Laboratory Testing for COVID-19

Biotech Centre for Viral Disease Emergency

National Institute for Viral Disease Control and Prevention

Chinese Centre for Disease Control and Prevention

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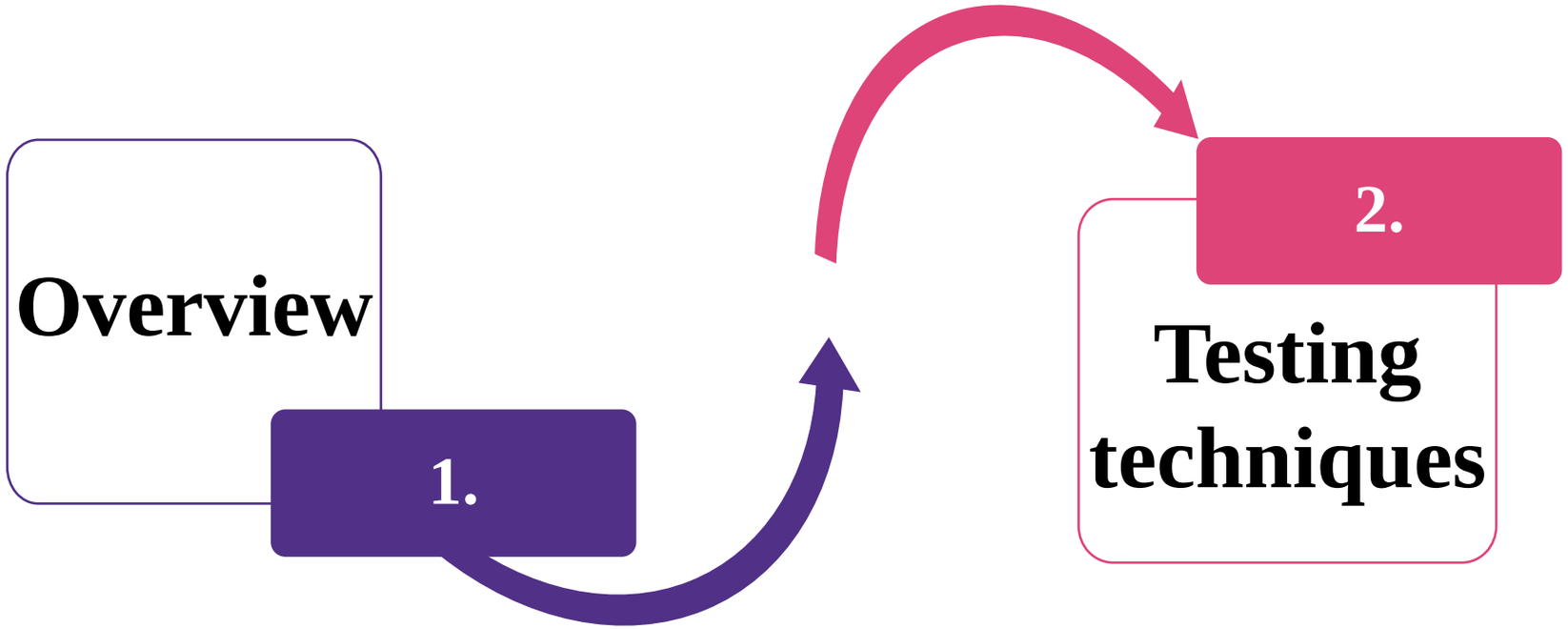
October 19, 2020

Table of contents




CDC
CHINESE CENTER FOR DISEASE CONTROl AND PREVENTION

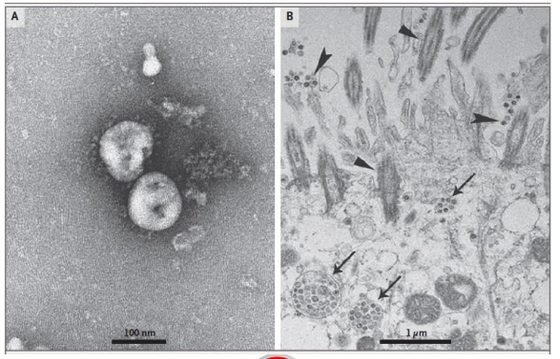

01
PART ONE

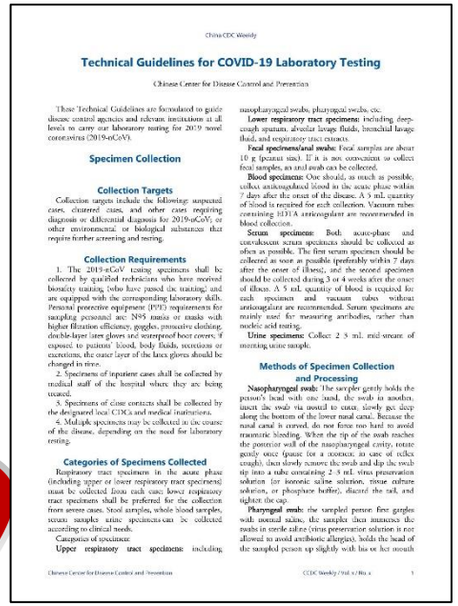
Overview

The identification of SARS-CoV-2

Jan 2, 2020
Samples arrived, RNA isolation and viral culture

Jan 7, 2020
Visualization of SARS-CoV-2 with transmission electron microscopy





Technical Guidelines for COVID-19 Laboratory Testing

Chinese Center for Disease Control and Prevention

These Technical Guidelines are formulated to guide disease control agencies and relevant institutions at all levels to carry out laboratory testing for 2019 novel coronavirus (2019-nCoV).

Specimen Collection

Collection Targets

Collection targets include the following: suspected cases, clustered cases, and other cases requiring diagnosis or differential diagnosis for 2019-nCoV; or other environmental or biological substances that require further screening and testing.

Collection Requirements

1. The 2019-nCoV 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) requirements for sampling personnel are: N95 masks or masks with higher filtration efficiency, goggles, protective clothing, double-layer latex gloves and waterproof boot covers; if exposed to patients' blood, body fluids, secretions or excretions, the outer layer of the latex gloves should be changed in time.

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 for laboratory testing.

Categories of Specimens 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, whole blood samples, serum samples, urine specimens can be collected according to clinical needs.

Categories of specimens:

Upper respiratory tract specimens: including nasopharyngeal swabs, pharyngeal swabs, etc.

Lower respiratory tract specimens: including deep-cough sputum, alveolar lavage fluids, bronchial lavage fluid, and respiratory tract extracts.

Fecal specimens/anal swabs: Fecal samples are about 10 g (peanut size). If it is not convenient to collect fecal samples, an anal swab can be collected.

Blood specimens: One should, as much as possible, collect anticoagulated blood in the acute phase within 7 days after the onset of the disease. A 5 mL quantity of blood is required for each collection. Vacuum tubes containing EDTA anticoagulant are recommended in blood collection.

Serum specimens: Both acute-phase and convalescent serum specimens should be collected as often 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 or 4 weeks after the onset of illness. A 5 mL quantity 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.

Urine specimens: Collect 2–5 mL mid-stream of morning urine sample.

Methods of Specimen Collection and Processing

Nasopharyngeal swab: The sampler gently holds the patient's head with one hand, the swab in another, inserts the swab via mouth to canal, slowly go 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 2–3 mL virus preservation solution (or isotonic saline solution, tissue culture solution, or phosphate buffer), discard the rod, and tighten the cap.

Pharyngeal swab: the sampled person first gargles with normal saline, the samples then immerse 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 his or her mouth

01

Jan 3, 2020
Whole genome sequencing

02

03

Jan 12, 2020
Sequences submitted to GISAID

04

Articles

Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding

Summary

Background In late December, 2019, patients presenting with viral pneumonia due to an unidentified microbial agent were reported in Wuhan, China. A novel coronavirus was subsequently identified as the causative pathogen, provisionally named 2019 novel coronavirus (2019-nCoV). As of Jan 21, 2020, more than 2000 cases of 2019-nCoV infection have been confirmed, most of which involved people living in or visiting Wuhan, and human-to-human transmission has been confirmed.

Methods We did next-generation sequencing of samples from bronchoalveolar lavage fluid and cultured isolates from nine inpatients, eight of whom had visited the Huanan seafood market in Wuhan. Complete and partial 2019-nCoV genome sequences were obtained from these individuals. Viral contigs were connected using Sanger sequencing to obtain the full-length genomes, with the terminal regions determined by rapid amplification of cDNA ends. Phylogenetic analysis of these 2019-nCoV genomes and those of other coronaviruses was used to determine the evolutionary history of the virus and help infer its likely origin. Homology modelling was done to explore the likely receptor-binding properties of the virus.

Findings The ten genome sequences of 2019-nCoV obtained from the nine patients were extremely similar, exhibiting more than 99·98% sequence identity. Notably, 2019-nCoV was closely related (with 88% identity) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in 2018 in Zhoushan, eastern China, but were more distinct from SARS-CoV (about 79%) and MERS-CoV (about 50%). Phylogenetic analysis revealed that 2019-nCoV fell within the subgenus Sarbecovirus of the genus Betacoronavirus, with a relatively long branch length to its closest relatives bat-SL-CoVZC45 and bat-SL-CoVZXC21, and was genetically distinct from SARS-CoV. Notably, homology modelling revealed that 2019-nCoV had a similar receptor-binding domain structure to that of SARS-CoV, despite amino acid variation at some key residues.

Interpretation 2019-nCoV is sufficiently divergent from SARS-CoV to be considered a new human-infecting betacoronavirus. Although our phylogenetic analysis suggests that bats might be the original host of this virus, an animal sold at the seafood market in Wuhan might represent an intermediate host facilitating the emergence of the virus in humans. Importantly, structural analysis suggests that 2019-nCoV might be able to bind to the angiotensin-converting enzyme 2 receptor in humans. The future evolution, adaptation, and spread of this virus warrant urgent investigation.

Funding National Key Research and Development Program of China, National Major Project for Control and Prevention of Infectious Disease in China, Chinese Academy of Sciences, Shandong First Medical University.

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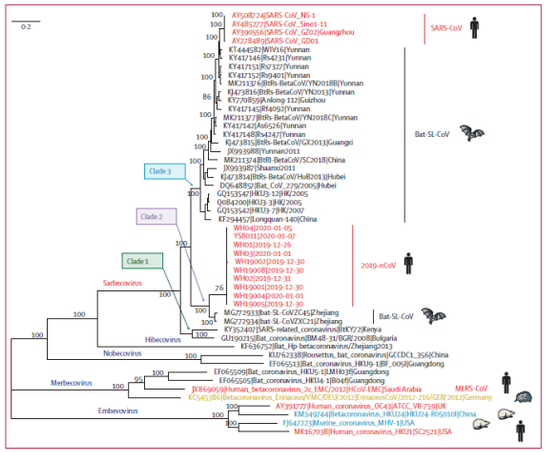
Introduction

Viruses of the family Coronaviridae possess a single, ... [illegible]



Timeline of the key events of the COVID-19 outbreak

Hu B., et al. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2020

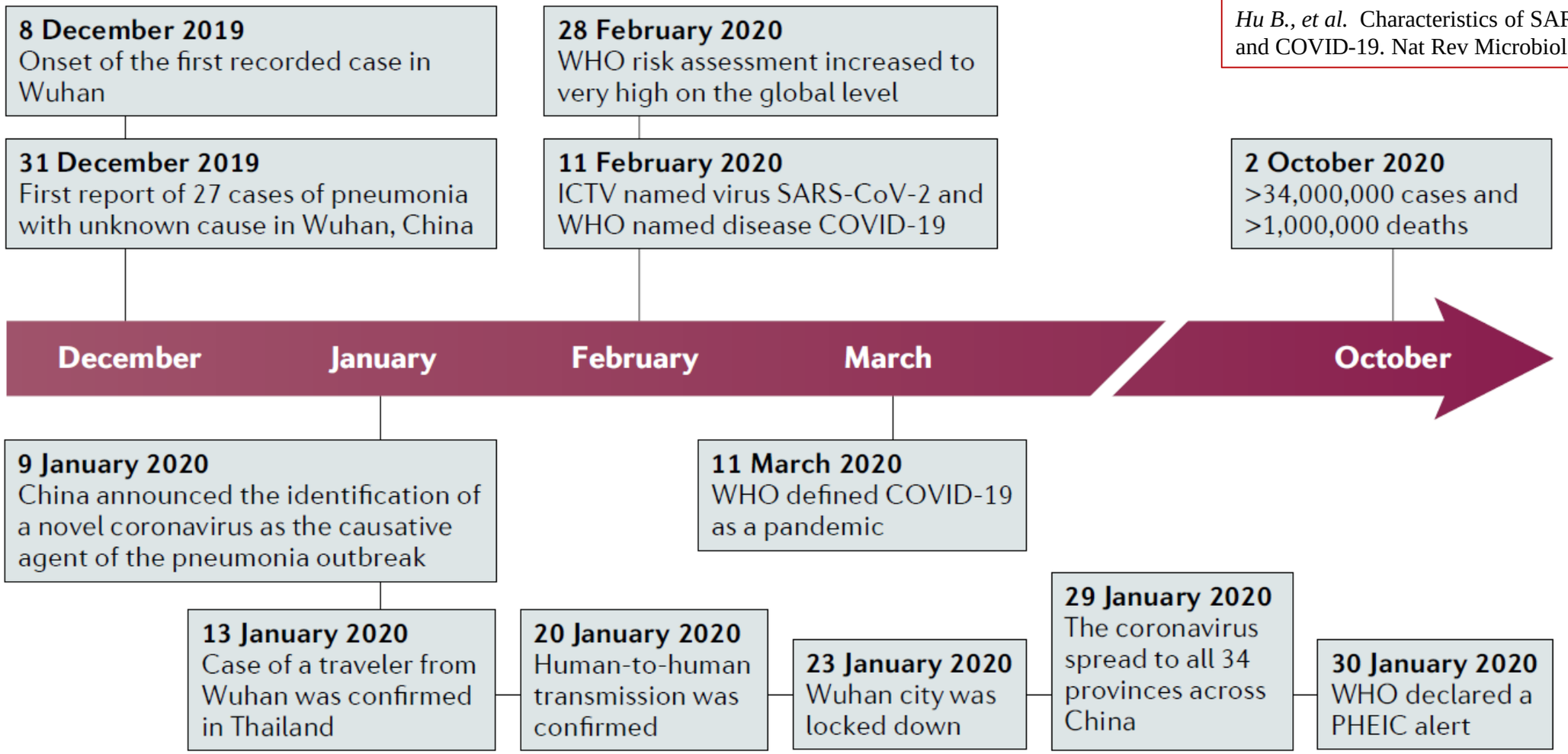


Fig. 1 | **Timeline of the key events of the COVID-19 outbreak.** The first recorded cases were reported in December 2019 in Wuhan, China. Over the course of the following 10 months, more than 30 million cases have been confirmed worldwide. COVID-19, coronavirus disease 2019; ICTV, International Committee on Taxonomy of Viruses; PHEIC, public health emergency of international concern; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

