



Department of Health
Research Institute for Tropical Medicine
National Reference Laboratories for Emerging Infectious Diseases
Filinvest Corporate City, Alabang, Muntinlupa City 1781



INTERIM GUIDELINES FOR SPECIMEN COLLECTION AND LABORATORY TESTING FOR CASE FINDING AND INVESTIGATION OF SEVERE RESPIRATORY DISEASE ASSOCIATED WITH NOVEL CORONAVIRUS

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1. BACKGROUND

The United Kingdom Health Protection Agency (UK HPA) informed the World Health Organization (WHO) of a case of acute respiratory syndrome with travel history to Saudi Arabia and Qatar on 22 September 2012. Laboratory testing conducted by the UK HPA confirmed the presence of the novel coronavirus, similar to the virus isolated from a fatal case in Saudi Arabia on July 2012 [1]. Limited information showed that the two cases had an acute, serious respiratory illness presenting with fever, cough, shortness of breath, and breathing difficulties. It is not clear if such clinical presentation is typical of the novel coronavirus infection [2].

The WHO released interim case definitions for case finding severe respiratory disease associated with novel coronavirus [3]. Laboratory confirmation is needed to classify a patient as a confirmed case of novel coronavirus infection. On the other hand, it should be kept in mind that there may be other potential primary aetiologies of community-acquired pneumonia aside from coronavirus like *Streptococcus pneumoniae*, *Hemophilus influenza* type B, *Legionella pneumophila*, other recognized primary bacterial pneumonias, influenza, and respiratory syncytial virus and testing for any of these other microorganisms may be needed as well to identify the etiologic agent of a patient presenting with community-acquired pneumonia.

This document provides the interim guidelines for sample collection, storage and transport and laboratory testing.

2. SAMPLE TYPES FOR COLLECTION

2.1. For patients falling under the WHO and DOH Interim Case Definitions for **Patient Under Investigation**, collect the following priority specimens for the molecular detection of the novel Coronavirus and for differential diagnosis of other respiratory pathogens:

- Nasopharyngeal swab (NPS) **AND** Oropharyngeal swab (OPS) in Virus Transport Medium¹ (VTM), collected within 14 days after the onset of symptoms

AND

- Nasopharyngeal swab (NPS) in Atypical Pneumonia Transport Medium¹ (APTM), collected within 14 days after the onset of symptoms

AND

- Sputum sample, collected within 7 days after the onset of symptoms.

AND

- Serum sample, preferably within 7 days after the onset of symptoms.

¹ VTM and APTM collection kits are available by request from the RITM Clinical Laboratory, Telephone Nos. (02) 8422442 or (02) 8072628 to 32 local 201.

2.2. Collect blood sample in BHI broth for patients with signs and symptoms of septicemia.

2.3. Other specimens which may be tested for the molecular detection of the novel coronavirus and differential diagnosis are as follows:

- Nasopharyngeal wash or aspirate in sterile containers, collected within 14 days after the onset of symptoms
- Lower respiratory tract bronchoalveolar lavage, tracheal aspirate or pleural fluid tap in sterile containers, collected within 14 days after the onset of symptoms
- Fresh tissue samples (e.g., lung tissues), collected as clinically indicated

2.4. Refer to a separate document for electron microscopy and histopathology diagnostics.

2.5. Other specimen types may be collected as indicated by the attending physician.

3. BIOSAFETY GUIDELINES DURING SAMPLE COLLECTION

Coronaviruses spread in a manner similar with other respiratory viruses – via large respiratory droplets and direct or indirect contact with infected secretions. Moreover, Coronaviruses may also be detected in feces and urine [4].

The following list summarizes the key biosafety considerations for healthcare personnel performing sample collection from a Patient Under Investigation [4].

- Standard precautions should be observed during sample collection, with careful attention to hand hygiene.
- In addition to standard precautions, biosafety measures should include:
 - Airborne precautions, including the use of fit-tested N95 respirator or higher.
 - Contact and droplet precautions, including use of long sleeve fluid repellent gown and latex or similar non-latex gloves with long tight-fitting cuffs and eye protection
- Gloves should be disposed and replaced with a fresh pair between patients.
- Standard precautions when handling any clinical waste, which must be placed in leak-proof clinical waste bags or bins and disposed of safely.

