To The Chief Wildlife Warden of all States and Union Territories,

Sub: Precautionary measures in the wake of confirmation of SARS-CoV-2 in a Tiger housed in the Bronx Zoo, New York - regarding

Ref: This office Circular F.No.25-1/2002-CZA(Vol.II)(AK)/2469/A/2020 dated 6.04.2020
2. This office Circular F.No.25-1/2002-CZA(Vol.II)(AK)/2469/A/2020 dated 24.03.2020
3. This office Circular F.No.25-1/2002-CZA(Vol.II)(AK)/2469/B/2020 dated 23.03.2020
4. This office Circular F.No.19-115/93-CZA(53)(Vol.XIV)(Pt.)/2427/2020 dated 13.03.2020

Sir,

In continuance to this office Circulars cited above, please find enclosed letter No.2-19/NRC ACTIVITY/2020-21/CWL dated 6th April, 2020 from the Director, Indian Veterinary Research Institute, Bareilly, wherein measures to be adopted by zoos are suggested.

Incidence of the SARS-CoV-2, if any, in captive animals housed in zoos shall be promptly reported to the designated government agencies including this office as well as the Director of Animal Husbandry Department of the respective State / Union Territory.

Yours sincerely

Encls. As above

(Dr. Sonali Ghosh)
Deputy Inspector General of Forests(CZA)

Copy to:
1. PS to Hon’ble Minister (EF&CC), Government of India
2. PS to Hon’ble Minister of State (EF&CC), Government of India
3. PPS to the Secretary to the Government of India (EF&CC)
4. PPS to the Director General of Forests and Special Secretary
5. The Director, Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, Uttar Pradesh, E-mail: dirivri@ivri.res.in, directorivri@gmail.com
6. The in-charge, Centre for Wildlife Conservation, Management and Disease Surveillance, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, E-mail: cwlincharge@gmail.com
7. The Officer in-charge of all recognised zoos
F. No. 2-19/NRC ACTIVITY/2020-21/CWL

Date: 06-04-2020

To
Member Secretary
CZA, New Delhi

Sub: SOP for Zoological Parks to control COVID-19 transmission to wild animals. Reg

Sir,

In continuation to the letter F. No. 25-1/2002-CZA (Vol.II)(AK)2469/B/2020 dated 06-04-2020 with the subject cited above, it is to inform that all the zoo authorities/administration is to be vigilant in lieu of the Bronx zoo, New York tiger episode dated 05-04-2020. Following biosafety and biosecurity measured to be adopted

1. All the vehicles at entrance and exit of the zoos must pass through tyre bath (0.5% sodium hypochlorite) or on 2m wide strip of dry slaked lime. Further all the vehicles to be sprayed or sanitized using 0.5% sodium hypochlorite.

2. All the personnel entering the zoo premises viz. zoo keepers, workers, sweepers coming from outside also the vehicles and personnel of feed and fodder supply, security guard, the meat contractors, vegetable and fruit suppliers and the administrative and managerial staff etc. must wash their hands with soap and water for 20sec and use hand sanitizer at the main entry of the gate. Closed packets to be sprayed with 65% ethanol/methanol/isopropanol and the vegetables to be thoroughly washed.

3. The zoo keepers to be screened at the gate for respiratory illness and to be enquired for any ailment of this kind in their family or neighbourhood. The ill persons should not be allowed to work in the premises rather they may be asked to remain indoor at home.

4. The keepers to be provided with dangri, gumboots, facemasks, heads covers, sanitizers and are not to be allowed to work with own clothings.

5. Coughing, spitting, urinating or cleaning nostrils in the enclosures should be strictly avoided.

6. Restrict the people movement in and around the zoo premises (morning or evening walks, all others, etc.)

7. Avoid unnecessary stress to the animals along with managerial decisions as per the climate.
8. The big cats should be monitored for any abnormal signs, viz., coughing, nasal discharge, respiratory distress, diarrhoea, anorexia, nervous symptoms and fever. If any visible symptoms are observed, the higher authority must be informed and the samples need to be collected as per WHO/OIE/ICMR guidelines (copy enclosed).

9. Samples to be collected from clinically ill big cats: blood in anticoagulant (5ml), serum (5ml) and nasal/oral/rectal swabs which to be transported under cold chain. Samples should be transported in leak-proof containers by considering all the biosafety measures (kindly refer WHO/OIE/ICMR guidelines for sample collection and dispatch).

10. The big cats to be provided with multivitamins, especially vit C, taurine and herbal immunomodulators like Restobal or any other make and the respiratory ailment to be treated symptomatically.

11. Disposal of biowaste and farm waste as per the SOP in place.

12. In case any symptoms are observed, Dr AM Pawde, Incharge, CWL, ICAR-IVRI should be contacted (Mob. 9412294363/7983309899, mail: cwlincharge@gmail.com).
Animal and environmental investigations to identify the zoonotic source of the COVID-19 Virus

Zoom conference, Friday 31 January 2020, 1pm (Paris time)

Participants: Billy Karesh (Chair – President OIE Wildlife Working Group), Casey Barton Behravesh (Centers for Disease Control, USA), Peter Ben Embarek (WHO), Etienne Bonbon (FAO), Stephane de La Rocque (WHO), Keith Hamilton (OIE), Hiroshi Kida (Hokkaido University), Jean-Claude Manuguerra (Institut Pasteur, Paris), Stefano Messori (OIE), Misheck Mulumba (OIE Scientific Commission for Animal Diseases), Malik Peiris (Hong Kong University), Dirk Pfeiffer (City University of Hong Kong), Primal Silva (Canadian Food Inspection Agency), Changchun Tu (Changchun Veterinary Research Institute) Sophie VonDobschuetz (FAO), Linfa Wang (Duke University, Singapore), Zheng Zengren (OIE Scientific Commission for Animal Diseases)

The purpose of the call was to discuss what is known about the role of animals in the emergence of Coronavirus Disease 2019, COVID-19 (caused by the SARS-CoV-2 virus (otherwise known as the COVID virus)) and to make preliminary recommendations relating to investigations at the human-animal ecosystems interface.