Coronavirus disease (COVID-19) Situation dashboard

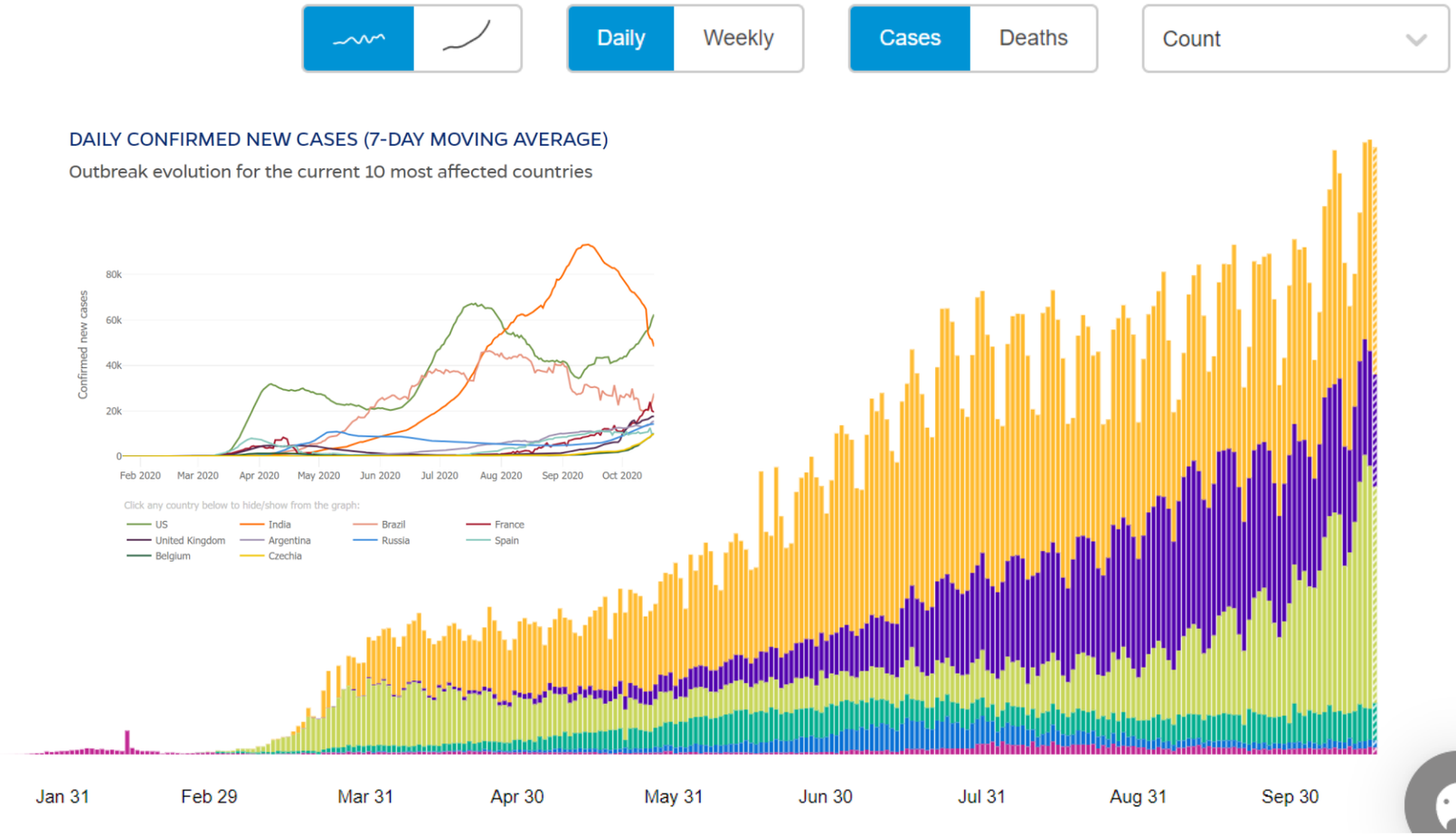
Globally, as of **18 October 2020**, there have been **39,596,858 confirmed cases** of COVID-19, including **1,107,374 deaths**, reported to WHO.

Situation by WHO Region

Americas	18,593,565 confirmed
South-East Asia	8,424,480 confirmed
Europe	7,889,116 confirmed
Eastern Mediterranean	2,749,606 confirmed
Africa	1,259,192 confirmed
Western Pacific	680,158 confirmed

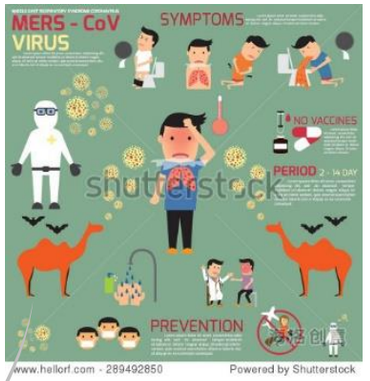
Source: World Health Organization

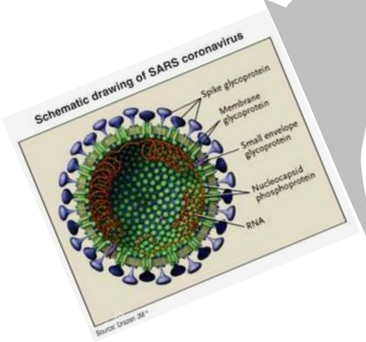
Data may be incomplete for the current day or week.



Symptoms of diseases caused by human coronavirus

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





HCoV-OC43
Hamre D, *et al.* 1966

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

HCoV-NL63
Lia van Hoek, *et al.* 2004

HCoV-HKU1
Patrick CYW, *et al.* 2005

MERS-CoV
Ali Moh Zaki, *et al.* 2012

SARS-CoV
Drosten C, *et al.* 2003

2019-nCoV
Zhu N, et al. 2020

02

PART TWO

Testing techniques

Laboratory testing techniques for COVID-19

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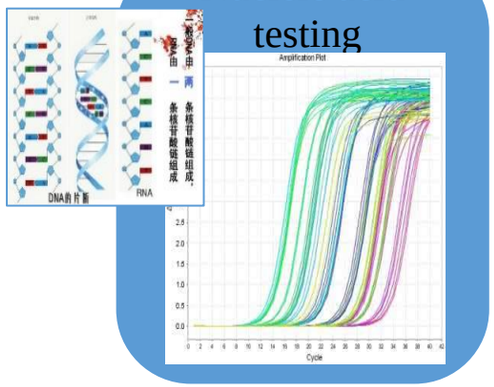
Nucleic acid testing



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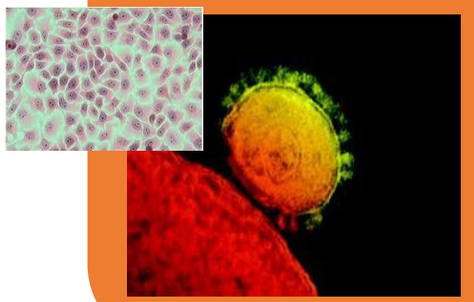
Viral isolation



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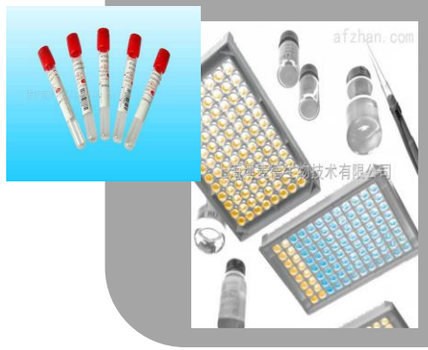
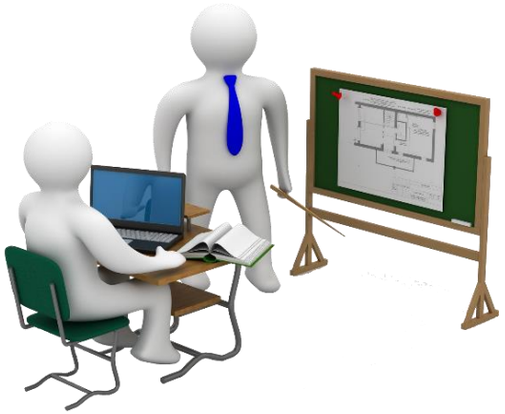
Serological testing



Contents



Part I Specimen collection requirements

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

1. Specimen collection targets

1
Suspected COVID-19 cases;

2
Others requiring diagnosis or differential diagnosis for COVID-19

2. Sample collection requirements

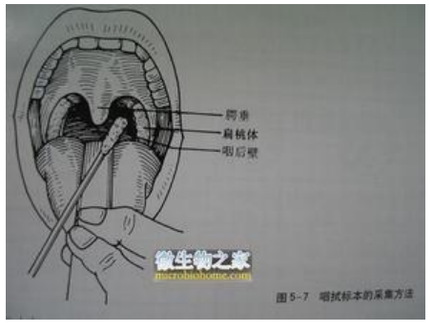
Sampling personnel

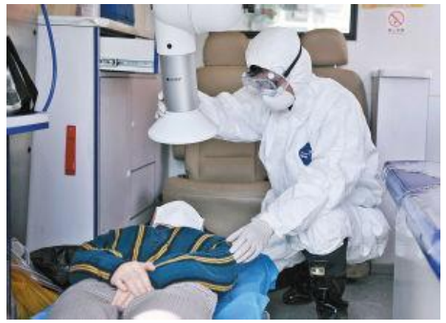
1. The COVID-2019 testing specimens shall be collected by qualified technicians who have passed biosafety training and are equipped with the corresponding laboratory skills. Personal protective equipment (PPE) is required for sampling personnel when performing the sampling
2. Specimens of inpatient cases 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. The 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: nasopharyngeal swabs, pharyngeal swabs etc.
2) Lower respiratory tract specimens: deep-cough sputum, alveolar lavage fluids, bronchial lavage fluid and respiratory tract extracts.
3) Fecal specimens: about 10 g (peanut size). If not convenient to collect, 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 morning urine sample.





CHINA CDC WEEKLY

CCDC 中国疾病预防控制中

Home

Article Navigati

Notes from

Yong Zhang[1,&]; C
Zijian Feng[3]; Gui
View author affili

The novel coronavirus (2019-nCoV) is spreading very fast in Hubei Province of China. As of February 14, 2020, 51,986 confirmed cases (including laborato in Hubei Province, and 1,318 of them died. Respi the most important routes of transmission of 201 coronavirus disease 2019 (COVID-19) cases, prev reasons for the rapid spread of this virus (*1*).

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-Shing 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

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【作者单位】State Key Laboratory for Emerging Infectious Diseases, Department of Microbiology, Carol Yu Centre for Infection, 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. Serial saliva viral load monitoring generally showed a declining tren 更多...

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Original article

Evaluating the accuracy of diffe

laboratory diagnosis and mo

2019-nCoV

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Li, Mingxia Zhang, Zhaoqin Wang, Li Xin
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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 Nanshan 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。

