Attachment 4

New coronavirus infected pneumonia laboratory Technical Guide to Detection

(2nd Edition)

To guide disease control departments at all levels and other relevant institutions to carry out the new coronavirus infection of pneumonia laboratory testing work, specially formulated this technical guide. This guide focuses on the now-mature and easy-to-implement nucleic acid detection methods.

First, specimen collection

(i) The acquisition object. New coronavirus infection pneumonia Suspected cases, suspected cluster case patients, others who need to diagnose or identify the new coronavirus infection, or other environmental or biological materials that require further screening and testing (e.g. traceability analysis).

(2) Specimens collection requirements.

1. Technicians engaged in the collection of new coronavirus test specimens should undergo biosafety training (qualified training) and have the appropriate experimental skills. Sampler Professional Protective Equipment (personal protective equipment, PPE) requirements: N95 masks, goggles, protective clothing, latex gloves, waterproof boots; Wear double-layer latex gloves when secreting or excreta.

2. Samples of inpatient cases are collected by the medical staff of the hospital in which they are located under the guidance of professionals from local CDC.

3. Close contact specimens are collected by local CDC authorities.

4. According to the needs of laboratory testing work, multiple samples can be combined with the course of the disease.

(iii) The type of specimen collection. Each case must be collected for acute respiratory and acute blood samples, and severe cases must be prioritized for lower respiratory tract specimens (e.g. bronchial or alveolar irrigation, etc.), which can be collected at intervals between clinical manifestations and samples.

Other research materials are collected according to design requirements.

Types of specimens:

1. Upper respiratory tract specimens: including pharynx swabs, nasal swabs, nasopharyngeal extracts.

2. Lower respiratory tract specimens: including deep cough sputum, respiratory extract, bronchial irrigation fluid, alveoli irrigation liquid, pulmonary tissue biopsy specimens.

3. Blood samples: Try to collect acute stage anticoagulant blood within 7 days of onset. The amount of 5 ml, with fasting blood as the best, is recommended to use a vacuum blood vessel containing anticoagulants to collect blood.

4. Serum specimens: Try to combine acute and recovery period double serum. The first serum should be collected as early as possible (preferably within 7 days of onset) and the second serum should be collected 3 to4 weeks after onset. The collection amount is 5 ml, with fasting blood as the best, it is recommended to use vacuum blood vessels.

(4) The method of specimen collection.

1. Phab swabs: Wipe the two-sided tonsils and the back wall of the swallowing side with a plastic rod swab with 2 polypropylene fiber heads, immerse the swab head in a tube containing 3 ml of sampling fluid, discard the tail, and tighten the tube cover.

2. Nasal swabs: Gently insert a plastic rod swab with a polypropylene fiber head into the nasal palate of the nasal passage, and slowly turn out after a moment of pause. Take a plastic rod swab from another polypropylene fiber head to collect the other side of the nostrils in the same way. The above two swabs are immersed in the same tube containing 3 ml of sampling fluid, the tail is discarded, and the tube cover is tightened.

3. Nasopharyngeal extracts or respiratory extracts: Extracting mucus from the nasopharynx or respiratory secretions from the trachea using a collector connected to a negative pressure pump. Insert the collector head into the nasal cavity or trachea, turn on the negative pressure, rotate the collector head and slowly exit, collect the extracted mucus, and rinse the collector 1 time with 3 ml of sample fluid (the collector can also be replaced with a pediatric catheter attached to a 50 ml syringe).

4. Deep cough sputum: After the patient is asked to cough

deep, collect the coughing sputum in a 50ml screw plastic tube containing 3 ml of sample fluid.

5. Bronchial lotion: Insert the collector head from the nostrils or trachea socket (about 30cm deep), inject 5 ml of physiological saline, turn on the negative pressure, rotate the collector's head and slowly exit. Collect extracted mucus and rinse the collector 1 time with sample dissonal (the collection can also be replaced by a pediatric catheter attached to a 50 ml syringe).

6. Alveoli irrigation lotion: after local anesthesia, the fiber bronchoscopy through the mouth or nasal pass into the branch of the right lung leaf or left lung tongue, its top into the branch of the bronchial branch opening, through the trachea biopsy hole slowly added sterile physiological saline, each time 30to50 ml,a totalof 100to250 ml,should notexceed 300 ml.