1. Review of current knowledge base

Many important questions remain unanswered about the animal origin of the COVID-19 virus. Although an animal source is probable, the scarcity of information leaves significant knowledge gaps, which leaves the door open for speculation and rumours. The lack of evidence also leads to, and in some ways necessitates, several assumptions being made.

From what is known, the COVID-19 virus isolated from humans shares 96% homology with beta coronaviruses isolated from multiple species of bats in the genus Rhinolophus (Yunnan, 2013). SARS-CoV isolated from humans shared 92% homology with SARS-like viruses that were circulating in bats. 90% of the SARS-like viruses from bats have been isolated from the Rhinolophus genus. The comparatively strong genetic sequence homology between the COVID-19 virus and beta coronavirus isolated from bats suggests that ancestors of the COVID-19 virus were circulating in bats in the Rhinolophus genus. Bats belonging to the Rhinolophus genus are widely distributed across Asia, the Middle East, Africa and Europe.

There is evidence that the transmission of SARS-CoV from an animal reservoir to humans involved an intermediate host (civets were implicated as an intermediate host for SARS-CoV). Owing to similarities between SARS-CoV and the COVID-19 virus, including the circumstances around their emergence, and considering the absence of other plausible theories an alternative assumption is being made that the transmission route of the COVID-19 virus to humans involved an intermediate animal host which has yet to be identified as opposed to direct bat to human transmission. The epidemiology of MERS shows how the role of an intermediate host can be more significant at the human animal interface than the original animal source of the virus. Thus, it is important to investigate the involvement of an intermediate host and to identify it.
Human epidemiological data links a high proportion of first- and second-generation human cases of COVID-19 to the Huanan Seafood Wholesale Market in Wuhan. An assumption is made that the COVID-19 virus was introduced to humans who visited or worked in the market. In the absence of detailed epidemiological data, several hypotheses exist for the introduction of the COVID-19 virus from animals to humans at the market. These include that 1. the virus was introduced to the human population from an animal source at the market and 2. that a human introduced the COVID-19 virus to the market (following exposure to the virus outside the market) and the virus was then amplified in animals which then infected humans.

There is only preliminary and incomplete information from investigations into the animal source at the market. This is understandable considering the importance and urgency of focusing on the public health response to contain the disease. However, information from these investigations is critical because it may hold the key to preventing further introductions of the virus into the human population, and it may also provide useful insights to reduce the risk of future spill over events from animals to humans.

In the absence of detailed information, the following assumptions are made. That a spill over event from animals to humans occurred at the Huanan Seafood Wholesale Market. The fact that wildlife was being sold at a seafood market indicates a possible route of introduction by wild species being brought into the market. It is likely that many different animal species were present in the market. Sampling investigations would likely have taken place several days (at least one incubation period) after animal-human exposure had occurred, by which time the source animals may no longer have been present in the market. It is known that samples were taken from several species of animal and that none of these samples tested positive, however information about the number of samples and species sampled is not available. However, several environmental (swab) samples did test positive and virus was isolated from environmental sample(s). It is not clear exactly how animals were incriminated from the positive environmental samples (apart from knowing that the swabs were taken from areas adjacent to where animals had been kept). The fact that the COVID-19 virus was easily isolated from environmental specimens taken at the Huanan Seafood Wholesale Market suggests that survivability of the virus in the environment is good and/or that viral load in the environment was high. In general, the COVID-19 virus and other SARS-like viruses appear to be stable; this has implications for contamination of and persistence in the environment and on fomites. Available information also suggests that it is relatively easy to culture and isolate the COVID-19 virus from specimens and that the virus grows well in Vero cells.

It is critical that important epidemiological and virological information which may explain the emergence and transmission of the COVID-19 virus from animals to humans is collected and preserved. The opportunity to understand this event must not be lost.

**General immediate recommendations:**

- The Advisory Group offers technical collaboration to support investigations into the animal source.

- Multisectoral one health collaboration including animal health, public health, wildlife experts should be encouraged.

- Immediate sharing of information from field investigations so far (including positive and negative results) should be encouraged.
2. Research priorities (broad categories):

**SURVEILLANCE AND RISK ASSESSMENT**

*Strategic objective:* To develop a better understanding of the key determinants of COVID-19 virus infection and transmission dynamics in animals (including at ecosystem level) and to humans to inform research, surveillance, and control.

Suggestions:

- **Identify the animal reservoir and intermediate host through surveillance/investigation strategies which consider:**
  
  - Evidence that ancestors of the COVID-19 virus circulate in bats from *Rhinolophus genus*.
  
  - The absence of information about an intermediate host, which could be any number of animal species (including wildlife, pests/vermin, domestic animals (companion or livestock), stray/feral animals).
  
  - In the absence of specific information, studies into the role of animals may need to consider a broad range of animal types and species. Where possible and appropriate, scientific information (epidemiological, virological, genetic etc.) may guide and support targeting of the investigations.
  
  - Broad serological surveillance is more likely to detect the COVID-19 virus in animals than virological surveillance alone (virological surveillance is too narrow). Serological studies can guide more specific targeted virological surveillance.
  
