



# Laboratory testing for COVID-19

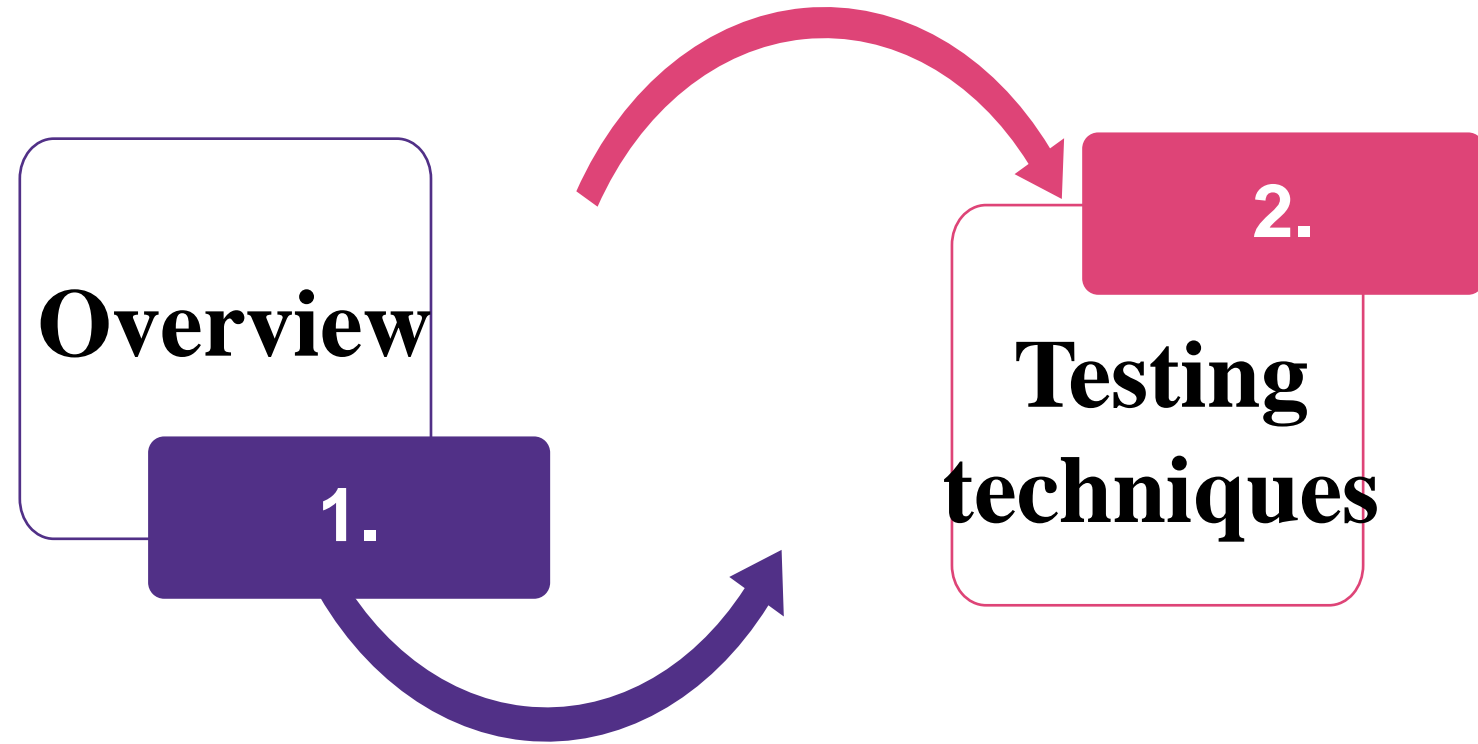
Emergency Response Technical Centre,

NIVD under China CDC

March 15<sup>th</sup>, 2020



# Table of content





# Overview

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# 1.1 The journey of discovery

In 1933, asthma attacks in chickens were discovered by Baudette et al. Infectious bronchitis virus or IBV was first isolated from chickens in 1937. Porcine transmissible gastroenteritis virus (TGEV)



## 1. Infectious bronchitis virus

## 2. B814 and HCoV-229E



In 1965, Tyrrell and Bynoe first used the human fetal nose and tracheal in vitro culture to isolate a strand of virus from the nasal wash of a common cold patient, which was named **B814**;

In 1966, Hamre and Procknow isolated a similar virus from human embryonic kidney cells and named the strain **229E**.

In 1967, McIntosh et al. isolated a batch of viruses from the human embryo trachea organ culture from an adult with a cold. OC43 was the major strand among them (organ culture, OC)



## 3. HCoV-OC43

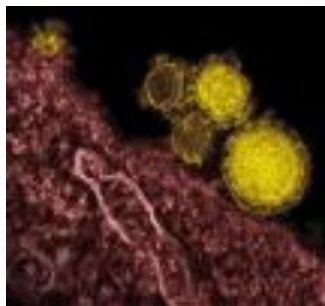
## 4. Discovery of SARS-CoV



2002, SARS-CoV;  
2004, NL-63;  
2005, HKU1  
2012, MERS-CoV  
2019, 2019-nCoV

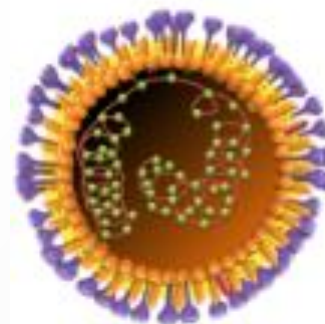


# 1.2 Naming



In 1968, June Almeida and Tyrrell performed morphological studies on these viruses. Electron microscopy observations revealed that the envelopes of these viruses had spikes that resemble the sun coronal, so they named these viruses **coronaviruses**.

01



In 1975, International Virus Nomenclature Committee or ICTV officially established Coronaviridae for the virus

02



# 1.3 Classification

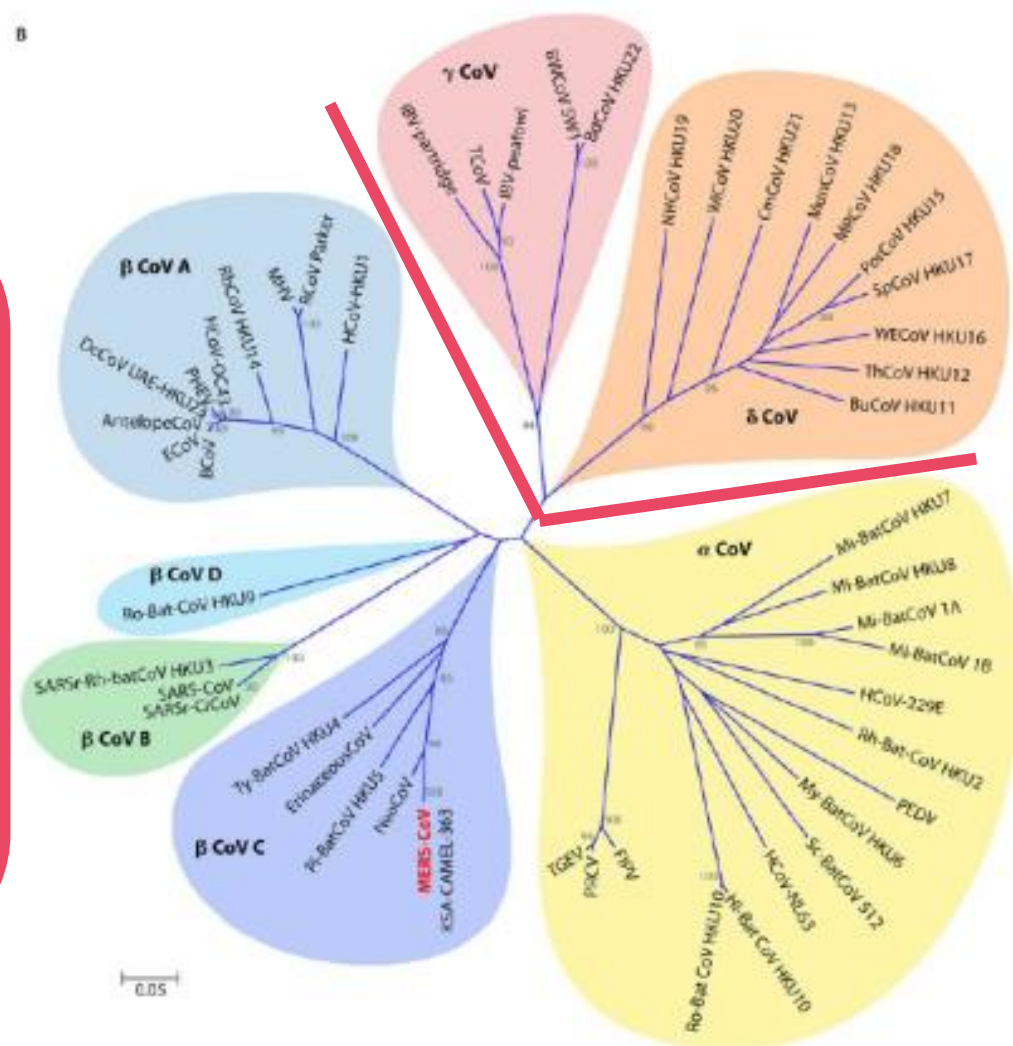
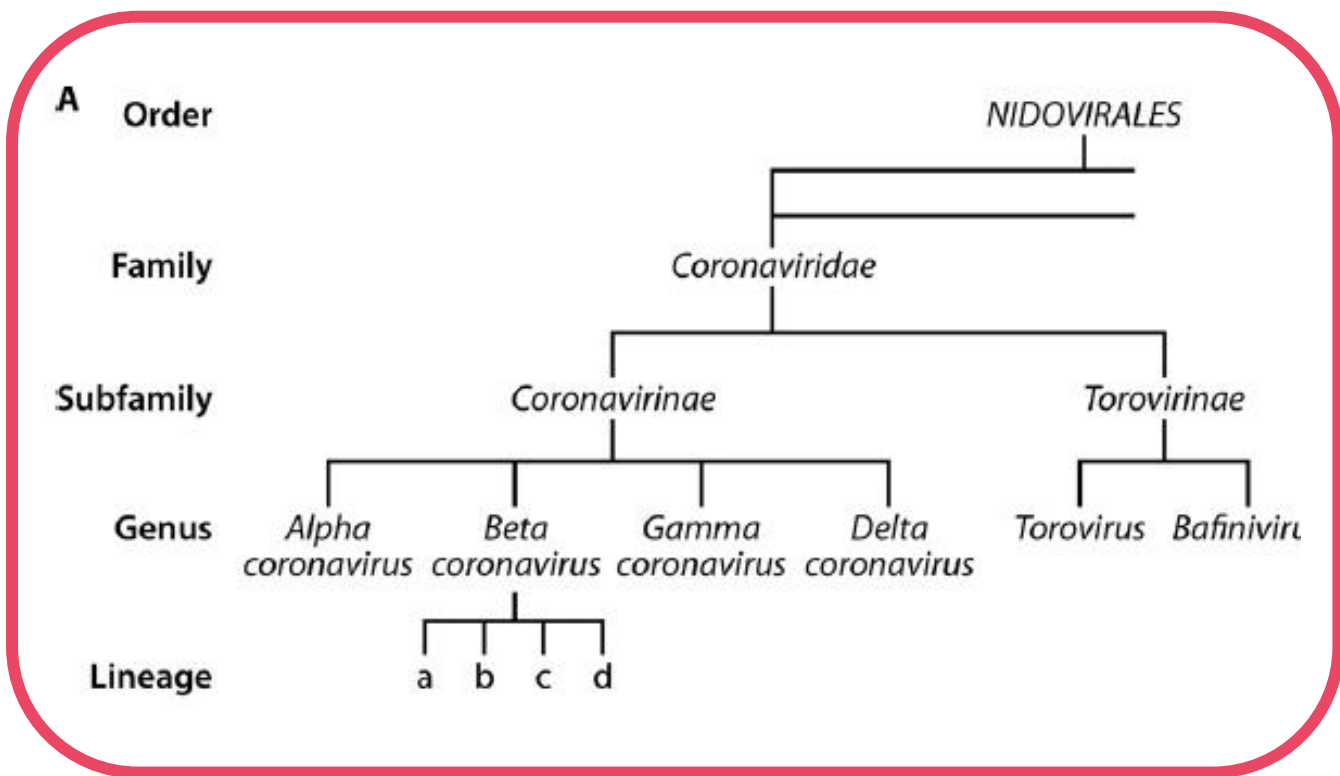