7. Blood samples: It is recommended to collect blood samples 5 ml using vacuum slug sacwases containing anticoagulants, set aside atroom temperature for 30 minutes,1500to2000rpm centrifugation for 10 minutes, collecting plasma and blood cells in sterile studs in plastic tubes.

8. Serum specimens: 5 mlof blood samples collected with

vacuum negative pressure blood vessels, 30 minutesat roomtemperature, 1500to2000 rpm centrifugation for 10 minutes, collected serum in a sterile stud plastic tube.

9. Other materials: Collected according to the design requirements specification.

(v) Specimens packaging. After collection, the specimen is installed in a biosecurity secondary laboratory biosecurity cabinet.

1. All specimens should be placed in a suitable screw cap with washers, cryo-resistant sample collection tube, tightened. The sample number, type, name and date of sampling are indicated outside the container.

2. Place the sealed specimens in a suitable plastic bag and place one specimen in each bag.

(vi) Specimens are preserved. Specimens used for virus isolation and nucleic acid testing should be tested as soon as possible, and specimens that can be detected within 24 hours can be stored at 4degrees C, and specimens that cannot be detected within 24 hours should be stored at -70degrees C or below (if not -70degrees C) The condition is stored in the refrigerator at -20degrees C). The serum can be stored for 3 days at 4degrees C

andcan be kept for a long time under-20degrees C. A special library or counter should be set up to store specimens separately. Repeated freezing and melting should be avoided during the shipment of specimens.

(VII) Specimens are sent for examination. Specimens should be collected as soon as possible to the laboratory, if the need for long-distance transport of specimens, it is recommended to use dry ice and other refrigeration methods for preservation.

1. Samples sent: Samples of the first positive test results, suspected cluster cases and cluster cases in each province (autonomous region, municipality directly under the central government), sent to the China Center for Disease Control and Prevention of Viral Disease Prevention and Control for test review, and attached samples to the test table (see annex).

2. Pathogens and specimen transport

2.1 Domestic transport

The transport packaging classification of the new coronavirus strain or other potentially infectious biological materials is category A, corresponding to the United Nations no. UN2814,and the packaging complies with the PI602 classification packaging

requirements of ICAO document Doc9284 Technical Rules for The Safe Transport ofDangerous Goods; the packaging complies with the PI650 classification packaging requirements of iCAO document Doc9284 Technical Rules for the Safe Transport ofDangerous Goods, and can be transported by other means of transport with reference to the above standard packaging.

The transport of new coronavirus strains or other potentially infectious materials shall be handled in accordance with the Regulations on transportation management of highly pathogenic pathogenic microorganism bacteria (toxic) bacteria (toxic) bacteria (toxic) bacteria (formerly Order No. 45 of the Ministry of Health) in accordance with the Regulations on transportation and management of highly pathogenic pathogenic microorganisms (toxic) organisms that can infect humans.

2.2 International transport

Where a new coronavirus strain or sample is transported internationally, the packaging shall be standardized, the relevant formalities shall be carried out in accordance with the Regulations on The Regulations on Health and Quarantine of Special Items in and out of The Country, and the relevant national and international

requirements shall be met.

Laboratory testing of new coronaviruses

The conventional detection method for new coronavirus infections is identified by real-time fluorescent RT-PCR. The testing of any new coronavirus must be operated by personnel trained in technical safety in a laboratory with the appropriate conditions. The nucleic acid detection methods in this guide are mainly for open read box 1a/b (open reading frame 1ab,ORF1ab) in the genome of the new coronavirus and nucleocapsidprotein, N.

In the laboratory to confirm a case is positive, the following conditions are met:

The 2 targets (ORF1ab, N) specific in the same specimen were positive for real-time fluorescence RT-PCR.

Negative results can not rule out a new type of coronavirus infection, it is necessary to rule out the possibility of false negative factors, including: poor sample quality, such as respiratory samples in the mouth and pharynx and other areas;

3. Real-time fluorescent RT-PCR method to detect new coronavirus nucleic acids

(a) The purpose. Standardize the real-time fluorescent

RT-PCR method to detect the working procedure stort of new coronavirus nucleic acids, to ensure the correct and reliable results of the experiment.

(II) Range . Suitable for real-time fluorescence RT-PCR method to detect new coronavirus nucleic acids.