  - Targeting surveillance to selected locations may improve likelihood of detection e.g. markets/farms where wildlife and other animal species (including domestic animals/livestock) are gathered (particularly markets with a link to the Huanan Seafood Wholesale Market). Sampling locations may include other points identified along the supply chain to and from the market. Investigation around markets should also consider that, many markets have already been closed to support control efforts.
  
  - Other types of animal (free ranging, feral, vermin) found in proximity to markets (and other relevant locations) should also be considered in investigations.
  
  - Strategies could include testing archived animal samples (serum, faeces etc.) collected from recent surveillance projects.
  
  - Positive environmental samples could be tested for genetic material belonging to animal species (using metagenomics or DNA bar coding techniques (DNA bar coding may be more efficient than sequencing the whole genome)). This approach may guide investigations to identify the source of environmental contamination.
  
  - *Rhinolophus* group has extensive range, concerted research in China has found >50 SARS-like CoV’s. Using biodiversity and host-phylogenetic diversity data sets to model targeting of sampling to increase likelihood of identifying range of reservoirs across Asia, Middle East and Europe.

- **Transmission pathways**
  
  - Investigate potential transmission pathways from animal reservoirs to intermediate hosts to humans.
  
  - Evaluate the role of intermediate hosts in amplifying the virus.
  
  - Investigate routes and duration of viral shedding from potential hosts.
  
  - Investigate viral persistence under a variety of environmental conditions.
- Testing of farmed wildlife, wildlife markets and wild animals of species other than bats that could be intermediate hosts to identify potential CoVs and possible transmission pathways to humans.
- Investigate the possibility for transmission from humans to animals (domestic animals).

**Host range**
- Investigate the possible animal host range of the COVID-19 virus (including use of field (serology) and laboratory studies).

**Dynamics of wildlife trade**
- Better understand the dynamics of wildlife trade e.g. origin of different wildlife species in markets, diversity of species, husbandry/production practices, contact/mixing of groups, supply chains etc.

**Possible role of livestock**
- As well as assessing the possible role of other types of animal (wildlife, stray animals), it will be important to consider the possible role of livestock, including the possibility for them to become infected by humans.

**Possible role of companion animals in epidemiology of human disease**
- Assess the potential role of pets and companion animals in the epidemiology of the disease in countries affected with human cases. Consider investigations/sampling of pets of humans suspected or confirmed with disease.

**DIAGNOSTICS**

*Strategic objective: To develop diagnostic tools (for use in animal species) that provide consistent optimal results in any setting.*

Suggestions:

**Serology**
- A fit for purpose serology test for use in different species would be a powerful tool in surveillance for the COVID-19 virus in animals (the utility of serology was demonstrated in SARS-CoV and Hendra virus investigations).
- Adapt and validate current serology test for antibodies to the COVID-19 virus used in humans to animal systems.
- Consider developing laboratory and field serology kits for animal investigations.
- Assess cross reactivity between the COVID-19 virus and other SARS-like viruses.
- Recombinant protein techniques can play a role in developing serological techniques.

**RT-PCR**
- RT-PCR platforms for the COVID19 virus have been developed and disseminated for use in humans.
- RT-PCR platforms for the COVID-19 virus need to be adapted to animal systems.
- RT-PCR tools need to be adapted to be fit for purpose e.g. For initial screening of animal surveillance samples, sensitivity will be more important than specificity, therefore for RT-
PCR screening tools, primers which span the whole subgroup of SARS-like viruses could be used (with SARS as a positive control). RT-PCR which are more specific to the COVID-19 virus could be used to differentiate viruses when samples are positive on screening.

- **Other tests**
  - Virus neutralisation, pseudo particle VN, and other tests may also be useful for detection in animal samples.

**PREVENTION AND CONTROL INTERVENTIONS**

*Strategic objective: To guide targeted and effective evidence-based interventions.*

Suggestions: In addition to the priorities listed under Surveillance and Risk Assessment above:

- **Collect baseline data to inform prevention and control strategies**
  - Conduct studies to develop a better understanding of the dynamics around illegal wildlife capture, transport, and trading, and current prevention strategies, considering:
    - Social science around criminal behaviour.
    - Social/marketing studies on consumer demand.
    - Existing international standards, agreements, legislation, and guidance around wildlife trade, markets etc.
    - Relevant stakeholders – NGOs, IOs, national government, public, criminals, traders.
    - Coordination between law enforcement, veterinary services, market inspectors/regulators.
    - Effectiveness of various interventions e.g. law enforcement, legislation, prosecution, risk communication, incentivisation of legal practices, certification.
    - Use of innovation and technology in criminal surveillance/prosecution – cameras, drones, identification of animals.
    - The management of wet markets in China, particularly in Wuhan.
  - Identify high-risk practices and behaviours (for spill over events) along the food/wildlife supply chain.

- **Assess drivers of high-risk practices**
  - Social and economic drivers of legal and illegal activities.
  - Value chains leading to human animal/wildlife/environmental exposure.

- **Develop strategies to reduce risk of spill over events**
  - Research to determine the most effective risk communication strategies which avoid stigmatisation and other unintended consequences.
  - Research to determine the most effective social and behavioural change (SBC) practices to improve hygiene practices at wet markets.
  - Research to determine most effective SBC practices to implement realistic and feasible strategies to encourage a high level of compliance at wet markets.
- Research to determine the strategy to strictly manage the wild animal farming and to stop the illegal transportation and trading as well as smuggling.

  - information from laboratory studies
    - In the absence of field data, animal laboratory studies could help to inform prevention and control strategies e.g. animal models.