图2 根据RdRp部分序列对50种冠状病毒进行进化树分析

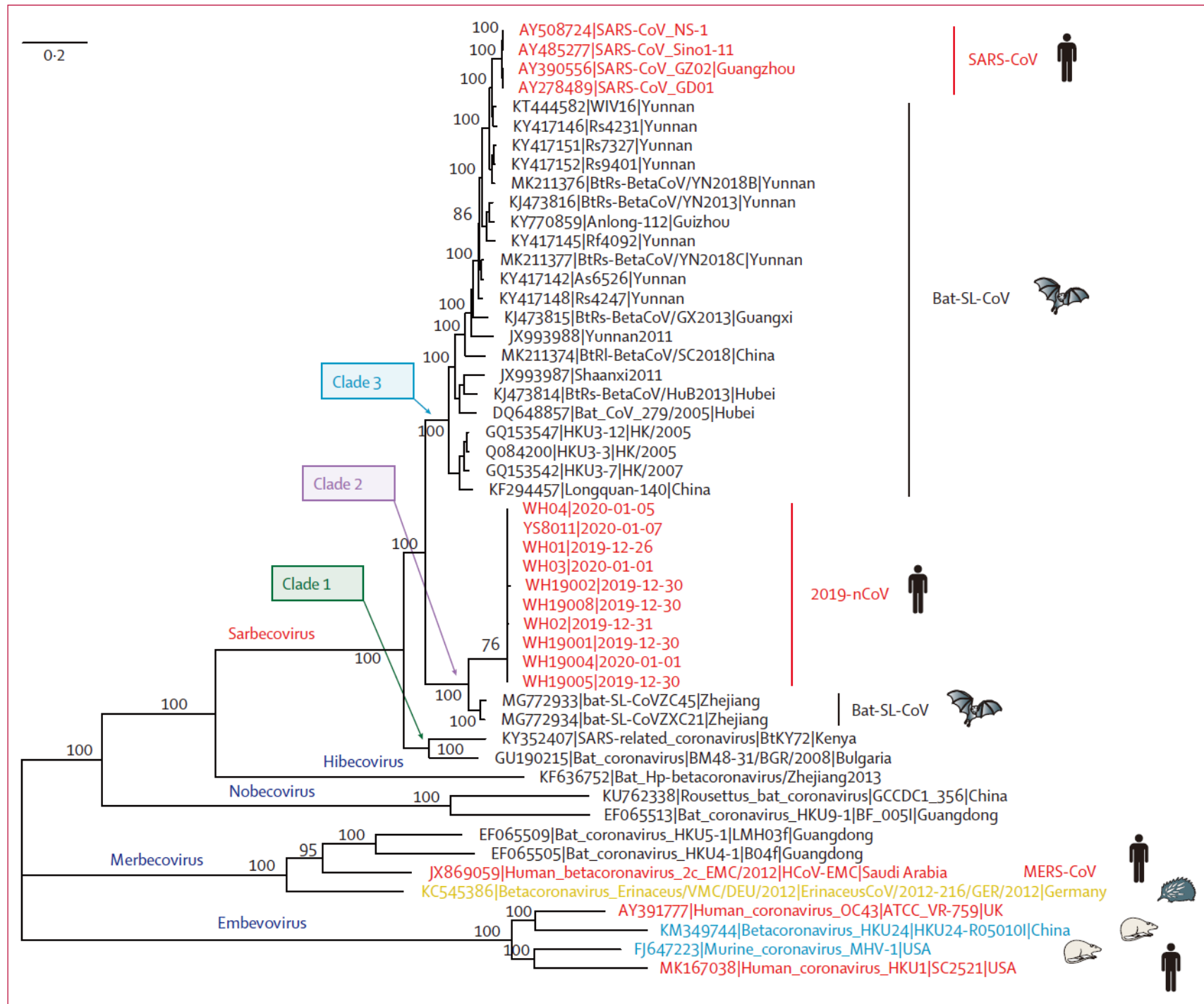
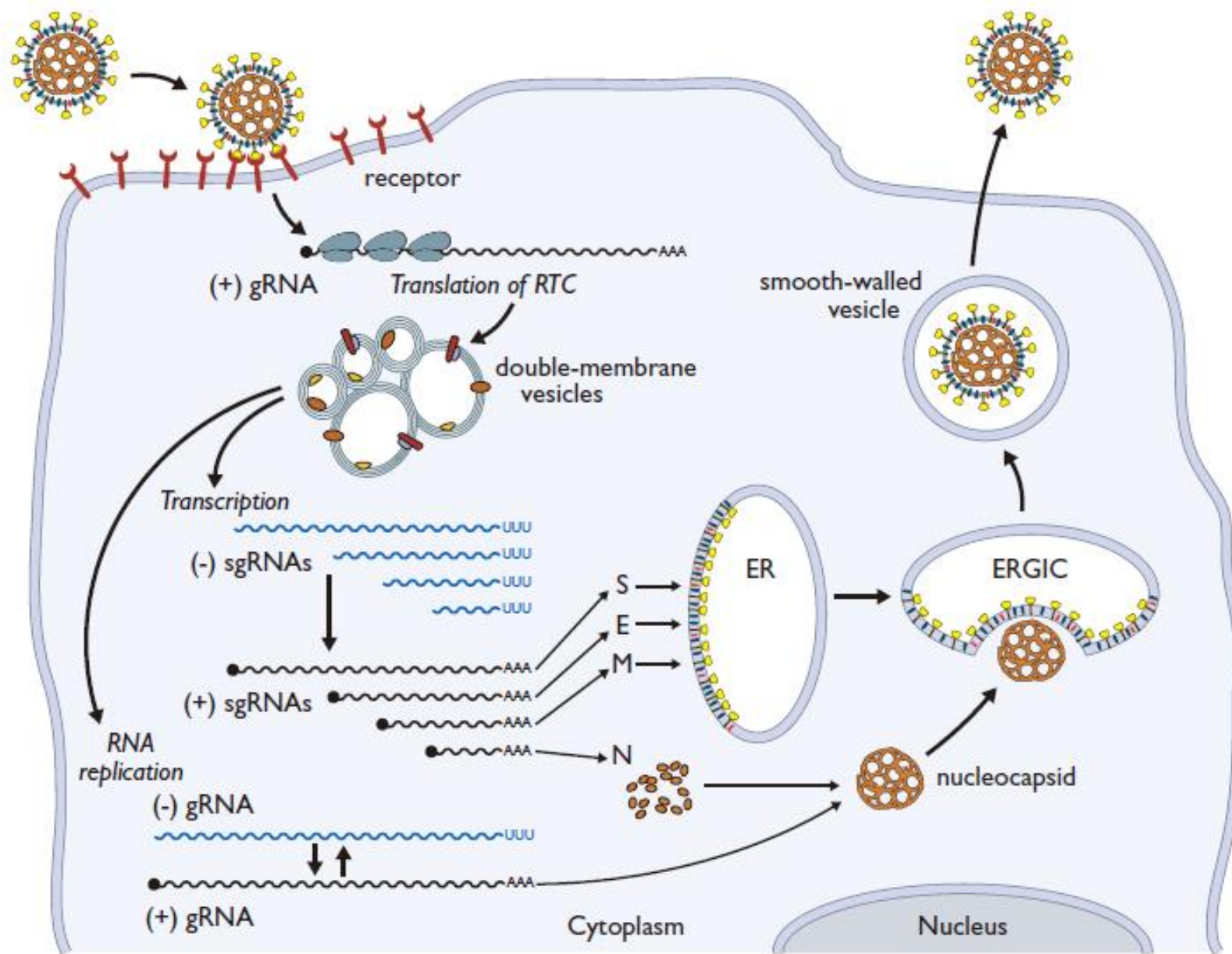


Figure 3: Phylogenetic analysis of full-length genomes of 2019-nCoV and representative viruses of the genus Betacoronavirus  
 2019-nCoV=2019 novel coronavirus. MERS-CoV=Middle East respiratory syndrome coronavirus. SARS-CoV=severe acute respiratory syndrome coronavirus.



# 1.4 Replication

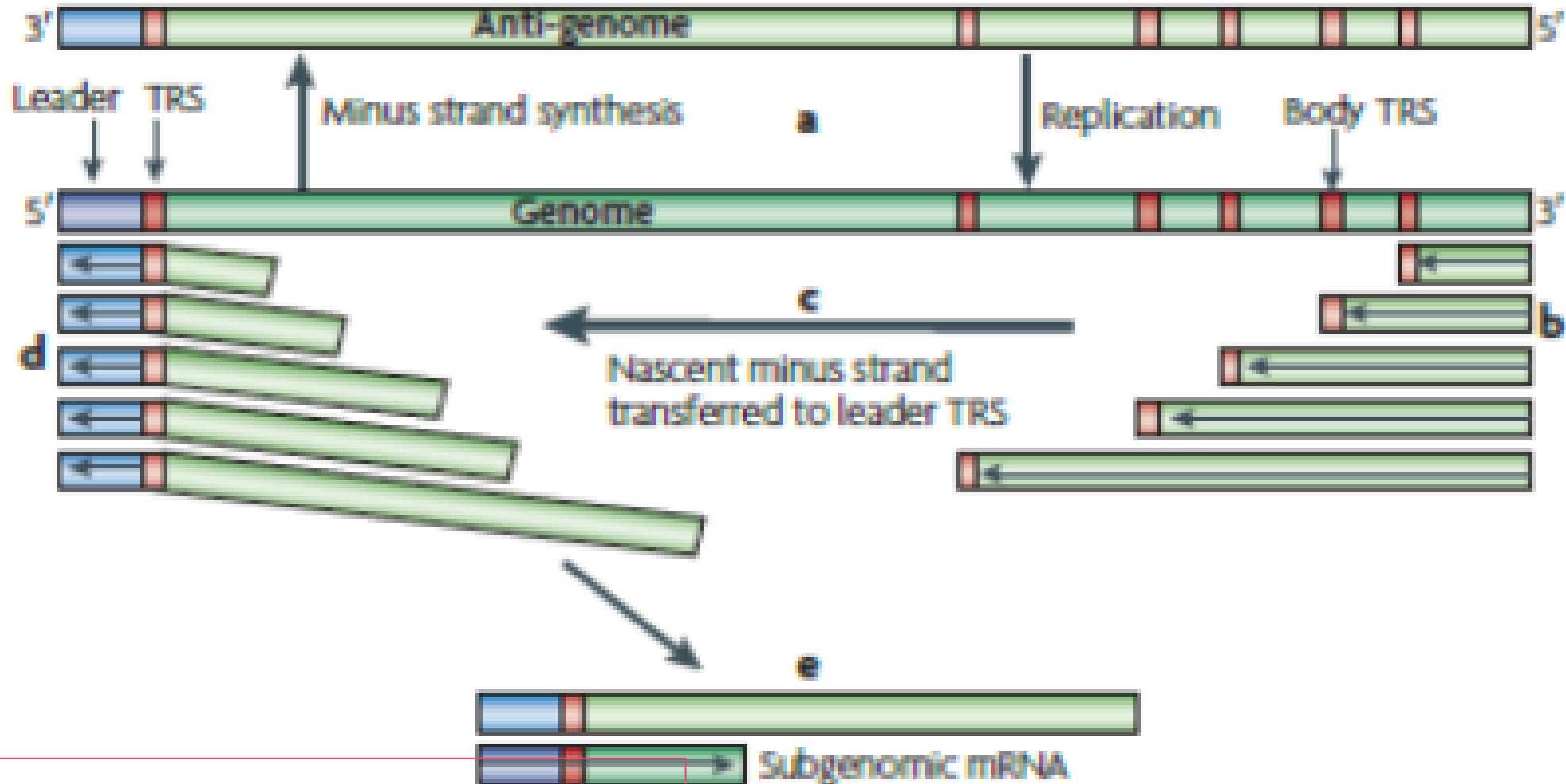


**FIGURE 28.6.** Overview of coronavirus replication (see text for details).





# 1.5 The unique intracytoplasmic discontinuous transcription pattern of the coronavirus



- 1. RdRp
- 2. Replicative intermediate
- 3. The RNA virus with the largest genome

**Prone to genome mutation and recombination**



# 1.6 Coronavirus structure



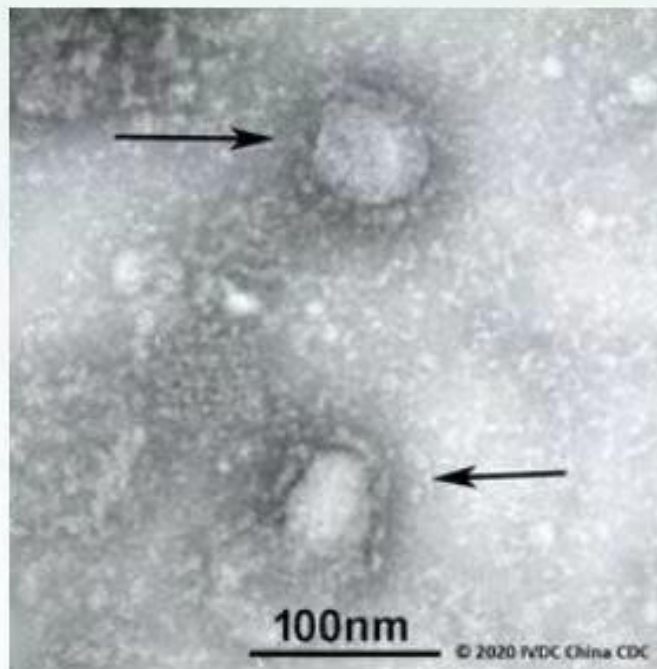
## In Focus

### China releases genetic sequence of newly discovered coronavirus from Wuhan

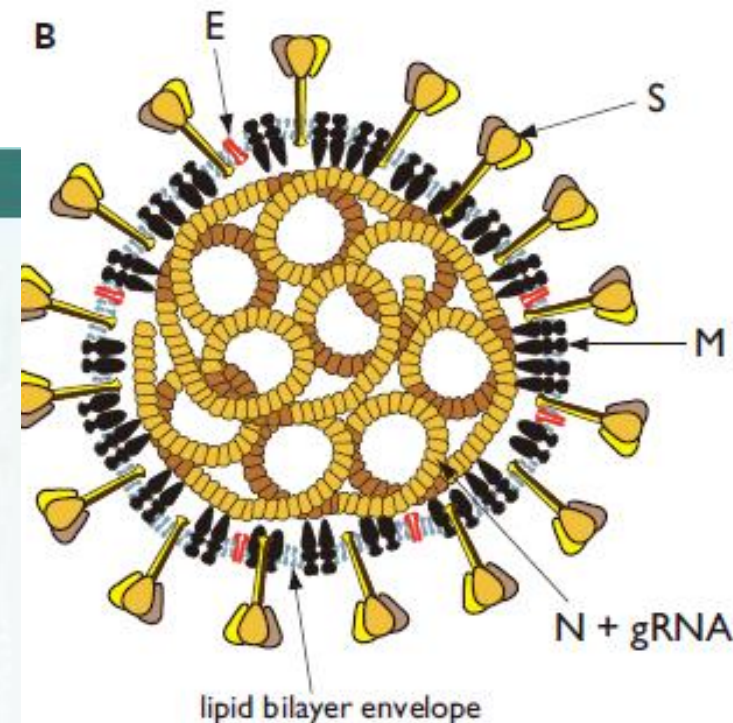
The Chinese Center for Disease Control and Prevention, the Chinese Academy of Science and the Chinese Academy of Medical Science released through the GISAID Initiative the genome sequence of a newly discovered coronavirus. The virus was identified during an outbreak in the city of Wuhan, where patients were suffering from respiratory illnesses such as pneumonia since late December 2019.

The genome sequence of this betacoronavirus is crucial to develop specific diagnostic tests and to identify potential intervention options.

> [read more and access the data](#)



Courtesy: IVDC, China CDC

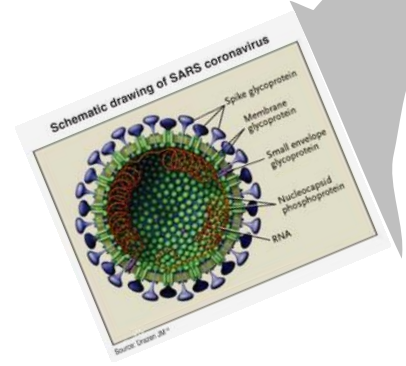


purified virions of mouse hepatitis virus (MHV), reconstituted, and Stanley Sawicki.) **B**: Schematic showing the protein; E, envelope protein; and N, nucleocapsid protein.



# 1.7 Symptoms of the diseases caused by human coronavirus

- Headache
- Fever
- Overall soreness and ache
- Flu symptoms
- Chills
- Dry cough
- Vomiting



HCoV-229E  
Tyrrell D.A.J., et al. 1965

HCoV-OC43  
Hamre D, et al. 1966

HCoV-NL63  
Lia van Hoek, et al. 2004

HCoV-HKU1  
Patrick CYW, et al. 2005

SARS-CoV  
Drosten C, et al. 2003

MERS-CoV  
Ali Moh Zaki, et al. 2012

2019-nCoV  
Zhu N, et al. 2020





# Testing techniques



# Laboratory testing techniques for COVID-19



Nucleic acid testing

The diagram shows DNA and RNA structures with labels 'DNA的组成' and 'RNA'. The amplification plot shows 'Amplification Plot' with 'Cycle' on the x-axis (0-40) and 'Amplification' on the y-axis (0.0-2.5). Multiple colored curves represent different samples, showing exponential amplification over time.



Viral isolation

The image shows a microscopic view of cells on the left and a large, spherical virus particle with a green outer layer and a yellow core on the right.



Serological testing

The image shows several microcentrifuge tubes with red caps and various laboratory equipment like a pipette, a tray of wells, and a calculator.

