Interim laboratory biosafety guidelines for the handling and transport of samples associated with the novel coronavirus 2019 (2019-nCoV)[1]

28 January 2020

In January 2020, the etiologic agent responsible for a cluster of severe pneumonia cases in Wuhan, China, was identified as a novel beta-coronavirus (2019-nCoV), distinct than SARS-CoV and MERS-CoV (1) (2) (3). The complete genomic sequence of this new agent is available and different detection protocols have been developed, although they have not been fully validated yet. However, in light of the possible introduction of a suspected case related to 2019-nCoV in the Region of the Americas, the Pan American Health Organization / World Health Organization (PAHO / WHO) recommends that Member States ensure its timely identification either by the shipment of the samples to national or reference laboratories, or the implementation of the molecular detection protocol for 2019-nCoV, depending on the laboratory's capacity.

To date, the pathogenic potential and transmission dynamics of 2019-nCoV is not fully understood. For this reason and in the light of the knowledge of other similar viruses (e.g., MERS-CoV, SARS-CoV), it is necessary to maintain and strengthen biosafety measures including personal protection procedures, to work with samples from suspected cases of respiratory pathogen infection.

General recommendations for working with potentially infectious material[2]

Laboratory personnel should wear appropriate personal protective equipment (PPE) that includes disposable gloves, surgical mask, anti-fluid gown, and eye protection when handling potentially infectious samples.

When collecting a respiratory sample from a suspected patient in the Intensive Care Unit, the use of N95 masks is highly recommended.

Any procedure with the potential to generate fine particle aerosols (for example, sample preparation with open tubes or vortexing) must be performed in a certified Class II biosafety cabinet (BSC). Appropriate physical restraint devices (e.g., centrifuge safety buckets and sealed rotors) should be used for centrifugation. Ideally, centrifuge rotors should be loaded and unloaded in a BSC. Any procedure within the laboratory which generates aerosols and is performed outside a BSC (or the cleaning up highly suspicious samples spilling, for example) must be performed using N95 mask.

After sample processing, decontaminate work surfaces and equipment with the appropriate disinfectants. To this end, use any registered hospital disinfectant. The manufacturer's recommendations for use/dilution (i.e., concentration), contact time and care in handling should be followed.

All disposable material must be autoclaved before final disposal.

[1] The recommendations made in this document can be subject to later modifications in accordance to the advances in the knowledge of the disease and the etiologic agent.

[2] These recommendations are based on the CDC's Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV): https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

Specific recommendations for the handling of samples that may contain 2019-nCoV

To avoid amplification and concentration of viral particles, it is **NOT recommended** to attempt viral isolation in cell culture.

The following procedures may be performed in BSL-2 facilities using standard work practices:

- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues
- Plate setup for molecular analysis of already extracted viral nucleic acids
- Electron microscopic studies with glutaraldehyde-fixed grids
- Routine (visual) examination of bacterial and mycotic cultures
- Routine staining and microscopic analysis of fixed smears
- Final packaging of specimens for transport to diagnostic laboratories for additional testing. Samples should already be in a sealed, decontaminated primary container.
- Inactivated specimens (specimens in nucleic acid extraction buffer)

The following procedures must be performed in a Class II BSC:

- Aliquoting and/or diluting samples
- Inoculating bacterial or mycological culture media
- Performing diagnostic tests that do not involve propagation of viral agents *in vitro* or *in vivo* (e.g., preparing slides for immunofluorescence)
- Nucleic acid extraction procedures involving potentially infected specimens
- Preparation and chemical- or heat-fixing of smears for microscopic analysis

International regulations for the safe transport of infectious substances

The safe packing, shipping and transport of samples that may contain 2019-nCoV must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations (4).