4. GUIDELINES FOR SPECIMEN COLLECTION

Effective and accurate diagnosis is highly dependent on the timing of specimen collection, appropriate clinical sample, and the condition during transport to the laboratory.

4.1. Nasopharyngeal and Oropharyngeal swabs

Refer to the document: **Guidelines for Specimen Collection, Storage and Transport for OPS and NPS** in the RITM website.

Collect nasopharyngeal swabs for VTM and APTM transport **separately**.

Label each specimen container with the Patient's Name, Age and Sex and Date of Specimen Collection. The label should remain attached to the specimen container under all conditions of storage and transport.

The information on the label must be legible and should match the information on the **RITM Coronavirus Outbreak Form**. Please fill out all the indicated fields in the form.

4.2. Serum

Collect 5mL to 10mL of whole blood in a serum separator tube. After clotting, centrifuge the tube and collect the resulting serum in polypropylene, external screw-capped vials with internal O-ring seals. If the vial has no internal O-ring seal, seal the vial tightly with the available cap and seal with Parafilm™ to prevent leakage. RITM requires a minimum amount of 500µL serum for testing and banking.

Label each specimen container with the Patient's Name, Age and Sex and Date of Specimen Collection. The label should remain attached to the specimen container under all conditions of storage and transport.

The information on the label must be legible and should match the information on the **RITM Coronavirus Outbreak Form**. Please fill out all the indicated fields in the form.

4.3. Sputum

Have the patient rinse the mouth with water. Let the patient expectorate deep cough sputum directly into a sterile screw-capped sputum collection cup or a sterile dry container.

Label each specimen container with the Patient's Name, Age and Sex and Date of Specimen Collection. The label should remain attached to the specimen container under all conditions of storage and transport.

The information on the label must be legible and should match the information on the **RITM Coronavirus Outbreak Form**. Please fill out all the indicated fields in the form.

5. SPECIMEN STORAGE AND TRANSPORT

The sending laboratory is responsible for the proper packaging of the specimens and in assuring that the specimen reaches RITM in good condition.

- 5.1. **For NPS and OPS samples:** Wrap the specimen tubes in tissue paper or any absorbent material; place upright in a separate 50 mL centrifuge tube or any leak/puncture proof container. Place the 50 mL tube in a resealable plastic bag (e.g., Ziplock™). The materials needed are provided in the VTM or APTM collection kit available from RITM (See Section 2.1 above).
- 5.2. **For serum, sputum and other samples:** Use screw-capped, polypropylene tubes or containers for transport. **Do not transport the samples in glass containers.** Individually place each sample container in a resealable plastic bag (e.g., Ziplock™).
- 5.3. Place **all the specimen containers except BHI** in a shipment/carrier box with at least four (4) frozen ice packs inside to maintain prescribed temperature. Put the frozen ice packs in first, at the bottom and at the sides of the carrier box; then place specimens at the middle so that they are surrounded by the ice packs. Cover the carrier box.
- 5.4. **BHI samples must be stored and transported in room temperature.** Do not include BHI samples inside the box with samples to be transported in cold chain.
- 5.5. Place the completely filled-up **RITM Coronavirus Outbreak Form** in a separate zip-locked plastic bag and put on top of the box and secure with tape.
- 5.6. Notify RITM before sending the specimen. RITM receives samples 24 hours a day, seven days a week.
- 5.7. Send to the Research Institute for Tropical Medicine (RITM) within 3 days of specimen collection:

**RESEARCH INSTITUTE FOR TROPICAL MEDICINE
CLINICAL LABORATORY**

Filinvest Corporate City, Alabang

Muntinlupa City 1781

Telephone Numbers: (02) 842 2442

(02) 807 2628 to 32 local 201

**SPECIMENS MUST BE SHIPPED WITHIN 48 HOURS (2 DAYS) AFTER
COLLECTION TO ENSURE ARRIVAL AT RITM WITHIN 72 HOURS (3 DAYS).**

- 5.8. **ALL** polystyrene foam (“styrofoam”) boxes used for specimen transport will be decontaminated and disposed of by RITM as infectious waste.