# Content

**2.1 Specimen collection requirements**

**2.2 Nucleic acid testing**

**2.3 Antibody testing**

**2.4 Biosafety requirements**





# Part I

## Specimen collection requirements

- Collection target
- Specimen categories
- Specimen packaging and preservation
- Requirements for the sampling personnel
- Specimen processing
- Specimen transportation



# 1. Specimen collection target



1  
Suspected COVID-19 cases;

2  
Others requiring diagnosis or differential diagnosis for SARS-CoV-2



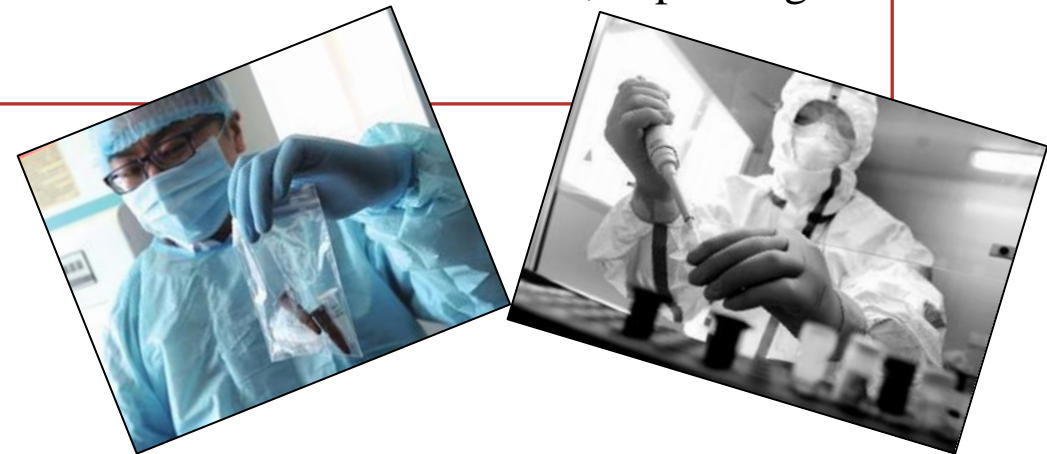




## 2. Sample collection requirements

### Sampling personnel

- 1. The SARS-CoV-2 testing specimens shall be collected by qualified technicians who have received biosafety training (who have passed the training) and are equipped with the corresponding laboratory skills. Personal protective equipment (PPE) is required for sampling personnel when performing the sampling
- 2. Specimens of inpatient cases shall be collected by medical staff of the hospital where they are being treated.
- 3. Specimens of close contacts shall be collected by the designated local CDCs and medical institutions.
- 4. Multiple specimens may be collected in the course of the disease, depending on the need of laboratory testing.

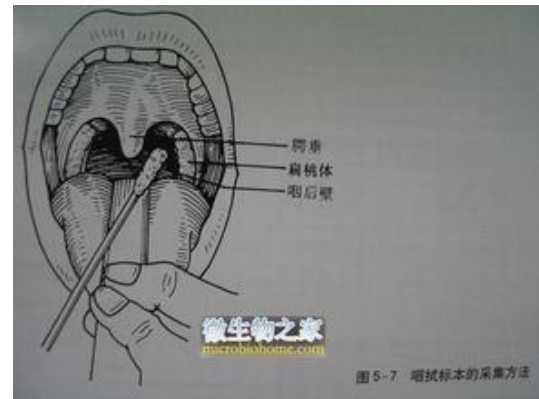


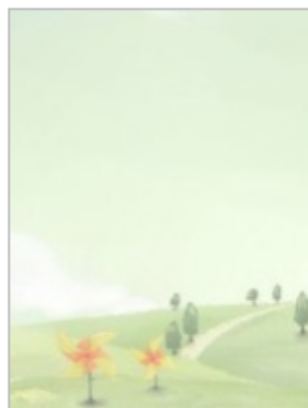


# 3. Categories of specimen collected

Respiratory tract specimens in the acute phase (including upper or lower respiratory tract specimens) must be collected from each case; lower respiratory tract specimens shall be preferred for the collection from severe cases. Stool samples, urine samples, whole blood samples and serum samples can be collected according to clinical needs.

- 1) Upper respiratory tract specimens: including nasopharyngeal swabs, pharyngeal swabs etc.
- 2) Lower respiratory tract specimens: including deep-cough sputum, alveolar lavage fluids, bronchial lavage fluid and respiratory tract extracts.
- 3) Fecal specimens: Fecal samples are about 10 g (peanut size). If it is not convenient to collect fecal samples, an anal swab can be collected.
- 4) Blood specimens: One should, as much as possible, collect anticoagulated blood in the acute phase within 7 days after the onset of the disease. 5 ml of blood is required for each collection. Vacuum tubes containing EDTA anticoagulant are recommended in blood collection.
- 5) Serum specimens: Both acute-phase and convalescent serum specimens should be collected as much as possible. The first serum specimen should be collected as soon as possible (preferably within 7 days after the onset of illness), and the second specimen should be collected during 3-4 weeks after the onset of illness. 5 ml of blood is required for each specimen and vacuum tubes without anticoagulant are recommended. Serum specimens are mainly used for measuring antibodies, rather than nucleic acid testing.
- 6) Urine specimens: Collect 2-3ml of mid-stream urine sample in the morning. (原文没有，请确认)





### Consistent detection of 2019 novel coronavirus in saliva.

【作者】 Kelvin Kai-Wang To;Owen Tak-Yin Tsang;Cyril Chik-Yan Yip;Kwok-Hung Chan;Tak-Chiu Wu;Jacky M C Chan;Wai-Shin g Leung;Thomas Shiu-Hong Chik;Chris Yau-Chung Choi;Darshana H Kandamby;Da 更多...

【刊名】 Clinical infectious diseases : an official publication of the Infectious Diseases Society of America

【出版日期】 2020

【doi】 10.1093/cid/ciaa149

【作者单位】 State Key Laboratory for Emerging Infectious Diseases, Department of Microbiology, Carol Yu Centre for Infectio n, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Admini... 更多...

【关键词】 2019 novel coronavirus;diagnostics;saliva;transmission;viral load

【摘要】 The 2019-novel-coronavirus (2019-nCoV) was detected in the self-collected saliva of 91.7% (11/12) of patients. Seria l saliva viral load monitoring generally showed a declining tren 更多...

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#### Original article

#### Evaluating the accuracy of differ ent laboratory diagnosis and mo nitoring of 2019-nCoV

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The novel coronavirus (2019-nCoV) is spreading very fast in Hubei Province of China. As of February 14, 2020, 51,986 confirmed cases (including laboratory-confirmed cases) were reported in Hubei Province, and 1,318 of them died. Respi ratory droplets are considered the most important routes of transmission of 2019-nCoV. However, the reasons for the rapid spread of this virus (1).

## 钟南山团队：从新冠肺炎患者尿液中分离出新冠病毒

Findings from the Zhong Nanshang Research Group: SARS-COV-2 isolated from the COVID-19 patient's urine



中国经济网

发布时间：02-22 12:58 | 中国经济网官方帐号

(原标题：#钟南山团队从尿液中分离出病毒#)

今日上午10时，广州市举行广州市科技战“疫”新闻通气会通报广州市防控新冠肺炎科研攻关工作的措施、成效等情况。在开展病毒溯源研究方面，钟南山院士团队专家、呼吸疾病国家重点实验室副主任赵金存教授团队联合广州海关首次从广州本地被感染的病例样本中成功分离出新型冠状病毒（COVID-19）毒株，为进一步开展疫苗和药物研究打下基础。其后，该团队又从新冠肺炎患者的粪便标本中分离出新型冠状病毒（COVID-19）。近日，他们再次从新冠肺炎患者尿液中分离出新冠病毒，这对公共卫生安全防控有重要的警示和指导意义。目前，相关课题组正在围绕病毒的致病机制和药物治疗靶点等开展研究。

#### 作者最新文章

“没有重来和等待的机会”  
一位ICU医生的值班日记

“我们对中国人民战胜疫情充满信心”  
(患难见真情 共同抗疫情)——外国媒体高度评价中国抗击疫情努力



# Pharynx structure

Applied anatomy of pharynx

I. Sections

1. Nasopharynx
2. Oropharynx
3. Laryngopharynx

## ● 咽的应用解剖

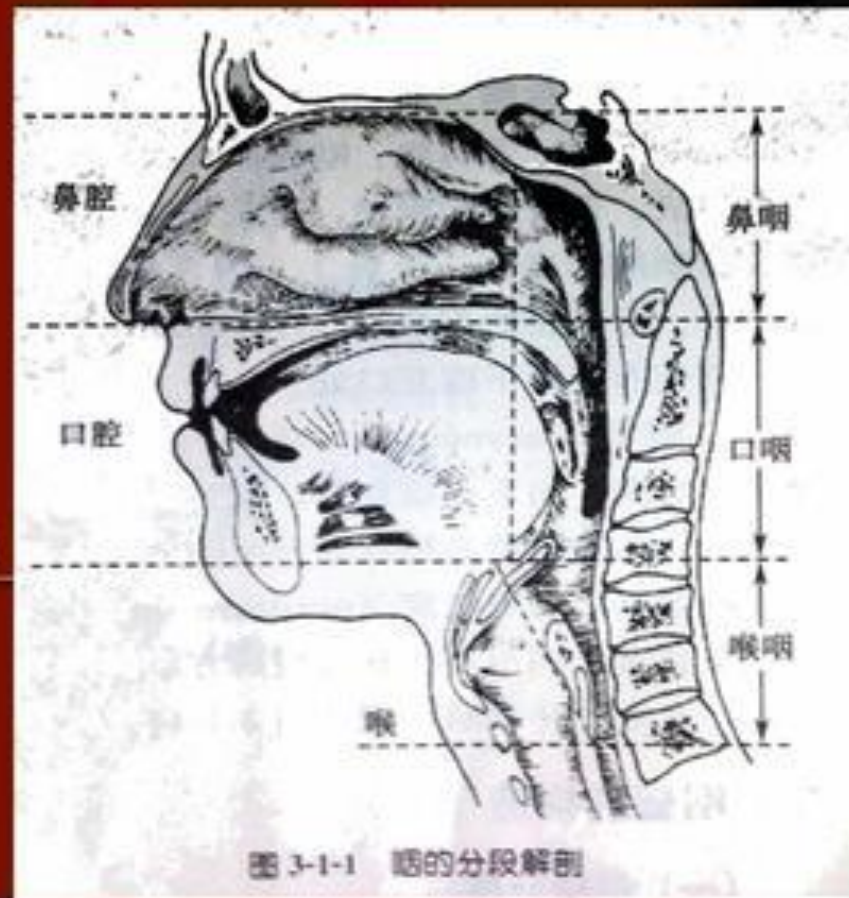
### 第一节咽的应用解剖

#### 一、分部

1鼻咽 (nasopharynx)

2口咽 (oropharynx)

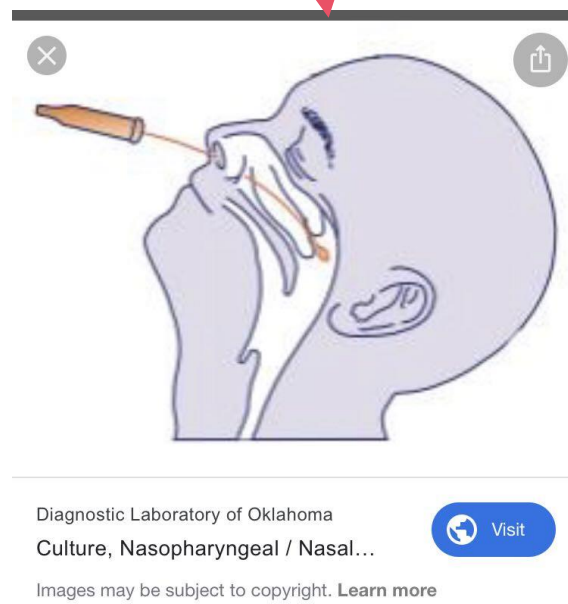
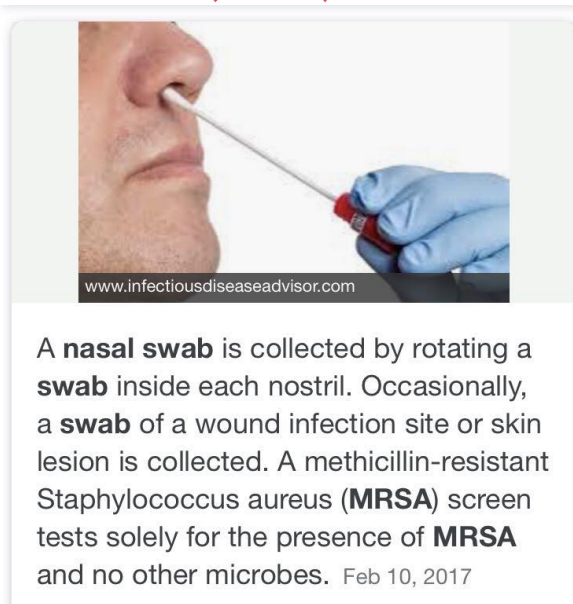
3喉咽 (laryngopharynx)





# Collection of nasopharyngeal swab

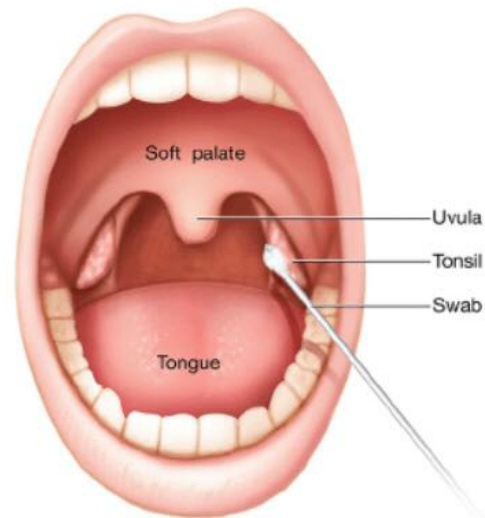
The sampler gently holds the person's head with one hand, the swab in another, insert the swab via nostril to enter, slowly get deep along the bottom of the lower nasal canal. Because the nasal canal is curved, do not force too hard to avoid traumatic bleeding. When the tip of the swab reaches the posterior wall of the nasopharyngeal cavity, rotate gently once (pause for a moment in case of reflex cough), then slowly remove the swab and dip the swab tip into a tube containing 2-3ml virus preservation solution (or isotonic saline solution, tissue culture solution or phosphate buffer), discard the tail and tighten the cap.





# Collection of pharyngeal swab

The sampled person first gargles with normal saline, the sampler immerses the swabs in sterile saline (virus preservation solution is not allowed to avoid antibiotic allergies), holds the head of the sampled person up slightly, with one's mouth wide open, making a sound "ah" to expose the lateral pharyngeal tonsils, insert the swabs, stick across the tongue roots, and wipe both sides of the pharyngeal tonsils with pressure at least 3 times, then wipe on the upper and lower walls of the pharynx for at least 3 times, and dip the swabs in a tube containing 2-3ml storage solution (or isotonic saline solution, tissue culture solution or phosphate buffer solution), discard the tail and tighten the cap. The pharyngeal swabs can also be placed in the same tube together with the nasopharyngeal swab.