Planning the logistics of the shipment

- Identify the name and contact details (phone and / or e-mail) of the technical officer for the event at the national reference laboratory, National Influenza Center (NIC), or international reference laboratory, who should be attentive until the shipment is received
- Notify the laboratory (NIC or reference laboratory) of the shipment of the sample
- Contact the transporting company to verify schedules and itineraries

Preparing the material for packaging

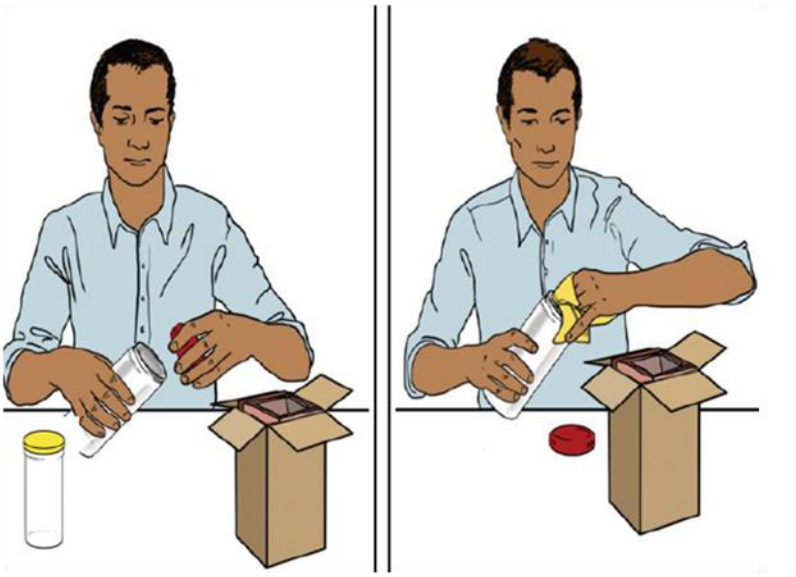
The transport of samples that may contain 2019-nCoV must use triple packaging and comply with international standards related to air transport of infectious substances: “Biological Substance, Category B”.

Absorbent material enough to absorb the entire liquid content
Rigid, leak-proof secondary container
Rigid shipping box (tertiary – outer-container). For air shipping, a P650 (Category B) should be used.

Packing the sample

1 **Open the secondary leak-proof container**. Be sure that the size of the container match the number of samples being shipped

2 **Insert the absorbent material.** There should be enough material to absorb all contents in primary container



3 **Wrap the primary container with cushioning material.** If packaging more than one sample, wrap each primary container individually

4 **Place the primary container(s) into the secondary container**

5 **Close the secondary container**

Packing of refrigerated samples (recommended)

6 **Place the secondary leak-proof container into a Styrofoam container and surround with ice packs**

7 **Place styrofoam container into the rigid shipping box (tertiary container)**

8 **Put laboratory form / letter and epidemiological questionnaire into an envelope**

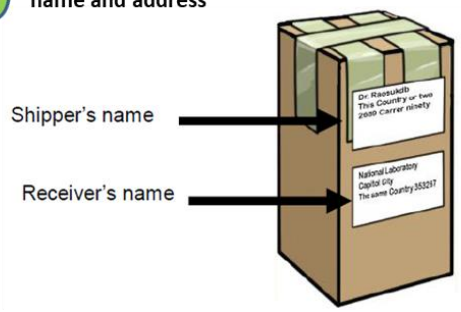
9 **Put laboratory form / letter and epidemiological questionnaire into the rigid shipping box**

10 **Close the top of the shipping box (tertiary container)**

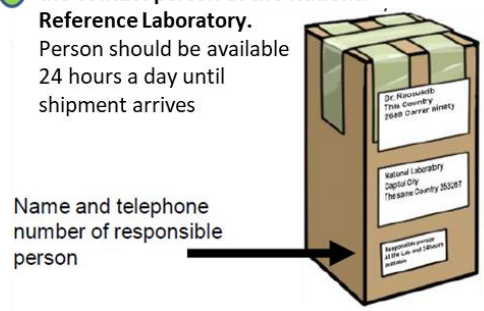
11 **Tape the box closed**

Marks and labels

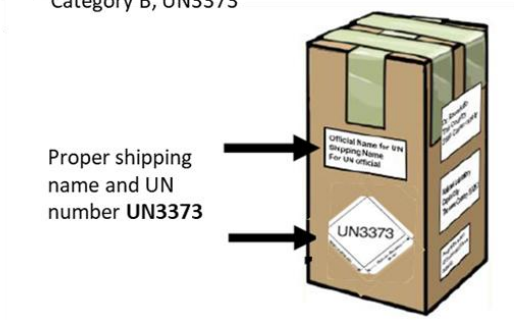
12 **Write (clearly) the precise name and address**



