A Practical Guide for SARS Laboratories: From sample collection to shipment

World Health Organization Regional Office for the Western Pacific
Manila, Philippines
23 Dec 2003

Key Points

- Clinical samples from SARS patients should be collected by properly trained personnel. In most instances, this will be members of the hospital/clinical staff. If unclear, hospitals should request guidance from the SARS-designated laboratories.
- In addition to standard precautions, strict infection control precautions are required in sample collection. Wear PPE including double gloves, lab gown and apron, safety eyewear, and N95 mask or higher. Dispose of gloves and replace with a fresh pair between patients.
- Discard all other disposable collecting materials, (e.g. cotton swabs, gauze) used at the bedside into a small bin lined with an autoclavable biohazard bag. Contaminated non-disposables should be disinfected or sterilised appropriately to inactivate potential virus particles.
- From the bedside to the hospital laboratory, label clinical samples with a unique patient ID number. A SARS laboratory request form(s) should accompany the clinical samples. Label all samples as 'suspected or probable SARS'' and include the day of onset of illness where possible.
- When sending samples from local laboratories to national or international reference laboratories, label each specimen with a unique tracking record number, enclose completed laboratory request forms, notify the receiving laboratory about a pending shipment, and have someone at the sending laboratory track and confirm safe arrival of the samples to their final destination.

SARS specimens should be handled according to appropriate Biosafety practices in order to avoid laboratory-related infections and spread of disease to close contacts. As the primary route of infection is thought to be via droplets, extreme caution must be exercised to eliminate the unguarded production of aerosols. Detailed information about containment facilities and Biosafety practices recommended in this document may be found in the WHO Laboratory Biosafety Manual, 2nd revised edition, available from the WHO web site. According to the latest findings, the etiologic agent responsible for the syndrome is a previously unknown coronavirus, currently called SARS coronavirus, or SARS-CoV. Accordingly, all laboratory work practices should be appropriate for work with viral agents, with particular emphasis on potential spread by droplets, air, and/or contaminated surfaces and objects. No procedure should be undertaken in which there is any doubt about the ability to adequately contain the specimen and prevent the uncontrolled release of the virus.

WHO Biosafety guidelines for handling SARS clinical specimens and materials derived from laboratory investigations of SARS:

SPECIMEN COLLECTION

A. DIRECT DETECTION (Viral or bacterial isolation and PCR detection)

1. *Respiratory tract specimens*

These can be collected at any time, but are best taken during the acute phase of illness.

a) Upper respiratory tract:

i) **Nasopharyngeal wash/aspirate SPECIMEN OF CHOICE FOR RESPIRATORY VIRUSES)-**
Have the patient sit with the head tilted slightly backward. Instill 1.5 ml of non-bacteriostatic sterile saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing (*e.g.* mucus trap tubing) with 2-3 ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate nasopharyngeal secretions. Repeat for the other nostril. Collect aspirates in sterile vial or mucus trap. Remove tubings and discard in plastic bag.

ii) Nasopharyngeal swabs or oropharyngeal swabs-
Use only sterile dacron or rayon swabs with plastic shafts. (Calcium alginate swabs, cotton swabs, or swabs with wooden sticks may inactivate some viruses or inhibit PCR testing).

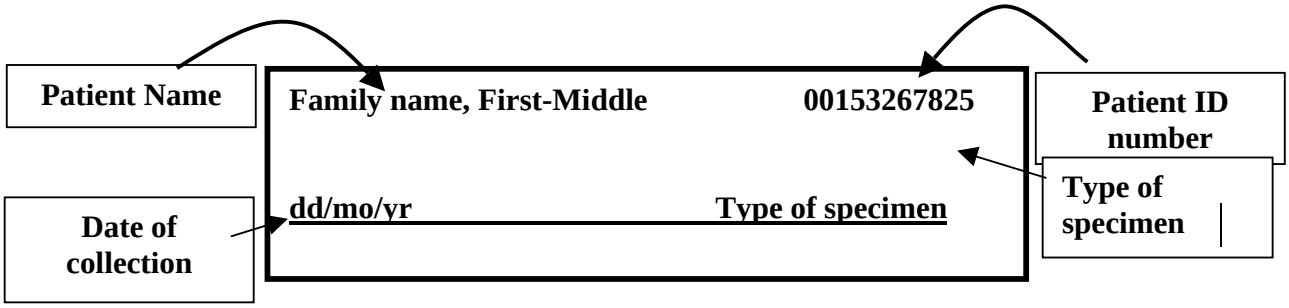
Nasopharyngeal swab Insert swab into nostril to the palate and leave in place for a few seconds to absorb secretions. Swab both nostrils.

Oropharyngeal swab Swab both posterior pharynx and deep inside the throat (tonsillar crypts, inner walls, sides, etc.), avoiding tongue. Apply a little force, taking large numbers of mucosal cells, as the virus may be intracellular.

- Place each swab immediately in a tube containing Virus Transport Media (VTM). Break the end of the swab that sticks out of the tube and close the tube tightly. If a swab with transport medium inside is used, place the swab back into the sheath and follow the directions for release of transport medium. DO NOT ALLOW ANY SWAB TO DRY OUT. Secure cap or top of the swab with parafilm to prevent leakage during transport.
- Remember these media contain antibiotics and are of no use for bacteriology or fungal investigations. In these instances bacterial and fungal specific swabs and media are appropriate.

Collect combined nose and throat swabs (usually in adults) or throat washes and place them in viral transport media (VTM). It is strongly recommended, wherever possible, instructions are given for self-collection.