(iii) Responsibilities.

Testing personnel: responsible for the test ingress with the samples tested in accordance with these testing rules.

Reviewer: Responsible for reviewing whether the inspection operation is standardized and whether the test results are accurate.

Department head: Responsible for the department's comprehensive management and inspection report audit.

(4) Sample reception and preparation. Check the name, gender, age, number and test items of the sample sat under inspection, the status of the sample to be inspected should be indicated, and the sample to be inspected should be stored in the refrigerator of -70degrees C.

(v) Detection project.

1. New coronavirus nucleic acid assays (real-time fluorescent

RT-PCR method)

Primersandprobes for the ORF1abandN gene regions of the new coronavirus esthe.

Target One (ORF1ab):

Forward Prime(F):CCCTGTGTGTTTTACACTTAA

Reverse Prime(R):ACGATTGTGCATCAGCTGA

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

Target 2(N):

Forward Prime(F):GGGGAACTTCTCCTTAGAAT

Reverse Prime(R):CAGACATTTGCTCTCAAGCTG

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

Nucleic acid extraction and real-time fluorescent RT-PCR reaction system Referto to the relevant manufacturer kit instruc tions.

2. Outcome judgment

Negative: No Ct value or Ct

value . . . .

Positive:Ct value, slt;37,can be reported aspositive.

Suspicious:The Ct value is between 37-40, it is recommended to repeat the experiment, if the Ct value is slt;40,theamplification curve has a significant peak, the sample is judged to be positive, otherwise it is negative.

The requirements of the biological safety experiment of pathogens

According to the current grasp of the biological characteristics of the new coronavirus, transmission characteristics, pathogenicity, clinical information and other information, taking into account the new coronavirus infection cluster and serious cases, and the characteristics of the death case, experimental activities temporarily according to the pathogenic microbial harm classification of the second type of pathogenic microorganisms to manage, the specific requirements are as follows:

(a) Virus culture . Refers to the isolation, culture, titration, middle testing, purification of live viruses and their proteins, viral freeze-dried and recombination tests of living viruses. Using live viruses or their infected cells (or cell extracts) for inactivated biochemical analysis, serological testing, immunological testing,

etc., such operations are treated as viral culture, which should be performed in biosecurity level 3(BioSecurity Level 3, BSL-3)laboratory with the qualifications to carry out the corresponding activities. When using viral cultures to extract nucleic acids, the addition of lysates or inactivators must be carried out under the same level of laboratory and protective conditions as viral culture, and reliable methods for inactivated viral cultures can be performed in BSL-2 or BSL-1 laboratories Operation.

(ii) Animal infection experiments. An experiment in which live virus estomers are infected. Operations should be performed in the Animal (animal)BSL-3 (ABSL-3) laboratory, which is qualified to carry out the appropriate activities.

(iii) The operation of uncultivated infectious materials. Uncultivated infectious materials in the use of reliable methods before inactivation of viral antigen detection, serological testing, nucleic acid testing, biochemical analysis and other operations, should be operated in the biosecurity secondary laboratory, but personal protective equipment reference to the protection requirements of the biosecurity tertiary laboratory. Human and animal tissue specimens that have not been reliably inactivated or fixed should be operated at a level of protection compared to viral

culture due to high viral content.

(iv) The operation of inactivated materials. Infectious materials or live viruses can be operated in the BSL-1 laboratory after inactivation using a reliable method.

(v) Operation of non-infectious materials. All operations against materials that are identified as non-infectious, including but not limited to infectious viral DNA or cDNA, should be performed in the BSL-1 laboratory.

Attachment: New coronavirus detection specimen sent to inspection table

Attachment

New coronavirus detection specimen sent to inspection table

Sample unit (stamp): Send sample date: year, month, send sample:

Specimens Number	Types of specimens	Name	Gender	Age	Date of onset	Date of treatment	Sample date	Whether the source of the sample is a clustercase	Detection date	Real-time fluorescence RT-PCR		Gene sequence homogeneity		Note
										Reagent manufacturers	Target Gene	Generation	Deep sequencing	

Gene sequence homologous , non-mandatory option, indicating the completion of a specific target gene sequence / whole genome sequence, and its homologous nature with the new coronavirus. Whether the source of the sample is a clustercase of s/he yes or no.