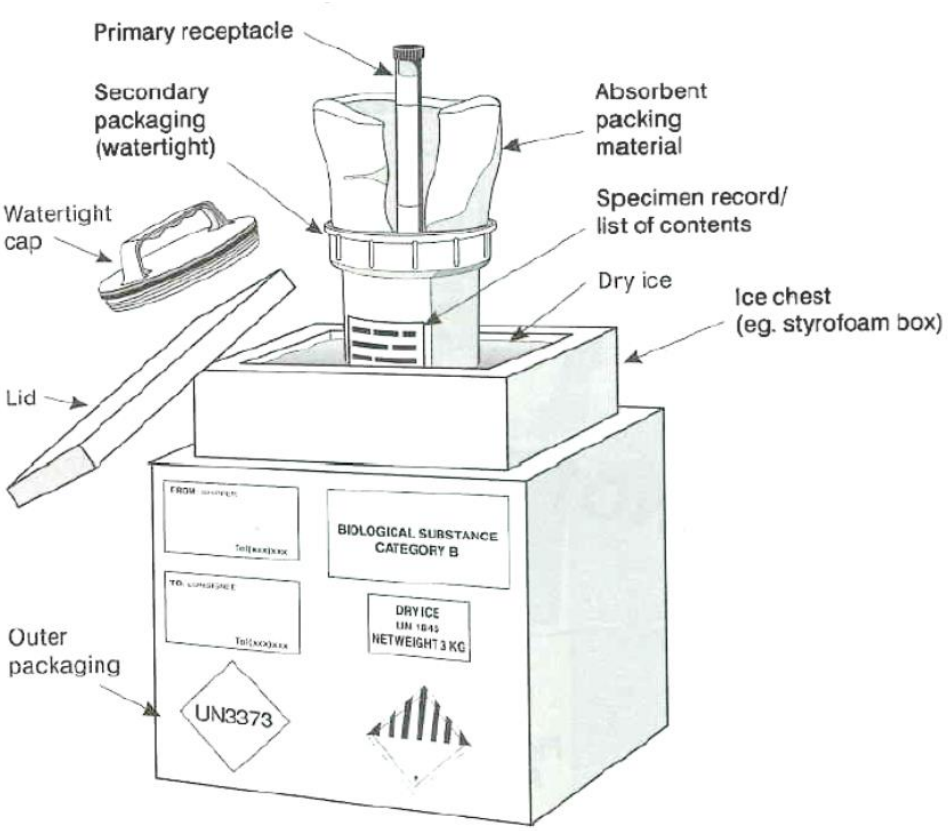
13 **Name and the telephone number of the contact person at the National Reference Laboratory.** Person should be available 24 hours a day until shipment arrives



14 **Write the proper shipping name and UN number:** "Biological substance, Category B, UN3373"



Mandatory triple packaging model for air shipments that can be used to comply with the P650 instruction for Biological Substances, Category B.



Documentation that must accompany the shipment (Biological substances, Cat. B)

For the transport of samples, it is important that the shipper prepares the required documentation according to the applicable regulations, in order to inform those who are going to transport the package (i.e., the carrier, the courier or the logistics specialist) about the way in which it was prepared and about its content.

