Laboratory Preparation for 2019 Novel Coronavirus

National Influenza Centre of Myanmar (NIC)

National Health Laboratory

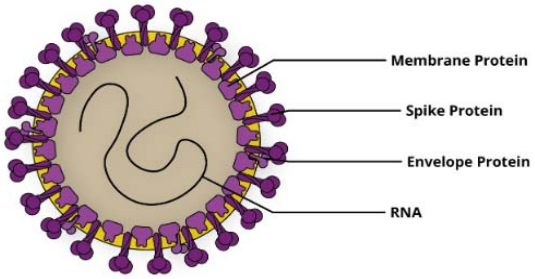
Date:24.1.2020

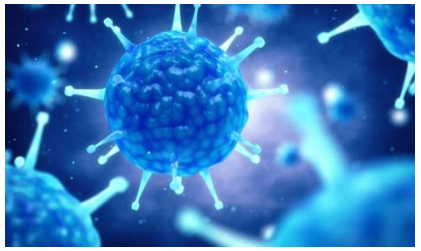
Overview

- Coronavirus
- Sample collection and shipment
- Lab Diagnosis
- Constraints

Coronavirus

Coronavirus





- Coronaviruses are named for the crown-like spikes on their surface
- Family *Coronaviridae*, subfamily *Coronavirinae*
- There are four genera - alpha, beta, gamma, and delta
- High frequency of recombination
- Common in a wide range of mammalian and avian species, most notably bats
- Human coronaviruses were first identified in the mid-1960s

Seven coronaviruses that can infect people

- Common human coronaviruses
  1. 229E (alpha coronavirus)
  2. NL63 (alpha coronavirus)
  3. OC43 (beta coronavirus)
  4. HKU1 (beta coronavirus)
- Other human coronaviruses
  5. MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome).
  6. SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome).
  7. 2019 Novel Coronavirus (2019-nCoV)

2019 Novel Coronavirus

- 2019- nCoV is a new strain that has not been previously identified in humans
- Zoonosis

Sample collection and shipment

Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases

Interim guidance
14 January 2020

WHO/2019-nCoV/laboratory/2020.2

World Health Organization

1. Introduction

The purpose of this document is to provide interim guidance to laboratories and stakeholders involved in laboratory testing of patients who meet the definition of suspected case of pneumonia associated with a novel coronavirus identified in Wuhan, China (See: Surveillance case definitions for human infection with novel coronavirus. Interim guidance).

Various existing WHO documents have been adapted for use in the drafting of this document, including WHO laboratory guidance for MERS-CoV (1-11). As information about the etiology, clinical manifestations and transmission of disease in the cluster of respiratory disease patients identified in Wuhan is evolving, WHO continues to monitor developments and will revise these recommendations as necessary.

The etiologic agent responsible for the cluster of pneumonia cases in Wuhan has been identified as a novel betacoronavirus, (in the same family as SARS-CoV and MERS-CoV) via next generation sequencing (NGS) from cultured virus or directly from samples received from several pneumonia patients. Electron microscopy revealed a virus with a characteristic crown morphology: a coronavirus. Working directly from sequence information, the team developed a series of genetic amplification (PCR) assays used by laboratories associated with the China CDC to detect several dozen cases as of today.

Full genome sequence data from the viruses have been shared officially with WHO and on the GISAID platform (https://www.gisaid.org/) and can inform the development of specific diagnostic tests for this emergent coronavirus. It is expected that validated PCR tests will become available soon. Until that time, the goals of diagnostic testing are to detect conventional causes of pneumonia early, to support disease control activities, and to work with reference laboratories that can perform pan coronavirus detection and directed sequencing.

2. Suspected case definition

For case definition see: WHO Surveillance case definitions for human infection with novel coronavirus.

3. Specimen collection and shipment

Rapid collection and testing of appropriate specimens from suspected cases is a priority and should be guided by a laboratory expert. As extensive testing is still needed to confirm the 2019-nCoV and the role of mixed infection has not been verified, multiple tests may need to be performed and sampling sufficient clinical material is recommended. Local guidelines should be followed regarding patient or guardian's informed consent for specimen collection, testing and potentially future research.

