

GUIDELINES FOR COVID-19 INVESTIGATION IN CAPTIVE WILD ANIMALS

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by



These guidelines are a set of recommendations from the Laboratory for the Conservation of Endangered Species (LaCONES), CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad. This contains scientifically validated protocols, shared in the interest of all. As new findings emerge and newer protocols are validated, those will be shared.

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INTRODUCTION: COVID-19 IN ANIMALS

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) outbreak happened at the end of 2019. The disease caused by the SARS-CoV-2 virus is called COVID-19. The disease has spread globally causing the COVID-19 pandemic in humans. SARS-CoV-2 has also been reported to affect wild animals in many parts of the world. Most of the reports suggest illness in carnivores. In view of the SARS-CoV-2 outbreak and transmission to wild animals, Central Zoo Authority of India (CZA) has provided timely advisories to zoos. LaCONES at CSIR-CCMB initiated testing for COVID-19 in wild animals after due authorization from CZA.

CSIR-CCMB has been actively involved in COVID-19 human sample testing and development of newer COVID-19 diagnostic and surveillance tools since April 2020. It is also a national biorepository of COVID-19 samples. It sequences the genomes of the SARS-CoV-2 prevalent in India.

Following the recent incidences of COVID-19 infection in big cats in zoos, LaCONES, CSIR-CCMB has framed guidelines for safe and efficient collection, packing and transport of samples for COVID-19 tests, and further investigations into the infections.

FREQUENTLY ASKED QUESTIONS ON SARS-COV-2 IN ANIMALS

What is SARS-CoV-2?

SARS-CoV-2 belongs to the family of coronaviruses that are known to infect mammals and birds. They do not always cause disease; many infected animals and birds are reservoirs. Reservoirs may show no disease symptoms, and appear perfectly healthy.

Where has SARS-CoV-2 come from?

The origin of SARS-CoV-2 still remains debatable. Most of the evidence suggests the mutant strains of virus originated from bats or pangolins, and a spillover event caused infection in humans.

Which animals can be infected with SARS-CoV-2?

In the last one year there have been reports of wild animals across the globe getting infected with SARS-CoV-2. These include non-human primates such as gorillas, large felids such as tigers, lions, pumas, cougars and snow leopards, and mustelids like ferrets, minks in an animal farm. Experimental research and reports also show that many mammals, including cats, dogs, bank voles, ferrets, fruit bats, hamsters, mink, pigs, rabbits, raccoon dogs, tree shrews, and white-tailed deer can be infected with the virus.

Can these animals spread the SARS-CoV-2 infection back to humans?

Current studies do not indicate that animals spread infection back to humans.

How do I know if the animals have gotten the SARS-CoV-2 infection?

SARS-CoV-2 symptoms in animals may include lack of appetite, anorexia, coughing, sneezing, wheezing, nasal discharge, fever, respiratory distress. If any or all the above symptoms are noticed, then samples must be collected and sent immediately for diagnostics including RT-PCR for SARS-CoV-2.

How should I collect samples from a live captive animal to test for SARS-CoV-2 infection?

Wear Personal Protective Equipment (PPE) before you go to the animal enclosure. Collect samples of nasal or oral swabs if possible, or nasal discharges/saliva/faecal samples or rectal swabs for COVID-19 testing. Use only sterile flocked nylon or polyester swabs for sample collection and store it in VTM. DO NOT use cotton swabs.

How should I collect samples from a post-mortem examination to test for SARS-CoV-2 infection?

Collect samples as early as possible after the animal's death. Wear PPE before approaching the carcass. Take deep throat, nasopharyngeal, tracheal and lung swabs.

How should I collect samples from a free ranging wild animal to test for SARS-CoV-2 infection?

Make repeated observations of the animal suspected to be infected. Mark any peculiar markings, scars etc for identification of animals. Take videos of the animal and take an expert opinion before any further steps are taken. Nasal/oropharyngeal swabs in VTM should be collected and sent for further testing.

How should an animal that has died due to infection with SARS COV-2 be handled and carcass disposed of?

Incineration is the best option. Handlers should wear PPE at all times when they are handling the carcass. If a transport vehicle is used to carry the infected carcass to the disposal site, it should be thoroughly disinfected before using it again.

How should I pack the sample to send it to a COVID-19 testing centre?