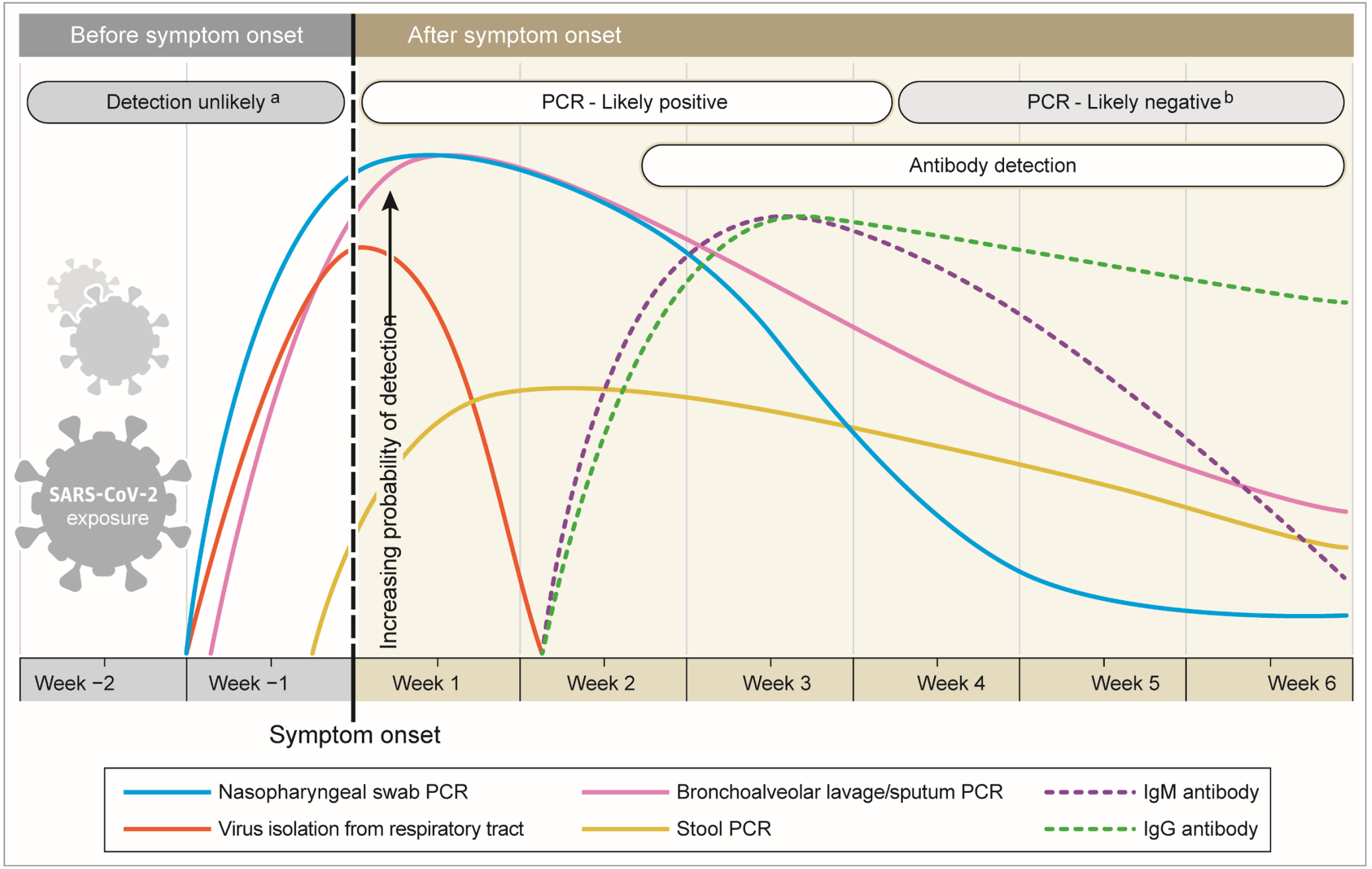
作者最新文章

"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

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

Relative to Symptom Onset

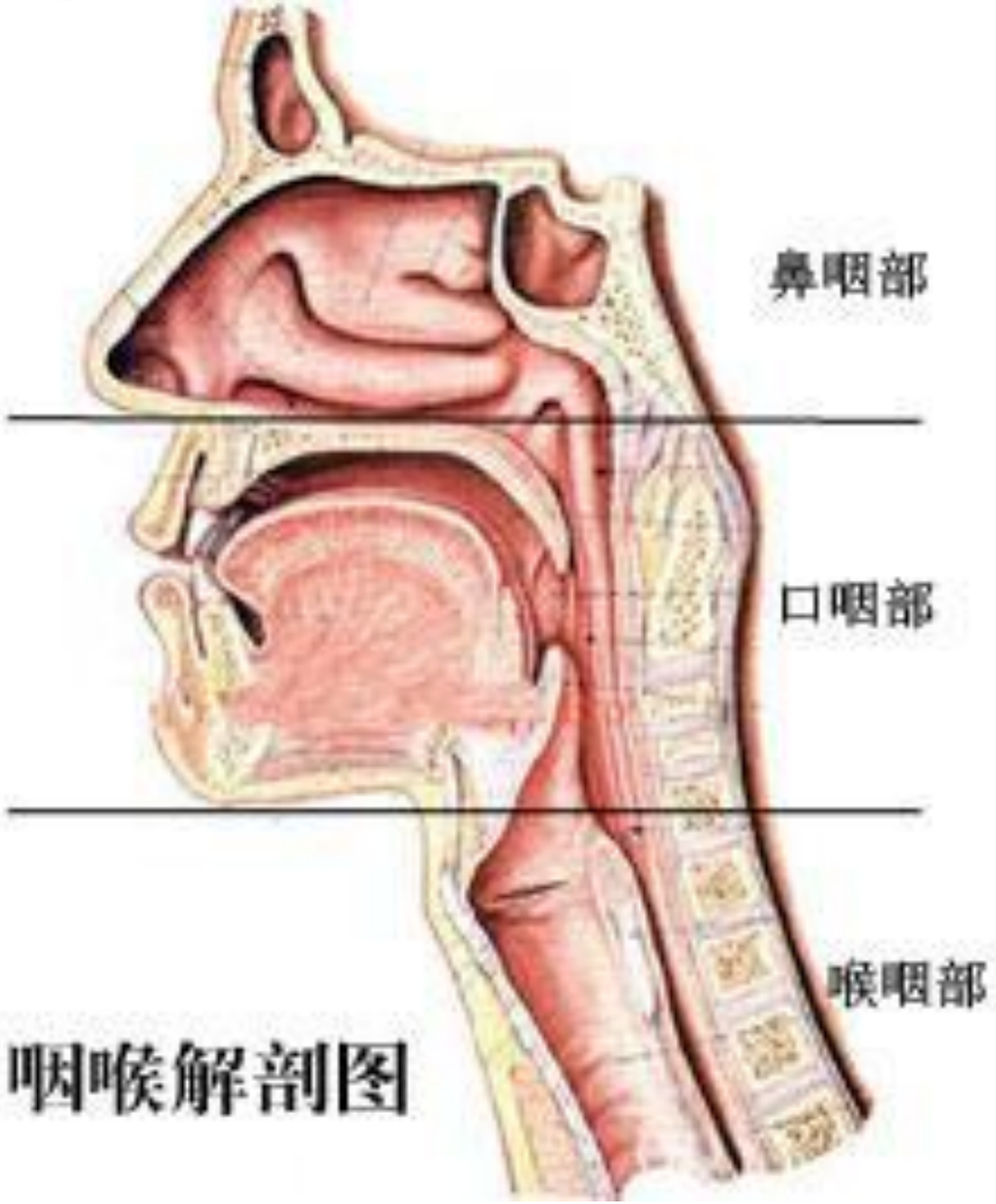


Sethuraman N, et al.
JAMA 2020

Pharynx structure

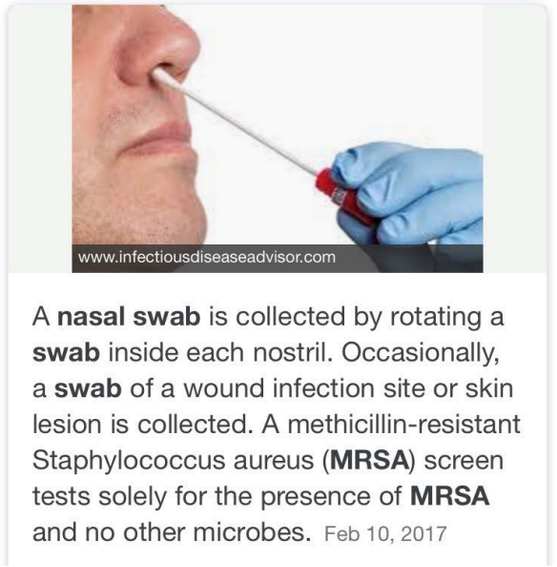
Anatomy of pharynx

1. Nasopharynx
2. Oropharynx
3. Laryngopharynx



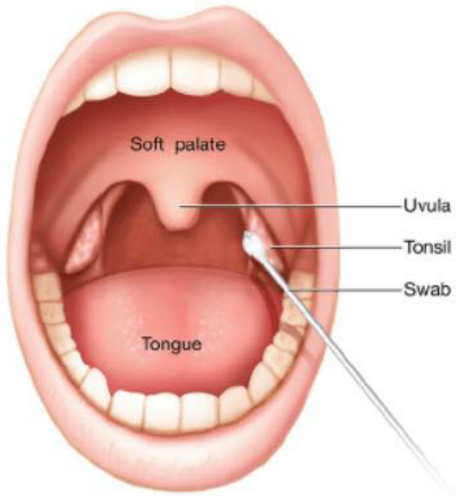
Collection of nasopharyngeal swab

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

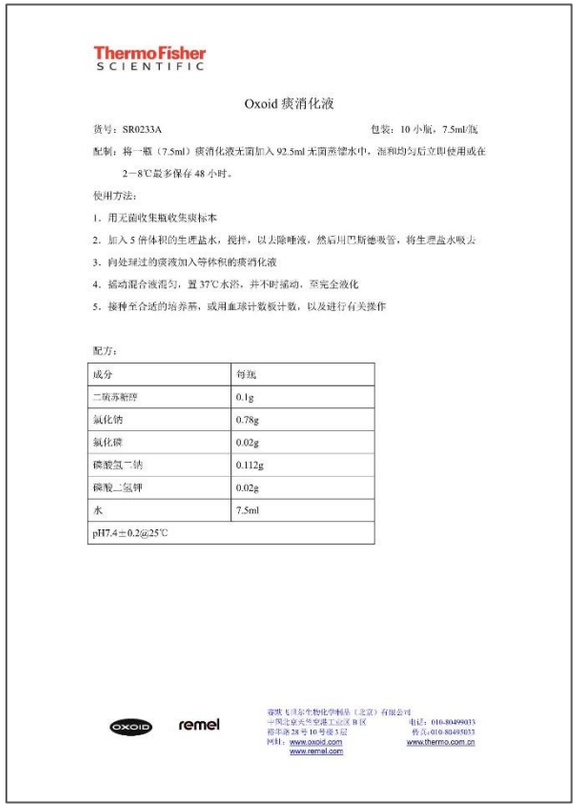
The person to be sampled first gargles with normal saline, the sampler immerses the swabs in sterile saline (virus preservation solution is not allowed to avoid antibiotic allergies), holds the head of the sampled person up slightly, with one’s mouth wide open, making a sound "ah" to expose the lateral 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 collection and 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 an equal volume of sputum digestion reagents.



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℃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. 摇动混合液混匀，置 37℃水浴，并不时摇动，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
二硫苏糖醇	0.1g
氯化钠	0.78g
氯化镁	0.02g
磷酸氢二钠	0.112g
磷酸二氢钾	0.02g
水	7.5ml
pH7.4±0.2@25℃	

OXOID　remel

Phosphate buffer containing 1 g/L of protease K or 0.1 g of dithiothreitol (DTT) 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 8,000 rpm for 5 minutes, absorb the supernatant for detection

Treatment solution for fecal specimen

211 g tris,
8.5 g sodium chloride,
1.1 g calcium chloride anhydrous or 1.47g calcium chloride containing crystalline water

dissolve into 800 ml deionized water, with the pH adjusted to 7.5 with hydrochloric acid, finally replenish 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.

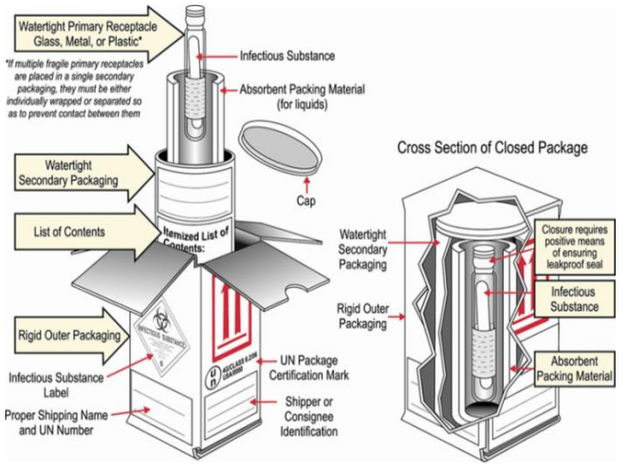
4. Specimen packaging and preservation

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.

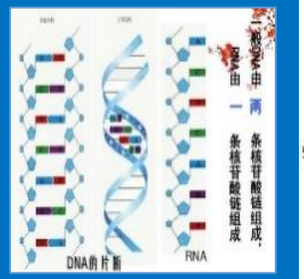
5. Specimen transportation

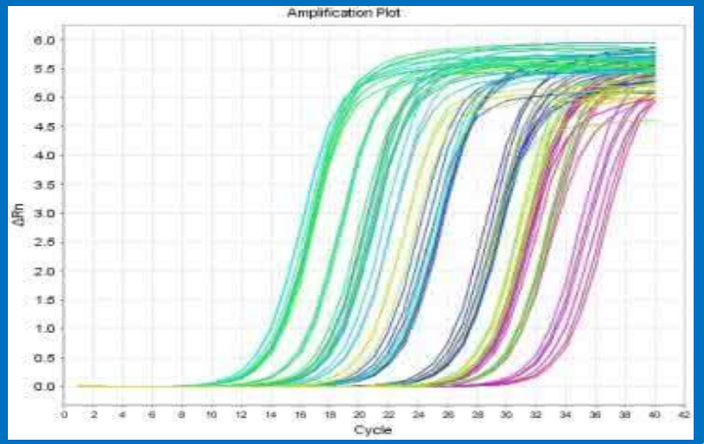
1. SARS-CoV-2 strains or other potentially infectious biological substances are subject to the packaging instructions for Category A substances assigned to UN2814, and the PI 602 of the Technical Instructions For The Safe Transport of Dangerous Goods by Air (Doc 9284) issued by ICAO
2. Environmental 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).



Laboratory testing techniques for COVID-19

Nucleic acid testing





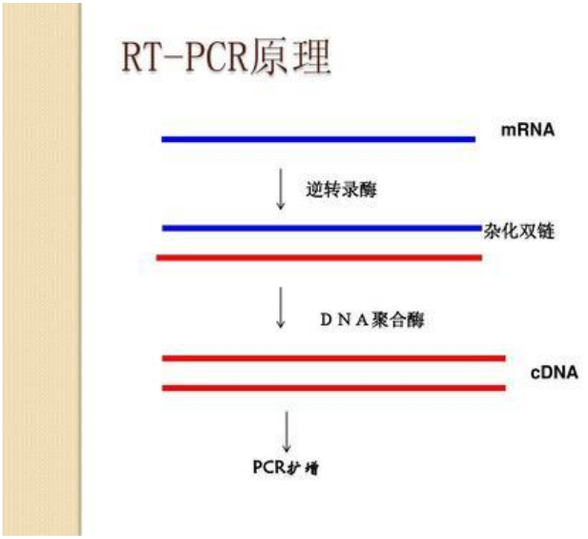
Serological testing

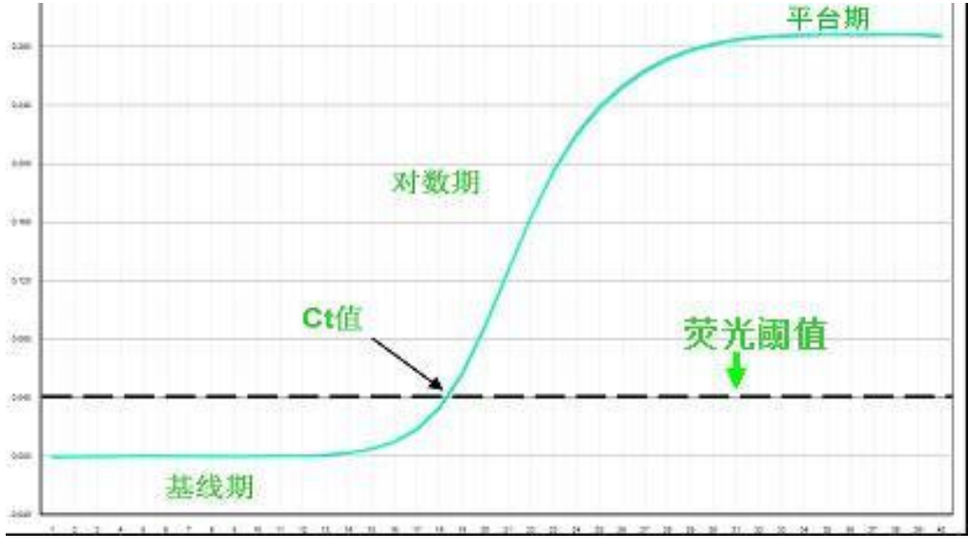
Part II

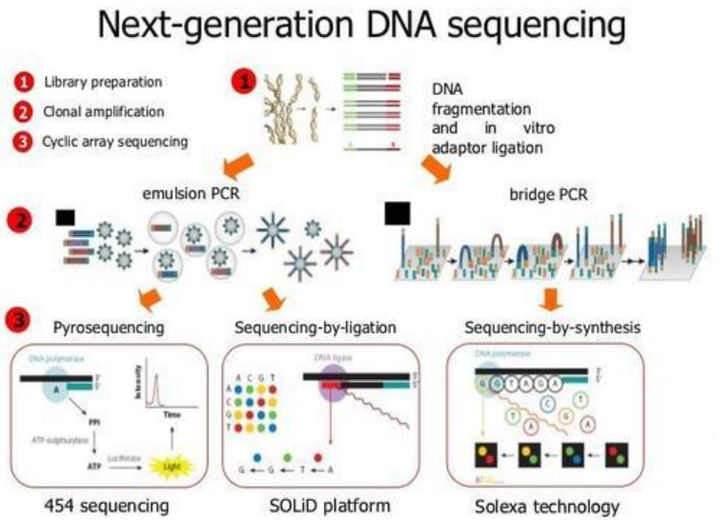
Nucleic acid testing

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

1. Nucleic acid testing techniques







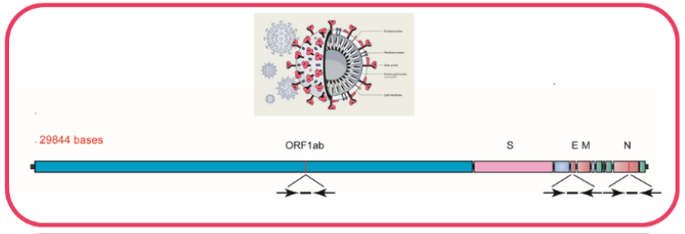
1. RT-PCR

2. Real time RT-PCR

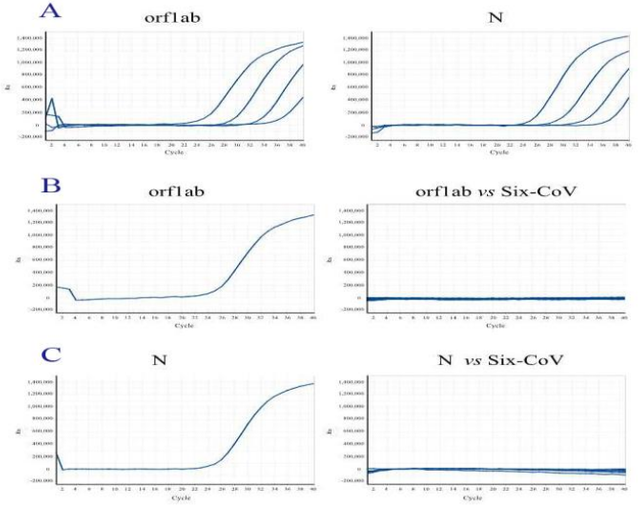
3. Sequencing

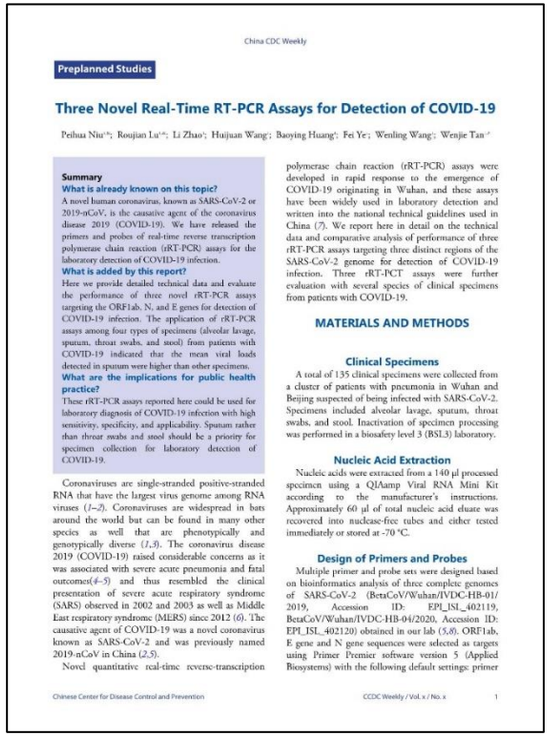
Real time RT-PCR established and shared on line in real time

Sensitivity of rRT-PCR targeted orf1ab and N (A) and specificity of ORF1ab (B) and N (C)-based rRT-PCR were evaluated rapidly.