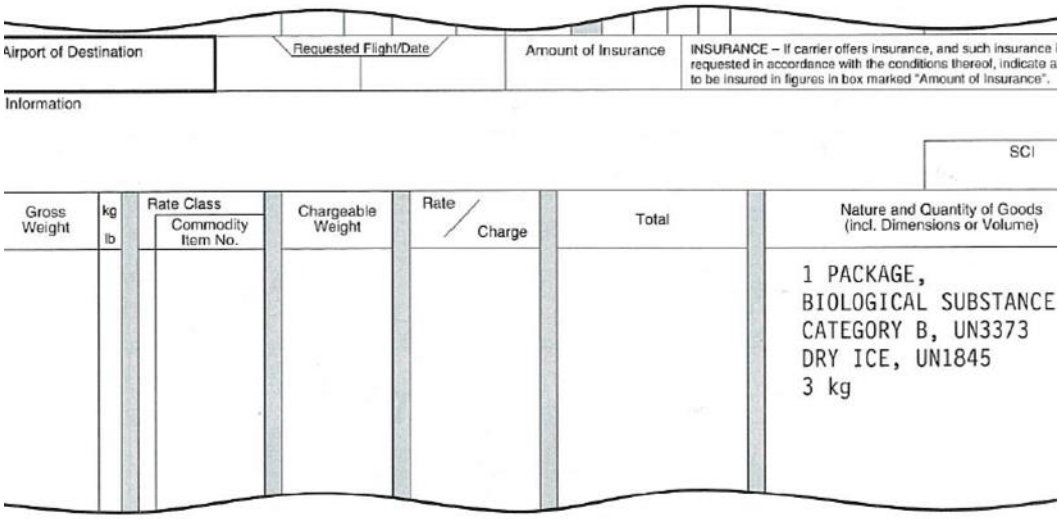
All information provided in transport documents must be easy to read and written so that it cannot be altered (for example, with permanent ink that cannot be easily removed).

- ### Air waybill