Pack them in leak-proof containers, seal the sample vials with parafilm

and then in ziplock packets. Label the sample vials/containers with sample ID, sample type, animal details, and name of the zoo). Samples that are not sealed properly or do not contain any labels will not be taken up for analyses. Place the ziplock packets on ice in a thermocol box. Label the thermocol box with sender's address and contact details, and consignee's address and contact details.

Where should I send the sample for testing?

There are four designated centres in India for testing animal samples for possible SARS CoV-2 infection. They are:

a. National Institute of High Security Animal Diseases (NIHSAD), Bhopal, Madhya Pradesh

(http://www.nihsad.nic.in/covid_dashboard.html)

b. National Research Centre on Equines (NRCE), Hisar, Haryana
(<http://nrce.gov.in/>)

c. Centre for Animal Disease Research and Diagnostic (CADARD), ICAR-Indian Veterinary Research Institute (IVRI), Bareilly, Uttar Pradesh
(<http://ivri.nic.in/Division/JD/Cadrad/default.aspx>)

d. Laboratory for Conservation of Endangered Species at CSIR-Centre for Cellular and Molecular Biology, Hyderabad, Telangana
(<http://covid-19.ccmb.res.in/>)

What food and medicine should I give to the animals until their test results arrive?

The decision is to be made by the Veterinarian-in-charge of the care of animals in the facility. There is no specific treatment regime standardised for SARS-COV-2 infection in wild animals. Symptomatic treatment is currently the preferred approach. Feed the animals their regular diet and with proper hygienic precautions: washing of meat with clean, running water at least two times and followed by a single wash with hot water, using a gentle stream for 2-3 minutes before feeding.

How should I store and stock food for the animals in case of a crisis?

Wash the meat thoroughly with hot water and then store in the cold storage. Procure the essential medicines, feed/water supplements well in advance.

What are the general hygiene sanitation measures for captive animals and animal handlers?

Animals: Separate the non-infected animals immediately from the suspected/infected animals. Keep the suspected/infected animals in open spaces, or spacious enclosures with good ventilation or in alternate cages. Do not cause the animals to become agitated or aggressive as this might promote spread of virus by aerosol to other animals. Observe all animals very closely. Do not move animals across zoos during the COVID-19 pandemic.

Keepers: Wear PPE at all times when working in animal cages. Inform the concerned zoo veterinarian/officer immediately if any signs of illness are noticed. Used PPE gear should be disposed of on site by incineration to reduce contact points. Regular training should be provided to animal keepers on following COVID-19 precautions and monitoring of animals.

STANDARD OPERATING PROCEDURE FOR COVID-19 INVESTIGATION IN ZOO ANIMALS

How should I collect samples from a live captive animal to test for SARS-CoV-2 infection?

Sample collection material:

i) Sterile flocked polyester/nylon swabs to be used. These are available commercially. DO NOT USE COTTON SWABS.

ii) Sterile collection vials with Viral Transport Medium for collecting discharges/secretions. Avoid the collection of soiled samples (eg saliva/discharges from the floor or any surface). Samples should not be contaminated with blood.

- Follow the appropriate biosafety protocols while sample collection and packing. Use appropriate Personal Protective Equipment (PPE): face shield, face mask, N-95 mask, head cover, goggles, hand gloves, shoe cover and coverall or gown.



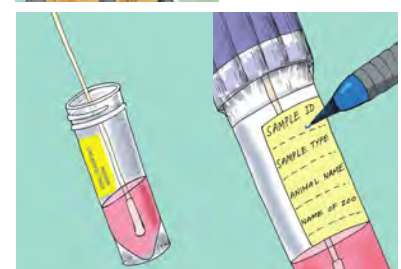
- Use proper sanitization protocols when sick animals are handled for sample collection. Give sufficient time to sanitize the restraining devices (chute/cages/immobilising equipment), if the same devices are to be used for another animal.



- Collect naso/oral swab samples in sample collection vials. Nasal discharges/saliva should be additionally collected.



- Seal all the collection vials. Label the sample vials/containers with sample ID, sample type, animal details, and name of the zoo). Vials that are not sealed properly or do not contain any labels will not be analysed.



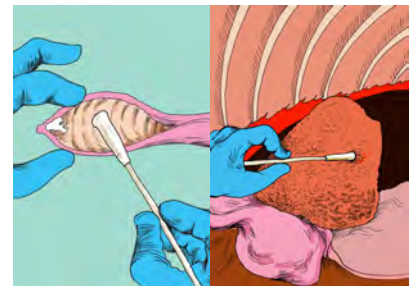
How should I collect samples from a post-mortem examination to test for SARS- CoV2 infection?