**HOST-PATHOGEN INTERACTION**

*Strategic objective:* To improve understanding of virus-host interactions and factors that impact on the interactions such as disease pathogenesis, transmissibility, and immune responses to better inform infection control.

Suggestions:

  - **Host pathogen studies**
    - Animal susceptibility - host range determination, receptor specificity/distribution in different species etc.
    - Cell line infections and animal experimental infections to understand transmission and pathogenicity.
    - Epidemiology of CoV in animal reservoirs, i.e. from bats to other species (viral load, routes of transmission).

  - **Behavioural risk**
    - Identify communities with high levels of exposure to bats and other key wildlife; analyse their risk behaviours; test samples from wildlife and people in these communities for serological evidence of the COVID-19 virus and other CoV spill over.
    - Inserting standardized key questions on wildlife exposure to be used during interview with suspected cases.

**SOCIODECONOMICS AND POLICY**

*Strategic objective:* To improve the effectiveness of detection, prevention and control measures through the integration of social, economic and institutional analyses of the environment affected.

Suggestions:

  - **Wildlife trade**
    - Define what is meant by wildlife (i.e. farmed wildlife vs. domestic animals/livestock etc.) in different contexts.
    - Characterize the wildlife trade value chain globally and regionally and how it is linked with China.
    - Policy/social research to regulate wildlife trading – innovation (cameras, drones etc.), collaboration with social scientists, law enforcement/ behaviour/demographic patterns.
    - Study of economic impact of removing wildlife from markets and market closures.
    - Analyses of the social impacts and economic analyses of different degrees of limiting wildlife trade for food: 1) complete ban; 2) partial ban (select species); 3) regulating and testing animals; 4) promoting only farmed wildlife as a source of food.
Wildlife capture vs. production

- Scenario analysis of whether or not farming wildlife reduces the risk of CoV emergence as compared to wild caught wildlife.

Wildlife consumption

- Survey of public to assesses knowledge, attitudes, and practices around wildlife consumption, geographic variation, and changing demographics.

Domestic animals

- Draw on research/risk communication already existing in this area relating to other zoonotic diseases (e.g. zoonotic influenza, Nipah, SARS, etc.) related to the breeding, keeping, selling and consumption of livestock.

3. Additional general notes:

There is a need to learn lessons from the introduction of the COVID-10 virus to the human population and from similar past events. A similar event in the future is inevitable.

Research

There is a need to highlight the limitations of research objectives in order to manage expectations on outcomes.

Risk mitigation strategies

It will be important to take a comprehensive long-term approach to risk mitigation strategies which aim to reduce the risk of spill over events.

Risk mitigation strategies need to feasible and consider cultural importance of certain high-risk practices. They need to adopt a multidisciplinary approach (vets, economists, food hygienists, microbiologists, social scientists, communication experts) and could include a package of risk mitigation measures targeted to the right stakeholders.

In risk communication there is a need to be clear about the current uncertainties around the role of animals in human outbreaks or animal species involved and it will be important to manage expectations e.g. risk can be reduced but not eliminated.

For short term, a key message is that the highest risk for COVID-19 virus infection is human to human transmission; identifying animal hosts is only an additional measure so that other (rare) spill over events can be reduced and similar human outbreaks prevented in future.

Risk communication can also build on material developed for other risk mitigation strategies (Ebola and wildlife/bushmeat, zoonotic avian influenza and live bird markets).

The spectrum of people at risk in different systems (field scientists, farmers, traders, consumers) needs to be considered in risk communication and other risk mitigation strategies.

Interventions need to be targeted for maximal positive impact (e.g. HACCP) and policies should avoid or manage unintended negative consequences (regulatory impact assessment).

Studies and guidance on wildlife trading and consumption should be adapted to both the global and regional levels i.e. global coverage whilst considering regional characteristics and specificities.

Strategies should be realistic and focus on risk reduction rather than elimination and should take lessons from other successful policy initiatives which led to behaviour change e.g. seat belts, smoking, diet.
Specimen, Packaging and Transport Guidelines for 2019 Novel Coronavirus (2019-nCoV)

Title: Specimen Collection, Packaging and Transport Guidelines for 2019 Novel Coronavirus (2019-nCoV)

Scope:
To be used by the Government health authorities/ hospitals/ clinicians/ laboratories planning to collect appropriate clinical samples as indicated for diagnosis of 2019-nCoV.

Purpose:
This document describes the information for collection, packaging and transport of clinical specimens to Influenza group at ICMR-National Institute of Virology (NIV), Pune, Maharashtra for diagnosis of 2019 Novel Coronavirus (2019-nCoV).

Responsibilities:
- The clinician should decide necessity for collection of clinical specimens for laboratory testing of 2019-nCoV only after following the case definition as given by the health authorities, Government of India.
- Appropriate clinical sample need to be collected by laboratory personnel/ health care worker trained in specimen collection in presence of a clinician.
- By following all biosafety precautions and using personal protective equipment (PPEs), clinical samples need to be sent to the designated laboratory (ICMR-NIV, Pune) by following standard triple packaging.

Selection of patient:
Any person who presents with Severe Acute Respiratory Illness (SARI) AND any one of the following i.e. a history of travel from Wuhan, China in 14 days prior to symptoms onset; disease in healthcare worker working in an environment of SARI patients; unusual or unexpected clinical course, especially sudden deterioration despite appropriate treatment; should be urgently investigated. Updated case definition need to be followed as per MOHFW, Govt of India which is available on the website www.mohfw.gov.in.