Target 1 (ORF1ab):
Forward primer (F): CCCTGTGGGTTTTACACTTAA
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'




China CDC Weekly

Preplanned Studies

Three Novel Real-Time RT-PCR Assays for Detection of COVID-19

Peihua Niu[&]; Roujian Lu[&]; Li Zhao; Huijuan Wang; Baoying Huang; Fei Ye; Wenling Wang; Wenjie Tan

Summary

What is already known on this topic?
A novel human coronavirus, known as SARS-CoV-2 or 2019-nCoV, is the causative agent of the coronavirus disease 2019 (COVID-19). We have released the primers and probes of real-time reverse transcription polymerase chain reaction (rRT-PCR) assays for the laboratory detection of COVID-19 infection.

What is added by this report?
Here we provide detailed technical data and evaluate the performance of three novel rRT-PCR assays targeting the ORF1ab, N, and E genes for detection of COVID-19 infection. The application of rRT-PCR assays among four types of specimens (alveolar lavage, sputum, throat swabs, and stool) from patients with COVID-19 indicated that the mean viral loads detected in sputum were higher than other specimens.

What are the implications for public health practice?
These rRT-PCR assays reported here could be used for laboratory diagnosis of COVID-19 infection with high sensitivity, specificity, and applicability. Sputum rather than throat swabs and stool should be a priority for specimen collection for laboratory detection of COVID-19.

Coronaviruses are single-stranded positive-stranded RNA that have the largest virus genome among RNA viruses (*1–2*). Coronaviruses are widespread in bats around the world but can be found in many other species as well that are phenotypically and genotypically diverse (*1,3*). The coronavirus disease 2019 (COVID-19) raised considerable concerns as it was associated with severe acute pneumonia and fatal outcomes(*4–5*) and thus resembled the clinical presentation of severe acute respiratory syndrome (SARS) observed in 2002 and 2003 as well as Middle East respiratory syndrome (MERS) since 2012 (*6*). The causative agent of COVID-19 was a novel coronavirus known as SARS-CoV-2 and was previously named 2019-nCoV in China (*2,5*).

Novel quantitative real-time reverse-transcription polymerase chain reaction (rRT-PCR) assays were developed in rapid response to the emergence of COVID-19 originating in Wuhan, and these assays have been widely used in laboratory detection and written into the national technical guidelines used in China (*7*). We report here in detail on the technical data and comparative analysis of performance of three rRT-PCR assays targeting three distinct regions of the SARS-CoV-2 genome for detection of COVID-19 infection. Three rRT-PCT assays were further evaluation with several species of clinical specimens from patients with COVID-19.

MATERIALS AND METHODS

Clinical Specimens

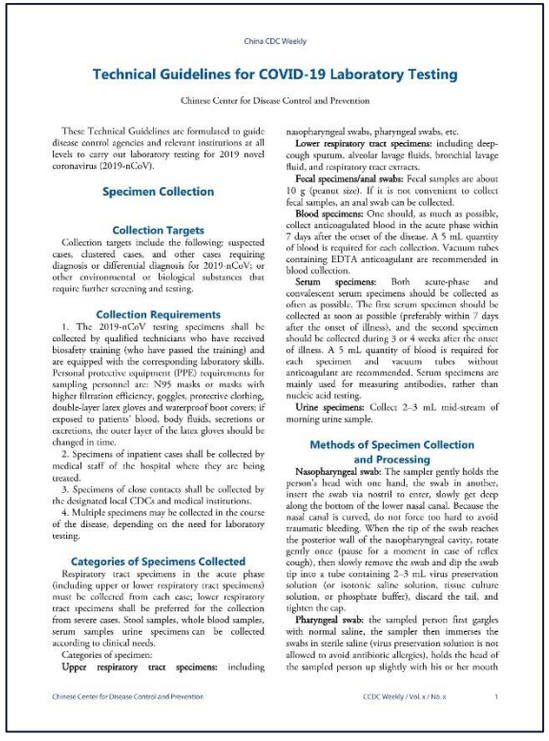
A total of 135 clinical specimens were collected from a cluster of patients with pneumonia in Wuhan and Beijing suspected of being infected with SARS-CoV-2. Specimens included alveolar lavage, sputum, throat swabs, and stool. Inactivation of specimen processing was performed in a biosafety level 3 (BSL3) laboratory.

Nucleic Acid Extraction

Nucleic acids were extracted from a 140 μl processed specimen using a QIAamp Viral RNA Mini Kit according to the manufacturer's instructions. Approximately 60 μl of total nucleic acid eluate was recovered into nuclease-free tubes and either tested immediately or stored at -70 °C.

Design of Primers and Probes

Multiple primer and probe sets were designed based on bioinformatics analysis of three complete genomes of SARS-CoV-2 (BetaCoV/Wuhan/IVDC-HB-01/2019, Accession ID: EPI_ISL_402119, BetaCoV/Wuhan/IVDC-HB-04/2020, Accession ID: EPI_ISL_402120) obtained in our lab (*5,8*). ORF1ab, E gene and N gene sequences were selected as targets using Primer Premier software version 5 (Applied Biosystems) with the following default settings: primer


China CDC Weekly

Technical Guidelines for COVID-19 Laboratory Testing

Chinese Center for Disease Control and Prevention

These Technical Guidelines are formulated to guide disease control agencies and relevant institutions at all levels to carry out laboratory testing for 2019 novel coronavirus (2019-nCoV).

Specimen Collection

Collection Targets

Collection targets include the following: suspected cases, clustered cases, and other cases requiring diagnosis or differential diagnosis for 2019-nCoV; or other environmental or biological substances that require further screening and testing.

Collection Requirements

1. The 2019-nCoV 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) requirements for sampling personnel are: N95 masks or masks with higher filtration efficiency, goggles, protective clothing, double-layer latex gloves and waterproof boot covers if exposed to patients' blood, body fluids, secretions or excretions, the outer layer of the latex gloves should be changed in time.
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 for laboratory testing.

Categories of Specimens 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, whole blood samples, serum samples urine specimens can be collected according to clinical needs.

Categories of specimen:

Upper respiratory tract specimens: including nasopharyngeal swabs, pharyngeal swabs, etc.

Lower respiratory tract specimens: including deep-cough sputum, alveolar lavage fluids, bronchial lavage fluid, and respiratory tract extracts.

Fecal specimens/anal swabs: Fecal samples are about 10 g (peanut size). If it is not convenient to collect fecal samples, an anal swab can be collected.

Blood specimens: One should, as much as possible, collect anticoagulated blood in the acute phase within 7 days after the onset of the disease. A 5 mL quantity of blood is required for each collection. Vacuum tubes containing EDTA anticoagulant are recommended in blood collection.

Serum specimens: Both acute-phase and convalescent serum specimens should be collected as often 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 or 4 weeks after the onset of illness. A 5 mL quantity 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.

Urine specimens: Collect 2–3 mL mid-stream of morning urine sample.

Methods of Specimen Collection and Processing

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 2–3 mL virus preservation solution (or isotonic saline solution, tissue culture solution, or phosphate buffer), discard the tail, and tighten the cap.

Pharyngeal swab: the sampled person first gargles with normal saline, the sampler then 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 his or her mouth


中国疾病预防控制中心
病毒病预防控制所
National Institute For Viral Disease Control and Prevention

首页 | 机构信息 | 党群建设 | 疾病控制 | 科技工作 | 教育培训 | 实验室管理 | 国际合作

新型冠状病毒核酸检测引物和探针序列（Specific primers and probes for detection 2019 novel coronavirus）

来源：病毒病所 发布时间：2020-01-21

1.新型冠状病毒核酸测定（实时荧光RT-PCR方法）

推荐选用针对新型冠状病毒的开放读码框1ab（open reading frame，ORF1ab）、核壳蛋白（nucleoprotein，N）基因区域的引物和探针。

Target 1（ORF1ab）：

正向引物（F）：CCCTGTGGGTTTTACACTTAA

反向引物（R）：ACGATTGTGCATCAGCTGA

荧光探针（P）：5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'

Target 2（N）：

正向引物（F）：GGGGAACTTCTCCTGCTAGAAT

反向引物（R）：CAGACATTTTGCTCTCAAGCTG

荧光探针（P）：5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'

可以核酸提取和实时荧光RT-PCR反应体系参考相关厂家试剂盒说明。

2.结果判断

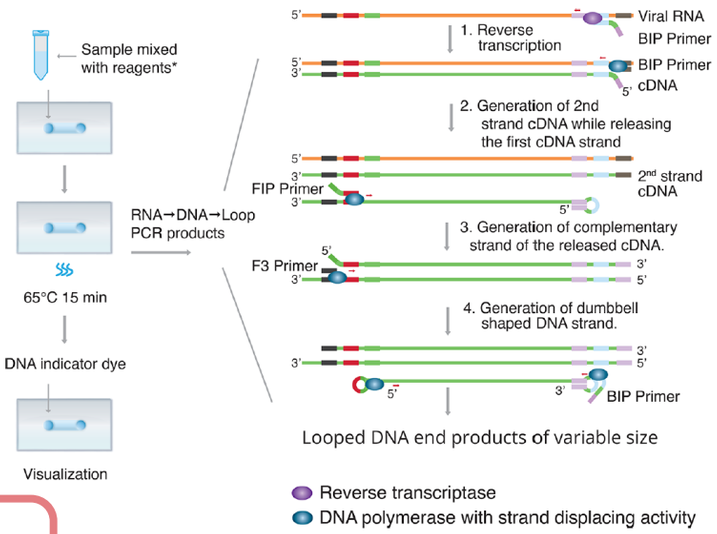
阴性：无Ct值或Ct为40。

阳性：Ct值<37，可报告为阳性。

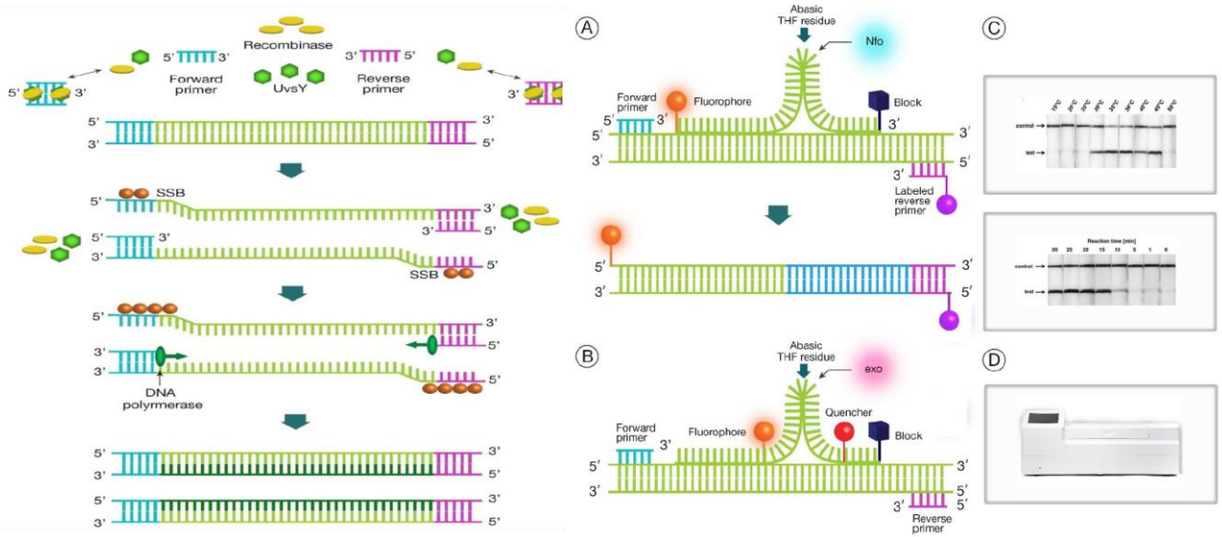
可疑：Ct值在37-40之间，建议复复实验，若复做结果Ct值<40，扩增曲线有明显起峰，该样本判断为阳性，否则为阴性。

SARS-CoV-2 specific real time RT-PCR detection technology was rapidly established and shared with the whole world after the first batch clinical samples were sequenced and detected in IVDC, China CDC. The detection technology including specific primers and probes were all contained in "**Technical Guidelines for COVID-19 Laboratory Testing"** drafted by IVDC, China CDC and available publicly, which lays foundation for the development of commercial detection kits.

RT-LAMP

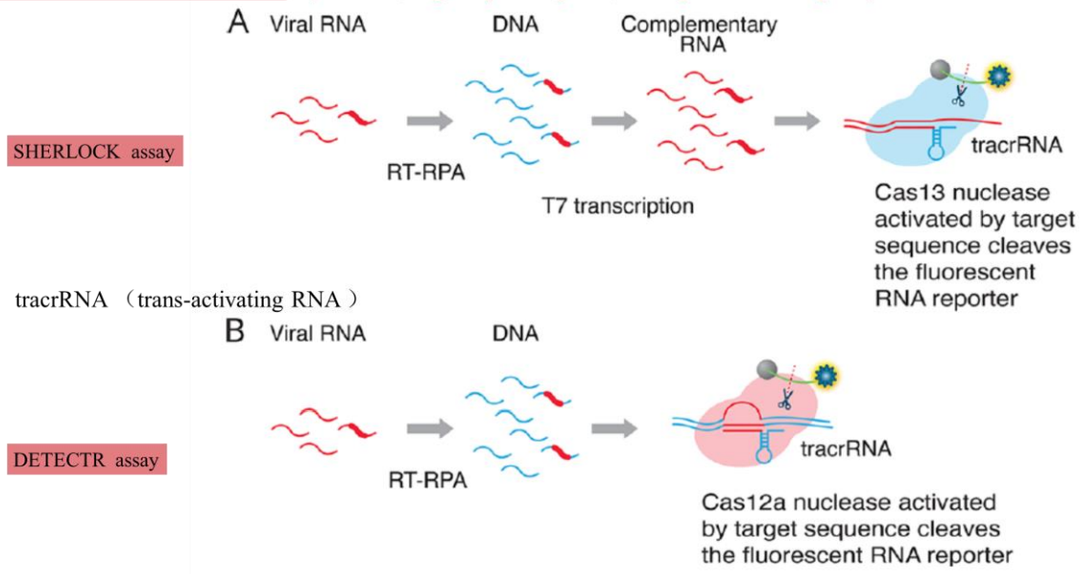


RT-RPA

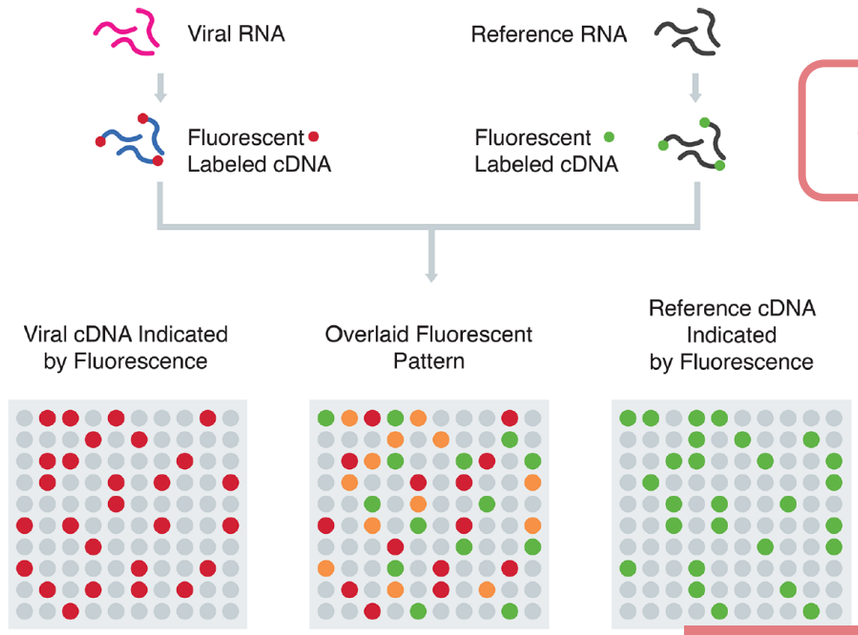


CRISPR

(clustered, regularly interspaced, short palindromic repeats)