6. LABORATORY TESTING AT RITM

Tests Available	Specimen Type	Days After Onset of Symptoms	Turnaround Time
Novel coronavirus-specific detection			
Novel coronavirus PCR detection	NPS and OPS in VTM	within 14 days	4 working days
	other respiratory samples	within 7 days	
Differential diagnosis			
Influenza A PCR detection and subtyping	NPS and OPS in VTM	within 7 days	4 working days
Influenza B PCR detection			
Respiratory Syncytial Virus PCR detection			
Atypical pneumonia PCR panel (<i>Mycoplasma</i> , <i>Legionella</i> and <i>Chlamydia</i>)	NPS in APTM	as clinically indicated, before antibiotic treatment	4 working days
Invasive bacterial diseases PCR panel (<i>H. influenzae</i> , <i>S. pneumonia</i> , <i>N. meningitidis</i>)	serum	as clinically indicated, before antibiotic treatment	4 working days
Aerobic culture	sputum and/or blood in BHI	as clinically indicated, before antibiotic treatment	4 working days
Electron microscopy ¹	fresh tissues and secretions	as clinically indicated	please see separate document
Histopathological examination ¹	fresh tissues and secretions	as clinically indicated	4 working days

¹ Please refer to a separate document for specimen collection, cost and turn-around time for electron microscopy and histopathology diagnostics.

7. FOLLOW-UP OF RESULTS

Testing results may be followed-up after the indicated turnaround time from the RITM Emergency Operations Center: **(02) 842 0936**. RITM will be releasing additional contact information as soon as these are available.

Results will be faxed to the official fax number of the referring institution or emailed as a *pdf* copy to the official email address of the referring institution. Alternately, results may be picked-up at RITM-DOH. As an institutional policy, RITM will not release official results by phone.

8. REFERRALS FROM PRIVATE INSTITUTIONS

Please refer to the **RITM Laboratory Examination Rates** (www.ritm.gov.ph/Announcements/RITM%20Laboratory%20Examination%20Rates%202011.pdf) for the testing fees. Fees for the respiratory swab collection kits and novel coronavirus detection PCR are indicated below:

Novel Coronavirus PCR detection	P 6,000.00
Negative Staining (for Electron Microscopy)	P 3,500.00
Tissue Processing (for Electron Microscopy)	P 5,000.00
Tissue Processing (for Histopathology)	P 350.00 per cassette of tissue
VTM Collection kit (contains VTM tube, OPS, NPS, tongue depressor, ziplock bag, absorbent material and 50mL tube as secondary container)	P 300.00
APTM Collection kit (contains APTM tube, NPS, , ziplock bag, absorbent material and 50mL tube as secondary container)	P 250.00

9. REFERENCES

- [1] World Health Organization, "Novel coronavirus infection - update," 25 September 2012. [Online]. Available: http://www.who.int/csr/don/2012_09_25/en/index.html. [Accessed 27 September 2012].
- [2] United Kingdom Health Protection Agency, "Questions and Answers - novel coronavirus September 2012," 24 September 2012. [Online]. Available: <http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/RespiratoryViruses/NovelCoronavirus/respqandanovelcoronavirusspet2012/>. [Accessed 27 September 2012].
- [3] World Health Organization, "Revised interim case definition – novel coronavirus," 29 September 2012. [Online]. Available: http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/index.html. [Accessed 3 October 2012].
- [4] United Kingdom Health Protection Agency, "Infection Control Advice - Suspected or Confirmed Novel Coronavirus Cases," 26 September 2012. [Online]. Available: http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317136232722. [Accessed 27 September 2012].
- [5] World Health Organization, "Novel coronavirus infection - update," 25 September 2012. [Online]. Available: http://www.who.int/csr/don/2012_09_25/en/index.html. [Accessed 27 September 2012].