Specimen collection details:
(Adapted from the WHO guidelines on 2019-nCoV):

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Collection materials</th>
<th>Transport to laboratory</th>
<th>Storage till testing</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal and oropharyngeal swab</td>
<td>Dacron or polyester flocked swabs*</td>
<td>4 °C</td>
<td>≤5 days: 4 °C</td>
<td>The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;5 days: -70 °C</td>
<td></td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>sterile container*</td>
<td>4 °C</td>
<td>≤48 hours: 4 °C</td>
<td>There may be some dilution of pathogen, but still a worthwhile specimen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;48 hours: -70 °C</td>
<td></td>
</tr>
<tr>
<td>Tracheal aspirate, nasopharyngeal aspirate or nasal wash</td>
<td>sterile container*</td>
<td>4 °C</td>
<td>≤48 hours: 4 °C</td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;48 hours: -70 °C</td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td>sterile container</td>
<td>4 °C</td>
<td>≤48 hours: 4 °C</td>
<td>Ensure the material is from the lower respiratory tract</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;48 hours: -70 °C</td>
<td></td>
</tr>
<tr>
<td>Tissue from biopsy or autopsy including from lung</td>
<td>sterile container with saline</td>
<td>4 °C</td>
<td>≤24 hours: 4 °C</td>
<td>Autopsy sample collection preferably to be avoided</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;24 hours: -70 °C</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>Serum separator tubes</td>
<td>4 °C</td>
<td>≤5 days: 4 °C</td>
<td>Collect paired samples:</td>
</tr>
<tr>
<td>(2 samples – acute and convalescent)</td>
<td>(adults: collect 3-5 ml whole blood)</td>
<td></td>
<td>&gt;5 days: -70 °C</td>
<td>• acute – first week of illness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• convalescent – 2 to 3 weeks later</td>
</tr>
</tbody>
</table>

*For transport of samples, for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens.

Specimen labelling and processing:
- Personal protective equipment (apron, hand gloves, face shield, N95 Masks etc.) need to be used and all biosafety precautions should be followed so as to protect individuals and the environment.
- Proper labelling (name/age/gender/specimen ID) need to be done on specimen container and other details of sender (name/address/phone number) on the outer container by mentioning “To be tested for 2019-nCoV”.
- For any queries, the nodal officer from ICMR-NIV Pune (Dr Yogesh K. Gurav, Scientist E) may be contacted (Phone 020-26006290 / 26006390; Email: gurav.yk@gmail.com/gurav.yk@gov.in) and need to be informed in advance before sending specimens to ICMR-NIV, Pune.
### Requirements for Clinical Samples Collection, Packaging and Transport

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sample vials and Virus Transport Medium (VTM)</td>
<td>Use sample vials and Virus Transport Medium (VTM)</td>
</tr>
<tr>
<td>2. Adsorbent material (cotton, tissue paper), paraffin, seizer, cello tape</td>
<td>Use adsorbent material (cotton, tissue paper), paraffin, seizer, cello tape</td>
</tr>
<tr>
<td>3. A leak-proof secondary container (e.g., ziplock pouch, cryobox, 50 mL centrifuge tube, plastic container)</td>
<td>Use a leak-proof secondary container (e.g., ziplock pouch, cryobox, 50 mL centrifuge tube, plastic container)</td>
</tr>
<tr>
<td>4. Hard-frozen Gel Packs</td>
<td>Use hard-frozen Gel Packs</td>
</tr>
<tr>
<td>5. A suitable outer container (e.g., thermocol box, ice-box, hard-board box)</td>
<td>Use a suitable outer container (e.g., thermocol box, ice-box, hard-board box) (minimum dimensions: 10 x 10 x 10 cm)</td>
</tr>
</tbody>
</table>

### Procedure for Specimen Packaging and Transport

1. **Use PPE while handling specimen**
2. **Seal the neck of the sample vials using parafilm**
3. **Cover the sample vials using absorbent material**
4. **Arrange primary container (vial) in secondary container**
5. **Placing the centrifuge tube inside a zip-lock pouch**
6. **Placing the zip-lock pouch inside a sturdy plastic container and seal the neck of the container**
7. **Using a thermocol box as an outer container and placing the secondary container within it, surrounded by hard-frozen gel packs**
8. **Placing the completed Specimen Referral Form (available on www.niv.co.in) and request letter inside a leak-proof, zip-lock pouch**
9. **Securing the zip-lock pouch with the Specimen Referral Form on the outer container**
10. **Attaching the labels:**
    - Senders’ address, contact number;
    - Consignee’s address /contact number;
    - Biological substance Category B;
    - ‘UN 3373’; Orientation label; Handle with care

### Documents to accompany:

1) Packaging list/proforma Invoice 2) Air way bill (for air transport) (to be prepared by sender or shipper) 3) Value equivalence document (for road/rail/sea transport)  [Note: 1. A vaccine-carrier/ice-box can also be used as an outer container 2. The minimum dimensions of the outer container should be 10 x 10 x 10 cm (length x width x height)]

### Routing of samples:

- Clinical specimens, official documents and Specimen request forms for testing of 2019-nCoV need to be sent to the ICMR-NIV address (The Director, ICMR-National Institute of Virology, 20-A, Dr Ambedkar Road, Pune, Maharashtra, Pin: 4110001).
- For shipment-related queries/information, kindly contact Dr Sumit Bharadwaj (Scientist B, Influenza Group) on email: sumitduttbhardwaj@gmail.com, phone 020-26006290/26006390
1. Introduction

Several coronaviruses can infect humans, the globally endemic human coronaviruses HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43 that tend to cause mild respiratory disease, and the zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) that have a higher case fatality rate. In December 2019, a cluster of patients with a novel coronavirus was identified in Wuhan, China (1). Initially tentatively named 2019 novel coronavirus (2019-nCoV), the virus has now been named SARS-CoV-2 by the International Committee of Taxonomy of Viruses (ICTV) (2). This virus can cause the disease named coronavirus disease 2019 (COVID-19). WHO refers to the virus as COVID-19 virus in its current documentation.