# Sputum treatment

## Deep cough sputum:

Ask the patient to cough deeply, and collect the sputum coughed up in a 50-ml screw-capped plastic tube containing 3 ml of sampling solution. If the sputum is not collected in the sampling solution, 2-3 ml of the sampling solution can be added into the tube before testing, or add sputum digestion reagents of equal volume of sputum.

**ThermoFisher**  
SCIENTIFIC

**Oxoid 痰消化液**

货号: SR0233A 包装: 10 小瓶, 7.5ml/瓶

配制: 将一瓶 (7.5ml) 痰消化液无菌加入 92.5ml 无菌蒸馏水中, 混和均匀后立即使用或在 2-8°C 最多保存 48 小时。

使用方法:

1. 用无菌收集瓶收集痰标本
2. 加入 5 倍体积的生理盐水, 搅拌, 以去除唾液, 然后用巴斯德吸管, 将生理盐水吸去
3. 向处理过的痰液加入等体积的痰消化液
4. 振荡混合液均匀, 置 37°C 水浴, 并不时振荡, 至完全液化
5. 接种至合适的培养基, 或用血球计数板计数, 以及进行有关操作

配方:

成分	每瓶
二巯苏糖醇	0.1g
氯化钠	0.78g
氯化钾	0.02g
磷酸氢二钠	0.112g
磷酸二氢钾	0.02g
水	7.5ml

pH7.4 ± 0.2 @ 25°C

OXOID remel

赛默飞世尔科学仪器(北京)有限公司  
中国北京怀柔区空港工业区内  
裕华路 28 号 10 号楼 3 层  
P411: www.oxoid.com  
www.remel.com

电话: 010-80489013  
传真: 010-80489013  
www.thermo.com.cn



Phosphate buffer containing 1 g/L of protease K

Phosphate buffer containing 0.1 g of dithiothreitol and 0.78 g of sodium chloride



# Fecal specimen processing

Take 1ml sample processing solution, pick up a little sample about the size of a soybean and add it into the tube, gently blow for 3-5 times, set aside at room temperature for 10 minutes, centrifuge at 8,000rpm for 5 minutes, absorb the supernatant for detectio



## **Treatment solution for the fecal specimen**

211g tris,  
8.5g sodium chloride,  
1.1 g calcium chloride anhydrous or 1.47g calcium chloride containing crystalline water, dissolved into 800 ml deionized water, with the pH adjusted to 7.5 with concentrated hydrochloric acid and replenishing with deionized water to 1000 ml.





# Anal swab

Gently insert the disinfectant cotton swab into the anus for 3-5cm in depth, then gently rotate and pull out, immediately put the swab into a 15-ml screw-capped sampling tube containing 3-5ml virus preservation solution, discard the tail and tighten the tube cover.



## 4. Specimen packaging and preservation

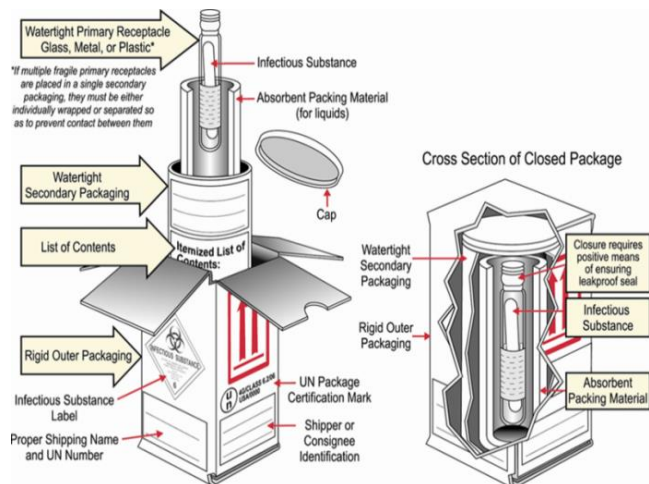
1. Collected specimens shall be packaged separately in a biosafety cabinet of a BSL-2 laboratory.
2. All specimens should be placed in an airtight freeze-tolerant sample collection tube of appropriate size, with a screw cap and a gasket inside. The sample number, category, name and sampling date should be indicated on the outside of the container.
3. Specimens kept in an airtight container should be sealed in a plastic bag of appropriate size, with each bag containing one specimen.

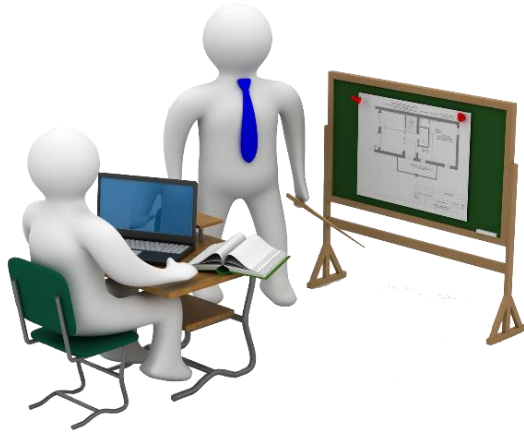
Specimens for virus isolation and nucleic acid detection purposes should be tested as soon as possible. Specimens to be tested within 24 hours can be stored at 4 °C; those that cannot be tested within 24 hours should be stored at -70 °C or below (specimens may be temporarily stored in -20 °C refrigerators in the absence of -70 °C storage condition). Serum can be stored at 4 °C for 3 days and below -20 °C for a longer period. A special depot or cabinet is required to store specimens separately.



# 5. Specimen transportation

1. SARS-CoV-2 strains or other potentially infectious biological substances are subject to the packaging instructions for Category A substances assigned to UN2814, and the PI 602 of the Technical Instructions For The Safe Transport of Dangerous Goods by Air (Doc 9284) issued by ICAO
2. environmental samples, assigned to UN3373, shall be transported in Category B packaging in accordance with the PI 650, Doc 9284; one may refer to the aforementioned standards for specimens to be transported in other modes of transportation.
3. A Permit of Transport is required for the transportation of the SARS-CoV-2 strains or other potentially infectious substances, according to the Transport Regulations on the Highly Pathogenic Microorganism (Virus) Strains and Specimens that are Pathogenic to Humans (Order No. 45, former Ministry of Health).





## Part II

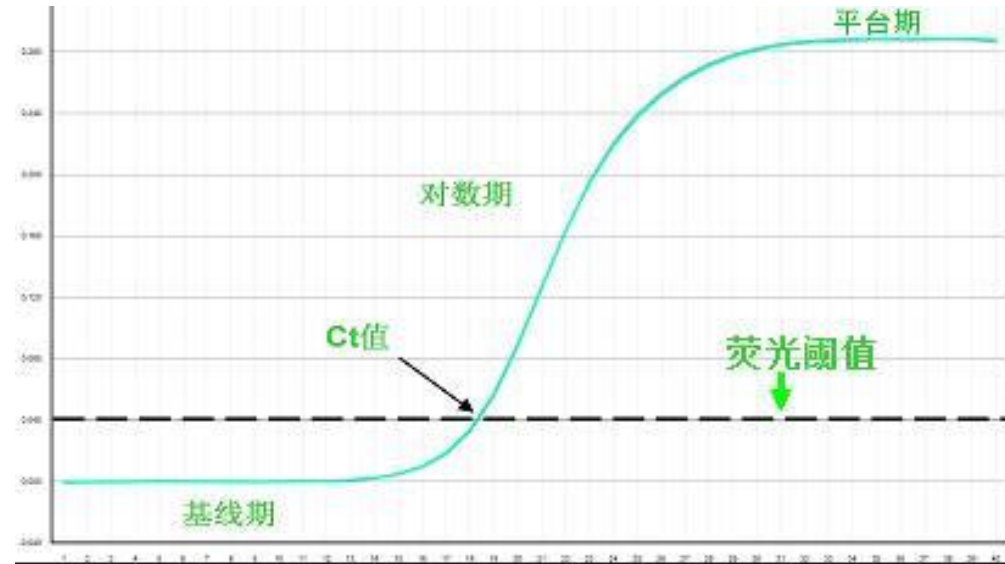
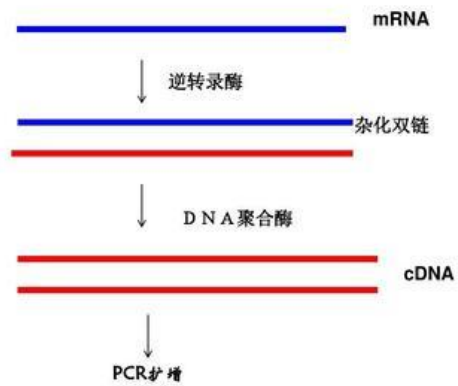
## Nucleic acid testing

- Technique
- Primer and probe
- Confirmation of positive specimens
- Principle
- Judgment of the testing results

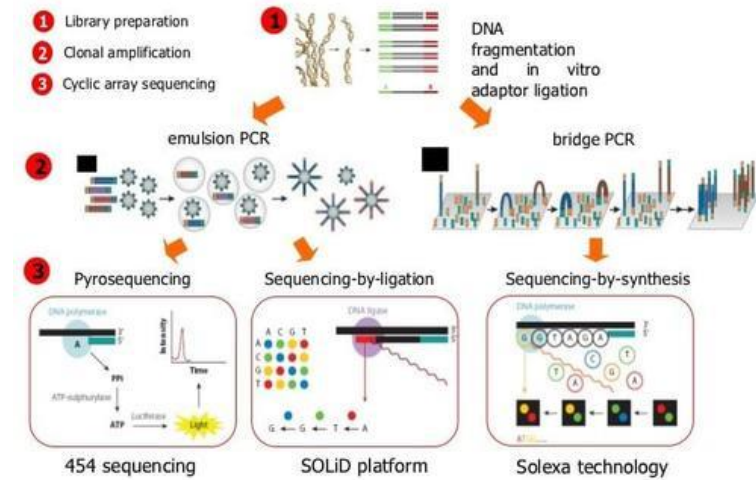


# 1. Nucleic acid testing techniques

## RT-PCR原理



## Next-generation DNA sequencing



1. RT-PCR

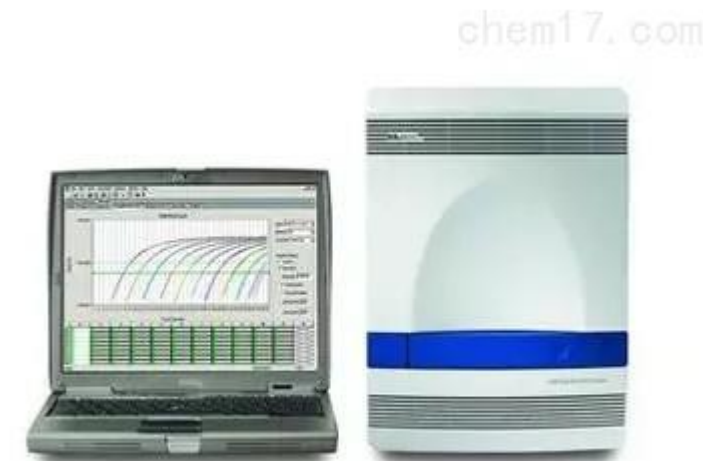
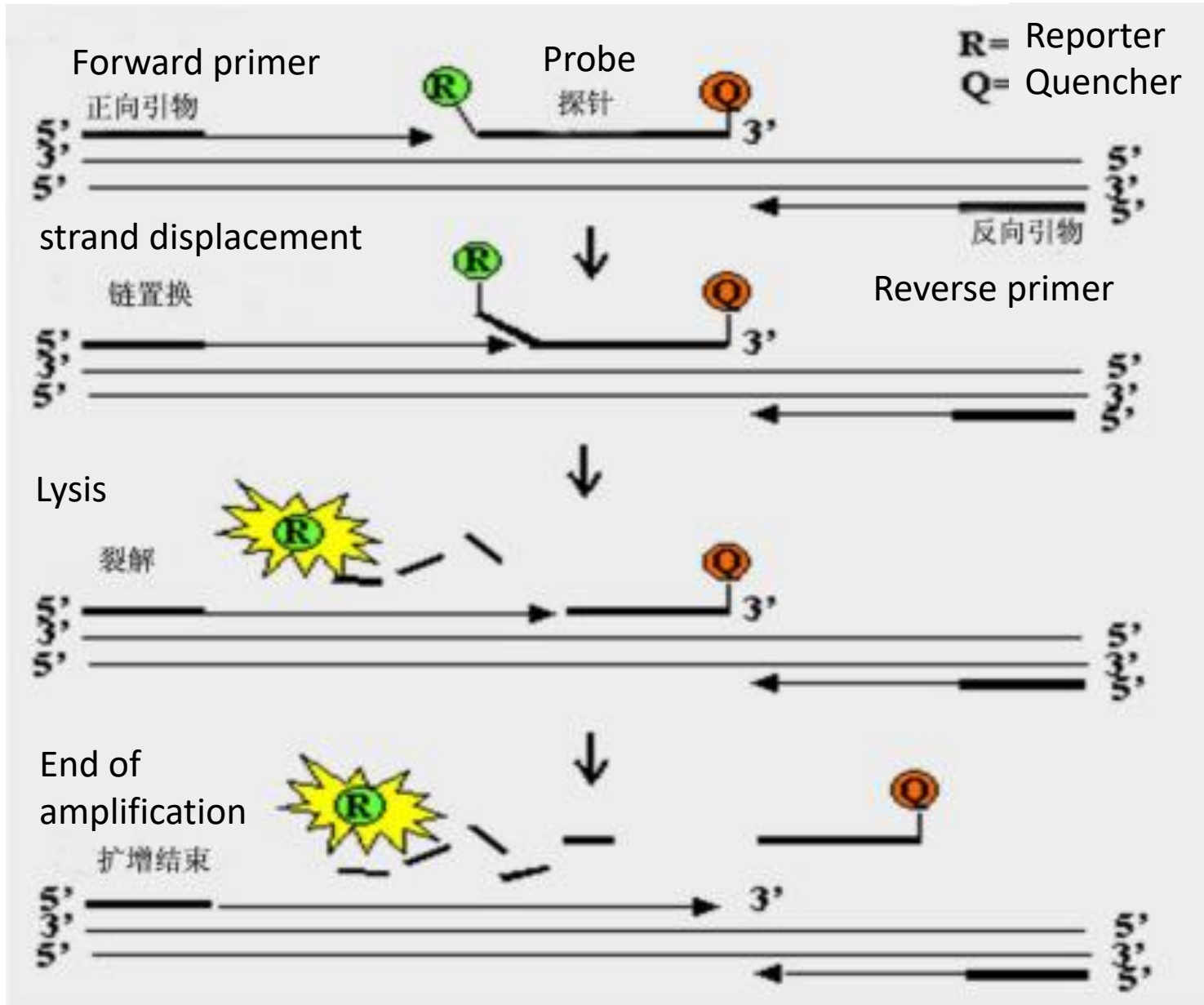
2. Real time RT-PCR