Assure SOPs are available, and the appropriate staff is trained and available for appropriate collection, specimen storage, packaging and transport. There is still limited information on the risk posed by the reported coronavirus found in Wuhan, but it would appear samples prepared for molecular testing could be handled as would samples of suspected human influenza (2, 7-9). Attempts to culture the virus require a higher level of biosecurity.

Samples to be collected (see Table 1 for details on sample collection and storage):

1. Respiratory material* (nasopharyngeal and oropharyngeal swab in ambulatory patients and sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease)
2. Serum for serological testing, acute sample and convalescent sample (this is additional to respiratory materials and can support the identification of the true agent, once serologic assay is available)

*Modifiable with information on whether upper or lower respiratory material is better for coronavirus detection.

A single negative test result, particularly if this is from an upper respiratory tract specimen, does not exclude infection. Repeat sampling and testing, lower respiratory specimen is strongly recommended in severe or progressive disease. A positive alternate pathogen does not necessarily rule out either, as little is yet known about the role of coinfections.

Reference 2, 3, 7

Table 1. Specimens to be collected from symptomatic patients
Guidance on specimen collection (adapted from reference 5)

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	4 °C	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
(Endo)tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	
Sputum	sterile container	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	4 °C	≤24 hours: 4 °C >24 hours: –70 °C	
Serum (2 samples acute and convalescent possibly 2-4 weeks after acute phase)	Serum separator tubes (adults: collect 3-5 ml whole blood)	4 °C	≤5 days: 4 °C >5 days: –70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weeks later
Whole blood	collection tube	4 °C	≤5 days: 4 °C >5 days: –70 °C	For antigen detection particularly in the first week of illness
Urine	urine collection container	4 °C	≤5 days: 4 °C >5 days: –70 °C	

*For transport of samples for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. For bacterial or fungal culture, transport dry or in a very small amount of sterile water. Avoid repeated freezing and thawing of specimens.

Aside from specific collection materials indicated in the table also assure other materials and equipment are available: e.g. transport containers and specimen collection bags and packaging, coolers and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, materials for decontamination of surfaces.

Safety procedures during sample collection and transport

All specimens collected for laboratory investigations should be regarded as potentially infectious, and HCWs who collect, or transport clinical specimens should adhere rigorously to infection prevention and control guidelines and national or international regulations for the transport of dangerous goods (infectious substances) to minimize the possibility of exposure to pathogens (14). Implement the appropriate infection prevention and control precautions, guidance on IPC for the 2019-nCoV has been drafted (11).

Assure good communication with the laboratory and provide needed information

To assure proper and fast processing of samples and to assure adequate biosafety measures in the laboratory, communication and information sharing is essential. Be sure you have alerted the laboratory of the urgency and situation before sending the sample. Also assure that specimens are correctly labelled, and diagnostic request forms are filled out properly and clinical information is provided (see box: information to be recorded).

Information to be recorded:

- Patient information – name, date of birth, sex and residential address, unique identification number, other useful information (e.g. patient hospital number, surveillance identification number, name of hospital, hospital address, room number, physicians' name and contact information, name and address for report recipient),
- Date and time of sample collection,
- Anatomical site and location of specimen collection,
- Tests requested.
- Clinical symptoms and relevant patient history (including vaccination and antimicrobial therapies received, epidemiological information, risk factors).

Samples to be collected

- Respiratory material (nasopharyngeal and oropharyngeal swab in ambulatory patients and sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease)
- Serum for serological testing, acute sample and convalescent sample (this is additional to respiratory materials and can support the identification of the true agent, once serologic assay is available)
  - *A single negative test result, particularly if this is from an upper respiratory tract specimen, does not exclude infection. Repeat sampling and testing, lower respiratory specimen is strongly recommended in severe or progressive disease. A positive alternate pathogen does not necessarily rule out either, as little is yet known about the role of coinfections.*

Novel Coronavirus (nCoV)ရောဂါသံသယလူနာထံမှ ဓါတ်ခွဲနမူနာများယူရန် လမ်းညွှန်ချက်

နှာခေါင်းတို့ပတ် (Nasopharyngeal Swab) ဓါတ်ခွဲနမူနာယူခြင်း

၁။ နှာခေါင်းတို့ပတ် ဓါတ်ခွဲနမူနာကို ရောဂါလက္ခဏာပြပြီး တတ်နိုင်သမျှ စောလျှင်စွာ ယူပါ။