The purpose of this document is to provide interim guidance to laboratories and stakeholders involved in COVID-19 virus laboratory testing of patients.

Existing WHO documents have been consulted for drafting this interim guidance, including the interim guidance on laboratory testing for MERS (3-9). Information on human infection with the COVID-19 virus is evolving and WHO continues to monitor developments and revise recommendations as necessary. Feedback is welcome and can be sent to WHElab@who.int.

2. Laboratory testing guiding principles for patients who meet the suspect case definition

The decision to test should be based on clinical and epidemiological factors and linked to an assessment of the likelihood of infection. PCR testing of asymptomatic or mildly symptomatic contacts can be considered in the assessment of individuals who have had contact with a COVID-19 case. Screening protocols should be adapted to the local situation. The case definitions are being regularly reviewed and updated as new information becomes available. For the WHO suspect case definition see: Global Surveillance for human infection with coronavirus disease (COVID-2019) (10).

Rapid collection and testing of appropriate specimens from patients meeting the suspect case definition for COVID-19 is a priority for clinical management and outbreak control and should be guided by a laboratory expert. Suspect cases should be screened for the virus with nucleic acid amplification tests (NAAT), such as RT-PCR.

If testing for COVID-19 is not yet available nationally, specimens should be referred. A list of WHO reference laboratories providing confirmatory testing for COVID-19 and shipment instructions are available in Section 4 of the following webpage: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance.

If case management requires, patients should be tested for other respiratory pathogens using routine laboratory procedures, as recommended in local management guidelines for community-acquired pneumonia. Additional testing should not delay testing for COVID-19. As co-infections can occur, all patients that meet the suspect case definition should be tested for COVID-19 virus regardless of whether another respiratory pathogen is found.
In an early study in Wuhan, the mean incubation period for COVID-19 was 5.2 days among 425 cases, though it varies widely between individuals (11,12,13). Virus shedding patterns are not yet well understood and further investigations are needed to better understand the timing, compartmentalization and quantity of viral shedding to inform optimal specimen collection. Though respiratory samples have the greatest yield, the virus can be detected in other specimens, including stool and blood (14,15,16). Local guidelines should be followed regarding patient or guardian’s informed consent for specimen collection, testing and potentially future research.

3. Specimen collection and shipment

Safety procedures during specimen collection
Ensure that adequate SOPs are in use and that staff are trained for appropriate specimen collection, storage, packaging and transport. All specimens collected for laboratory investigations should be regarded as potentially infectious.

Ensure that health care workers who collect specimens adhere rigorously to infection prevention and control guidelines. Specific WHO interim guidance has been published: “Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected, interim guidance, January 2020” (17) and “WHO interim guidance for laboratory biosafety related to 2019-nCoV” (18).

Specimens to be collected
At minimum, respiratory material should be collected:

- **upper respiratory specimens:** nasopharyngeal and oropharyngeal swab or wash in ambulatory patients
- and/or **lower respiratory specimens:** sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. (Note high risk of aerosolization; adhere strictly to infection prevention and control procedures).

Additional clinical specimens may be collected as COVID-19 virus has been detected in blood and stool, as had the coronaviruses responsible for SARS and MERS (14,16,19-21). The duration and frequency of shedding of COVID-19 virus in stool and potentially in urine is unknown. In case of patients who are deceased, consider autopsy material including lung tissue. In surviving patients, paired serum (acute and convalescent) can be useful to retrospectively define cases as serological assays become available.

Further recommendations on materials to collect, including the testing of asymptomatic individuals, can be found in Table 1.

Packaging and shipment of clinical specimens
Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation is essential. Specimens which can be delivered promptly to the laboratory can be stored and shipped at 2-8°C. When there is likely to be a delay in specimens reaching the laboratory, the use of viral transport medium is strongly recommended. Specimens may be frozen to - 20°C or ideally -70°C and shipped on dry ice if further delays are expected (see Table 2). It is important to avoid repeated freezing and thawing of specimens.

Transport of specimens within national borders should comply with applicable national regulations. International transport of potentially COVID-19 virus containing samples should follow the UN Model Regulations, and any other applicable regulations depending on the mode of transport being used. More information may be found in the “WHO Guidance on regulations for the Transport of
Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases

Infectious Substances 2019-2020”(22) and “WHO interim guidance for laboratory biosafety related to 2019-nCoV”(18).

Ensure good communication with the laboratory and provide needed information
Alerting the laboratory before sending specimens encourages proper and timely processing of samples and timely reporting. Specimens should be correctly labelled and accompanied by a diagnostic request form (template provided in Annex I).

4. Laboratory testing for COVID-19 virus

Laboratories undertaking testing for COVID-19 virus should adhere strictly to appropriate biosafety practices.

Nucleic acid amplification tests (NAAT) for COVID-19 virus
Routine confirmation of cases of COVID-19 is based on detection of unique sequences of virus RNA by NAAT such as real-time reverse-transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary. The viral genes targeted so far include the N, E, S and RdRP genes. Examples of protocols used may be found here. RNA extraction should be done in a biosafety cabinet in a BSL-2 or equivalent facility. Heat treatment of samples prior to RNA extraction is not recommended.