3. Sequencing

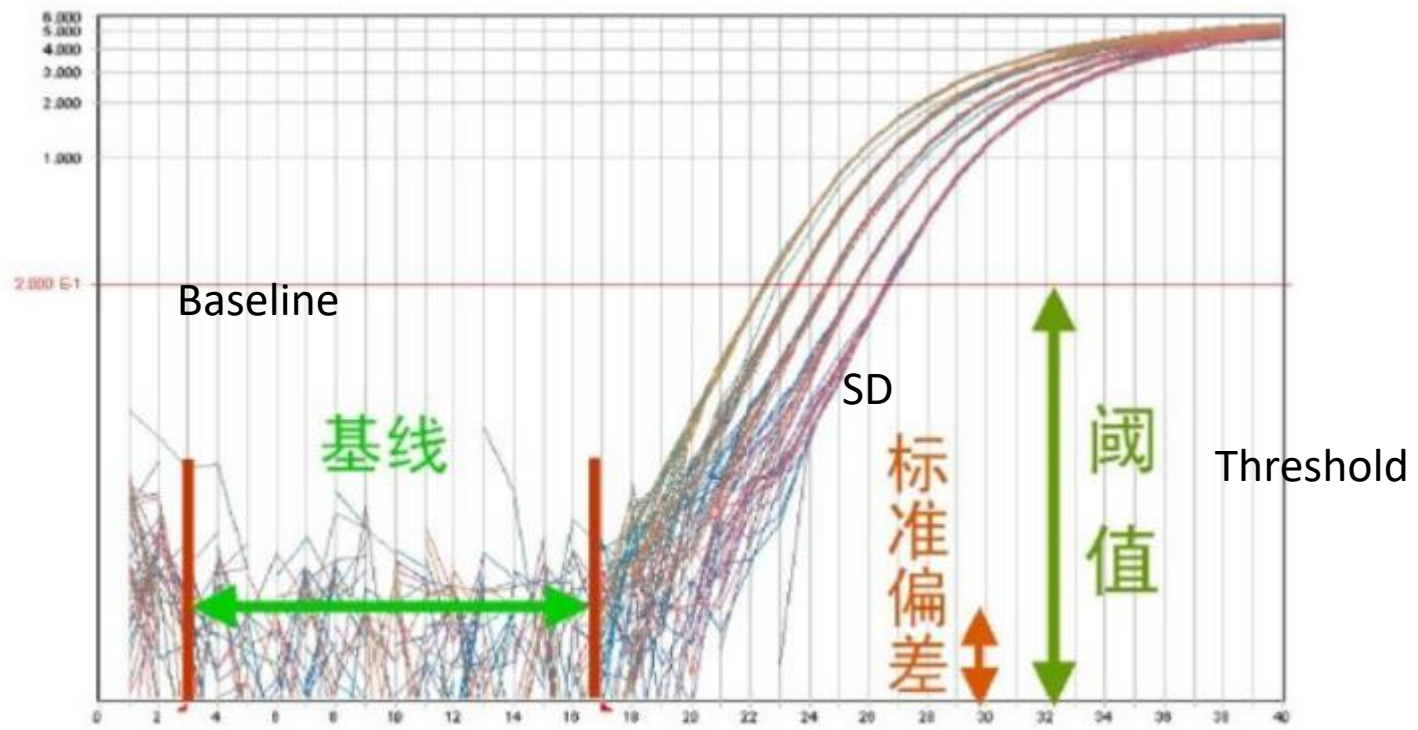


## 2. Real time RT-PCR

# Principle



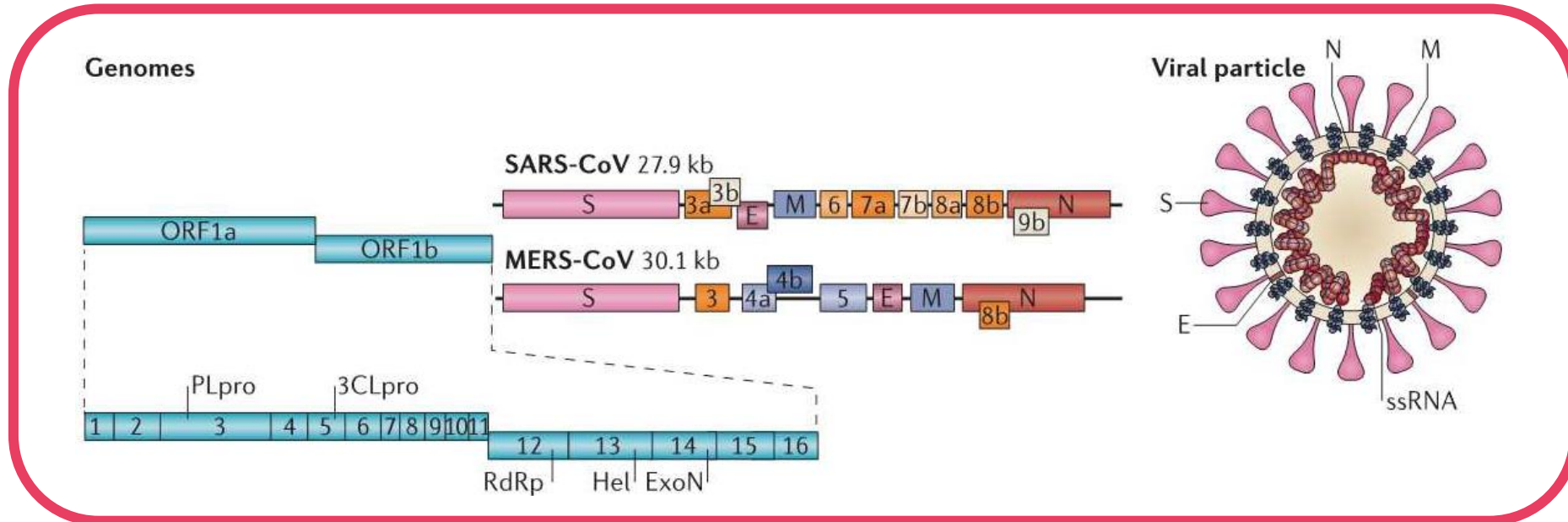
chem17.com



1. Baseline: In the first few cycles of the PCR amplification reaction, the fluorescence signal is close to a straight line as it does not change significantly. Then, such a straight line is the baseline;
2. Fluorescence threshold: Generally, the fluorescence signal of the first 15 cycles of PCR reaction is used as the fluorescence background signal. The fluorescence threshold is 10 times the standard deviation of the fluorescence signal of the first 3-15 cycles. The fluorescence threshold is set in the exponential phase of PCR amplification.
3. Ct value: indicates the number of cycles that the fluorescence signal in each PCR reaction tube undergoes when the threshold is met. The Ct value of each template has a linear relationship with the logarithm of the initial copy number; a standard curve can be developed based on the known initial copy number, the x coordinate represents the logarithm of the initial copy number, and the y coordinate represents the Ct value.



# Primer and probe of the SARS-CoV-2 nucleic acid assay



Target 1 (ORF1ab):

Forward primer (F): CCCTGTGGGTTTTACTTAA

Reverse primer (R): ACGATTGTGCATCAGCTGA

Fluorescent probe (P): 5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'

Target 2 (N):

Forward primer (F): GGGGAACTTCTCCTGCTAGAAT

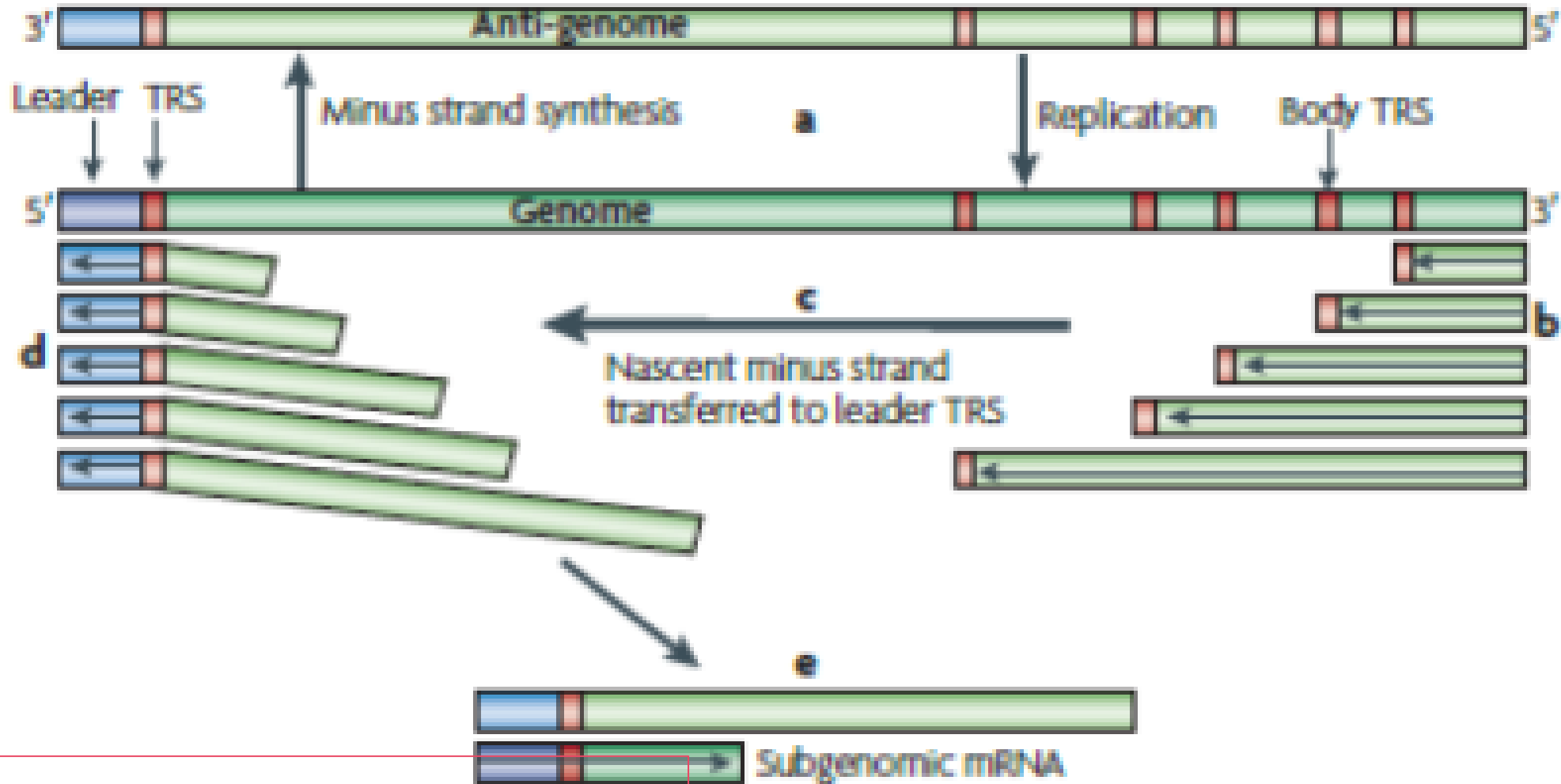
Reverse primer (R): CAGACATTTTGCTCTCAAGCTG

Fluorescent probe (P): 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'





## 1.5 The unique intracytoplasmic discontinuous transcription pattern of the coronavirus



**Prone to genome mutation and recombination**

1. RdRp
2. Replicative intermediate
3. The RNA virus with the largest genome



## 5. Judgment of the fluorescence quantitative RT-PCR assay results

Reverse transcription	42 °C	5 min	1 cycle
Initial denaturation	95 °C	10 s	1 cycle
PCR	95°C	10 s	40 cycles
	60°C (Collect fluorescence)	45 s	

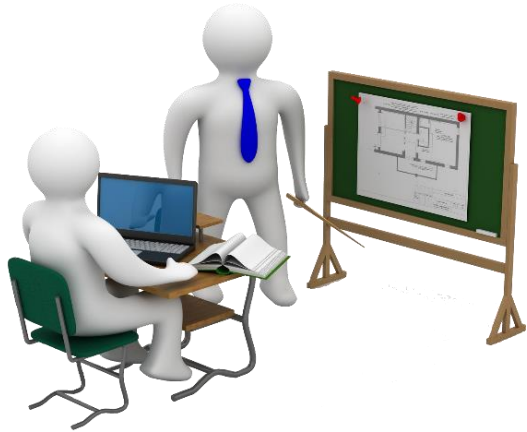
1. Negative: no Ct value or Ct value is 40.
2. Positive: Ct value <37.
3. Repeated experiments are recommended should Ct value range between 37 and 40. If the Ct value reads <40 and the amplification curve has obvious peaks, the sample should be considered being tested positive, otherwise it should be considered as negative.



# Confirmation of COVID-19 positive cases

To confirm a case as positive in the laboratory, one of the following criteria shall be met:

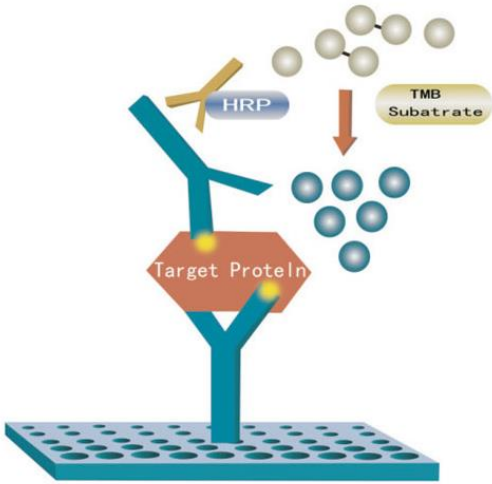
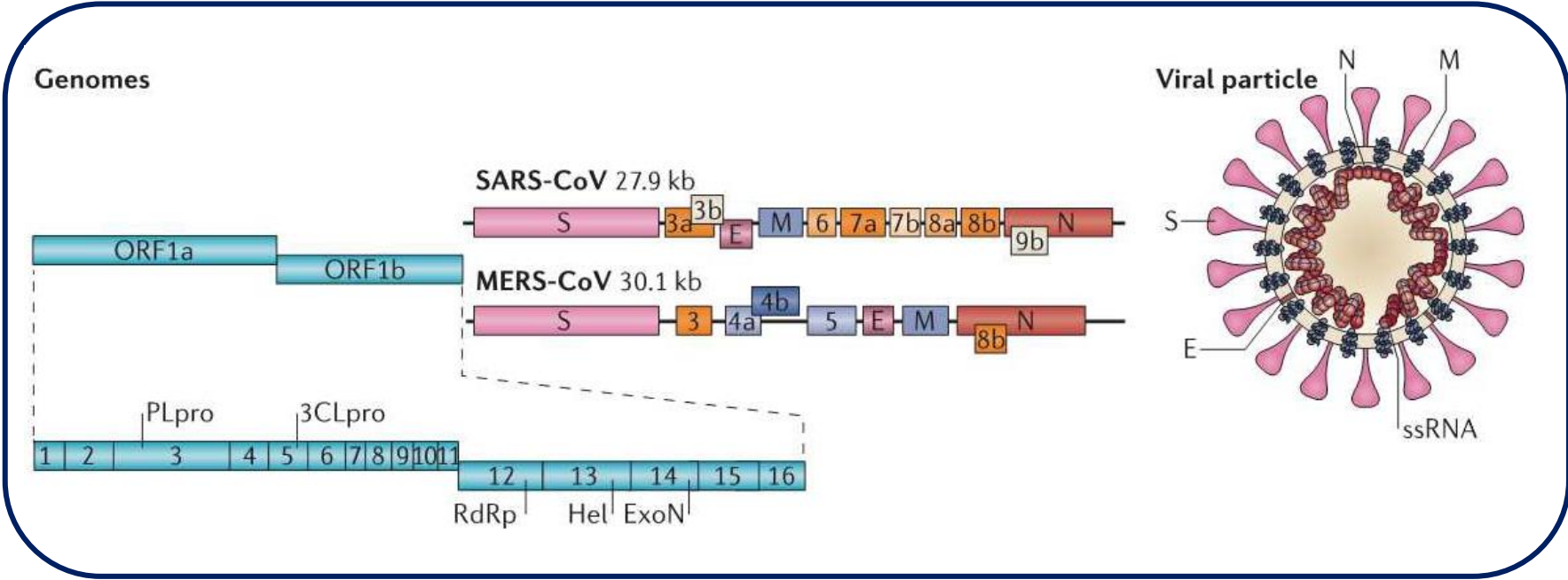
1. The real-time fluorescence-based RT-PCR assay of the 2019-nCoV in the same specimen shows that the two targets, ORF1ab and Protein N, are both positive. In case of the result showing positive for one target, then samples shall be re-collected for another test. If it is still positive for a single target, it is determined to be positive.
2. The real-time fluorescence-based RT-PCR assay of two types of specimens show one single target positive at the same time, or one target positive in two samples of the same type, it could be determined as positive.