၂။ Virus Transport Medium Tube တွင်ပါသော sterile swab stick ကို အသုံးပြု၍ နှာခေါင်းအတွင်း အဆုံးထိ (Nasopharyngeal wall) ထိရောက်အောင် ထည့်ပါ ။

၃။နှာခေါင်းအတွင်း၌ စက္ကန့်အနည်းငယ်ထားပြီး Swab အိုးတံကိုဖြည်းဖြည်းချင်းလှည့်၍ ထုတ်ပါ ။

၄။လက်ယာဘက် နှာခေါင်းထဲသို့အရင်ထည့်၍ Specimen ကို အရင်ယူပါ။ ထို့နောက် ၎င်း swab ဖြင့် ပင် လက်ဝဲဘက် နှာခေါင်းထဲသို့ ထည့်၍ Specimen ကို ထပ်ယူပါ။

၅။ Swab ကို Virus Transport Medium Tube ထဲသို့ ထည့်ပါ။ Tube အဖုံးပိတ်၍ ရုံအောင် အပေါ်မှ ရှည်ထွက်နေသော အိုးတံ(swab stick) ကိုအနေတော်ချိုးပါ။ အရည်များ ဓာင်စေရန် သတိထားပါ။ Tube အဖုံးပိတ်၍ အဖုံးပိုမိုလုံခြုံမှုရှိစေရန် ၎င်းအဖုံးပေါ်တွင် စက္ကူပလာစတာဖြင့် ပတ်ပေးပါ။

၆။ နှာခေါင်းတို့ပတ် ဓါတ်ခွဲနမူနာအား ယူပြီးလျှင်ပြီးချင်း (+4) °C တွင် သိမ်းပါ။

၇။ ထို့နောက် အချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာဌာနသို့ ရေခဲပုံးဖြင့် (၄၈)နာရီအတွင်းအရောက် ပို့ရပါမည်။

အာခေါင်တို့ပတ် (Oropharyngeal Swab) ဓါတ်ခွဲနမူနာယူခြင်း

၁။ အာခေါင်တို့ပတ် ဓါတ်ခွဲနမူနာကို ရောဂါလက္ခဏာပြပြီး တတ်နိုင်သမျှ စောလျှင်စွာ ယူပါ။

၂။ Virus Transport Medium Tube တွင်ပါသော sterile swab stick ကိုအသုံးပြု၍ အာသီး (tonsils) များနှင့် လည်ချောင်းအနောက်ဘက် (Posterior pharyngeal wall) တို့ကို ပွတ်၍ ယူပါ။ နမူနာယူသည့်အခါတွင် ပါးစပ်အတွင်းနံရံများ၊ သွား နှင့် လျှာ တို့ကို swab ဖြင့် မထိမိစေရန် သတိပြုပါ။

၃။ Swab ကို Virus Transport Medium Tube ထဲသို့ ထည့်ပါ။ Tube အဖုံးပိတ်၍ ရုံအောင် အပေါ်မှ ရှည်ထွက်နေသော အိုးတံ(swab stick) ကိုအနေတော်ချိုးပါ။ အရည်များ ဓာင်စေရန် သတိထားပါ။ Tube အဖုံးပိတ်၍ အဖုံးပိုမိုလုံခြုံမှုရှိစေရန် ၎င်းအဖုံးပေါ်တွင် စက္ကူပလာစတာ ဖြင့် ပတ်ပေးပါ။

၄။ အာခေါင်တို့ပတ် ဓါတ်ခွဲနမူနာအား ယူပြီးလျှင်ပြီးချင်း (+4) °C တွင် သိမ်းပါ။

၅။ ထို့နောက် အချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာဌာနသို့ ရေခဲပုံးဖြင့်(၄၈)နာရီအတွင်း အရောက် ပို့ရပါမည်။

သလိပ်(Sputum) ဓါတ်ခွဲနမူနာယူခြင်း

၁။ ရင်ခေါင်းအတွင်းမှ ဟပ်ထုတ်လိုက်သော သလိပ်နမူနာကို ရောဂါလက္ခဏာပြပြီး တတ်နိုင်သမျှ စောလျှင်စွာ ယူပါ။

