Laboratory Guideline for Pandemic Influenza Diagnosis at Medical Institutions

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Pandemic Influenza Experts Advisory Committee

Laboratory Guidelines for Pandemic Influenza Diagnosis at Medical Institutions

Designated medical institutions for infectious diseases etc.

Preparations

○**Viral transport media**

Make media using PBS etc. at local health institutes

○**Supply viral transport media**

Allocate and supply media to designated medical institutions for infectious diseases etc. from local health institutes

○**Preserve viral transport media**

Preserve media at medical institutions at 4°C or -20°C

Taking clinical samples

○**Protect health care workers**

Prepare PPE to prevent infection

○**Types of required clinical samples**

Pharyngeal lavage fluid, nasal cavity lavage fluid, blood etc.

○**Sampling timing**

Appropriate timing for each sample

○**Preserve clinical samples**

Appropriate temperatures & media for each sample and day of storage

○**Labeling**

Use a test request form based on the infectious disease surveillance system

Transporting samples

○ **Standards for containers and indications of samples**

Three-layer structured containers that prevents the leak of samples

○**Indications and transport modes of samples**

Transport samples based on the WHO's Guidance on Transport Regulations for Infectious Substances

Register & Report | **Register & Report** | **Register & Report**

Health centers, local health institutes, National Institute of Infectious Diseases etc.

Laboratory Guidelines for Pandemic Influenza Diagnosis at Medical Institutions

1. Objectives

To diagnose pandemic influenza adequately, appropriate samples must be taken from (suspected) patients at appropriate timings, and preserved appropriately until they are transported to laboratories. At the same time, it is important to make preparations and establish schemes for protecting health care workers and preventing hospital infections. These guidelines aim to provide guidance on performing the abovementioned process appropriately. These guidelines assume immediately before and after Pandemic Phase 4, and shall not apply to situations where the number of samples increases to the level exceeding the capacity of laboratories. These guidelines may also apply to H5N1 influenza.

2. Preparations for sampling

(1) Prepare viral transport media (VTM)

○ Local health institutes shall make viral transport media (VTM) in the following composition, allocate and supply them to health centers and designated medical institutions for infectious diseases etc. where (suspected) patients are examined or hospitalized in each prefecture. Prefectural governments shall make decision on the abovementioned process, considering geographic conditions and communication channels of the relevant health centers and medical institutions. Local health institutes shall also provide instructions on appropriate preservation, and retain VTM through appropriate collaboration.

○ Composition of VTM:

Apply a final concentration of 0.5% BSA, penicillin (100 – 500 U/mL), streptomycin (100 – 500 μg/mL), gentamicin (100 μg/mL) and amphotericin B (2 μg/mL) to commercial cell cultivation medium (e.g. MEM, 199) or PBS.

○ VTM shall be made by the liter, and dispensed in one or two milliliters following filter sterilization, and preserved at 4°C or -20°C.

* Physiological saline shall not be used because pH becomes instable and inactivates virus.

3. Types of required clinical samples and methods for sampling

○ As a rule, clinical samples shall be taken by health care workers at medical institutions capable of sufficient infection prevention, including designated medical institutions for infectious diseases where (suspected) patients are examined or hospitalized, medical institutions having tuberculosis beds or otherwise requested by prefectural governments to provide beds (hereafter “cooperating medical institutions”).

○ If a (suspected) patient is designated for hospitalization, and if the hospitalizing medical institution is far from the consulting medical institution, it may be an option for a health center official to visit the consulting medical institution, take the (suspected) patient’s sample, and transport the sample and the (suspected) patient to the hospitalizing medical institution at the same time.

(1) Protecting health care workers

Health care workers who consult and take samples from (suspected) patients are at high risks of infection through heavy contact with them. Therefore, health care workers must wear a set of personal protective equipment (PPE) to protect themselves from droplet infection due to coughs and hiccups of (suspected) patients.

- Gown
- Gloves
- Goggles or face shields
- Mask (N95 or equivalent)
- Also consider wearing a rubber apron and rubber boots as necessary.

* Health care workers who handled samples taken from patients without wearing sufficient protective equipment shall be targeted by health monitoring and/or preventive administration of antiviral drugs (See the Proactive Epidemiological Research Guidelines for Pandemic Influenza for details).

References:

- “4. Infection prevention at different departments of medical institutions,” Guidelines for Infection Prevention at Medical Facilities
- “Use of Personal Protective Equipment (PPE) pertaining to Contacts with Patients in Countermeasures against Avian (H5N1) and Pandemic Influenza (Phases 3 to 5),”

  Infectious Disease Surveillance Center, National Institute of Infectious Diseases

  http://idsc.nih.go.jp/disease/influenza/05pandemic.html

(2) Types of samples

1) Samples for identifying pathogens and genetic testing

Pharyngeal lavage fluid or swab, nasal cavity lavage fluid swab, endotracheal lavage fluid, alveolar lavage fluid etc.

2) Samples for identifying antibodies

Blood

(3) Methods for sampling

1) Sampling for identifying pathogens

○ Patients manifesting pandemic influenza symptoms etc.

Of pharyngeal lavage fluid or swab, nasal cavity lavage fluid swab, endotracheal lavage fluid and alveolar lavage fluid, it is recommended to take pharyngeal lavage fluid or swab, and/or nasal cavity lavage fluid swab.