Laboratory confirmation of cases by NAAT in areas with no known COVID-19 virus circulation
To consider a case as laboratory-confirmed by NAAT in an area with no COVID-19 virus circulation, one of the following conditions need to be met:

- A positive NAAT result for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus using a validated assay (as at present no other SARS-like coronaviruses are circulating in the human population it can be debated whether it has to be COVID-19 or SARS-like coronavirus specific); OR
- One positive NAAT result for the presence of betacoronavirus, and COVID-19 virus further identified by sequencing partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used.

When there are discordant results, the patient should be resampled and, if appropriate, sequencing of the virus from the original specimen or of an amplicon generated from an appropriate NAAT assay, different from the NAAT assay initially used, should be obtained to provide a reliable test result. Laboratories are urged to seek confirmation of any surprising results in an international reference laboratory.

Laboratory confirmed case by NAAT in areas with established COVID-19 virus circulation
In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which for example screening by rRT-PCR of a single discriminatory target is considered sufficient.

One or more negative results do not rule out the possibility of COVID-19 virus infection. A number of factors could lead to a negative result in an infected individual, including:

- poor quality of the specimen, containing little patient material (as a control, consider determining whether there is adequate human DNA in the sample by including a human target in the PCR testing)
- the specimen was collected late or very early in the infection
- the specimen was not handled and shipped appropriately
- technical reasons inherent in the test, e.g. virus mutation or PCR inhibition.

If a negative result is obtained from a patient with a high index of suspicion for COVID-19 virus infection, particularly when only upper respiratory tract specimens were collected, additional specimens, including from the lower respiratory tract if possible, should be collected and tested.

Each NAAT run should include both external and internal controls, and laboratories are encouraged to participate in external quality assessment schemes when they become available. It is also recommended to laboratories who order their own primers and probes to perform entry testing/validation on functionality and potential contaminants.
Laboratories with limited experience in testing for COVID-19 virus are encouraged to work with laboratories with more experience with this pathogen to have their initial test results confirmed and to improve their own performance.

For laboratories testing for COVID-19 virus in countries where COVID-19 was not previously circulating, WHO advises the confirmation of testing results for:

- the first 5 positive specimens,
- the first 10 negative specimens (collected from patients that fit the case definition)

by referring them to one of the WHO reference laboratories providing confirmatory testing for COVID-19. For national COVID-19 laboratories that require support with specimen shipment to one of the reference laboratories for testing confirmation, a WHO shipment fund is available. Please refer to the WHO website for the most updated list of reference laboratories and shipment instructions.

Serological testing
Serological surveys can aid investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of an outbreak. In cases where NAAT assays are negative and there is a strong epidemiological link to COVID-19 infection, paired serum samples (in the acute and convalescent phase) could support diagnosis once validated serology tests are available. Serum samples can be stored for these purposes.

Cross reactivity to other coronaviruses can be challenging (24) but commercial and non-commercial serological tests are currently under development. Some studies with COVID-19 serological data on clinical samples have been published (25,26).

Viral sequencing
In addition to providing confirmation of the presence of the virus, regular sequencing of a percentage of specimens from clinical cases can be useful to monitor for viral genome mutations that might affect the performance of medical countermeasures, including diagnostic tests. Virus whole genome sequencing can also inform molecular epidemiology studies. Many public-access databases for deposition of genetic sequence data are available, including GISAID, which is intended to protect the rights of the submitting party (27).

Viral culture
Virus isolation is not recommended as a routine diagnostic procedure.

6. Reporting of cases and test results

Laboratories should follow national reporting requirements. In general, all test results, positive or negative, should be immediately reported to national authorities. States Parties to the IHR are reminded of their obligations to share with WHO relevant public health information for events for which they notified WHO, using the decision instrument in Annex 1 of the IHR (2005) (28).

7. Research toward improved detection of COVID-19 virus

Many aspects of the virus and disease are still not understood. A better understanding will be needed to provide improved guidance. For example:

Viral dynamics: optimal timing and type of clinical material to sample for molecular testing

- Dynamic of immunological response
- Disease severity in various populations, e.g. by age.
- The relationship between viral concentration and disease severity
- The duration of shedding, and relation to clinical picture (e.g. clinical recovery occurs with viral clearing, or shedding persists despite clinical improvement)
- Development and validation of useful serological assays
- Comparative studies of available molecular and serological assays
- Optimal percentage of positive cases that requires sequencing to monitor mutations that might affect the performance of molecular tests.

WHO encourages the sharing of data to better understand and thus manage the COVID-19 outbreak, and to develop countermeasures.
### Table 1. Specimens to be collected from symptomatic patients and contacts

<table>
<thead>
<tr>
<th>Test</th>
<th>Type of sample</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAAT</td>
<td>Lower respiratory tract</td>
<td>Collect on presentation. Possibly repeated sampling to monitor clearance. Further research needed to determine effectiveness and reliability of repeated sampling.</td>
</tr>
<tr>
<td></td>
<td>- sputum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- aspirate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- lavage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Upper respiratory tract</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- nasopharyngeal and oropharyngeal swabs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- nasopharyngeal wash/nasopharyngeal aspirate</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Consider stools, whole blood, urine, and if diseased, material from autopsy</strong></td>
<td></td>
</tr>
<tr>
<td>NAAT</td>
<td>Serum for serological testing once validated and available</td>
<td>Paired samples are necessary for confirmation with the initial sample collected in the first week of illness and the second ideally collected 2-4 weeks later (optimal timing for convalescent sample needs to be established).</td>
</tr>
<tr>
<td><strong>Contact</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAAT</td>
<td>Nasopharyngeal and oropharyngeal swabs</td>
<td>Within incubation period of last documented contact.</td>
</tr>
<tr>
<td>Serology</td>
<td>Serum for serological testing once validated and available</td>
<td>Baseline serum taken as early as possible within incubation period of contact and convalescent serum taken 2-4 weeks after last contact (optimal timing for convalescent sample needs to be established).</td>
</tr>
</tbody>
</table>
Table 2. Specimen collection and storage (adapted from ref 6 and ref 29,30)

