Establishment of local cut-off

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China CDC MODPAD

SARS-CoV-2
Nucleic Acid Detection
(PCR-Fluorescence Probing)
CONTENTS

1 About the Virus
2 Diagnostic Strategy
3 Our solution
COVID-19 is an infectious disease caused by SARS-CoV-2, a novel coronavirus that can cause illness in animals or humans. In humans there are several known coronaviruses that cause respiratory infections. These coronaviruses range from the common cold to more severe diseases such as SARS, MERS, and COVID-19.
COVID-19

Envelope

Genetic material (Single RNA strand)

Protein spikes

Infected person

TRANSMISSION

Droplets

Contact

aerosol
Wide range of symptoms reported:

- Fever
- Cough
- Shortness of breath or difficulty breathing
- Headache
- Nasal congestion
- Muscle pain
- Sore throat
- Loss of smell or taste
- Diarrhea (may be present in some patients)
I. Currently, care for patients is primarily supportive:
   - Relieve symptoms
   - Manage respiratory, and other organ, failure

II. There are no specific antiviral treatments currently licensed for COVID-19

III. No vaccine is currently available
I. Real-time RT-PCR testing
II. Antibody detection
III. Antigen detection
IV. Blood Test
V. CT
From the first edition to the eighth edition, COVID-19 diagnosis and treatment protocol always proposed that fluorescence real-time RT PCR detection is the gold standard of diagnosis.

confirmed cases should be tested positive by fluorescent rRT-PCR for nucleic acid of SARS-CoV-2.
Case Management

- Suspected case exclusion:
  - The SARS-CoV-2 nucleic acid test was negative for two consecutive samples (the sampling interval was at least 24h apart)
  - SARS-CoV-2 specific IgM and IgG antibodies remained negative 7 days after onset

Post-discharge management

- Discharge standards:
  - The nucleic acid test results of two consecutive sputum and nasopharyngeal swabs and other respiratory tract specimens were negative (sampling interval was at least 24h)
  - Follow-up and examination of respiratory specimens were strengthened after discharge

Asymptomatic infection management

- quarantine for 14 days
- In principle, a person can be released from quarantine after 14 days if nucleic acid tests of SARS-CoV-2 are negative
REAL-TIME PCR TESTING

Step 1. Collect sample
Step 2. Nucleic acid extraction
Step 3. Prepare master mix
Step 4. Run the program

The whole process took around 90 minutes
Notice:

1. The technicians engaged in SARS-CoV-2 specimen collection should have biosafety training and relevant experimental skills. The quality of specimen is important in Real-Time PCR test.
2. The virus detection rate in sputum samples is high. But the sputum samples are usually very thick. So here are some good practice:
   1) 1g/L Phosphate Buffer for Protease K
   2) Phosphate Buffer with 0.1g Dithiothreitol (DTT) and 0.78g Sodium Chloride (table 1)

<table>
<thead>
<tr>
<th>Component</th>
<th>Mass/Volume</th>
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</thead>
<tbody>
<tr>
<td>Dithiothreitol</td>
<td>0.1g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.78g</td>
</tr>
<tr>
<td>PCl3</td>
<td>0.02g</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>0.112g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.02g</td>
</tr>
<tr>
<td>H₂O</td>
<td>7.5ml</td>
</tr>
</tbody>
</table>

PH: 7.4 ± 0.2 (25 °C)
REAL-TIME PCR TESTING

3. Stool specimen: 1ml specimen processing solution was taken, and a stool specimen about the size of soybean was added to the tube. Vertex 5 mins and centrifuged at 8000 rpm for 5 minutes, and the supernatant was kept for detection. (table 2)

4. Sample preservation: Specimens used for virus isolation and nucleic acid testing should be tested as soon as possible, and those that can be tested within 24 hours can be stored at 4°C; Specimens that cannot be detected within 24 hours should be stored at or below -70°C (or temporarily stored at -20°C if no -70°C is available). Serum specimens can be stored at 4°C for 3 days and below -20°C for a longer time.