An air waybill must accompany all shipments made by air (Cat. A, Cat. B and exempt material).

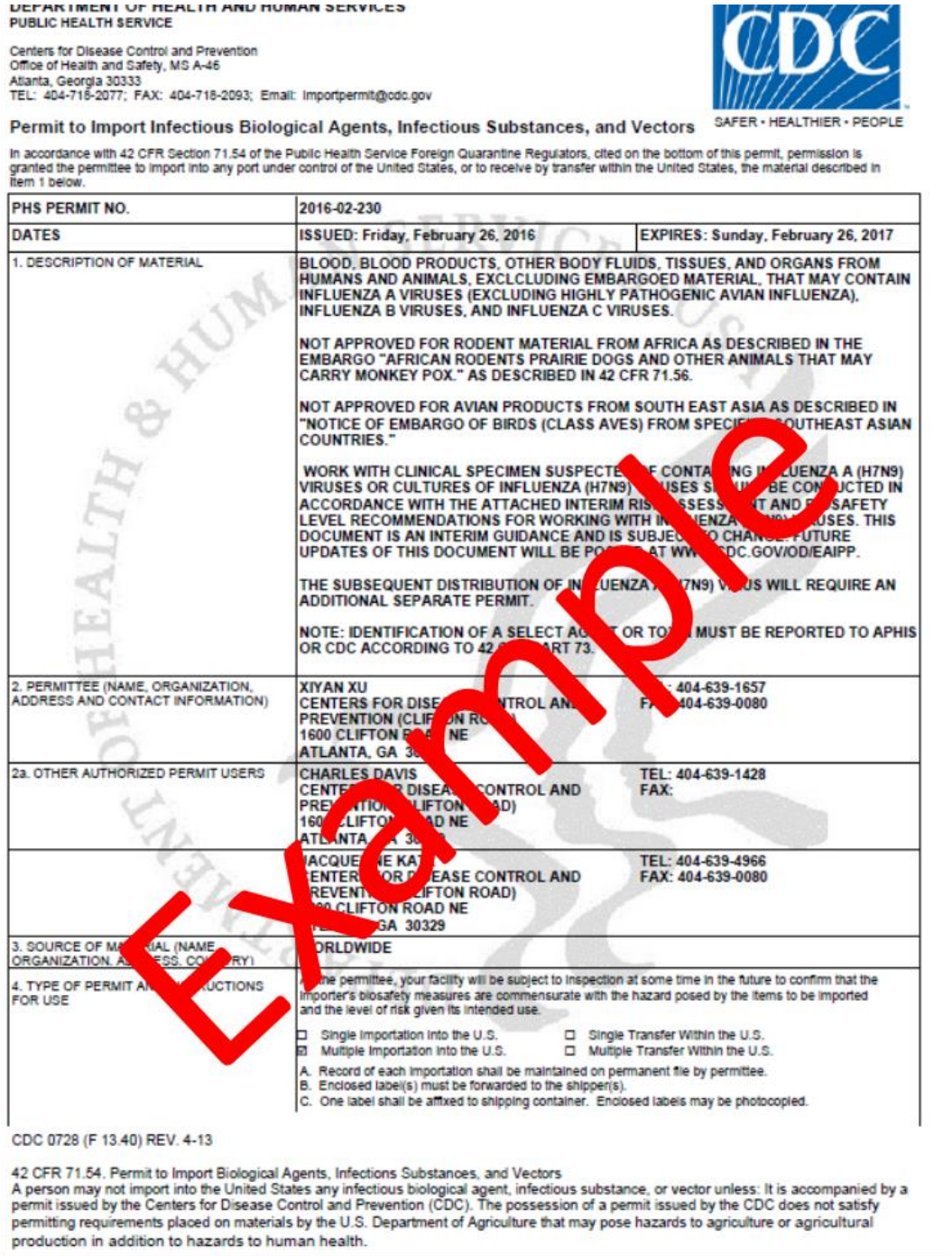
The shipper or transport company will be responsible for completing the information in the air waybill.