Nucleic acid hybridization



Figures from ACS Cent. Sci. 2020, 6, 591−605

2. List of SARS-CoV-2 nucleic acids test kits approved by the NMPA

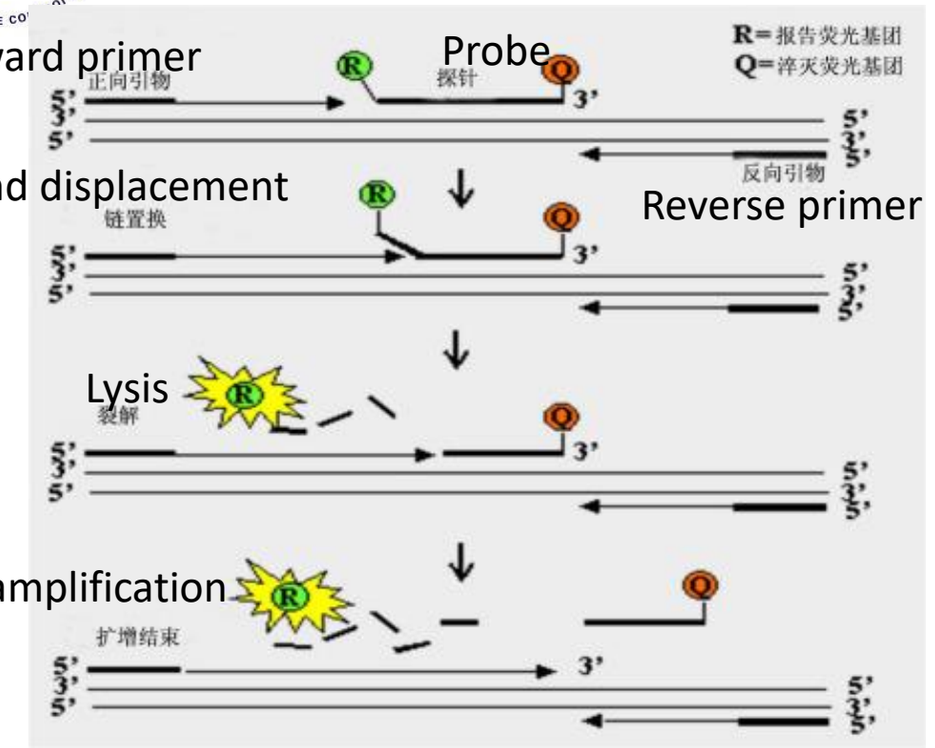
No	Registration company	Detection principle	Targets	Approved No	Approval date (YY-MM-DD)
1	Shanghai ZJ Bio-Tech Co., Ltd.	Real time RT-PCR	ORF1ab, E, N	20203400057	2020-01-26
2	Shanghai GeneoDX Biotech Co., LTD	Real time RT-PCR	ORF1ab, N	20203400058	2020-01-26
3	BGI Biotechnology (Wuhan) CO., LTD	Real time RT-PCR	ORF1ab	20203400060	2020-01-26
4	Daan Gene Co., Ltd. of Sun Yat-Sen University	Real time RT-PCR	ORF1ab, N	20203400063	2020-01-28
5	Sansure Biotech Inc.	Real time RT-PCR	ORF1ab, N	20203400064	2020-01-28
6	Shanghai BioGerm Medical Biotechnology Co., Ltd.	Real time RT-PCR	ORF1ab, N	20203400065	2020-01-31
7	Beijing Applied Biological Technologies Co., Ltd.	Real time RT-PCR	ORF1ab, E, N	20203400179	2020-02-27
8	Maccura Biotechnology Co., Ltd.	Real time RT-PCR	ORF1ab, E, N	20203400184	2020-03-01
9	Wuhan EasyDiagnosis Biomedicine Co. Ltd	Real time RT-PCR	ORF1ab, N	20203400212	2020-03-12
10	Shanghai Fosun Long March Medical Science Co., Ltd.	Real time RT-PCR	ORF1ab, E, N	20203400299	2020-03-24
11	Beijng Kinghawk Pharmaceutical Co., Ltd.	Real time RT-PCR	ORF1ab, N,	20203400322	2020-04-03
12	Jiangsu Bioperfectus Technologies Co., Ltd	Real time RT-PCR	ORF1ab, N	20203400384	2020-04-16
13	Zhejiang Oriental genetic biological products Co., Ltd	Real time RT-PCR	ORF1ab, N	20203400520	2020-05-21
14	Shenzhen United Medical Science and Technology Co., Ltd.	Real time RT-PCR	ORF1ab	20203400535	2020-06-05
15	Beijing NaGene Diagnosis Reagent Co., Ltd	Real time RT-PCR	ORF1ab, N	20203400537	2020-06-09
16	Coyote Biotechnology CO., LTD	Real time RT-PCR	ORF1ab, N	20203400644	2020-07-13
17	BGI Biotechnology (Wuhan) CO., LTD	Sequencing	2019-nCoV	20203400059	2020-01-26
18	Chengdu CapitalBio Jingxin Biotechnology Co., Ltd.	Isothermal Amplification on Disk Chip	2019-nCoV S, N Influenza A, New Influenza A H1N1 Virus (2009) Influenza A H3N2, Influenza B, RSV	20203400178	2020-02-22
19	Ustar Biotechnologies (Hangzhou), Ltd.	Isothermal Amplification of RNA-Real time RT-PCR	ORF1ab, N	20203400241	2020-03-16
20	Anbio (Xiamen) Biotechnology Co., Ltd	Hybrid Capture-Immunofluorescence Assay	ORF1ab, N, E	20203400298	2020-03-24
21	Rendu (Shanghai) Biotechnology Co., Ltd	RNA Capture Probe	ORF1ab	20203400300	2020-03-26
22	Wuhan Zhongzhi Biotechnologies Inc.	Isothermal Amplification of RNA-Gold Probes Chromatography	ORF1ab, E	20203400301	2020-03-31
23	Wuhan Zhongzhi Biotechnologies Inc.	Isothermal Amplification of RNA-dual amplification	ORF1ab, E	20203400302	2020-03-31

Comparison of detection methods for nucleic acids

Method	Sample	Detected material	Key features
RT-PCR	• Nasopharyngeal swab • Oropharyngeal swab • Bronchoalveolar lavage • Tracheal aspirates • Saliva	Viral RNA	• Duration: 2–5 days • Accuracy: High • Primary use: Gold standard diagnostic test • Cost: High (Reagents and Equipment) • Major limitations: Time and cross reactivity with other viruses (false positives)
Emerging Methods			
Isothermal amplification • RT-LAMP • RT-RPA	Blood (finger stick)	Viral RNA	• Duration: Minutes (<30 min) • Accuracy: To be determined • Primary use: Rapid screening • Cost: Medium (Specific reagents) • Major limitations: Requires validation
CRISPR/Cas13a	Blood (finger stick)	Viral RNA	• Duration: Minutes • Accuracy: To be determined • Use: Rapid diagnosis • Cost: Low • Major limitations: Requires validation
NGS	Blood (finger stick)	Viral RNA	• Duration: Hours–days • Accuracy: High • Primary use: Genomic profiling of virus • Cost: High (Reagents and Equipment) • Major limitations: Cost, mainly used for genetic mapping rather than diagnostic

D'Cruz RJ, Currier AW and Sampson VB (2020) Laboratory Testing Methods for Novel Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2).
Front. Cell Dev. Biol. 8:468.
doi: 10.3389/fcell.2020.00468