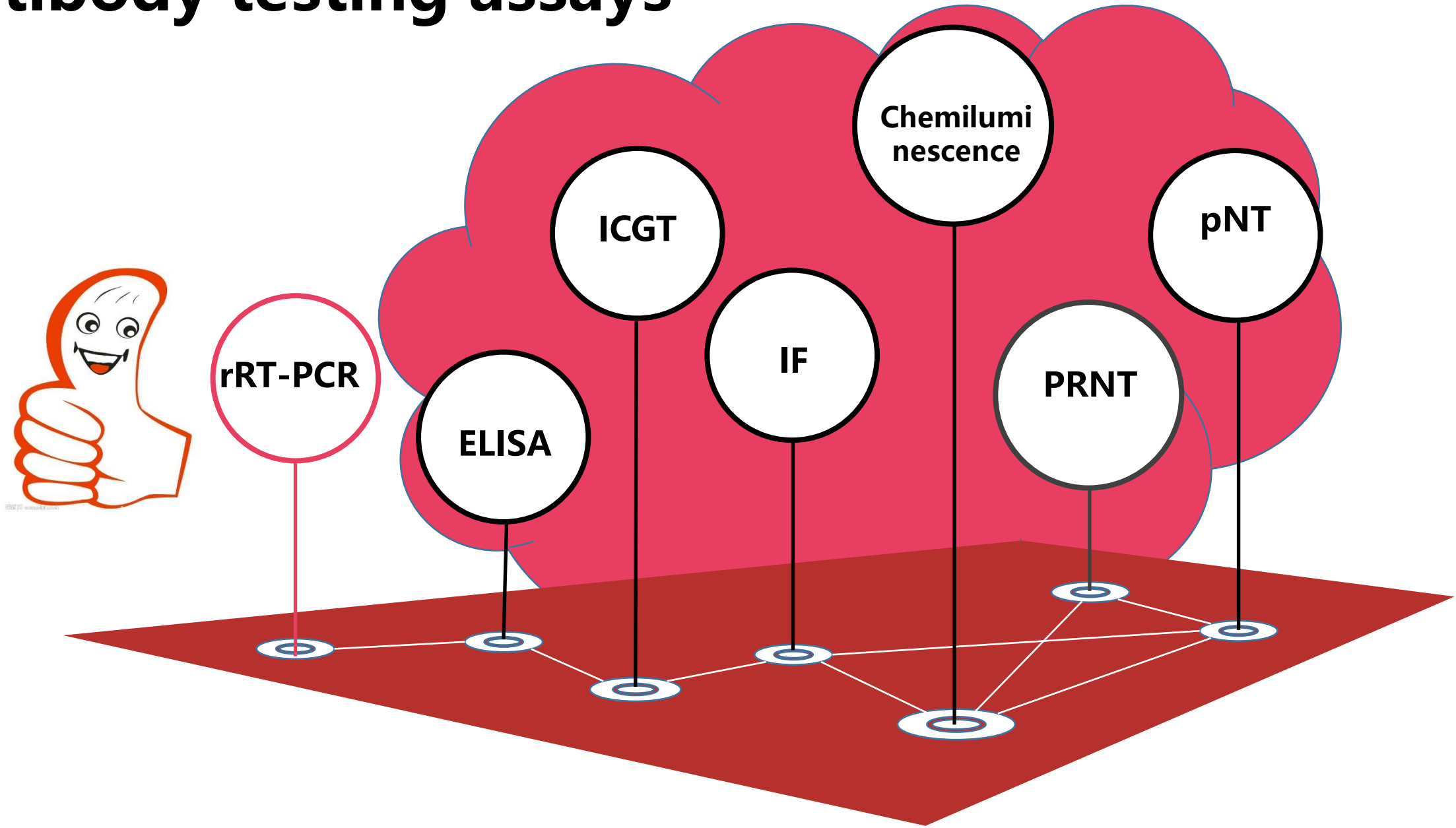
## Part III

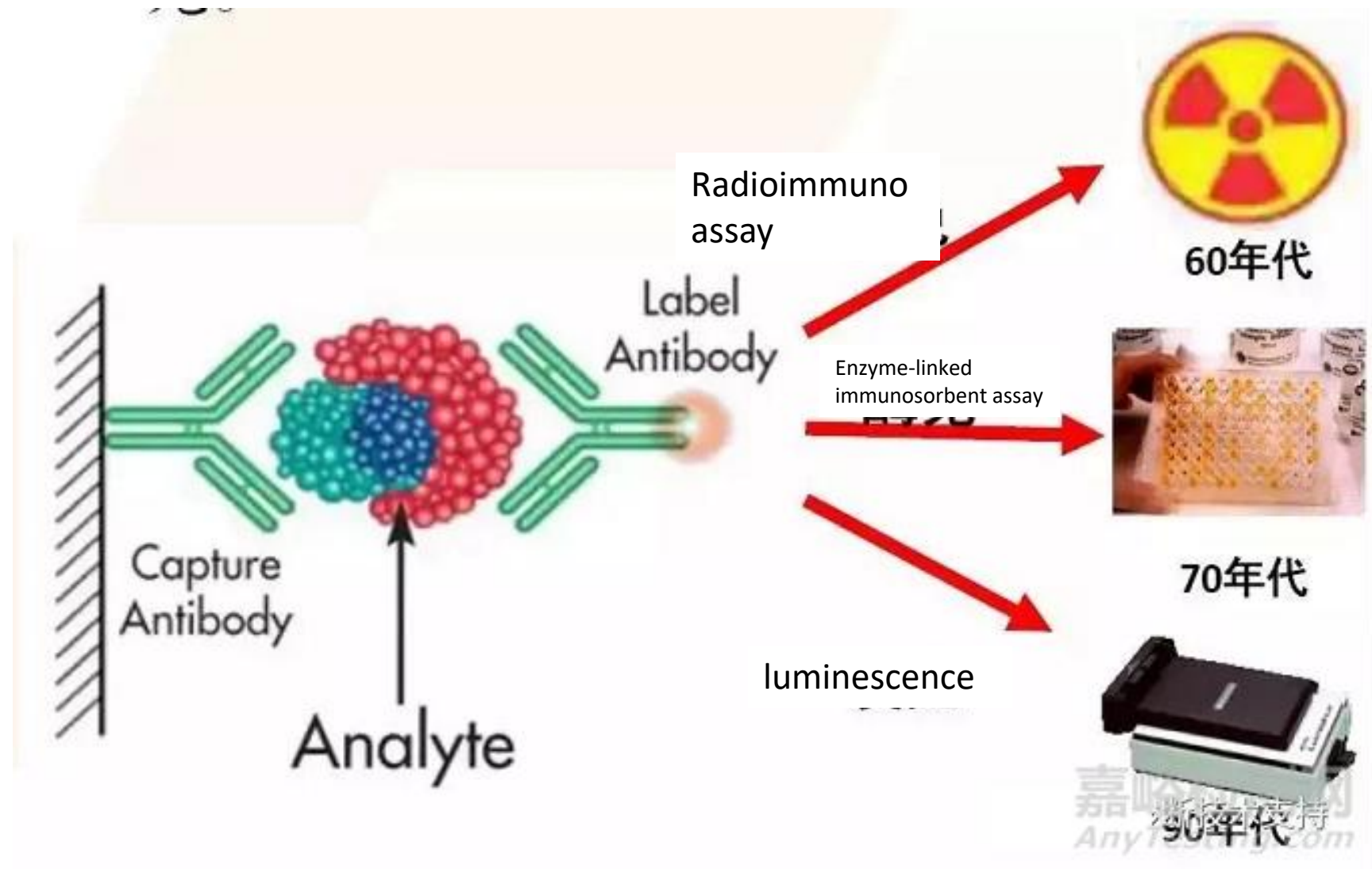
## Antibody testing

- 抗体检测方法
  - 胶体金法检测抗体
  - ELISA原理
  - 核酸检测和抗体检测时间的选择
- 
- Antibody testing methods
  - ELISA's principle
  - Colloidal gold antibody testing
  - How to choose the nucleic acid test against the testing window