Table 2 Stool specimen processing solution formula

<table>
<thead>
<tr>
<th>Component</th>
<th>Mass/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>1.211g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>8.5g</td>
</tr>
<tr>
<td>Anhydrous Calcium Chloride</td>
<td>1.1g</td>
</tr>
<tr>
<td>H₂O</td>
<td>800ml</td>
</tr>
<tr>
<td>PH: 7.5 (25°C)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjust pH to 7.5 with concentrated hydrochloric acid and replenish to 1000 mL with deionized water.
REAL-TIME PCR TESTING

Notice:

5. Reporting result: For SARS-CoV-2 targets: Ct value $\leq 38$ is considered positive(+) ; Ct value $> 40$ is considered negative(-) ; $38 < Ct \leq 40$ is considered diagnostic gray zone. For RNase P: Ct value $\leq 38$ is considered positive (+); Ct value $> 38$ is considered negative (-)

6. Reporting positive
   (1) ORF1ab and N gene both positive.
   (2) One of two target genes is positive. resampling and retesting, if positive again, then positive

7. Trouble shooting in Reporting
   (1) One target gene is in the diagnostic gray zone and the other target gene is negative. (2) Both of two target genes is in the gray zone. Under these circumstances, repeating nucleic acid extraction is suggested and then amplifies simultaneously with previous template. If both results show positive then report positive, otherwise report suspicious. When suspicious is reported, consider the following actions: (1) change other manufacturer’s kit or different method with superior sensitivity such as digital PCR to further confirm. (2) Repeat sampling or collect specimen from different parts of the patient and repeat the test.

8. Reporting negative
   Report “negative” when none of both target genes shows result. It is possible due to low viral load and should be analyzed by combining clinical sign. Repeat sampling or collect specimen from different parts of the patient and repeat the test when clinical sign and other examinations are high suspected.
WHERE TO DO THE TEST

I. PCR Laboratory
   • CDC Center
   • Public & Private Labs
COVID-19 Coronavirus Real Time PCR Kit

**Features:**

- **Multiplex** real-time PCR technology
- **ORF1ab and N-genes** target region
- Genes of RnaseP Internal Control (IC)
- **72 minutes** Assay runtime
- Clinical Sensitivity: 94.9%
- Clinical Specificity: 98.7%
- Limit Of Detection: **350 copies/mL**
- Sample Input Volume: **5 μL**
- Storage Temperature: **-20±5℃**
- Transportation Temperature: **-25~8℃**
- Shelf Time : 6 months

**Sample Type:**

- Nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs.
- Nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum specimens.

Approved by CE, NMPA, FDA-EUA, WHO-EUL

<table>
<thead>
<tr>
<th>Catalog</th>
<th>Package</th>
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<tr>
<td>JC10223-1NW</td>
<td>50 Tests/Kit</td>
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</table>
COVID-19 Coronavirus Real Time PCR Kit

Main advantage

- Shorter time, more efficient: The runtime within **72 minutes** which normally 90-150 minutes

- Large size sample validation: more than **9000000 samples**

- Better performance parameter: Clinical Sensitivity: **94.9%** Clinical Specificity: **98.7%**

- Lower detection limit: The LOD is **350 copies/mL**, which make less chance to miss any low concentration of the samples

COVID-19 Coronavirus Real Time PCR Kit
For further information kindly check:

https://www.medrxiv.org/content/10.1101/2020.02.12.20022327v2
CERTIFICATES AND EVALUATION

Evaluation in Russia

Certification in Australia

Certification in Thailand
BIOPERFECTUS

China's first mobile nucleic acid testing laboratory

Help Xinjiang fight COVID-19

Support Chinese Medical Team to help Guyana

Donation by WHO
Approved by National Institutes for Food and Drug Control (national reference product)

Approved by Quality SuBeijing Center for Medical Device pervision and Testing of State Food and Drug Administration

BioGerm’s 2019-nCoV Nucleic Acid Detection Kit has already tested more than 3 million specimens in China at 600 units

Now, the national and provincial CDC, Beijing and Shanghai customs all use BioGerm's PCR testing products for detection of entry personnel for the 2019-nCoV

>280 hospitals

>300 CDC

>50 customs and ports

>12 medical laboratories all over China
Thank you!