Summary of nucleic acids detection for SARS-CoV-2



Forward primer

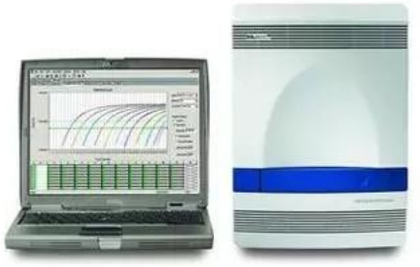
Probe

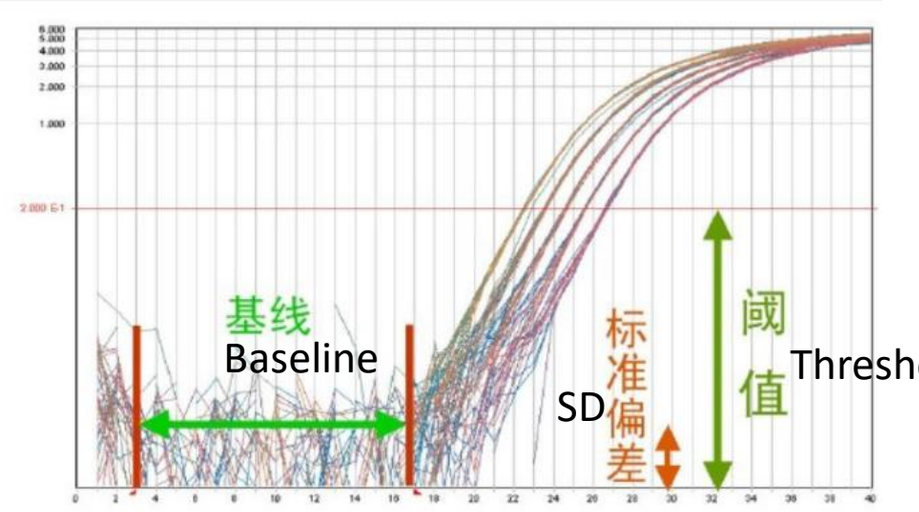
strand displacement

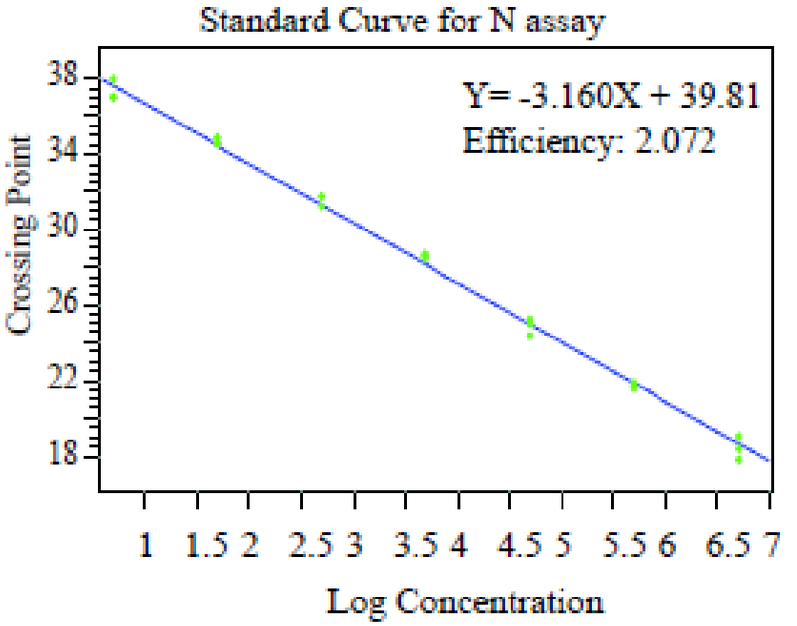
Reverse primer

Lysis

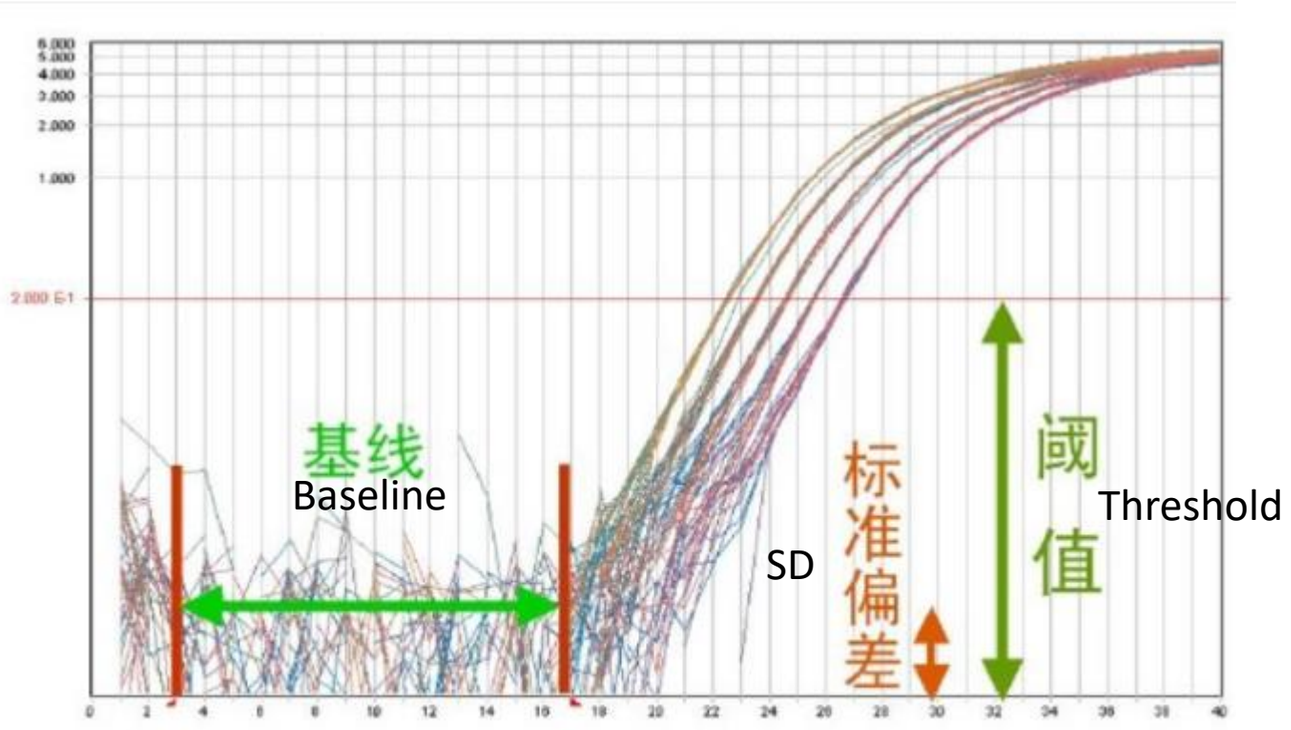
End of amplification

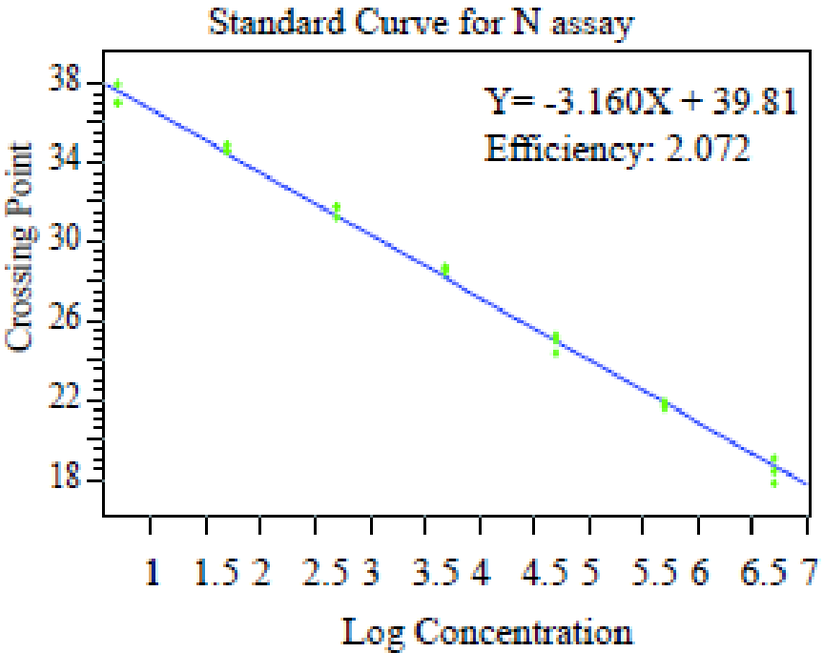






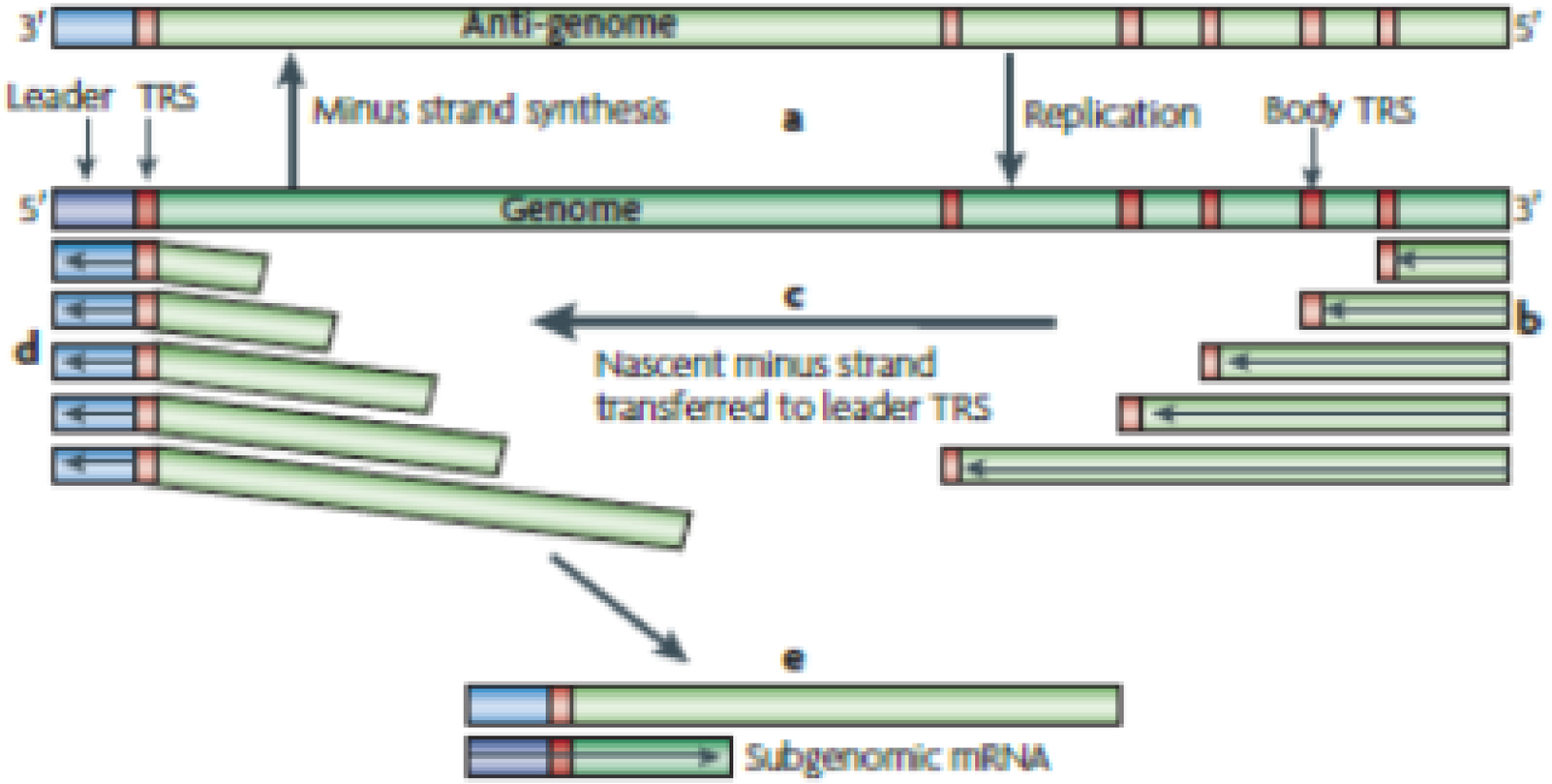
Several detection methods, such as rRT-PCR, Isothermal Amplification, Hybrid Capture-Immunofluorescence Assay, and probe-based RNA Capture have been developed to detect nucleic acids of the 2019-nCoV. Among all the approved nucleic acid detection kits, novel rRT-PCR techniques were developed in rapid response to the emergence of COVID-19 in China, and have been written into the technical guidelines (Chinese Center for Disease Control and Prevention 2020) and widely used.





1. Baseline: In the first few cycles of PCR amplification reaction, the fluorescence signal is close to a straight line as it does not change significantly. Thus, the baseline is a horizontal line.
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 of 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.

4. Unique intracytoplasmic discontinuous transcription pattern of coronavirus



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

Prone to genome mutation and recombination

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

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

1. Negative: no Ct value or Ct value is 40.

2. Positive: Ct value < 37.

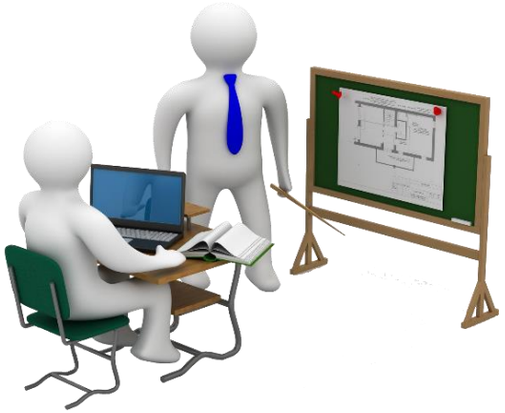
3. Repeated experiments are recommended should Ct value range between 37 and 40. If the Ct value reads <40 and the amplification curve has obvious peaks, the sample should be considered being tested positive, otherwise it should be considered as negative.

6. Confirmation of COVID-19 positive cases

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

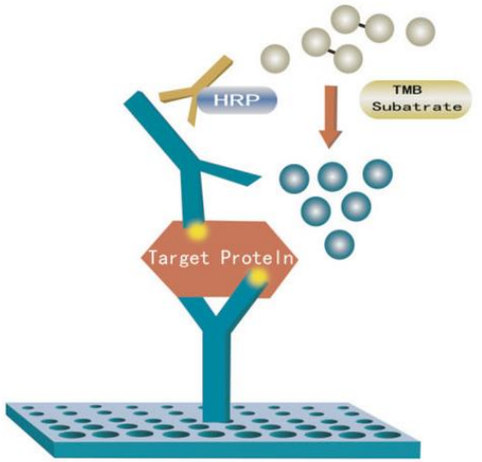
1. The real-time fluorescence-based RT-PCR assay of the 2019-nCoV in the same specimen shows that the two targets, ORF1ab and Protein N, are both positive. In case of the result showing positive for one target, then samples shall be re-collected for another test. If it is still positive for a single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

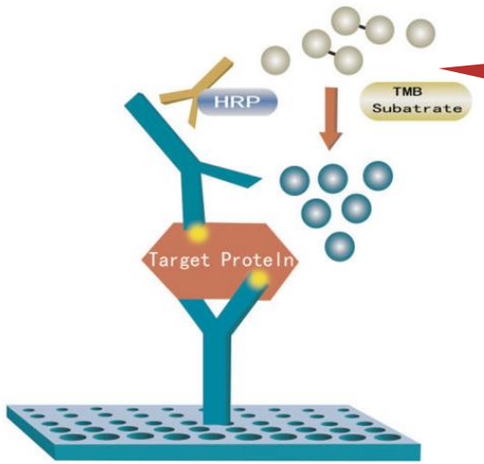


Part III Antibody testing

- Antibody testing methods
- ELISA’s principle
- Colloidal gold antibody testing
- Time points for nucleic acid and antibody detection

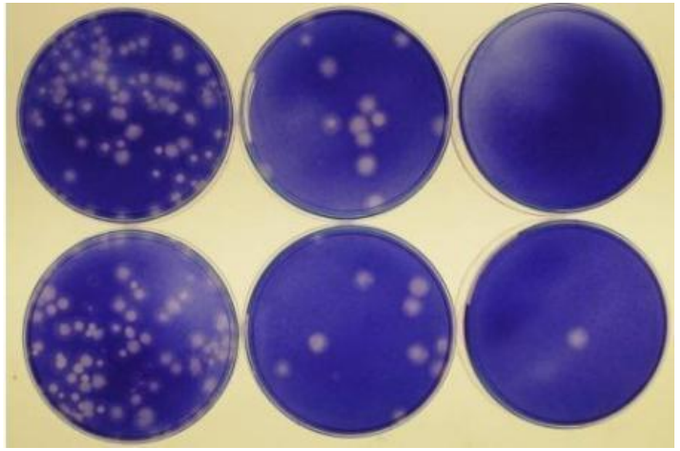


1. Antibody testing assays

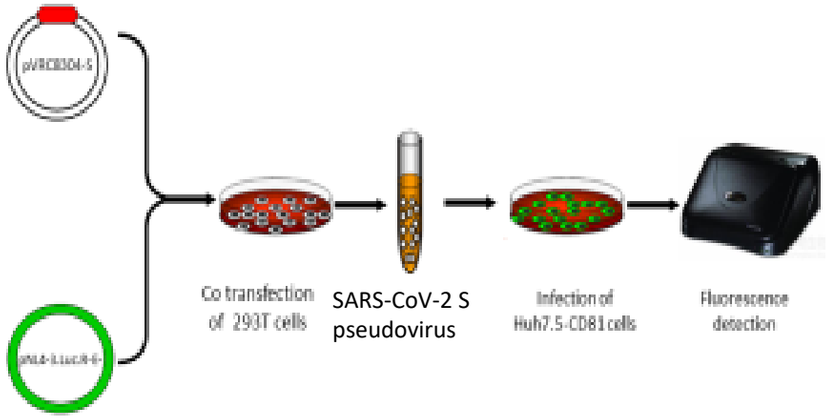


Antibody detection

1.PRNT



2.ppNT

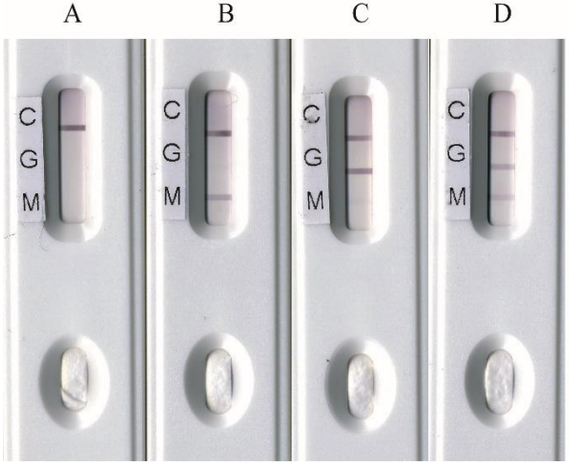


The antibody detection test used for supplementary detection of suspected cases with negative SARS-CoV-2 nucleic acid.

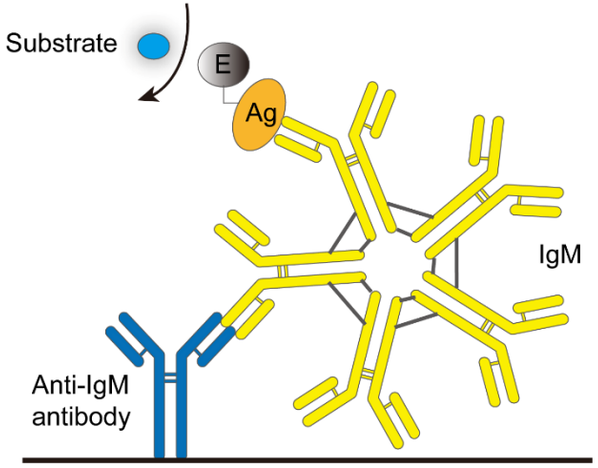
IgM：Appeared about 5 days POI and peaked about 2-3 weeks POI;

IgG：Appeared about 7-14 days POI, increased gradually and kept a long time.

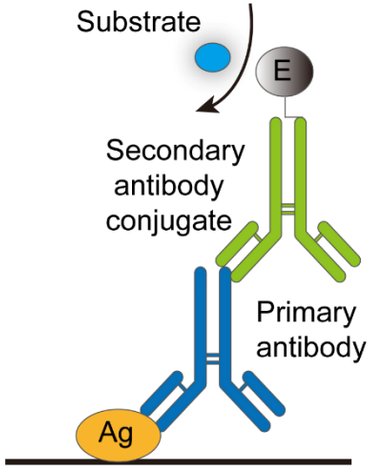
3. Colloidal gold lateral flow immunochromatography

