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

Wenling Wang, PhD
wangwl@ivdc.chinacdc.cn

October 19, 2020
Table of contents

1. Overview

2. Testing techniques
The identification of SARS-CoV-2

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

Jan 3, 2020
Whole genome sequencing

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

Jan 12, 2020
Sequences submitted to GISAID
Timeline of the key events of the COVID-19 outbreak

8 December 2019
Onset of the first recorded case in Wuhan

31 December 2019
First report of 27 cases of pneumonia with unknown cause in Wuhan, China

11 February 2020
ICTV named virus SARS-CoV-2 and WHO named disease COVID-19

28 February 2020
WHO risk assessment increased to very high on the global level

2 October 2020
>34,000,000 cases and >1,000,000 deaths

9 January 2020
China announced the identification of a novel coronavirus as the causative agent of the pneumonia outbreak

11 March 2020
WHO defined COVID-19 as a pandemic

13 January 2020
Case of a traveler from Wuhan was confirmed in Thailand

20 January 2020
Human-to-human transmission was confirmed

23 January 2020
Wuhan city was locked down

29 January 2020
The coronavirus spread to all 34 provinces across China

30 January 2020
WHO declared a PHEIC alert

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.
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 cases
- South-East Asia: 8,424,480 confirmed cases
- Europe: 7,889,116 confirmed cases
- Eastern Mediterranean: 2,749,606 confirmed cases
- Africa: 1,259,192 confirmed cases
- Western Pacific: 680,158 confirmed cases

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
PART TWO

Testing techniques
Laboratory testing techniques for COVID-19

- Nucleic acid testing
- Viral isolation
- Serological testing
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
- 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

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.
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 Wai; Jacky M.C. Chan; Wai-Shing Leung; Thomas Shiu-Hong Chik; Chris Yau-Chung Choi; Daphne H. K. Ramadan;

【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 Infection, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region; more...

【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 trend.

The novel coronavirus (2019-nCoV) is spreading very fast in Hubei Province of China. As of February 14, 2020, 51,086 confirmed cases (including laboratory-confirmed cases) were reported in Hubei Province, and 1,318 of them died. Respiratory tracts are believed to be the most important routes of transmission of this disease. The clinical presentation of this novel coronavirus disease 2019 (COVID-19) cases, potential reasons for the rapid spread of this virus (1).
Figure. Estimated Variation Over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection Relative to Symptom Onset

Before symptom onset

Detection unlikely a

After symptom onset

PCR - Likely positive

PCR - Likely negative b

Antibody detection

Symptom onset

Week -2  Week -1  Week 1  Week 2  Week 3  Week 4  Week 5  Week 6

Nasopharyngeal swab PCR
Bronchoalveolar lavage/sputum PCR
Virus isolation from respiratory tract
Stool PCR
IgM antibody
IgG antibody

Sethuraman N, et al. JAMA 2020
Anatomy of pharynx
1. Nasopharynx
2. Oropharynx
3. Laryngopharynx
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.

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.
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.

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

- Sample mixed with reagents
- RNA–DNA-Loop PCR products
- 65°C 15 min
- DNA indicator dye
- Visualization

Looped DNA end products of variable size
- Reverse transcriptase
- DNA polymerase with strand displacing activity

RT-RPA

- Fig. 1. Reverse transcription
- Fig. 2. Generation of 3rd strand cDNA while releasing the first cDNA strand
- Fig. 3. Generation of complementary strand of the released cDNA
- Fig. 4. Generation of dumbbell shaped DNA strand

CRISPR

(clustered, regularly interspaced, short palindromic repeats)

SHERLOCK assay

tracrRNA (trans-activating RNA)

DETECTR assay

Viral RNA → DNA → Complementary RNA

B

Viral RNA → DNA → tracrRNA

A

Viral RNA → DNA → tracrRNA

Viral cDNA indicated by Fluorescence

Overlaid Fluorescent Pattern

Reference cDNA indicated by Fluorescence

Positive indicator of virus-specific nucleic acid

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

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

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>Detected material</th>
<th>Key features</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR</td>
<td>Nasopharyngeal swab</td>
<td>Viral RNA</td>
<td>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)</td>
</tr>
<tr>
<td></td>
<td>Oropharyngeal swab</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bronchoalveolar lavage</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tracheal aspirates</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emerging Methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isothermal amplification</td>
<td>Blood (finger stick)</td>
<td>Viral RNA</td>
<td>Duration: Minutes (&lt;30 min), Accuracy: To be determined, Primary use: Rapid screening, Cost: Medium (Specific reagents), Major limitations: Requires validation</td>
</tr>
<tr>
<td>RT-LAMP</td>
<td>Blood (finger stick)</td>
<td>Viral RNA</td>
<td>Duration: Minutes, Accuracy: To be determined, Use: Rapid diagnosis, Cost: Low, Major limitations: Requires validation</td>
</tr>
<tr>
<td>RT-RPA</td>
<td>Blood (finger stick)</td>
<td>Viral RNA</td>
<td>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</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Time</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse transcription</td>
<td>42 °C</td>
<td>5 min</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Initial denaturation</td>
<td>95 °C</td>
<td>10 s</td>
<td>1 cycle</td>
</tr>
<tr>
<td>PCR</td>
<td>95°C</td>
<td>10 s</td>
<td>40 cycles</td>
</tr>
<tr>
<td></td>
<td>60°C (Collect fluorescence)</td>
<td>45 s</td>
<td></td>
</tr>
</tbody>
</table>

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
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.
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+/IgG+
Magnetic particle-based chemiluminescent immunoassay

- **Indirect chemiluminescent immunoassay**
  - Antibody (Ab)
  - Enzyme labeled secondary Ab

- **Competitive chemiluminescent immunoassay**
  - Antigen (Ag)
  - Enzyme labeled competitive Ab

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

- **Solid-phase antigen**
- **Incubation, washing**
- **Antibody to be tested**
- **Incubation**
- **Washing**
- **Enzyme-labelled antibody**
- **Substrate**
- **Colorimetric**

Indirect antibody testing
3. Principle for colloid gold testing

Sample Addition → Binding Pad → Testing line → QC line → Water absorbent materials

Sample pad → Chromatography membrane (NC nitrocellulose membrane) → PVC board

Positive
Negative
Null
Null
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

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

Incubation

Total antibody level

Incubation

Incubation

Initial antibody response

Secondary antibody response

IgM + IgG +

IgM - IgG +
Negative results of the nucleic acid assay

感染活跃期
Active phase

感染早期
Early phase

恢复期
Recovery period

感染早期
Early phase

1. IgM+ IgG-
2. IgM- IgG+
3. IgM+ IgG-
4. IgM- IgG+

图示了初次及再次免疫应答抗体产生的一般规律。
# Interpretation of SAR-Cov-2 nucleic/antibody testing results

For reference only. The clinical judgment should prevail.

<table>
<thead>
<tr>
<th>No.</th>
<th>Nucleic acid</th>
<th>IgM</th>
<th>IgG</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Patients may be during the &quot;window period&quot; of 2019-nCoV infection, typically 2 weeks</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>May be at early infection phage of 2019-nCoV</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>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.</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>The patient is during the active infection, a certain immunity to 2019-nCov has already been developed.</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>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.</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>The patients have recovered and the virus has been cleared. The IgG could be maintained for a long in the blood.</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>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.</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>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.</td>
</tr>
</tbody>
</table>
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
- Nitrile gloves
- Waterproof boot cover
- Protective clothing
- Positive pressure breathing apparatus
- Face shield
- Headgear
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
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!