Sample collection material:

Sterile swabs, sample collection vials containing Viral Transport Medium (VTM)

Sample collection from a post-mortem examination should follow these steps:

- Wear PPE and carry a pre-sanitised sample collection kit before approaching the carcass.
- Collect the samples in sample collection vials as early as possible after the death of the animal. Take deep throat swabs and nasopharyngeal swabs. Take a swab from the lungs. Avoid sampling if the trachea or nasal passages are contaminated with stomach contents.
- Send the samples to the testing laboratory as early as possible after collection. Send dry swabs, if the time to reach the testing lab is less than 24-48h. If it will take more than 48h, send the samples in 2-3 ml of VTM (2-3ml). VTM is available commercially.



How should I pack the sample to send it to a COVID-19 testing centre?

Packing material:

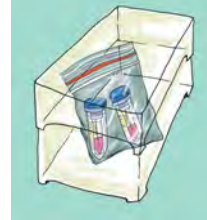
- Adsorbent material to prevent the contamination from any leakage: Cotton/tissue paper
- Leak proof container (2nd): 50 ml centrifuge tubes/ plastic containers to place the swab collection vials/containers (1st container).
- Cool packs: frozen gel packs
- Leak proof container (3rd): plastic containers/cryovial boxes
- Outer box: thermocol box/ vaccine carrier/ice box/hard cardboard box to place the 3rd container (with 1st and 2nd) and ice packs.
- Parafilm to seal the sample collection vials.
- Cello tape (transparent) and scissors
- Permanent markers (micro tip marker for labeling sample details on sample vial/container; large tip markers for labeling outer box.

Packing of samples collected in VTM should follow these steps:

- Place the swab in the sample collection vial and fasten the cap (1st container). Please ensure that the swab does not touch the outer surface of the container while placing the swab into the container.
- Seal the neck of sample tube with parafilm and wrap the tube with cotton/tissue paper.
- Place the 1st container in a 50 ml centrifuge (2nd) tube and seal the neck with parafilm.



- Keep the centrifuge tube in the sterile zip lock plastic cover.
- Place the sterile zip-lock cover with the sample in a plastic container (3rd container).
- Keep the 3rd container along with ice packs in a thermocol box/ice box/cardboard box (outer box) and seal the outer container with cello tape properly.
- Label the outer box with sender's address and contact details, and consignee's address and contact details.
- Sample information sheets (with animal details) should be enclosed.
- Send the samples through an authorized person to the testing laboratory.



Dry swab samples without VTM can be sent if the samples reach testing centres within 24 hours. Packing dry swab samples should follow the same steps as the samples in VTM.

Representative Sample Information Sheet

CASE No.
For use at CCMB

Sample Information Sheet for Covid-19

(Only For Zoo Animals)

Please fill the form and submit along with sample(s). If required enclose additional information with this form

1. Name & Address of the Zoo/Park:

2. Details of the animal

Species		Sex	
Animal I.D (Name/No)		Age	

3. Nature of sample (mark with √)

1. Live Animal Screening	
2. Post-mortem*	

* Samples should be collected immediately after death, give date and time

4. Clinical Signs: Y/N; If yes, give date of recording:

Loss of appetite		Sneezing		Cough		Dyspnoea		Fever	
Diarrhoea									
Any other specific symptoms:									

5. Pre-existing medical conditions please specify with date (eg. Bacterial, viral, parasitic diseases, tumours etc.)

6. Was the animal ever exposure to laboratory confirmed COVID-19 symptomatic or asymptomatic person (s) (animal keepers/attendants/doctors) or animals?

7. Investigation details, if done with date:

8. Treatment details if given:

9. Specimen details:

- (i) Date and time of Collection:
- (ii) Sample I.D:
- (iii) Sample Types:

Nasopharyngeal swab		Saliva swab		Deep throat swab [#]	
Oropharyngeal swab		Faecal sample*		Lung swab [#]	
Nasal swab				Lung piece [#]	
Nasal Discharges					

*optional, and should be accompanied with swab samples in VTM. [#] pertains to post-mortem samples (without blood contamination).