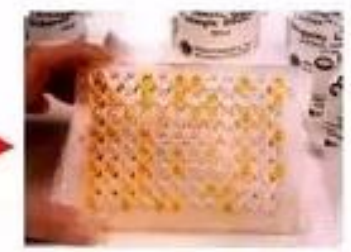
# ➤➤ 1. Antibody testing assays





60年代

1960's



70年代

1970's

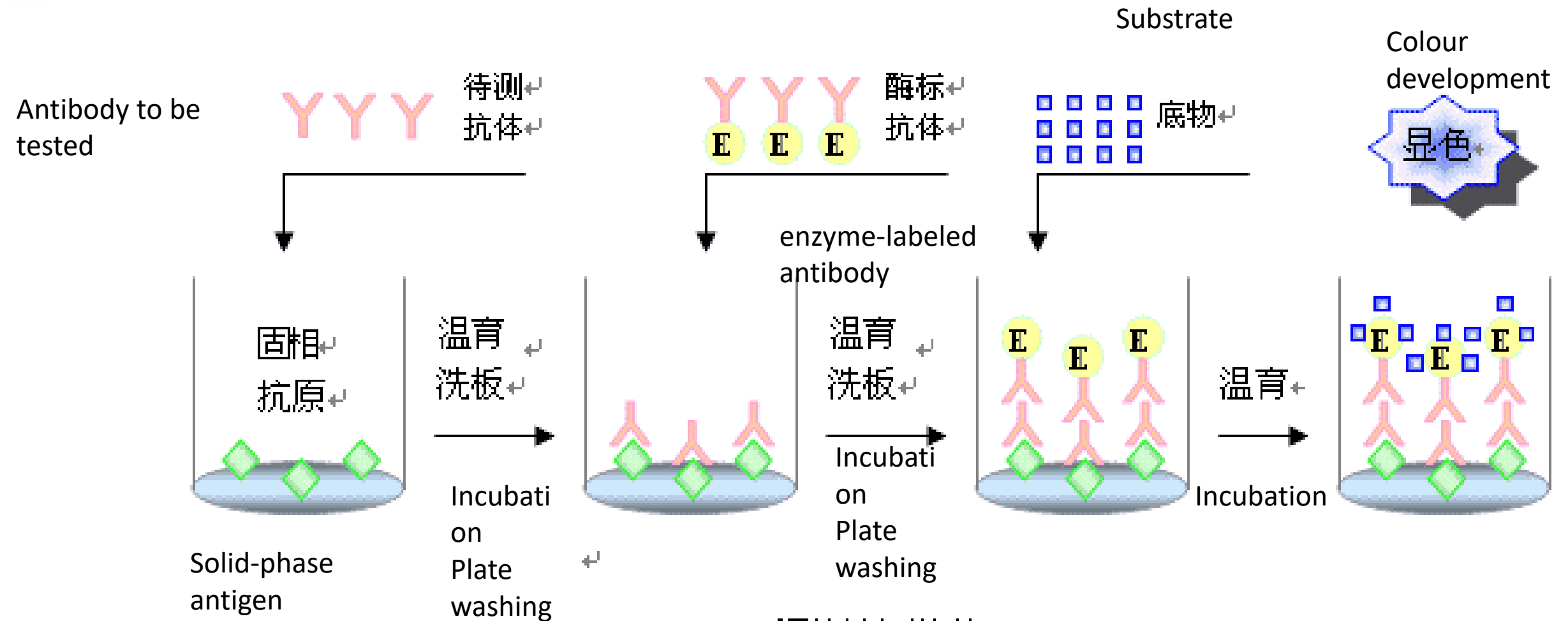


90年代

1990's



# 2. Principle for indirect ELISA



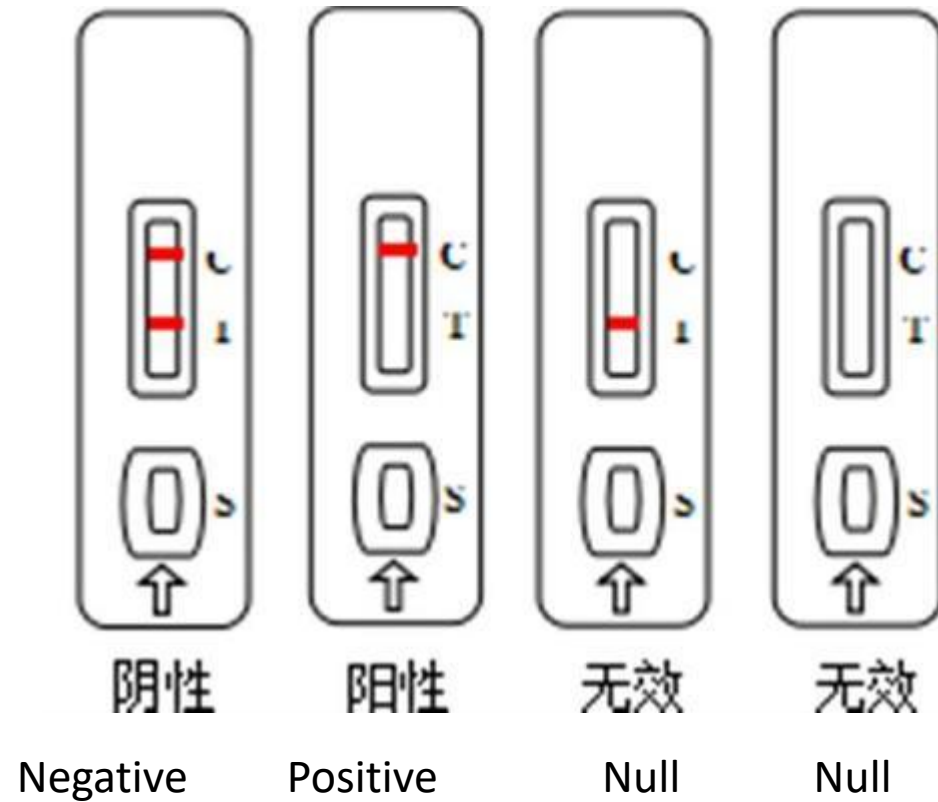
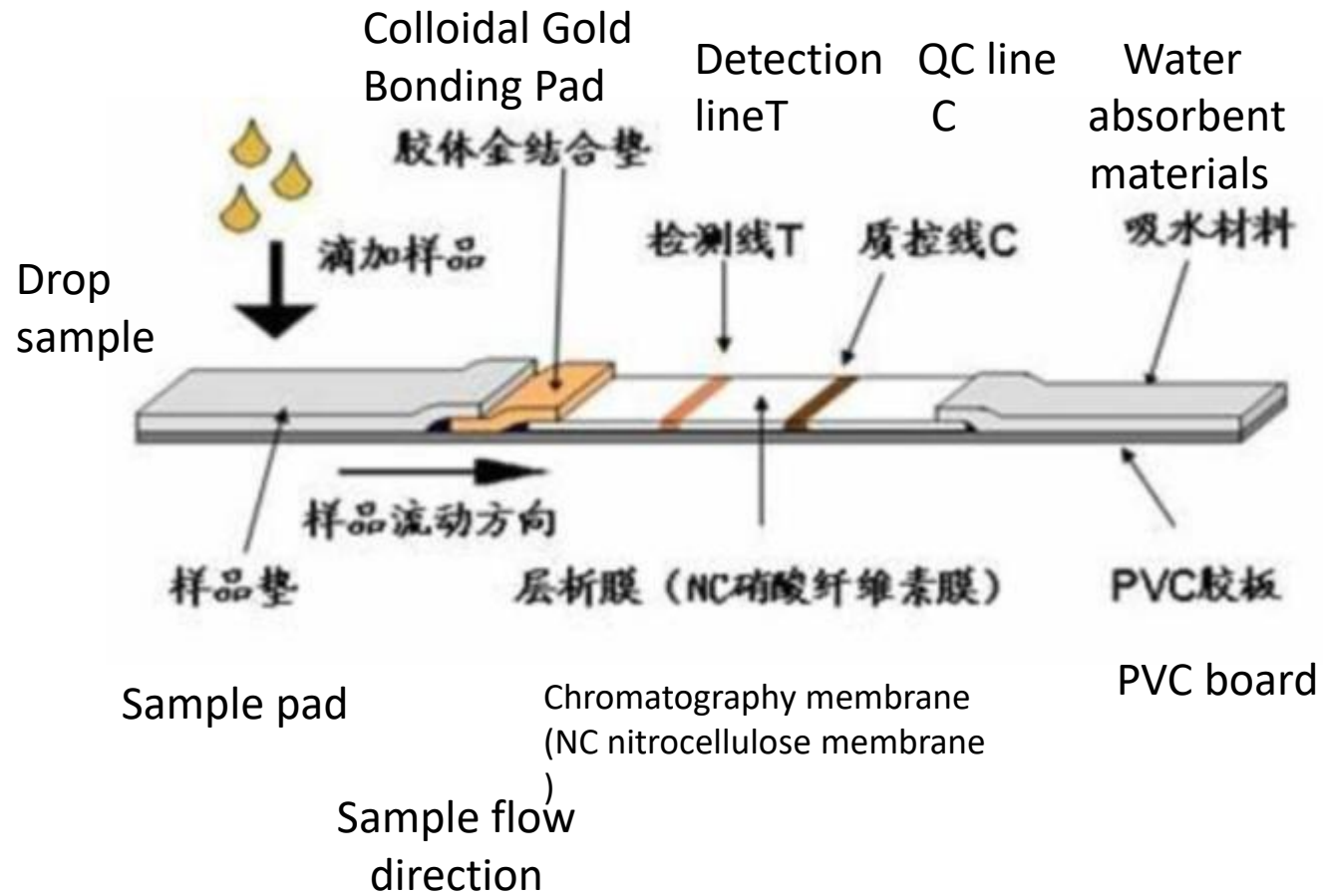
间接法测抗体

Indirect antibody testing





# 3. Principle for colloid gold testing







# Serum antibody tests for SARS-CoV-2

- Serum antibody tests are used as supplementary tests for cases of negative 2019-nCoV nucleic acid tests, and used in conjunction with nucleic acid tests in the diagnosis of suspected cases, or used in serological surveys and past exposure surveys of concerned population groups. Laboratory confirmed positive cases need to meet one of the following two conditions:
  1. Serum IgM antibodies and IgG antibodies to 2019-nCov are positive;
  2. Serum IgG antibodies to 2019-nCov turn from negative to positive or the IgG antibody titers of recovery period are 4 times or more higher than that of acute phase.

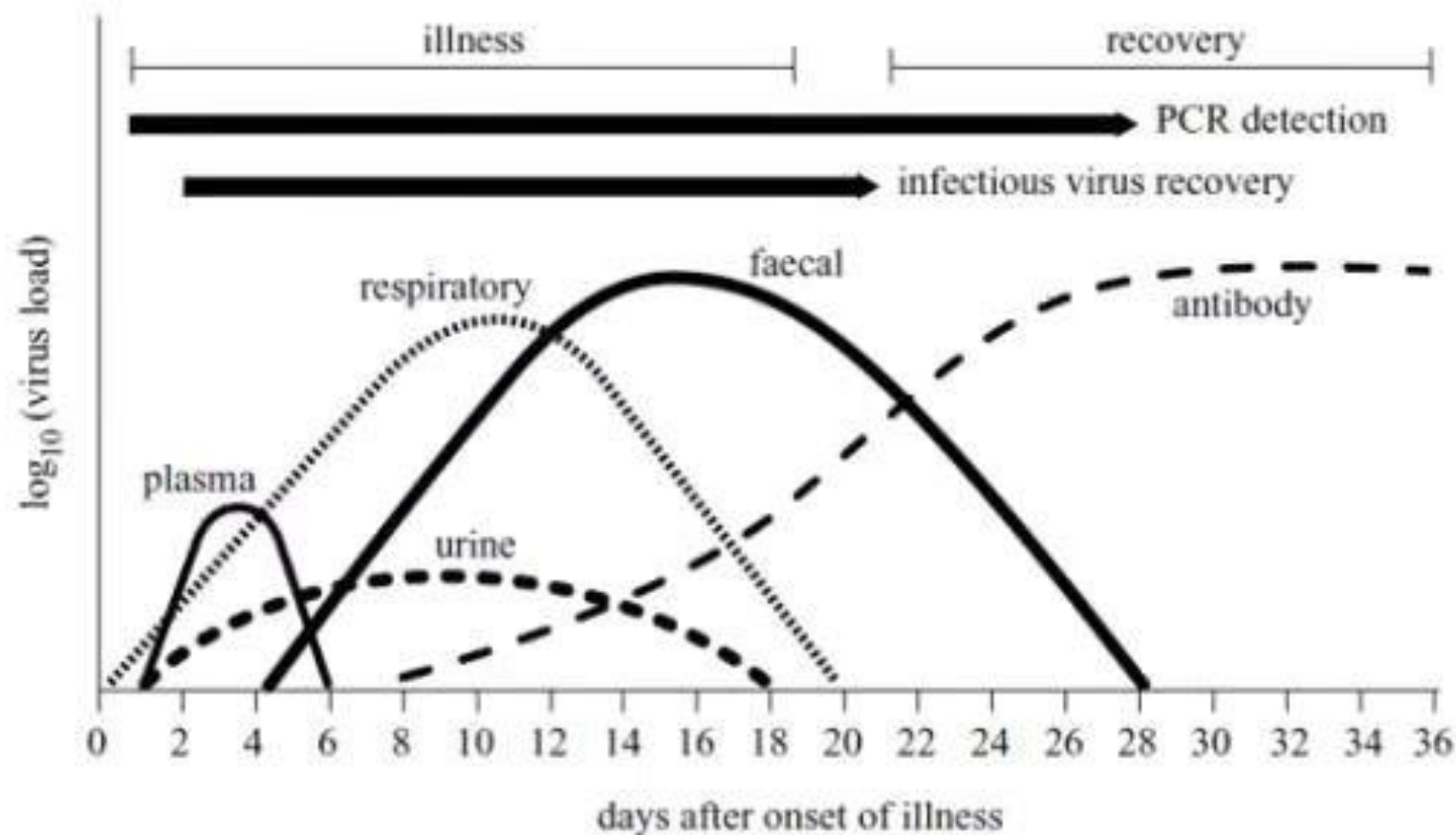


Figure 1. Schematic diagram of the course of virus shedding and detection in body fluids during SARS illness and recovery. Onset of illness is taken to be the onset of symptomatic fever.

SARS发病(以发热为首发症状)后, 病毒排出的时间分布, 及各种标本检测出病毒的时间分布

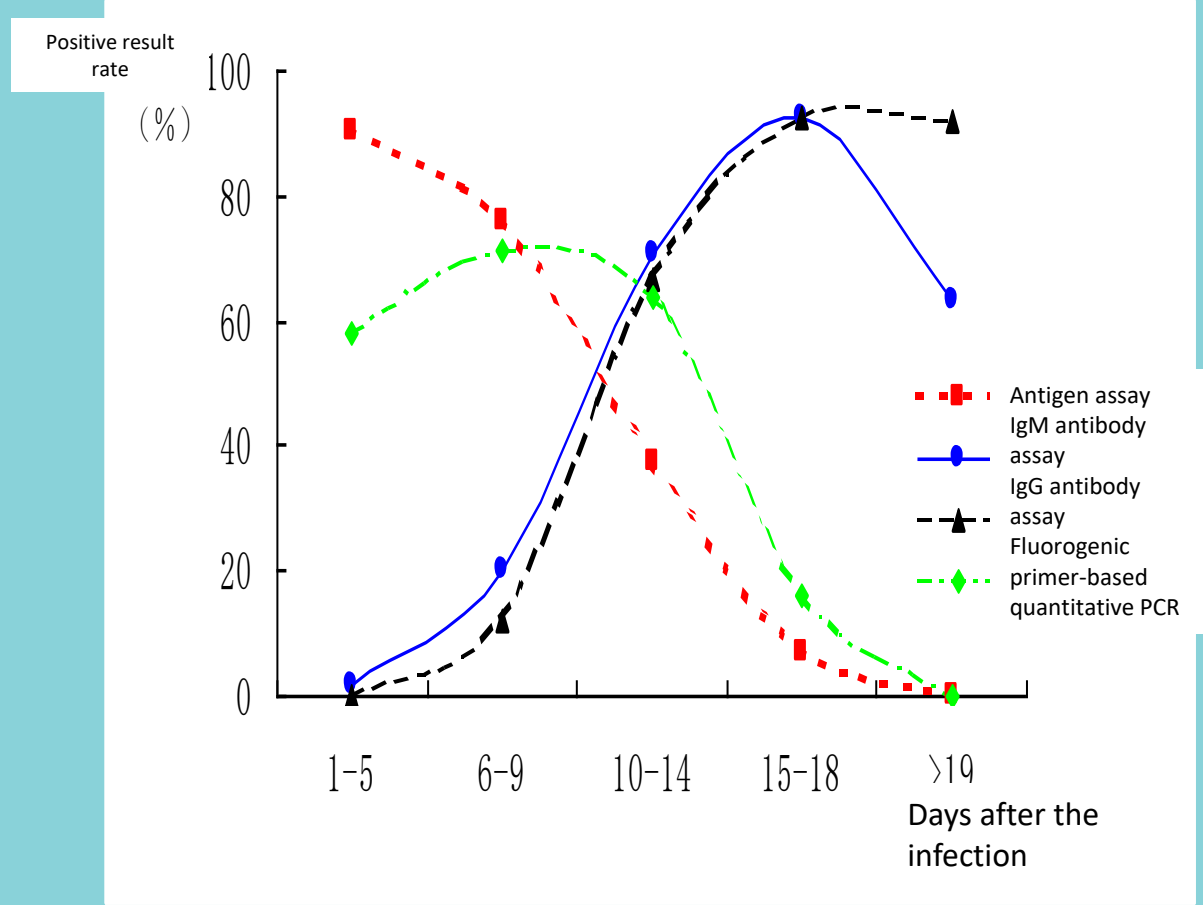


# Overview of the laboratory testing methods for SARS-CoV

Common testing method	Time required	Interpretation of the testing results and their implications
ELISA test for the virus nuclear protein (N) antigen	4 hours	Serum samples collected during 1—10 days after the disease onset (3-5 days most sensitive). The positive test result carries diagnosis significance. It is a sensitive test used for early diagnosis, with stable results and minor impacts from the quality of the specimen.
Real-time PCR assay for the viral nucleic acid	6 hours	Pharynx and anal swabs and serum specimens collected during 3-10 days since the disease onset. The positive results from multiple specimens have diagnosis significance. The method is applied to the early diagnosis, with stable results and good sensitivity. However, the specimens' quality exerts a major impact on the results.
Routine PCR assay for the viral nucleic acid	6 hours	Ditto, can be used for nucleic acid sequencing which has diagnosis significance. The method is applied for early diagnosis and is a stable and sensitive test. However, it can be impacted by the specimen's quality.
ELISA and fluorescence detection for IgG and IgM	4 hours	Only the serum collected during 10 days after the disease onset can yield results. The 4-times increase or the result turning positive carry diagnosis significance. The method is applied to mid and late stage diagnosis with stable results.
Neutralizing antibody assay	3-5 days	Only the serum collected during 10-22 days after the disease onset can yield results, with 4-time increase having diagnosis significance. The method should be applied to the mid and late stage diagnosis, with stable results.
Viral isolation	5-10 days	The pharynx and anal swabs and serum specimens collected during 1-10 days after the disease onset have diagnosis significance. The method is used for the mid and late stage diagnosis with major impacts from the samples' quality.