* These samples are used to for isolating virus and identifying pathogens by PCR.

* Take two of these samples respectively to prepare for reexamination. Issue separate test request slips in advance, using the National Epidemiological Surveillance of Infectious Diseases (NESID) support system to research suspected cases. Attach labels to individual samples, and indicate No.1, No.2 etc. to identify samples taken from the same (suspected) patients.

2) Blood samples for identifying antibodies

○ To diagnose infection correctly, it is important to take blood serum samples in both acute and convalescence phases.

(4) Timings of sampling

Correct diagnosis depends on timings of sampling. Therefore, sampling must be performed at adequate timings.

1) Samples for identifying pathogens

○ It is recommended to take samples for identifying pathogens during four days after symptoms manifested. Virus concentration is the largest in this period.

○ Even if a laboratory only conducts a genetic test, it is recommended to perform sampling in an early stage after symptoms manifested. Although PCR is considered capable of identifying genes from samples taken ten to fourteen days after symptoms manifested, the test proves negative in many cases.

2) Blood serum for identifying antibodies

○ For identifying antibodies, it is recommended to take blood serum samples in both acute (within one week after symptoms manifested) and convalescence (four weeks after symptoms manifested) phases.

(5) Preservation of samples

Even if samples are taken appropriately, viruses and genes contained in samples get inactivated if preserved inappropriately, making correct diagnosis difficult. Therefore, preservation of samples becomes another important factor.

1) Preservation of samples for isolating virus

○ If a test is performed in a short term: If a test is performed within seven days, samples shall be preserved in a refrigerator (4°C), not to be frozen.

○ If it takes time before testing: If it takes more than seven days before testing, samples shall be preserved in a freezer at -70°C or lower. The frozen status shall be retained during transport by packing samples with dry ice.

* It is strictly forbidden to preserve samples at a room temperature or at -20°C even for a short time.

2) Preservation of samples for genetic testing

○ It is strongly recommended to preserve samples for PCR genetic testing at -70°C or lower. (Preservation at -20°C or 4°C is acceptable for a short time.)

3) Sample transport media

○ A swab sample shall be taken from a (suspected) patient using a sterilized swab, and soaked in one or two milliliters of viral transport medium (VTM). Bend and throw away the stick, and keep the swab part soaked in VTM.

○ VTM shall be dispensed in one or two milliliters following filter sterilization, and preserved at 4°C or -20°C. Physiological saline shall not be used because pH becomes instable and inactivates virus.

4) Preservation of blood serum for identifying antibodies

○ It is recommended to preserve blood serum samples at -70°C or lower or at -20°C, but preservation at 4°C is acceptable for a short time.

(6) Labelling

Indications on labels attached to samples must accord with information registered in the National Epidemiological Surveillance of Infectious Diseases (NESID) support system to research suspected cases. Therefore, the following points must be noted in the labeling process.

○ Tests, test requests and test results shall be registered using the NESID support system to research suspected cases, until strategic early response is discontinued.

○ Each sample shall be attached with a test request slip issued using the NESID support system to research suspected cases, which is brought by a health center official. It is also required to attach a document of the infectious disease occurrence trend research pathogen surveillance.

○ For details of operation, see the section of support system to research suspected cases in the Surveillance Guidelines.

○ After strategic early response is discontinued, operations shall follow the section of "virological surveillance during pandemic" in the Surveillance Guidelines.

Labelling:

- Each label must indicate information including the ID number, type of sample, date of sampling, patient's initials etc. The ID number is issued automatically by the support system to research suspected cases. (See the manual for the support system to research suspected cases.)

4. Transporting samples

Clinical samples collected from (suspected) patients are categorized B[1]. When a sample is transported to a laboratory, it must be put in a three-layer structured container that will prevent the leak of sample in the case of damage to the container.

○ During transport, the same temperatures as during the preservation of samples must be retained. (See 3. (5) "Preservation of samples.")

○ See the WHO's Guidance on Transport Regulations for Infectious Substances, September 2005, the Japanese version supervised by the National Institute of Infectious Diseases in 2006, at:

http://www.nih.go.jp/niid/Biosafety/transportation/guidance_transport.pdf, for standards for the three-layer containers used for sample transportation, indications on outer cases and transport methods. Details will be specified later on the domestic transport of pathogens and samples.

5. Disinfection and prevention of cross contamination

○ To protect health care workers and to prevent hospital infections and cross contaminations, health care workers and sampling sites shall be disinfected appropriately, after taking samples from (suspected) patients.

[1] Category B

Category of infectious substances specified by the WHO's Guidance on Transport Regulations for Infectious Substances, which provides standards for transportation etc. for each category.

○ See “Attachment 1, Disinfection of Pandemic Influenza Virus,” Guidelines for Infection Prevention at Medical Facilities, for disinfectants and methods of disinfection.

6. Testing flow (Attachment)

○ At present, laboratory testing shall follow the Influenza Virus (H5N1) Guidelines – Phase 3 –. If pandemic influenza occurs, case definitions shall be established anew, reviewing methods and schemes for diagnosis. Laboratory testing flow shall also be reviewed as appropriate as cases and findings are accumulated to a certain extent.

○ Samples collected from (suspected) patients shall be tested at local health institutes, while considering the use of private laboratories as necessary.

(Attachment)

Testing Flow and Scheme