Model of air waybill for shipping of Biological Substances, Cat. B with dry ice (recommended)



- **Import permit**

In some instances, the laboratory that receives the sample requires a permit to import the biological material. A valid import permit must be requested in advance from the institution and should indicate the details of the person responsible for receiving the package.



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Centers for Disease Control and Prevention
Office of Health and Safety, MS A-46
Atlanta, Georgia 30333
TEL: 404-718-2077; FAX: 404-718-2093; Email: Importpermit@cdc.gov

CDC
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Permit to Import Infectious Biological Agents, Infectious Substances, and Vectors

In accordance with 42 CFR Section 71.54 of the Public Health Service Foreign Quarantine Regulators, cited on the bottom of this permit, permission is granted the permittee to import into any port under control of the United States, or to receive by transfer within the United States, the material described in item 1 below.

PHS PERMIT NO.	2016-02-230	
DATES	ISSUED: Friday, February 26, 2016	EXPIRES: Sunday, February 26, 2017
1. DESCRIPTION OF MATERIAL	BLOOD, BLOOD PRODUCTS, OTHER BODY FLUIDS, TISSUES, AND ORGANS FROM HUMANS AND ANIMALS, EXCLCLUDING EMBARGOED MATERIAL, THAT MAY CONTAIN INFLUENZA A VIRUSES (EXCLUDING HIGHLY PATHOGENIC AVIAN INFLUENZA), INFLUENZA B VIRUSES, AND INFLUENZA C VIRUSES. NOT APPROVED FOR RODENT MATERIAL FROM AFRICA AS DESCRIBED IN THE EMBARGO "AFRICAN RODENTS PRAIRIE DOGS AND OTHER ANIMALS THAT MAY CARRY MONKEY POX." AS DESCRIBED IN 42 CFR 71.56. NOT APPROVED FOR AVIAN PRODUCTS FROM SOUTH EAST ASIA AS DESCRIBED IN "NOTICE OF EMBARGO OF BIRDS (CLASS AVES) FROM SPECIFIED SOUTHEAST ASIAN COUNTRIES." WORK WITH CLINICAL SPECIMEN SUSPECTED OF CONTAINING INFLUENZA A (H7N9) VIRUSES OR CULTURES OF INFLUENZA (H7N9) VIRUSES SHOULD BE CONDUCTED IN ACCORDANCE WITH THE ATTACHED INTERIM RISK ASSESSMENT AND BIOSAFETY LEVEL RECOMMENDATIONS FOR WORKING WITH INFLUENZA A VIRUSES. THIS DOCUMENT IS AN INTERIM GUIDANCE AND IS SUBJECT TO CHANGE. FUTURE UPDATES OF THIS DOCUMENT WILL BE POSTED AT WWW.CDC.GOV/OD/EAIPP. THE SUBSEQUENT DISTRIBUTION OF INFLUENZA A (H7N9) VIRUS WILL REQUIRE AN ADDITIONAL SEPARATE PERMIT. NOTE: IDENTIFICATION OF A SELECT AGENT OR TOXIN MUST BE REPORTED TO APHIS OR CDC ACCORDING TO 42 CFR PART 73.	
2. PERMITTEE (NAME, ORGANIZATION, ADDRESS AND CONTACT INFORMATION)	XIYAN XU CENTERS FOR DISEASE CONTROL AND PREVENTION (CLIFTON ROAD) 1600 CLIFTON ROAD NE ATLANTA, GA 30329	TEL: 404-639-1657 FAX: 404-639-0080
2a. OTHER AUTHORIZED PERMIT USERS	CHARLES DAVIS CENTERS FOR DISEASE CONTROL AND PREVENTION (CLIFTON ROAD) 1600 CLIFTON ROAD NE ATLANTA, GA 30329	TEL: 404-639-1428 FAX:
	JACQUELINE KATZ CENTERS FOR DISEASE CONTROL AND PREVENTION (CLIFTON ROAD) 1600 CLIFTON ROAD NE ATLANTA, GA 30329	TEL: 404-639-4966 FAX: 404-639-0080
3. SOURCE OF MATERIAL (NAME, ORGANIZATION, ADDRESS, COUNTRY)	WORLDWIDE	
4. TYPE OF PERMIT AND INSTRUCTIONS FOR USE	As the permittee, your facility will be subject to inspection at some time in the future to confirm that the importer's biosafety measures are commensurate with the hazard posed by the items to be imported and the level of risk given its intended use. ☐ Single Importation into the U.S. ☐ Single Transfer Within the U.S. ☑ Multiple Importation into the U.S. ☐ Multiple Transfer Within the U.S. A. Record of each importation shall be maintained on permanent file by permittee. B. Enclosed label(s) must be forwarded to the shipper(s). C. One label shall be affixed to shipping container. Enclosed labels may be photocopied.	