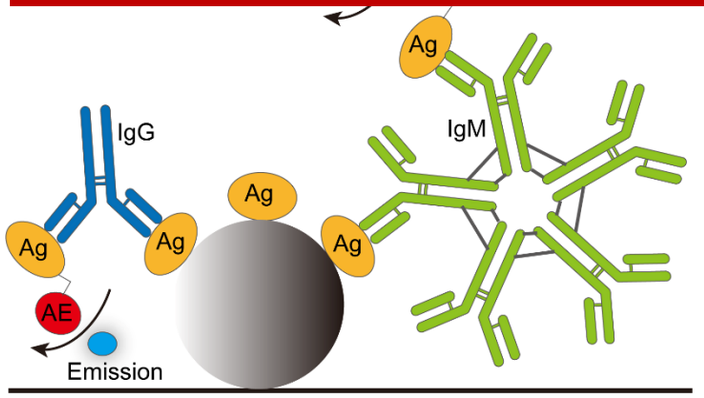


4. Capturing ELISA for IgM detection

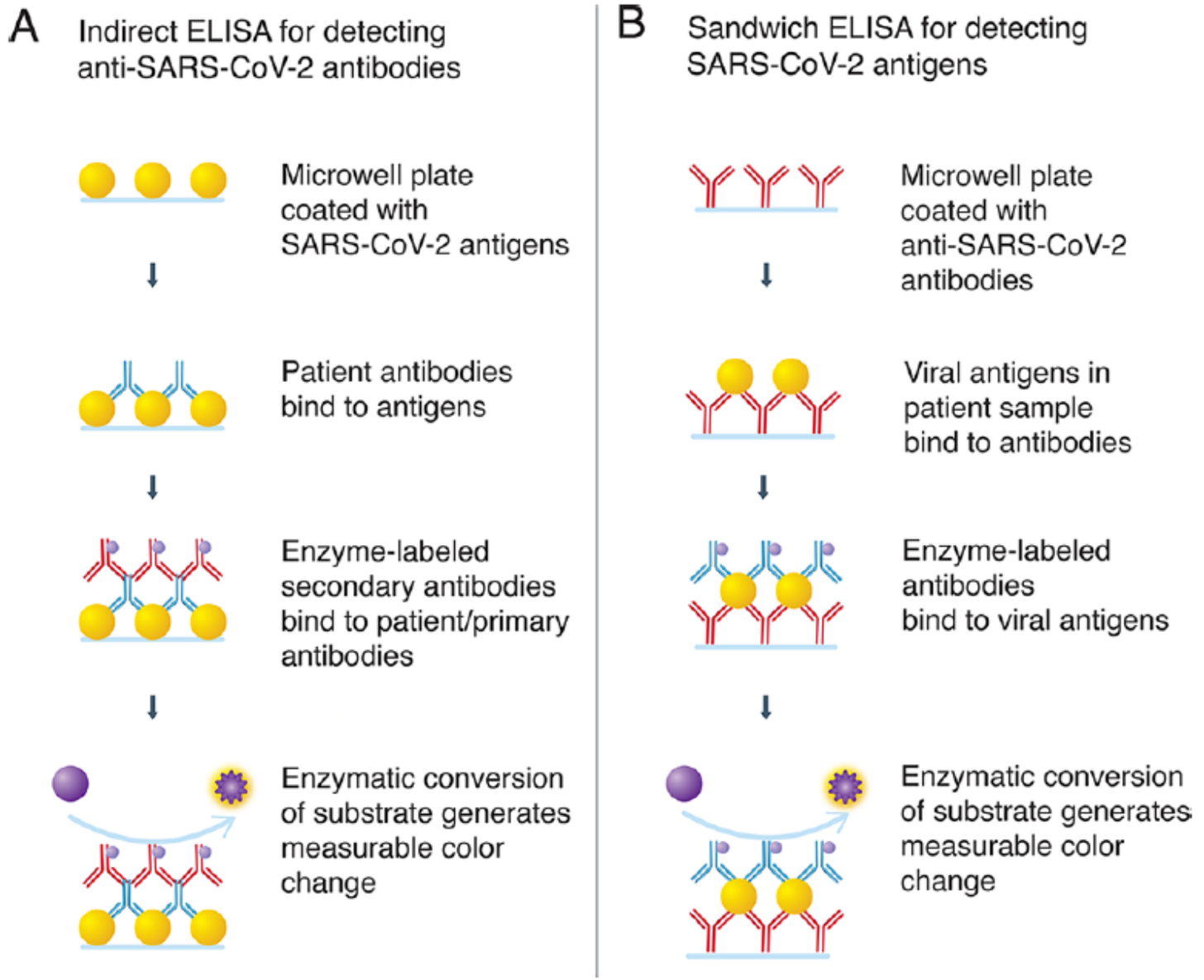


5. Indirect ELISA for Ig detection





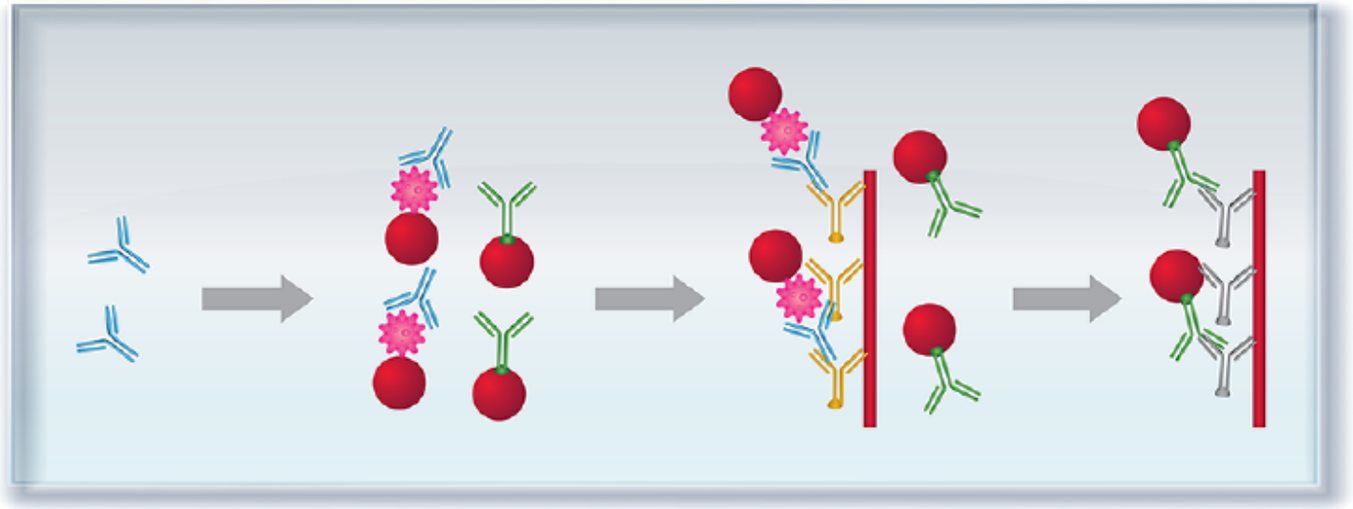
ELISA



ACS Cent. Sci. 2020, 6, 591−605

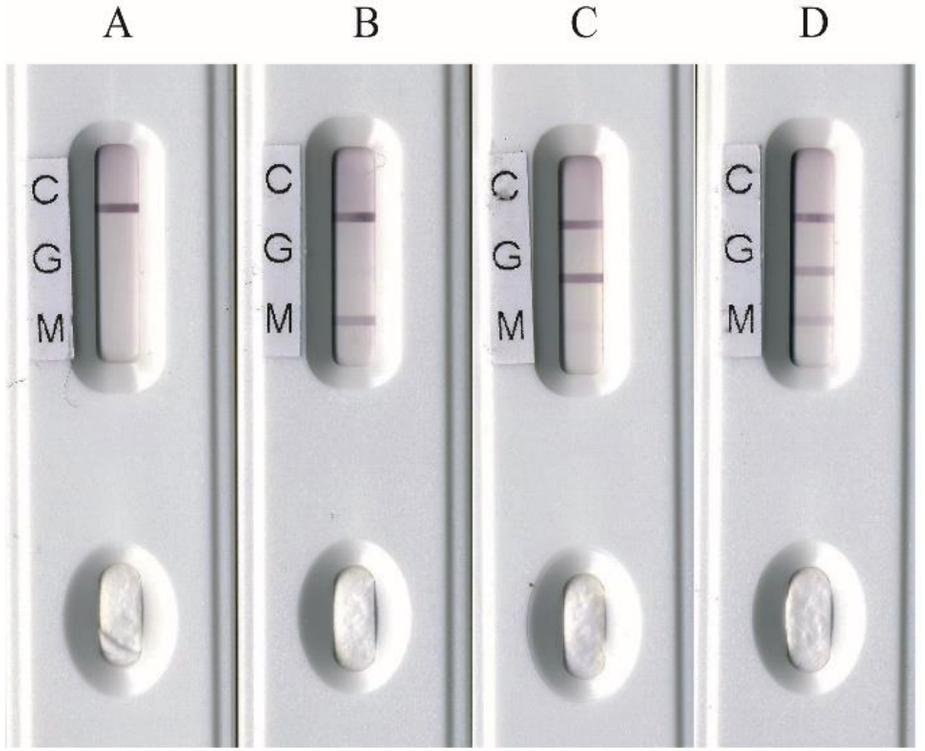
Figure 5. ELISA assays detecting antibodies (A) or antigens (B).

Colloidal gold lateral flow immunochromatography



Lateral Capillary Flow (Nitrocellulose Membrane)

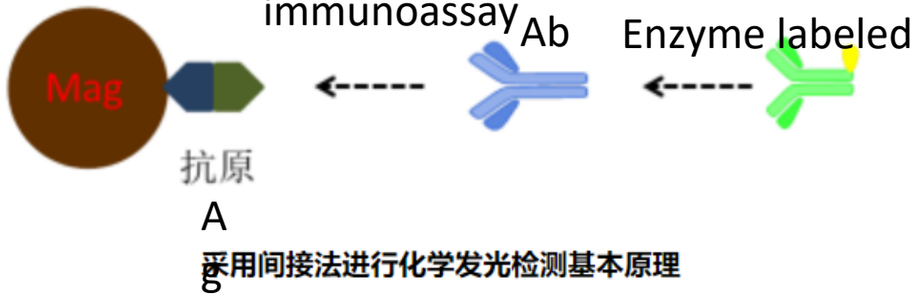
- Human anti-SARS-CoV-2 antibody
- SARS-CoV-2 antigen
- Tag
- Control antibody to validate assay
- Immobilized anti-human antibody
- Immobilized antibody against control antibody



Null IgM + IgG+ IgM+/Ig

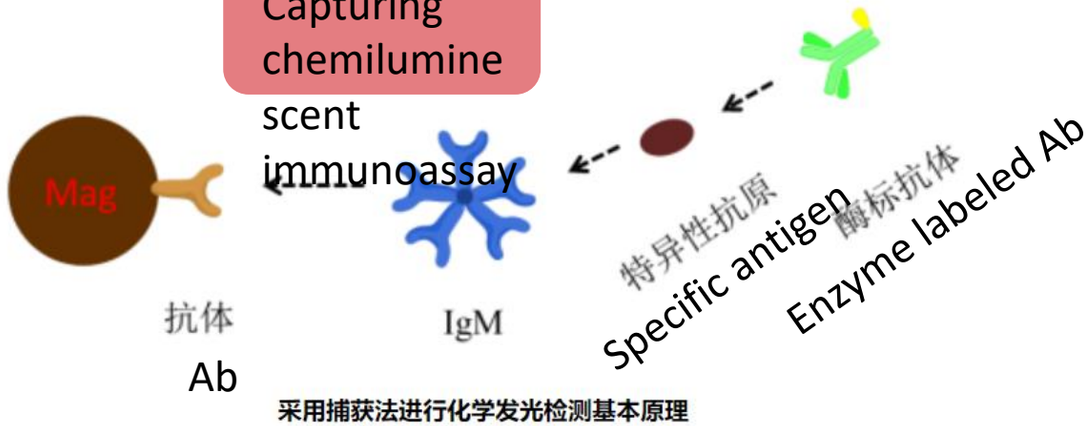
Magnetic particle-based chemiluminescent immunoassay