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Collection materials</th>
<th>Storage temperature until testing in-country laboratory</th>
<th>Recommended temperature for shipment according to expected shipment time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal and oropharyngeal swab</td>
<td>Dacron or polyester flocked swabs*</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 5 days &lt;br&gt;−70 °C (dry ice) if &gt; 5 days</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>Sterile container *</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 2 days &lt;br&gt;−70 °C (dry ice) if &gt; 2 days</td>
</tr>
<tr>
<td>(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspiration</td>
<td>Sterile container *</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 2 days &lt;br&gt;−70 °C (dry ice) if &gt; 2 days</td>
</tr>
<tr>
<td>Sputum</td>
<td>Sterile container</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 2 days &lt;br&gt;−70 °C (dry ice) if &gt; 2 days</td>
</tr>
<tr>
<td>Tissue from biopsy or autopsy including from lung</td>
<td>Sterile container with saline or VTM</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 24 hours &lt;br&gt;−70 °C (dry ice) if &gt; 24 hours</td>
</tr>
<tr>
<td>Serum</td>
<td>Serum separator tubes (adults: collect 3-5 ml whole blood)</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 5 days &lt;br&gt;−70 °C (dry ice) if &gt; 5 days</td>
</tr>
<tr>
<td>Whole blood</td>
<td>Collection tube</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 5 days &lt;br&gt;−70 °C (dry ice) if &gt; 5 days</td>
</tr>
<tr>
<td>Stool</td>
<td>Stool container</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 5 days &lt;br&gt;−70 °C (dry ice) if &gt; 5 days</td>
</tr>
<tr>
<td>Urine</td>
<td>Urine collection container</td>
<td>2-8 °C</td>
<td>2-8 °C if ≤ 5 days &lt;br&gt;−70 °C (dry ice) if &gt; 5 days</td>
</tr>
</tbody>
</table>

* For transport of samples for viral detection, use viral transport medium (VTM) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens. If VTM is not available sterile saline may be used in place of VTM (in such case, duration of sample storage at 2-8 °C may be different from what is indicated above).

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 etc.
Acknowledgements

The following people contributed to the drafting of the evolving versions of this guidance document: Katrin Leitmeyer, European Center for Disease Control, Maria Zambon, Public Health England, UK; Christian Drosten, Charité - Universitätsmedizin Berlin, Germany; Marion Koopmans, Erasmus MC, Rotterdam, The Netherlands; Leo Poon, Hong Kong University, China, Hong Kong SAR; George Gao, Chinese CDC, China.


8. References


27) GISAID.org (https://www.gisaid.org/), accessed on 19 February 2020


Annex I
COVID-19 virus LABORATORY TEST REQUEST FORM

<table>
<thead>
<tr>
<th>Submitter information</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAME OF SUBMITTING HOSPITAL, LABORATORY, or OTHER FACILITY*</td>
</tr>
<tr>
<td>Physician</td>
</tr>
<tr>
<td>Address</td>
</tr>
<tr>
<td>Phone number</td>
</tr>
<tr>
<td>Case definition*: ☐ Suspect case ☐ Probable case</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient info</th>
</tr>
</thead>
<tbody>
<tr>
<td>First name</td>
</tr>
<tr>
<td>Last name</td>
</tr>
<tr>
<td>Patient ID number</td>
</tr>
<tr>
<td>Date of Birth</td>
</tr>
<tr>
<td>Age:</td>
</tr>
<tr>
<td>Address</td>
</tr>
<tr>
<td>Sex ☐ Male ☐ Female ☐ Unknown</td>
</tr>
<tr>
<td>Phone number</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specimen information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
</tr>
<tr>
<td>☐ Nasopharyngeal and oropharyngeal swab</td>
</tr>
<tr>
<td>☐ Bronchoalveolar lavage</td>
</tr>
<tr>
<td>☐ Endotracheal aspirate</td>
</tr>
<tr>
<td>☐ Nasopharyngeal aspirate</td>
</tr>
<tr>
<td>☐ Nasal wash</td>
</tr>
<tr>
<td>☐ Sputum</td>
</tr>
<tr>
<td>☐ Lung tissue</td>
</tr>
<tr>
<td>☐ Serum</td>
</tr>
<tr>
<td>☐ Whole blood</td>
</tr>
<tr>
<td>☐ Urine</td>
</tr>
<tr>
<td>☐ Stool</td>
</tr>
<tr>
<td>☐ Other: ....</td>
</tr>
</tbody>
</table>

All specimens collected should be regarded as potentially infectious and you must contact the reference laboratory before sending samples. All samples must be sent in accordance with category B transport requirements.

<table>
<thead>
<tr>
<th>Please tick the box if your clinical sample is post mortem</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Time of collection</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Priority status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical details</td>
</tr>
<tr>
<td>Date of symptom onset:</td>
</tr>
<tr>
<td>Has the patient had a recent history of travelling to an affected area? ☐ Yes ☐ No</td>
</tr>
<tr>
<td>Country</td>
</tr>
<tr>
<td>Return date</td>
</tr>
</tbody>
</table>

| Has the patient had contact with a confirmed case? ☐ Yes ☐ No ☐ Unknown |
| Other exposure: |

| Additional Comments |

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WHO reference number: WHO/COVID-19/laboratory/2020.4

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1 Form in accordance with ISO 15189:2012 requirements