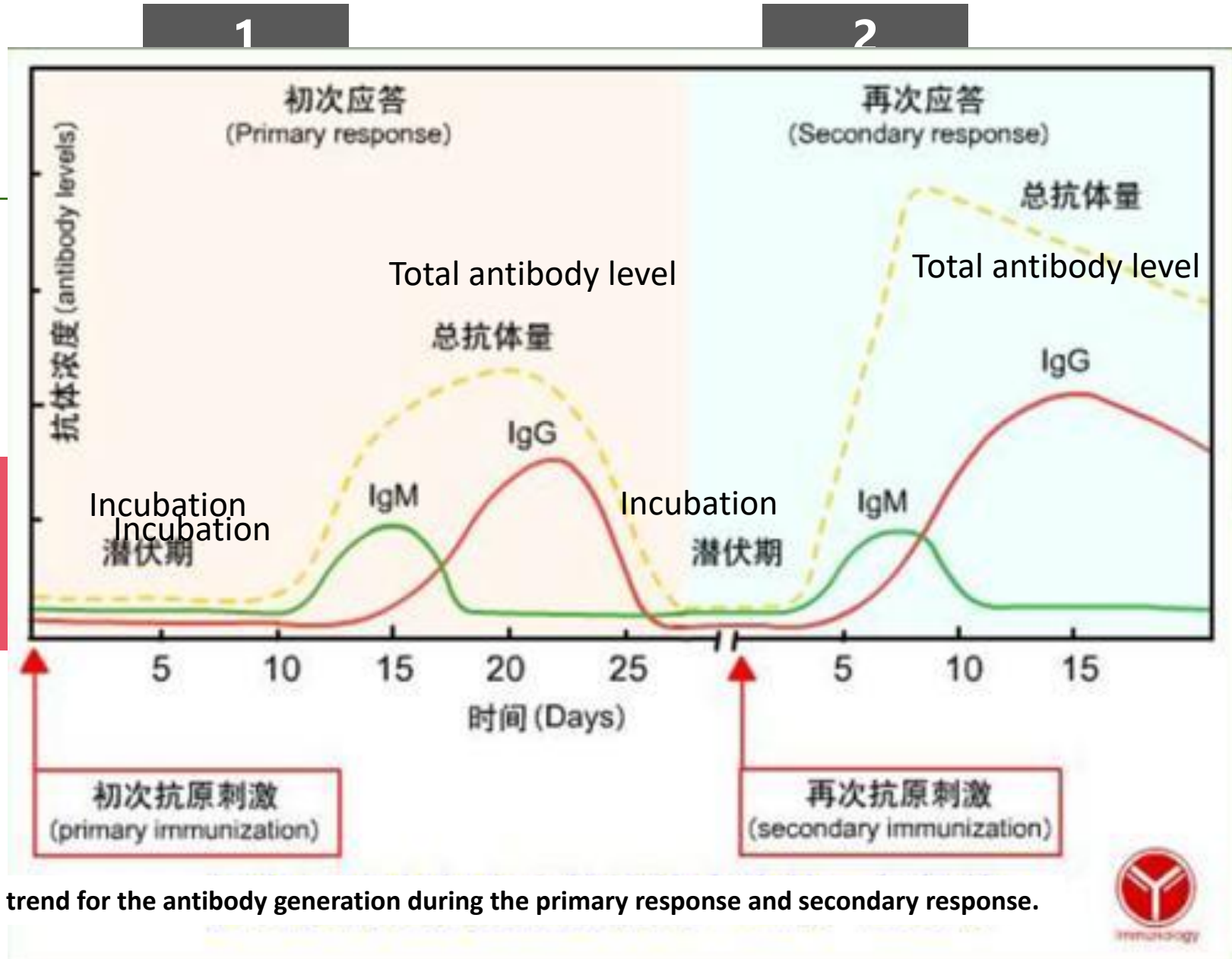


# SARS-CoV post-infection test markers





# Analysis of the test results



General trend for the antibody generation during the primary response and secondary response.

IgM +  
IgG +

IgM -  
IgG +

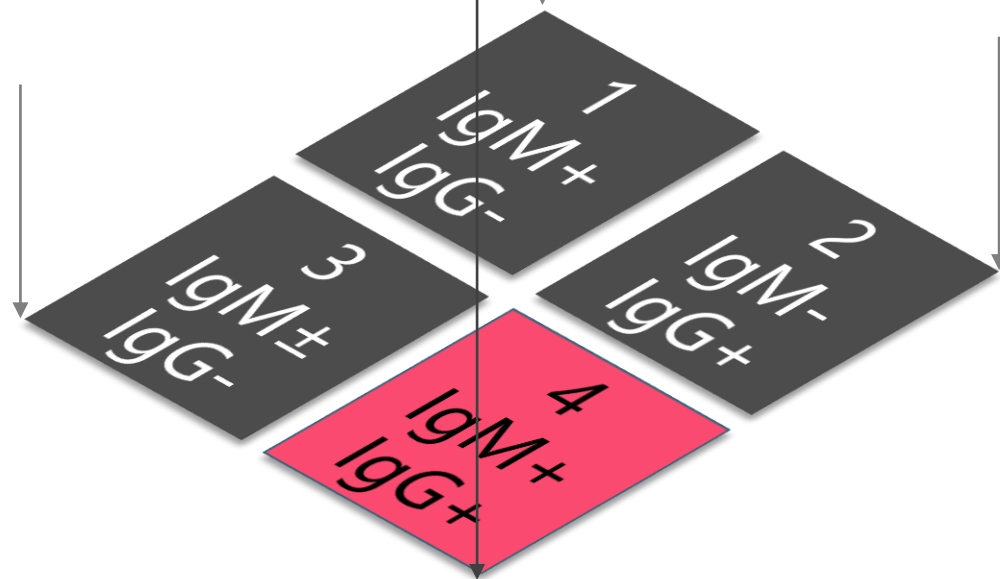




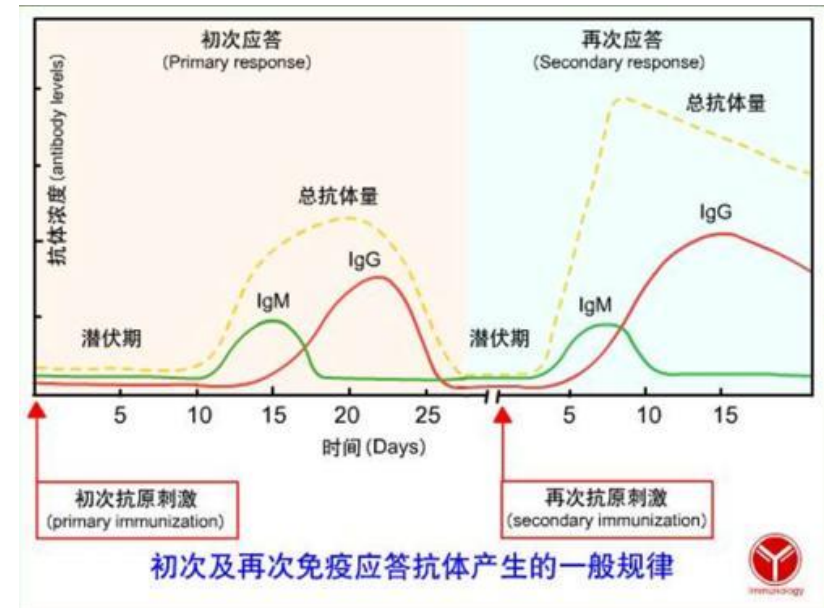
# Negative result from the nucleic acid assay

Active infection  
**感染活跃期**  
Early infection  
**感染早期**

Early infection  
**感染早期**



**恢复期**  
Recovery period







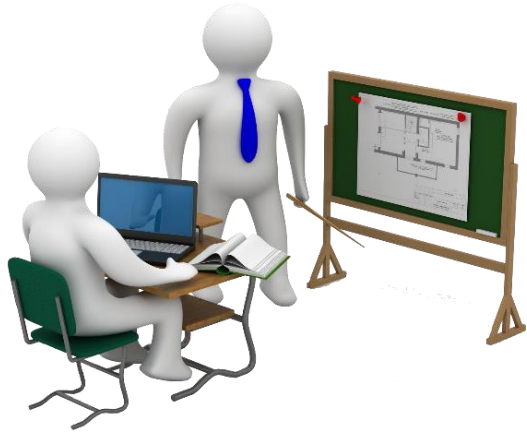
## Interpretation of the SAR-Cov-2 nucleic/antibody testing results

### 新冠肺炎核酸/抗体检测解读

No.	Nucleic acid	IgM	IgG	解读
1	+	-	-	Patients may be during the "window period" of nCoV infection in 2019, typically 2 weeks
2	+	+	-	May be at 2019-nCoV early infection
3	+	-	+	May be during the mid and late infection stage or recurrent infection. When the IgG antibody in the recovery period increases by 4 times or more compared with the acute phase, a recurrent infection can be diagnosed.
4	+	+	+	The patient is during the active infection, but its body has already developed a certain immunity to 2019-nCoV.
5	-	+	-	The patient's likely to be in the acute phase of 2019-nCoV infection. At this time, you need to question the results of nucleic acid testing. Other diseases such as rheumatoid factors have been found to cause weak IgM positive or positive tests.
6	-	-	+	May have been infected with 2019-nCoV in the past, but the patient has been recovered or the virus has been cleared. The IgG produced by the immune response is maintained for a long time and is still detected in the blood.
7	-	±	-	First infection with a very low viral load, and during an early stage. Thus, the viral load is lower than the lower limit of nucleic acid detection. The body produces a small amount of IgM antibodies, and has not yet produced IgG; a false positive result is caused by the patient's own rheumatoid factor
8	-	+	+	Recently infected 2019-nCoV and are in the recovery period. The internal virus is cleared, but the IgM has not been reduced to the lower limit of detection; or the nucleic acid test result is false negative, with the patient being in the active infection period

仅供大家参考，以临床综合判定为准！

For reference only. The clinical judgment should prevail



## Part IV

## Bio-safety requirements

- 总论
- 动物感染实验
- 灭活材料的操作
- 病毒培养
- 未经培养的感染性材料的操作

General introduction

Animal infection experiments

Operations of inactivated materials

Viral culture

Operations of the uncultured infectious substances



# Bio-safety requirements for the COVID-19 laboratory activities

According to the biological features, epidemiological characteristics, clinical data and other available information concerning the SARS-CoV-2, the pathogen shall be temporally managed as **Category B pathogens** and microorganisms based on its hazards.





# Bio-safety requirements for laboratory activities

## 1) Viral culture

Viral culture refers to operations such as virus isolation, culture, titration, neutralization test, purification of live virus and its protein, lyophilization of virus, and recombination test to produce live virus. The above operations should be performed in a biosafety cabinet of a BSL-3 laboratory. When viral medium is used to extract nucleic acid, the addition of lysing agent or inactivating agent must be performed under the same level of laboratory and protective conditions as viral culture. Laboratories shall report to the National Health Commission for approval and obtain relevant qualifications before carrying out the corresponding activities.





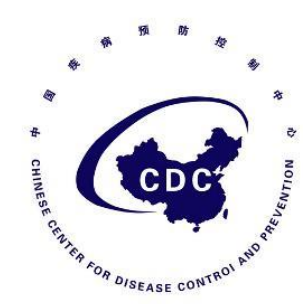
## 2) Animal infection experiment

Animal infection experiment refers to operations such as infecting animals with live viruses, sampling of infected animals, processing and testing of infectious samples, special test for infected animals, disposal of infected animal excrement, etc., which should be performed in a biosafety cabinet of a BSL-3 laboratory. Laboratories shall report to the National Health Commission for approval and obtain relevant qualifications before carrying out the corresponding activities.



### 3) Operation of uncultured infectious substances

The operation of uncultured infectious substances refers to viral antigen detection, serological testing, nucleic acid extraction, biochemical analysis, inactivation of clinical samples and other operations performed on uncultured infectious substances before inactivation through a reliable method. The operation should be performed in a BSL-2 laboratory, with personal protective equipment subject to BSL-3 laboratory protection requirements.

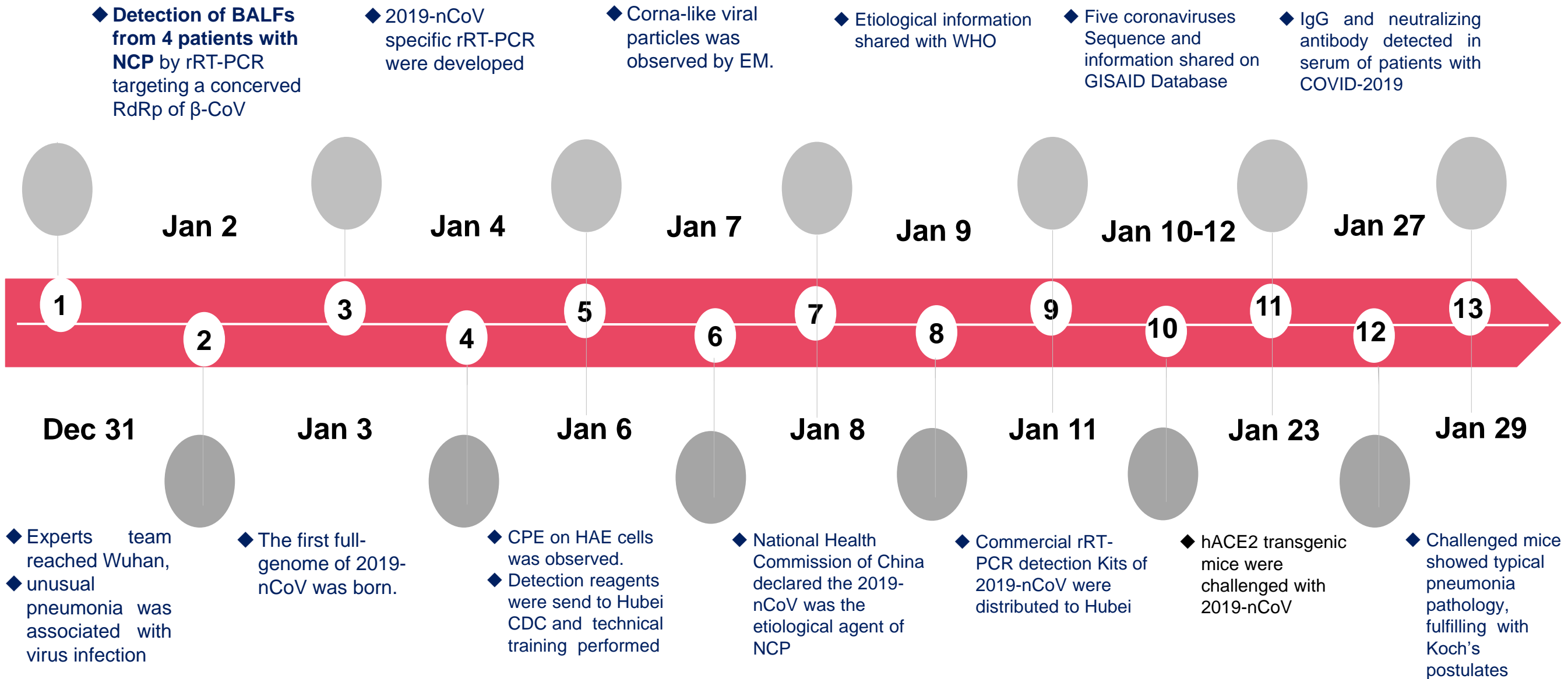


## 4) Operation of inactivated substances

After reliable inactivation of infectious substances or live viruses, operations such as nucleic acid testing, antigen testing, serological testing and biochemical analysis should be performed in a BSL-2 laboratory. Molecular cloning and other operations not involving live pathogenic viruses may be carried out in a BSL-1 laboratory.



# Vital milestones for the early laboratory work







Thank you!