၂။ သလိပ်နမူနာကို တစ်ခါသုံး Sterile Container ထဲသို့ သန့်ရှင်းစွာခံယူပါ။

၃။ ခံယူထားသော သလိပ်နမူနာ ကို (+4) °C တွင် စေ့တွေ့သိမ်းထားပါ။

၄။ သလိပ်နမူနာကို ခံယူပြီး (၄၈)နာရီအတွင်း ရေခဲပုံးဖြင့် အချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာ ဌာနသို့ ဖြစ်နိုင်သမျှ စောလျှင်စွာ ပို့ပါ။

Bronchoalveolar Lavage၊ Endotracheal Aspirate ဓါတ်ခွဲနမူနာယူခြင်း

၁။ Resipirator တပ်ထားရသောလူနာထံမှ Bronchoalveolar Lavage၊ Endotracheal Aspirate နမူနာကို ရောဂါလက္ခဏာပြပြီး တတ်နိုင်သမျှ စောလျှင်စွာ ယူပါ။

၂။ ဓာတ်ခွဲနမူနာကို ပိုးသတ်ထားသော Sterile Container ထဲသို့ သန့်ရှင်းစွာခံယူပါ။

၃။ ၎င်းဓာတ်ခွဲနမူနာကို (+4) °C တွင် စေ့တွေ့ သိမ်းထားပါ။

၄။ ထို့နောက် ဓာတ်ခွဲနမူနာကို (၄၈)နာရီအတွင်း ရေခဲပုံးဖြင့် အချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာ ဌာနသို့ ဖြစ်နိုင်သမျှ စောလျှင်စွာပို့ပါ။

သွေးရည်ကြည်(Serum)၊ သွေး(Whole Blood) ဓါတ်ခွဲ နမူနာယူခြင်း

၁။ ရောဂါလက္ခဏာပြပြီး ပထမဆတ်အတွင်း Acute Serum ကို လည်းကောင်း၊ ပထမအကြိမ်ယူပြီး သည့်နေ့မှ (၂)ပတ် အကြာတွင် Convalescent Serum ကို လည်းကောင်း ၊ ဓာတ်ခွဲစစ်ဆေးရန် အတွက် အဖုံး အနီရောင် Plain Tube ဖြင့် ယူရမည်။

၂။ အနီးအပူရှိန်တွင် သွေးကိုခဲအောင် (၁) နာရီခန့် ထားပါ။

၃။ အချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာဌာနသို့ မပို့မီ (+4) °C တွင် စေ့တွေ့သိမ်းထားပါ။

၄။ သွေးပုလင်းကို (၄၈)နာရီအတွင်း ရေခဲပုံးဖြင့် အချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာဌာနသို့ ဖြစ်နိုင်သမျှ စောလျှင်စွာပို့ပါ ။

Novel Coronavirus (nCoV) ဓါတ်ခွဲနမူနာများအား လုံးအတွက် လိုက်နာဆောင်ရွက်ရမည့် အချက်များ

၁။ ဓါတ်ခွဲနမူနာ ယူရာတွင် Personal Protective Equipment (PPE) အပြည့်အစုံ ဝတ်ပါ။

၂။ ဓါတ်ခွဲနမူနာပုလင်းပေါ်တွင် label ကို သေချာစွာကပ်ပါ။ လူနာအမည်၊ အသက်၊ ကျား/မ၊ ရောဂါလက္ခဏာစတင်ဖြစ်သည့်နေ့ (Date of onset of symptoms) ၊ ဓာတ်ခွဲနမူနာအမျိုးအစား နှင့် ဓာတ်ခွဲနမူနာယူသည့်ရက်စွဲကို ပြည့်စုံစွာ ရေးပါ။

၃။ ဓါတ်ခွဲနမူနာပုလင်းကို ဓစ်ပါသောပလပ်စတစ်အိတ်တွင်ထည့်၍လုံခြုံစွာပိတ်ပါ။ ထို့နောက်အဆိုပါ ပလပ်စတစ်အိတ်အား ဝက်အူရစ်ပါသောပလပ်စတစ်ဘူးကြီးထဲသို့ ထောင်လျက်အနေအထားဖြင့် ထည့်ပါ။ ပလပ်စတစ်ဘူးအဖုံးကို သေချာစွာပိတ်ပါ။ ၎င်းပလပ်စတစ်ဘူးအား ဓဟိုကူးစက်မှုထုတ်ပေးထားသော ရေခဲပုံးတွင် ရေခဲနေသော ice packs များနှင့် အတူထည့်ပါ။ ရေခဲတုံးကို မသုံးပါနှင့်။ အကယ်၍ ရေခဲတုံးမှလွဲ၍ သုံးစရာမရှိလျှင် ရေခဲတုံးကို ရေလုံသော ပလပ်စတစ်အိတ်ထဲထည့်၍ သားရေကွင်း နှင့် လုံအောင်ချည်ပါ။