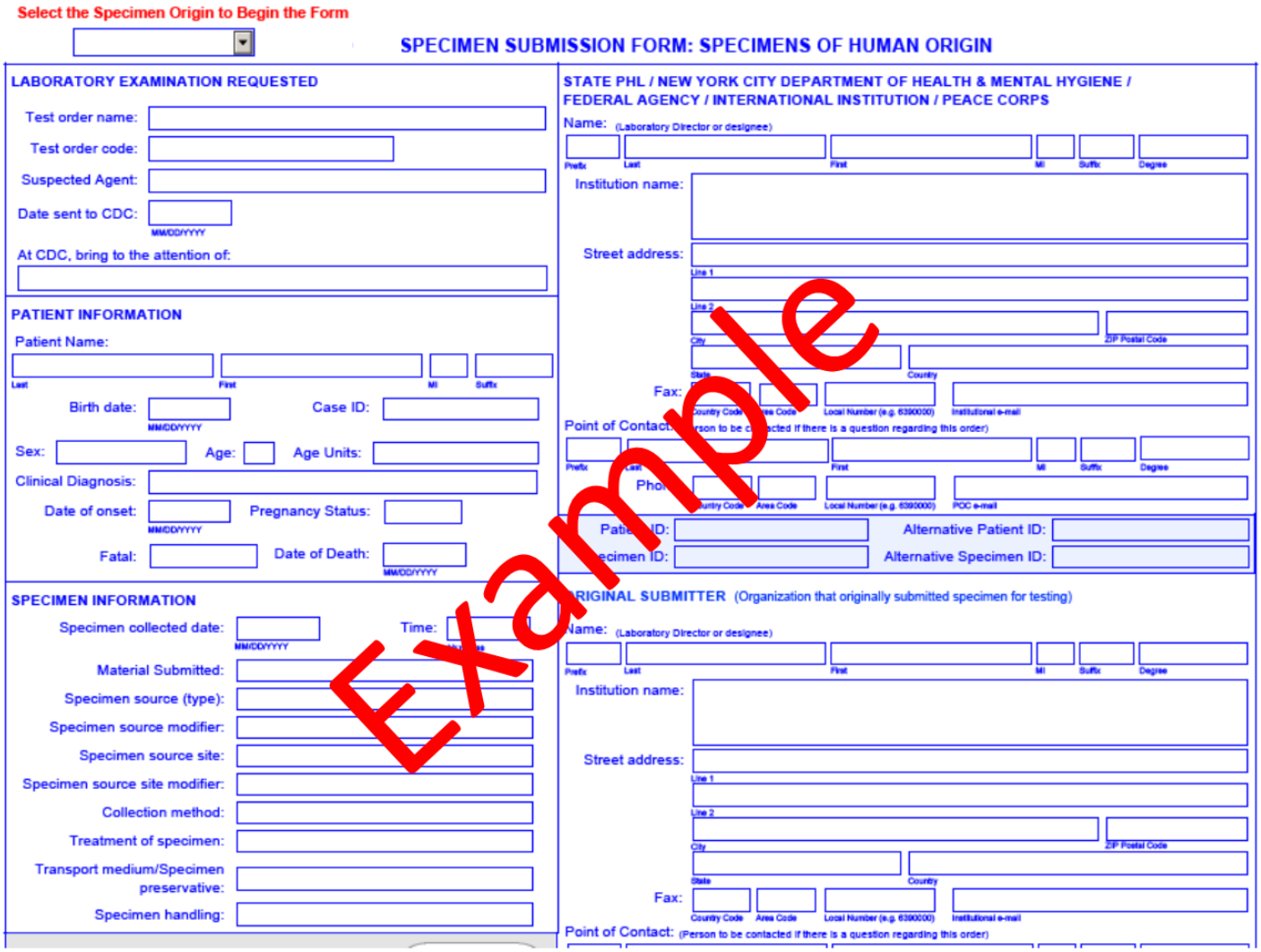
Example

CDC 0728 (F 13.40) REV. 4-13

42 CFR 71.54. Permit to Import Biological Agents, Infections Substances, and Vectors
A person may not import into the United States any infectious biological agent, infectious substance, or vector unless: It is accompanied by a permit issued by the Centers for Disease Control and Prevention (CDC). The possession of a permit issued by the CDC does not satisfy permitting requirements placed on materials by the U.S. Department of Agriculture that may pose hazards to agriculture or agricultural production in addition to hazards to human health.

- ## Sample Submission Form

To facilitate the identification, analysis and monitoring of cases, it is important to provide the reference laboratory with detailed information regarding each sample. There are different formats that can be used to summarize epidemiological and clinical data.



References

1. **Hui, DSC and Zumla, A.** Severe Acute Respiratory Syndrome - Historical, Epidemiologic; and Clinical Features. [book auth.] HW Boucher, A Zumla and DSC Hui. *Emerging and Re-emerging Infectious Diseases - Clinics Revew Articles .* Philadelphia : Elsevier, 2019, pp. 869-889.

2. **Drosten , C, et al.** Severe acute respiratory syndrome: identification of the etiological agent. *Trends Mol Med.* 2003, Vol. 9, pp. 325-7.

3. **GISAID.** Newly discovered betacoronavirus, Wuhan 2019-2020. *GISAID EpiFlu - Global Initiative on Sharing All Influenza Data.* [Online] January 2020. https://platform.gisaid.org/epi3/frontend#414223.

4. **World Health Organization.** Guidance on regulations for the transport of infectious substances 2019–2020. [Online] 2019. [Cited: January 28, 2020.] https://www.who.int/ihr/publications/WHO-WHE-CPI-2019.20/en/.