Indirect chemilumine scent immunoassay



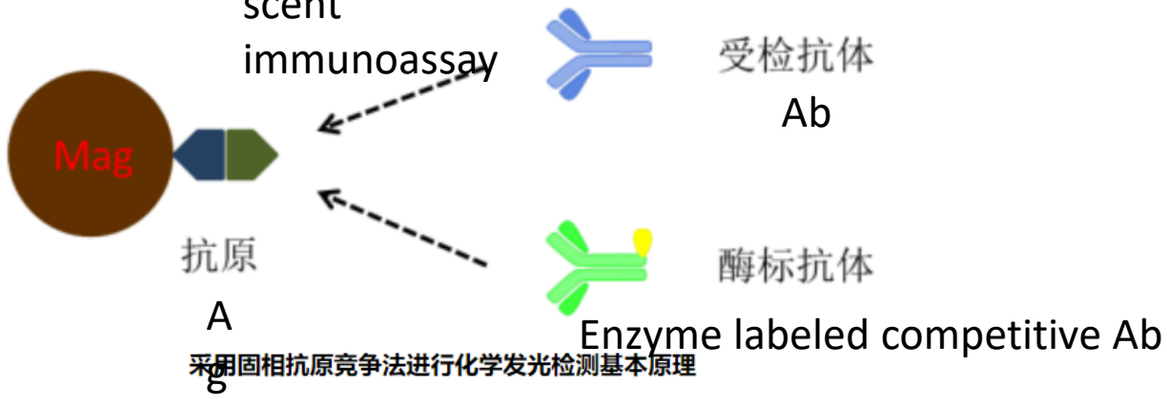
采用间接法进行化学发光检测基本原理

Capturing chemilumine scent immunoassay

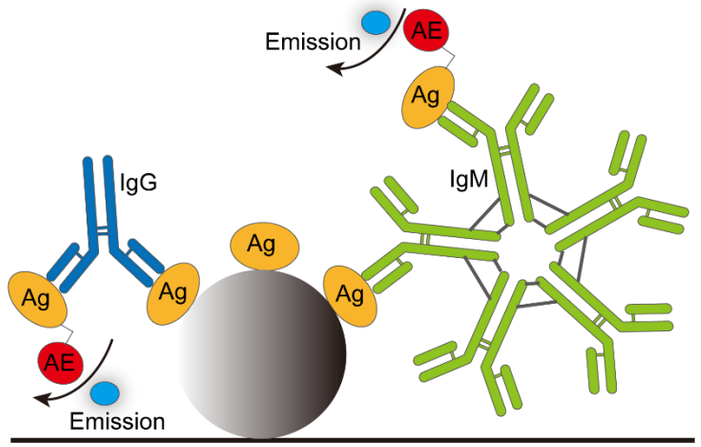


采用捕获法进行化学发光检测基本原理

Competitive chemilumine scent immunoassay



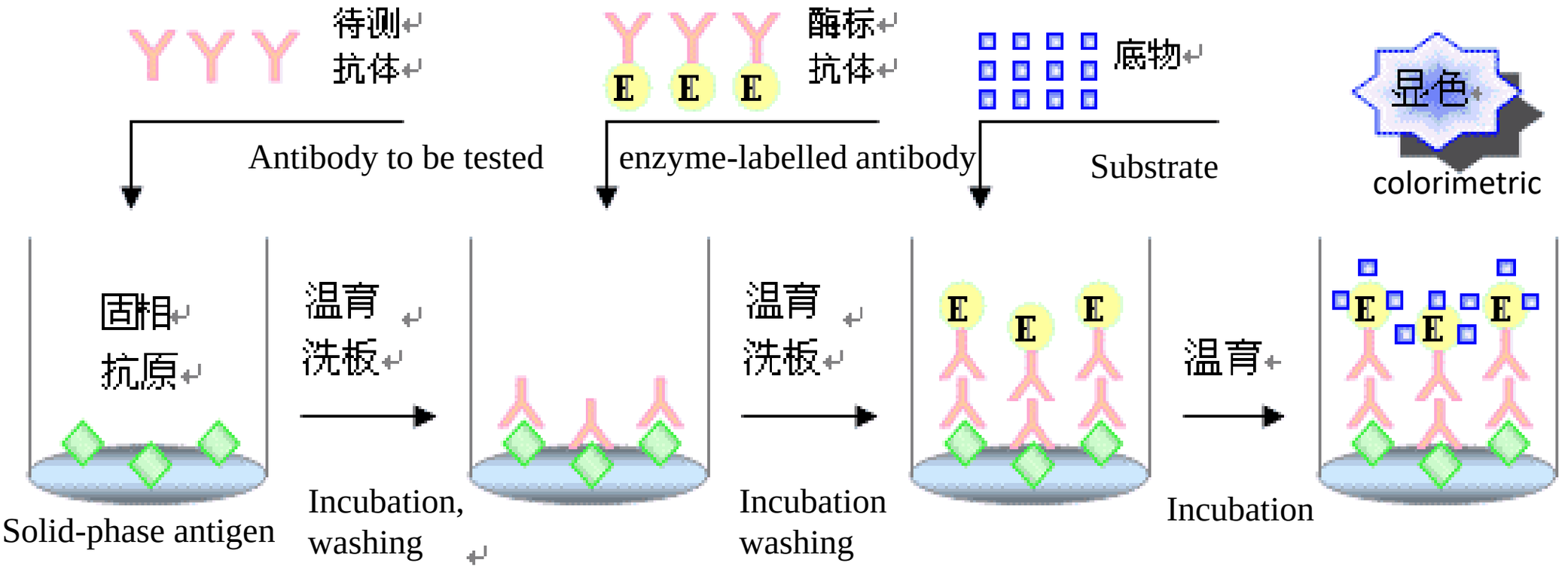
采用固相抗原竞争法进行化学发光检测基本原理



List of SARS-CoV-2 antibody test kits approved by NMPA of China

No	Registration company	Detection principle	Targets	Approved No	Approval date (YY-MM-DD)
1	Guangzhou Wondfo Biotech CO., Ltd.	GICA	IgM/IgG	20203400176	2020-02-22
2	Innovita (Tangshan) Biological Technology Co., Ltd	GICA	IgM/IgG	20203400177	2020-02-22
3	Guangdong Hexin Health Technology Co., Ltd	GICA	IgM	20203400199	2020-03-11
4	Vazyme (Nanjing) Biotech Co., Ltd	GICA	IgM/IgG	20203400239	2020-03-13
5	Zhuhai Livzon Diagnostics Inc	GICA	IgM/IgG	20203400240	2020-03-14
6	Shanghai Outdo Biotech Co., Ltd.	GICA	IgM/IgG	20203400367	2020-04-10
7	Beijing Zinxing Sihuan Biotech Co., Ltd	GICA	IgM	20203400457	2020-05-08
8	Bioscience (Chongqing) Diagnostic Technology Co., Ltd	MPCLIA	IgM	20203400182	2020-02-29
9	Bioscience (Chongqing) Diagnostic Technology Co., Ltd	MPCLIA	IgG	20203400183	2020-02-29
10	Xiamen InnodxBiotech Co. Ltd.	MPCLIA	IgM/IgG	20203400198	2020-03-06
11	Dynamiker Biotechnology (Tianjin) Co., Ltd.	MPCLIA	IgG	20203400365	2020-04-10
12	Dynamiker Biotechnology (Tianjin) Co., Ltd.	MPCLIA	IgM	20203400366	2020-04-10
13	Zhengzhou Autobio Diagnostics Co., Ltd	MPCLIA	IgM	20203400494	2020-05-15
14	Zhengzhou Autobio Diagnostics Co., Ltd	MPCLIA	IgG	20203400495	2020-05-15
15	Maccura Biotechnology Co., Ltd.	CLIA	IgG	20203400496	2020-05-18
16	Maccura Biotechnology Co., Ltd.	CLIA	IgM	20203400497	2020-05-18
17	Bioscience (Tianjin) Diagnostic Technology Co., Ltd	CLIA	IgG	20203400498	2020-05-19
18	Bioscience (Tianjin) Diagnostic Technology Co., Ltd	CLIA	IgM	20203400499	2020-05-19
19	Beijing Hotgen Biotech Co., Ltd.	UPICT	IgM/IgG	20203400523	2020-05-25
20	Beijng Kinghawk Pharmaceutical Co., Ltd.	QDFIC	IgM/IgG	20203400536	2020-06-09
21	BGI Biotechnology (Beijing) CO., LTD	ELISA	IgM/IgG	20203400567	2020-06-17

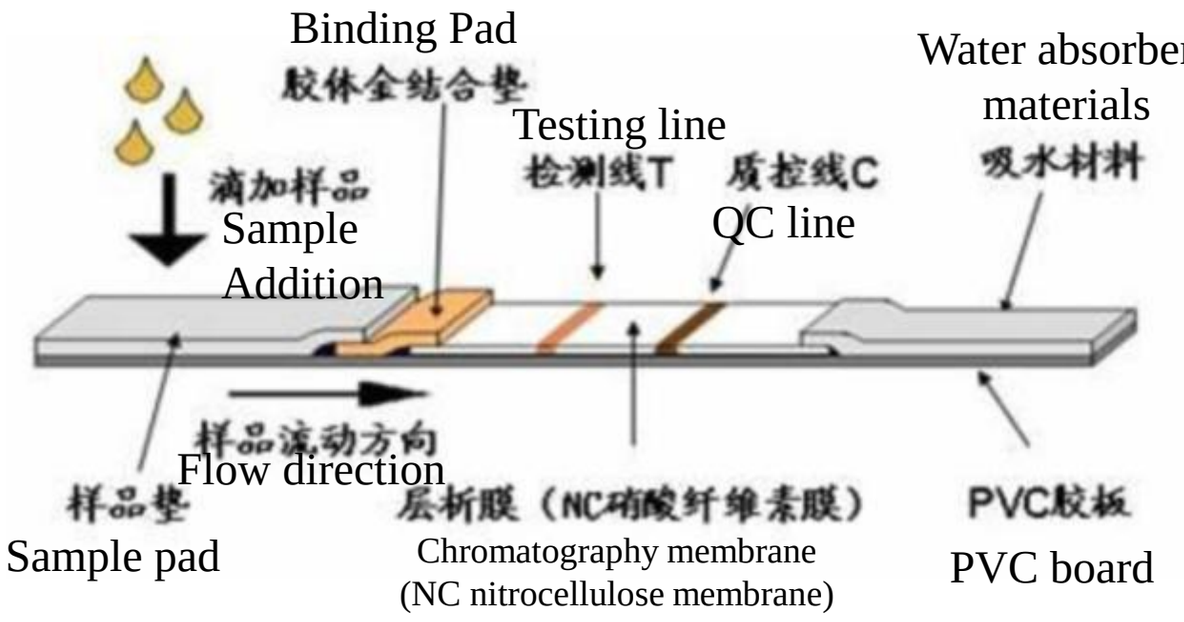
2. Principle for indirect ELISA

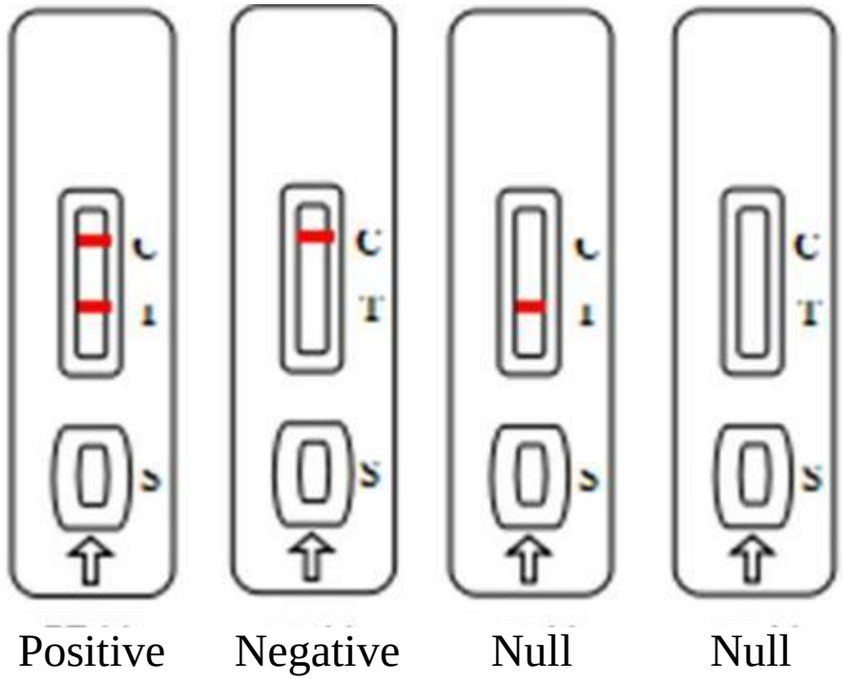


间接法测抗体

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:

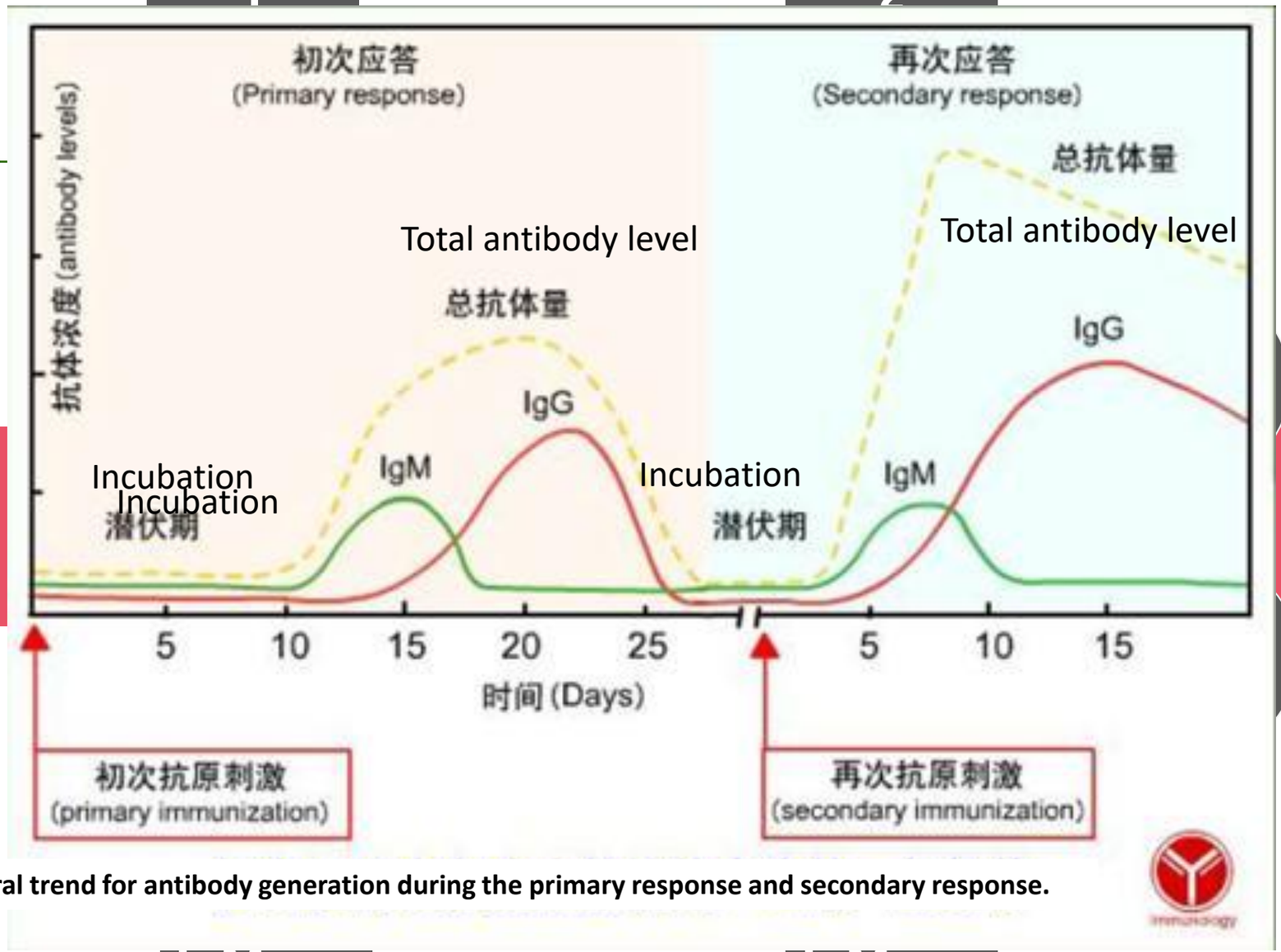
1. Serum IgM antibodies and/or IgG antibodies to 2019-nCov are positive;

2. Serum IgG antibodies to 2019-nCov turn from negative to positive or the IgG antibody titres during recovery period are 4 times or higher than that in acute phase.

Analysis of the test results

1

2



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

IgM +
IgG +

IgM -
IgG +

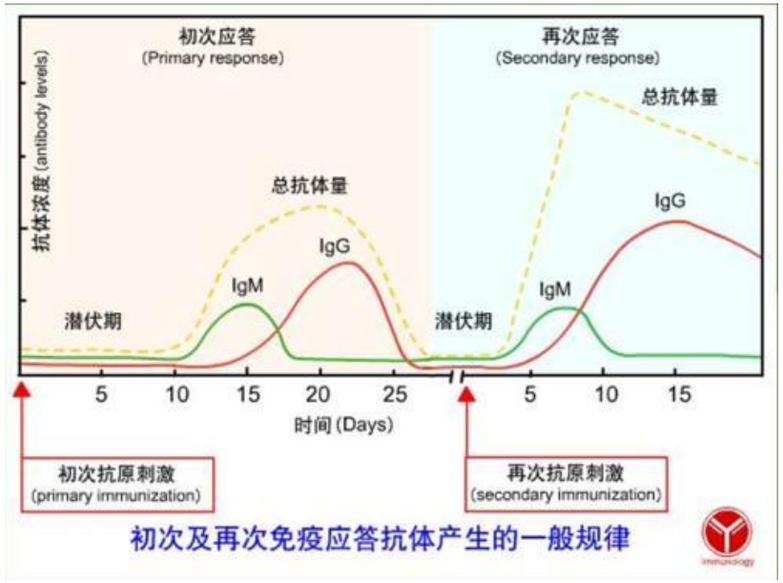
Negative results of the nucleic acid assay

感染活跃期

Active phase

Early phase

感染早期



感染早期

Early phase

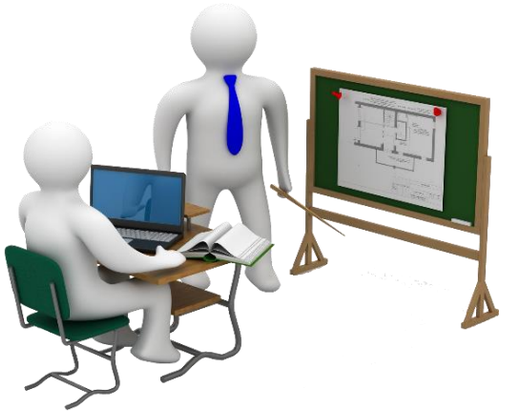
恢复期

Recovery period

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

For reference only. The clinical judgment should prevail

No.	Nucleic acid	IgM	IgG	Interpretation
1	+	-	-	Patients may be during the "window period" of 2019-nCoV infection, typically 2 weeks
2	+	+	-	May be at early infection phage of 2019-nCoV
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, a certain immunity to 2019-nCov has already been developed .
5	-	+	-	The patient’s likely to be in the acute phase of 2019-nCoV infection. Nucleic acid testing resulted should be confirmed firstly. Other factors such as rheumatoid factors have been found to cause weak IgM positive or positive tests.
6	-	-	+	The patients have recovered and the virus has been cleared. The IgG could be maintained for a long in the blood.
7	-	±	-	First infection of less virus and be during an early stage. Thus, the viral load is lower than the lower limit of nucleic acid detection. A small amount of IgM has been produced while IgG have not; a false positive result might be caused by rheumatoid factor.
8	-	+	+	The patients were recently infected with 2019-nCoV and are during the recovery period. The virus has been cleared, but the IgM has not reduced to the lower limit of detection; or the nucleic acid test result might be false negative, the patient is indeed in the active infection phage.



Part IV

Bio-safety requirements

- General introduction
- Viral culture
- Animal infection experiments
- Operations of the uncultured infectious substances
- Operations of inactivated materials

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 is 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.

PPE

Masks

Latex gloves

Face shield

Nitrile gloves

Protective clothing

Headgear

Waterproof boot cover

Positive pressure breathing apparatus

Waste disposal requirements

- Experimental waste should be handled and autoclaved immediately
- Clinical specimens should be handled and autoclaved immediately after detection
- SOP for laboratory waste disposal should be prepared in advance
- Selection, preparation and use of chemical disinfectants
- Use and maintenance of physical disinfection equipment
- Dispose of all kinds of experimental materials and instruments, especially sharps

Laboratory or clinical waste disposal

- Cleaning up： Surface of experimental equipment should be sprayed or cleaned with 75% ethanol or sodium hypochlorite containing 1 g/L effective chlorine.
- Medical Waste: should be autoclaved before transfer out of laboratory.
- Sharps: include syringes, needles, knife, metals, disposable scalpels, blades, and glasses, etc., should be put in the sharps container made of hard material, packaged into two layers of medical waste bags, sealed tightly and labelled clearly before leaving the ward, and then be transferred in closed containers.

Thank you!