10. Name and contact details of the authorized persons:

1.	Name of the Veterinary Officer	
	Mobile No	
	Mail I.D	
2.	Name of the Director/Curator	
	Mobile No	
	Mail I.D	

11. The information provided above is as per our records.

Date:

Authorized zoo personnel

Representative Sample Information Sheet (filled)

CASE No.
For use at CCMB

Sample Information Sheet for Covid-19

(Only For Zoo Animals)

Please fill the form and submit along with sample(s). If required enclose additional information with this form

1. Name & Address of the Zoo/Park:

Name: ABC Zoo/Park	City: XXXXXXXX	PIN: XXXXXX
State: XXXXXXXX		

2. Details of the animal

Species	Lion	Sex	M
Animal I.D (Name/No)	Raju	Age	18

3. Nature of sample (mark with ✓)

1. Live Animal Screening	✓
2. Post-mortem*	

* Samples should be collected immediately after death, give date and time

4. Clinical Signs: Y/N; If yes, give date of recording: 14-04-2021

Loss of appetite	✓	Sneezing		Cough	✓	Dyspnoea	✓	Fever	
Diarrhoea									

Any other specific symptoms:

5. Pre-existing medical conditions please specify with date (eg. Bacterial, viral, parasitic diseases, tumours etc.) nil

6. Was the animal ever exposure to laboratory confirmed COVID-19 symptomatic or asymptomatic person (s) (animal keepers/attendants/doctors) or animals? No

7. Investigation details, if done with date: No

8. Treatment details if given: symptomatic

9. Specimen details:

- (i) Date and time of Collection: 15-04-2021
- (ii) Sample I.D: XX/XX/ABC/2021 (refers to zoo/park)
- (iii) Sample Types:

Nasopharyngeal swab		Saliva swab		Deep throat swab [#]	
Oropharyngeal swab		Faecal sample*		Lung swab [#]	
Nasal swab	✓			Lung Piece [#]	
Nasal Discharges	✓				

*optional, and should be accompanied with swab samples in VTM. [#] pertains to post-mortem samples (without blood contamination).

10. Name and contact details of the authorized persons:

1.	Name of the Veterinary Officer	XX XXXXX XXXXX
	Mobile No	XXXXXXXXXX
	Mail I.D	XXXXXXXX@XXXXX.com
2.	Name of the Director/Curator	XX XXXXX XXXXX
	Mobile No	XXXXXXXXXX
	Mail I.D	XXXXXXXX@XXXXX.com

11. The information provided above is as per our records.

Date:

Authorized zoo personnel

What should be done to prevent the further spread of SARS CoV-2 in captive wild animals?

For animals:

- Separate the non-infected animals immediately from the suspected/infected animals. Keep the suspected/infected animals in open spaces, or spacious enclosures with good ventilation or in alternate cages.
- Clean their water tubs daily with appropriate disinfectant. Use separate cleaning material (buckets/mugs/brushes, floor mops /wipers etc.) for suspected/infected animals. The cleaning material should be disinfected after their use and dried in sunlight.
- Isolate the suspected/infected animal from other animals.
- Provide appropriate diet and feed/water supplements that increase immunity to captive animals.
- Wash food, especially meat, with hot water for 2-3 min before feeding the animals. WASHING UNDER A GENTLE STREAM OF WATER IS ESSENTIAL. Persons handling the feed should strictly follow all COVID-19 precautions.
- Incinerate the leftover meat and fecal material from the animal enclosures.
- Monitor their health closely by keeping a log of food intake, urination, defaecation, activity and looking for any abnormal behaviour and respiratory symptoms.
- Do not cause the animals to become agitated or aggressive as this might promote spread of virus by aerosol to other animals.
- Do not move animals across zoos during the COVID-19 pandemic.

For zoo staff:

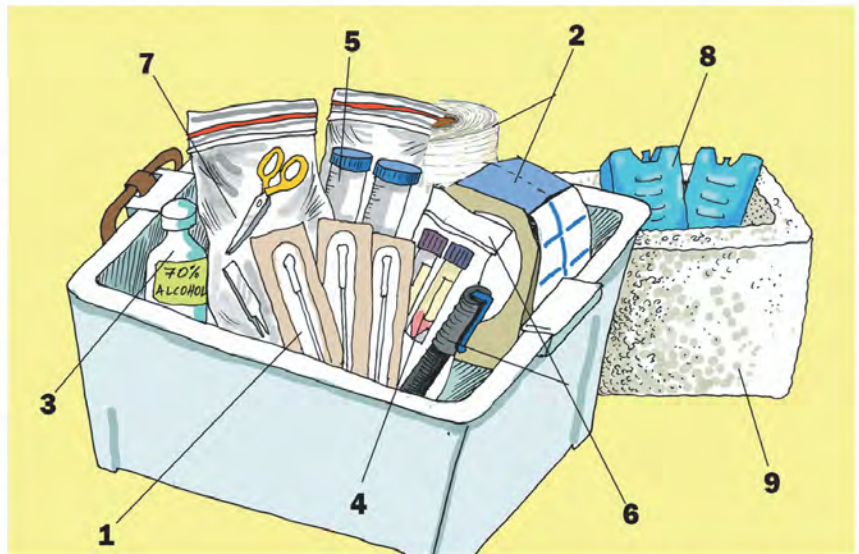
- Minimise contact between zoo-keepers and animals (both captive and free-ranging). We know that many human beings are asymptomatic carriers of the virus.
- Assign different animal keepers and caregivers for infected and non infected animals.
- Inform the concerned zoo veterinarian/officer immediately if any signs of illness are noticed.
- Test animal keepers and animal care staff including veterinarians regularly even if they don't show symptoms.
- Animal handlers should wear Personal Protective Equipment (PPE): Face Shield, Face mask, N-95 mask, head cover, goggles, hand gloves, shoe cover and coverall or gown when they go to animal enclosures.
- Encourage staggered working hours for the workers at the zoo. Maintain physical distancing among the workers at the zoo.
- Ensure vaccination of zoo staff, forest frontline staff and forest fringe communities on priority.
- Form COVID response teams (covid-free Veterinarian, Assistant Curator (ACF level officer), zoo keeper and helper) within the set-up so that there is swift action, proper implementation of COVID precautions and continuous monitoring.

RESOURCES FOR ANIMAL SAMPLE COLLECTION AND PACKING

THINGS YOU SHOULD HAVE FOR

SAMPLE COLLECTION AND PACKAGING

PPE



1) NYLON SWAB



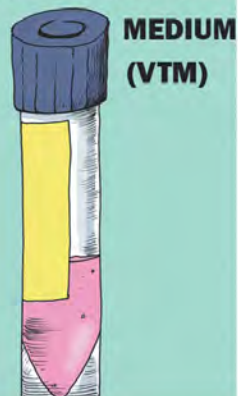
2) TISSUE PAPER



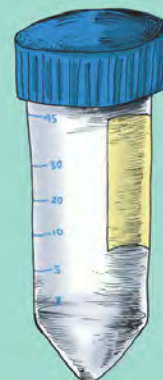
3) 70% ALCOHOL



4) VIRAL TRANSPORT



5) 50 ML TEST TUBE



6) ZIP-LOCK AND ...



7) SCISSORS



8) ICE PACK



9) THERMOCOL BOX



SAMPLE COLLECTION

DO

1) WEAR PPE DURING SAMPLE COLLECTION



2) COLLECT NASAL SWAB SAMPLE



3) COLLECT ORAL SWAB AND SALIVA



4) COLLECT RECTAL SWAB

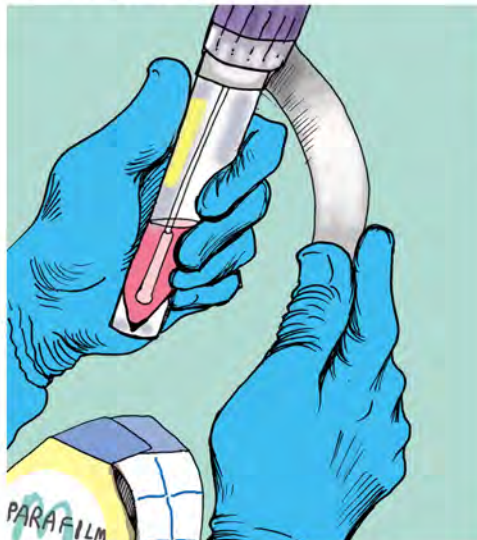


5) COLLECT SWAB IN STERILE VTM VIAL



**VTM = VIRAL
TRANSPORT
MEDIUM**

6) SEAL ALL THE COLLECTION VIALS WITH PARAFILM



7) LABEL ALL THE COLLECTION VIALS



SAMPLE COLLECTION

DON'T

**DO NOT USE
COTTON SWAB**



**AVOID COLLECTING SAMPLES
FROM FLOOR OR
ANY SURFACE**



**DO NOT COLLECT
SAMPLE WITH
BLOOD CONTAMINATION**



**DO NOT USE SAMPLE
WITH NO LABEL AND
SEALING**



Veterinarians can decide the protocol to restrain live animals for sample collection. Fecal samples alone do not yield very reliable results for SARS-CoV-2 detection. So, rectal samples must be accompanied by nasal and/or oral swab samples.