၄။ ဓါတ်ခွဲနမူနာများကို ယူပြီးပြီးချင်းနှင့် အချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာဌာနသို့ မပို့နိုင်မီ (+4) °C အတွင်းတွင် သိမ်းထားပါ။

၅။ Case investigation and laboratory request forms များတွင် လိုအပ်သောအချက်အလက်များကို တိကျစွာလင်မှန်ကန်စွာ ဖြည့်စွက်ပါ။*(ဥပမာ- ရောဂါလက္ခဏာစတင်ဖြစ်သည့်နေ့ (Date of onset of symptoms) ၊ဓါတ်ခွဲနမူနာယူသည့်နေ့၊လူနာ၏အဖြဲတမ်းနေရပ်လိပ်စာ၊ ပေးပို့သည့် ဆေးရုံအမည်)

၆။ ဓါတ်ခွဲနမူနာများကိုအချိုးသားကျန်းမာရေးဓာတ်ခွဲမှုဆိုင်ရာဌာနသို့ ပိတ်ရက်မရှိပေးပို့နိုင်ပါသည်။

National Health Laboratory
35,Maw Kun Tike St, Dagon Tsp, Yangon
Phone No: 01-371957 Ext: 124
Fax: 01-371925

- Already distributed to State and Regional Hospital Labs by email
- NHL FB page and Website

2019-nCoV Lab Request Form

Novel Coronavirus (nCoV) Suspected Cases Laboratory request Form

Please complete this form carefully and circle the response.

1. Report/Investigation Information: Name of Investigator(s): _____

Name of Hospital : _____

Date - Case Reported: ____/____/____

Date - Case Investigated: ____/____/____

2. Case Identification: Patient's Name: _____

Date of Birth: __ / __ / __ Age: years _____ months _____ Sex: _____

Father's Name: _____ Mother's Name: _____

Full Permanent Address: State/Region: ---------- Township: _____

Village/ward: _____ Street No. & House No _____

Phone No. _____

3. Hospitalization: Yes / No Date of Hospitalization: ___ / ___ / ___

Name of Hospital: _____ Hospital Registration Number: _____

Clinical Diagnosis: _____

Outcome: Recovered completely / Death / Unknown

4. Travel History within last two weeks: Yes / No

Where : _____ When __/__/___

5. Poultry Contact History within last two weeks : Yes / No

6. Sign and Symptoms: Date of onset of first symptoms: ___/___/___

Fever: Yes / No / Unknown Cough: Yes / No / Unknown

Lower respiratory tract involvement ; dyspnea: Yes / No / Unknown or difficulty breathing : Yes / No

Upper respiratory tract involvement ; sore throat : Yes / No / Unknown or coryza: Yes / No

Other symptoms : _____

7. Comorbid conditions : Heart Disease ( ) Asthma ( ) Chronic Lung Disease ( ) Liver Disease ( )
Immunocompromised ( ) Pregnancy ( ) Other (Specify) : _____

