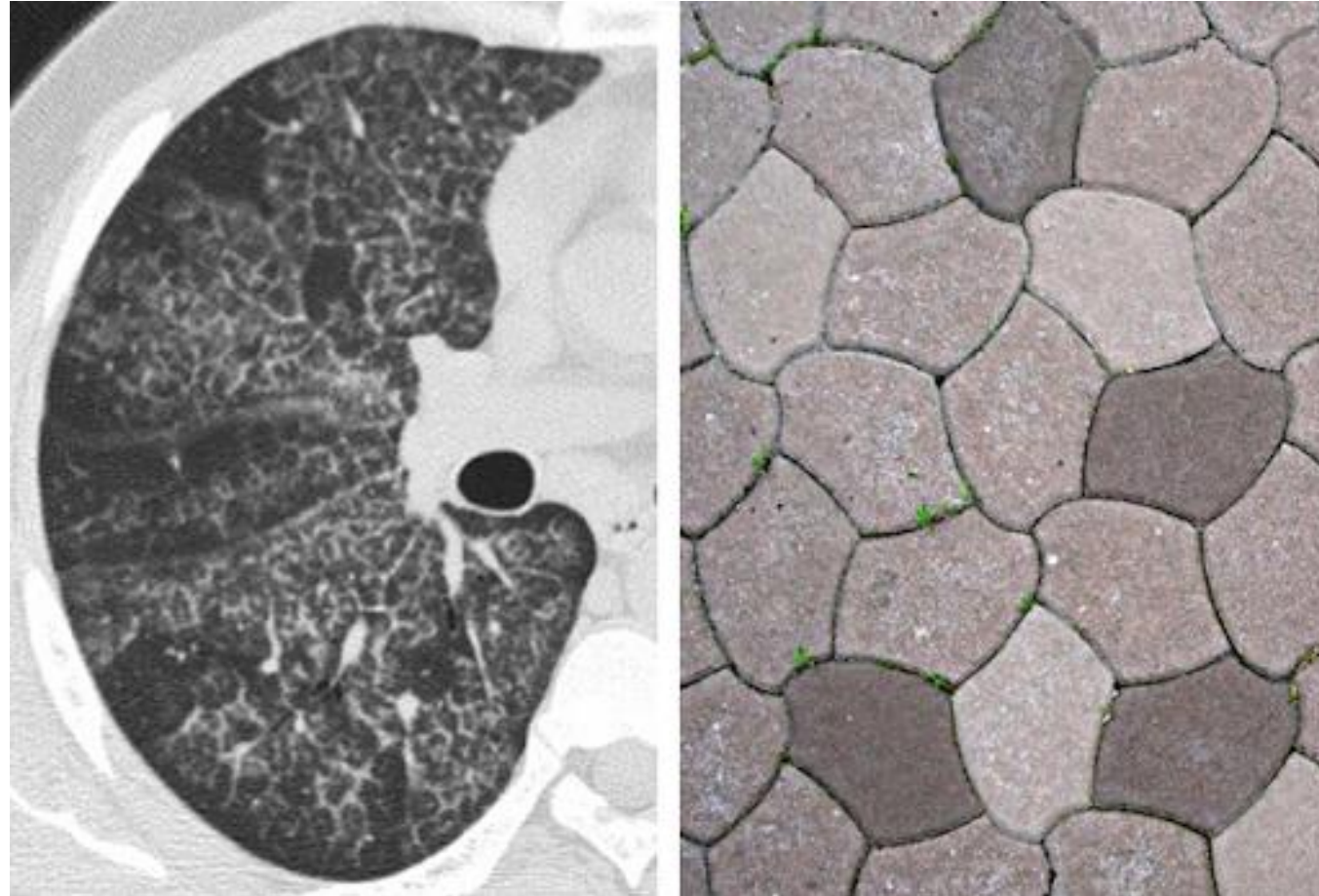
Halo Sign or Reverse Halo Sign
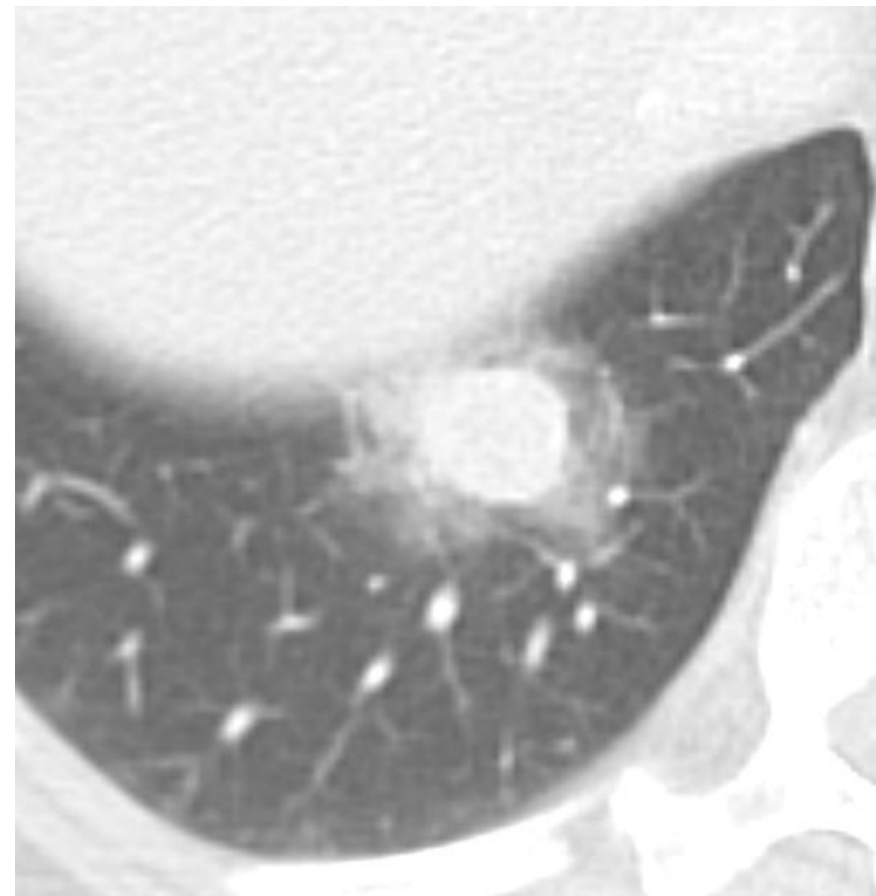
سی تی اسکن ریه

- کدورتهای Ground Glass معمولا در **روزهای اول** دیده می شود و با پیشرفت بیماری، Consolidation اضافه میشود.

- معمولا **لنفادنوپاتی** دیده نمیشود و **پلورال افیوژن** نادر و خفیف است.

- البته یک CT **نرمال** در اوایل رد کننده بیماری نیست

Thanks for your attention