SAMPLE PACKING DO

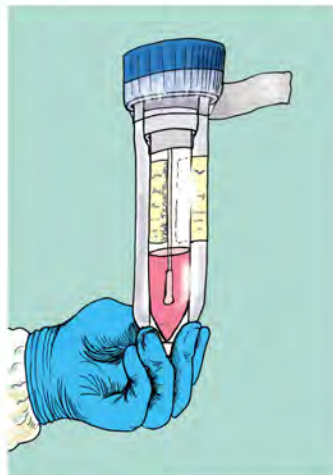
1) SEND THE SAMPLE IN VIRAL TRANSPORT MEDIUM



2) SEAL THE SAMPLE TUBE WITH PARAFILM



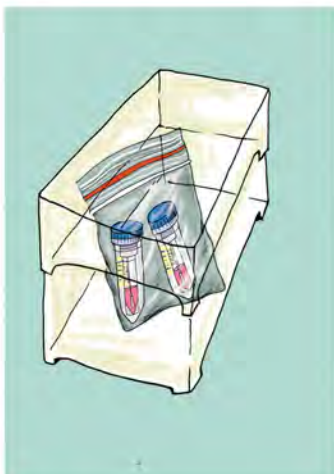
3) PLACE THE SAMPLE TUBE IN 50 ML FALCON, SEAL WITH PARAFILM



4) PLACE THE TUBES IN STERILE ZIP-LOCK



5) PLACE THE ZIP-LOCK IN A PLASTIC CONTAINER



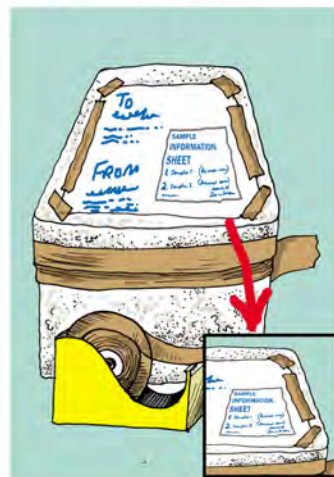
6) PLACE THE PLASTIC BOX IN A THERMOCOL BOX



7) PUT THE ICE PACKS IN THE THERMOCOL BOX



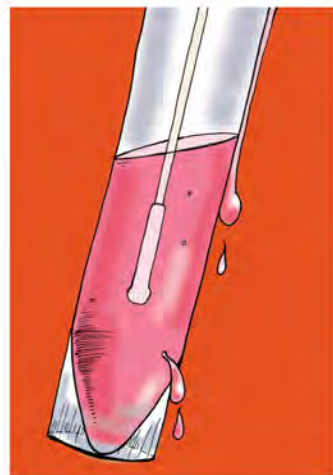
8) SEAL THE THERMOCOL BOX, LABEL WITH SAMPLE INFORMATION SHEET



SWAB SHOULD NOT TOUCH THE OUTER SURFACE OF THE COLLECTION TUBE



DO NOT USE TUBES WITH ANY LEAKAGE



DO NOT SEND SAMPLE WITH NO LABEL OR PARAFILM SEALING



SAMPLE PACKING DON'T

SAMPLE COLLECTION FROM DEAD ANIMALS

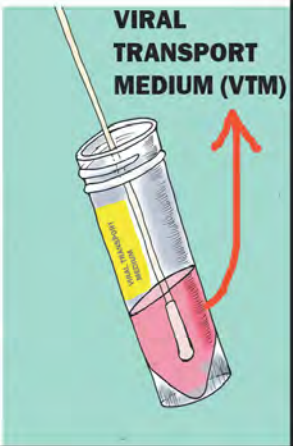
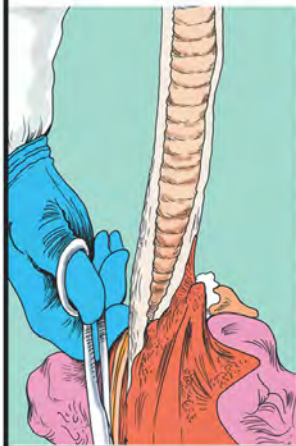
WEAR PPE



CARRY PRE-SANITIZED SAMPLE COLLECTION KIT



SAMPLE COLLECTION FROM TRACHEA



SAMPLE COLLECTION FROM LUNG