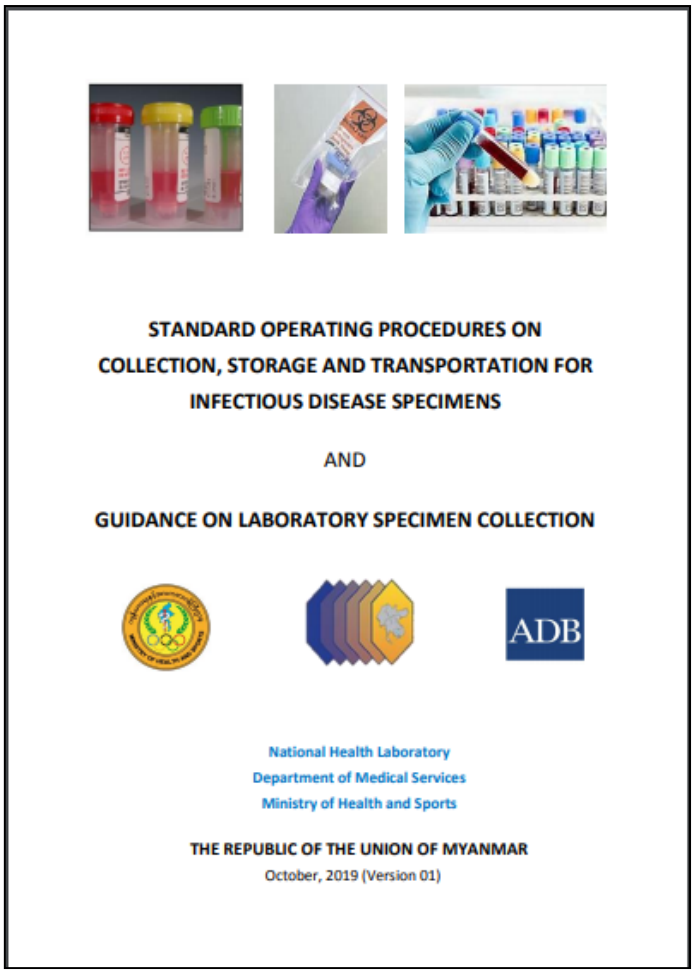
8. Specimen Collection:

	Date Collected	Date Sent to Lab	Date of Result	Laboratory Results
Nasopharyngeal Swab	__/__/__	__/__/__	__/__/__	Positive / Negative
Oropharyngeal Swab	__/__/__	__/__/__	__/__/__	Positive / Negative
Sputum	__/__/__	__/__/__	__/__/__	Positive / Negative
Bronchoalveolar Lavage	__/__/__	__/__/__	__/__/__	Positive / Negative
Endotracheal Aspirate	__/__/__	__/__/__	__/__/__	Positive / Negative
Serum	__/__/__	__/__/__	__/__/__	Positive / Negative
Whole blood	__/__/__	__/__/__	__/__/__	Positive / Negative

8. Case Classification: Lab confirmed Novel Coronavirus / Discard

9. Signature of responsible person filling the form _____

- Already distributed to State and Regional Hospital Labs by email
- NHL FB page and Website



Training Workshop on Infectious Substance Transport
25th - 26th September 2019

Receipt of Samples

- Assign NHL staff to provide 24-7 service for receipt of the samples
- Arrange the samples shipment to Reference lab as soon as possible

Lab Diagnosis

- According to SEARO's suggestion, plan to send the suspected samples to National Institute of Health (NIH), Department of Medical Sciences, Thailand
- NIH, Thailand agrees to test suspected samples from Myanmar
- No charges to pay for testing
- WCO already discussed with World courier for shipment
- The shipment charges will be covered by WCO
- Turn around time – NIH said – results will be 2 days after the receipt of samples

Constraints of NIC Myanmar

- Limited HR
- Require the particular PCR reagents and primers & probes for 2019- nCoV
- NIC is only (enhanced) Biosafety level 2 and handlings of novel coronavirus samples with current facilities are much be challenging to Biosafety and Biosecurity

Expecting capacities in NIC, Myanmar

- Already have well-set molecular facilities in NIC, Myanmar
- Can perform in house lab diagnosis if specific reagents (primers and probes) with validated controls but require to send the samples to reference lab for confirmation
- Smart BSL 3 (2 rooms) are going to arise in NHL in 2022

23 January 2020 by WHO

- The Emergency Committee convened by the WHO Director-General under the International Health Regulations regarding the outbreak of novel coronavirus 2019 advised on 23 January 2020 that
  - The event did not constitute a Public Health Emergency of International Concern (PHEIC)
- Of 584 cases now reported, 575 of them and all 17 deaths have been in China

References

- Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR, Berlin, 13.01.2020
- Interim guidance on Laboratory testing for 2019-nCoV in suspected human cases,14.01.2020
- Coronavirus 101 presented by Respiratory Viruses Branch, Division of Viral Diseases, National Center for Immunizations and Respiratory Diseases, Centers for Disease Control and Prevention

Thank you for your kind attention