All specimens should be labeled with patient's full name, unique ID number, date of collection and specimen type. The information on the label must be legible and should match the information on the SARS Laboratory Referral Form (an example of the form is attached as an addendum to this document. The label must remain attached to the specimen under all conditions of storage and transport. Place specimens in zip-lock plastic bags, and attach a second identical label to the outside of the plastic bag. An example of a label is shown below:



b) Lower respiratory tract:

- Collection of bronchoalveolar lavage, tracheal aspirate, pleural fluid. If these specimens have been obtained, half should be centrifuged and the cell-pellet fixed in formalin. The taking of these specimens pose an additional risk to staff. Aspirates and lavages require appropriate personal protection and should comply with requirements for high risk procedures.
- Remaining unspun fluid should be placed in sterile screw cap o-ring vials, and sealed with parafilm.
- Specimens should be labeled as noted above and placed into a zip lock plastic specimen bag.

2. Stool specimens collect at any time, but best on 10th day of illness or later.

- Collect about the size of a thumbnail (formed stool) or 10-50 ml (if diarrhea) into a sterile, wide mouth, screw capped receptacle. Secure cap, seal with parafilm, and place in zip lock plastic bag.
- All specimens should be labeled with patient's full name, unique ID number, date of collection and specimen type. The information on the label must be legible and should match the information on the SARS Laboratory Referral Form. The label must remain attached to the specimen under all conditions of storage and transport. Place specimens in zip-lock plastic bags, and attach a second identical label to the outside of the plastic bag

3. Other body fluids- please refer to the WHO document "Sampling for Severe Acute Respiratory Syndrome (SARS) Diagnostic Tests" (www.who.int/entity/csr/sars/sampling)

Confine requests to one or two optimally taken specimens as multiple specimens from patients may overwhelm laboratory resources.

Hospitals dealing with suspect or probable SARS should undertake the routine investigations for other pathogens causing the illness / atypical pneumonia available in their laboratory.

Urine, blood and other routine samples may be collected and transported as routine samples.

Routine biochemistry, haematology, bacteriology and other testing should be carried out at the facility managing the suspect patient using BSL2 precautions and in accordance with the guidelines for specimen handling found at http://www.who.int/csr/sars/biosafety2003_04_25/en/print.html

B. SEROLOGY TESTING

1. Blood/serum specimens

a) The timely collection of paired blood samples is very important!

- Collect an acute illness sample at first contact with the patient at days 7, 14, 28 and 90 after onset where possible.
- Collect blood on discharge if collection of a convalescent sample is unlikely.

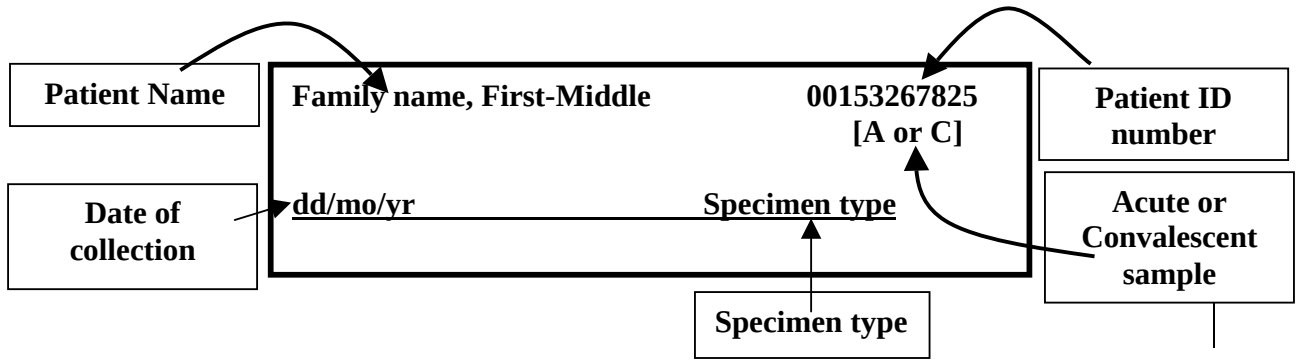
b) Serum collection

- Collect 5 - 10 ml of whole blood in a serum separator tube. Allow blood to clot.
- Centrifuge for around 2000 rpm for 8 minutes in a biocontainment centrifuge (sealed buckets and/or rotor) and separate the serum. Separation of serum avoids problems with haemolysed samples.
- Transfer and aliquot serum aseptically, plastic vials with external screw caps (preferably with internal O-rings). Aliquots should be a minimum of 200 microlitres and a maximum of 500 microlitres. Aliquot a total serum volume $\geq$ 1.5 ml.
- Close vials tightly and secure caps with parafilm to prevent leakage.

c) Labeling serum specimens

- All specimens should be labeled with patient's full name, unique ID number, and date of collection. The information on the label must be legible and should match the information on the SARS Laboratory Referral Form*. The label must remain attached to the specimen under all conditions of storage and transport.
- Place each specimen into a zip-lock plastic bag, and attach a second identical label to the outside of the plastic bag.
- Ensure that the dates of collection are correct.
- In addition, indicate the **ACUTE** illness sample as **A** and the **CONVALESCENT** sample as **C**.

An example of a label is shown below:



C. TISSUE SPECIMENS

Fixed tissues (formalin fixed or paraffin embedded) from all major organs (e.g. lung, trachea, heart, spleen, liver, brain, kidney, adrenals)

Formalin fixed tissue is not considered a biohazard or chemical hazard.

Store and ship fixed tissue at room temperature. ***DO NOT FREEZE FIXED TISSUES***

Fresh frozen tissues from lung and upper airway (e.g., trachea, bronchus)

Specimens should be collected aseptically as soon as possible after death. Technique and time will impact risk of post-mortem contamination. Use separate sterile instrument for each collection site. Place each specimen in separate sterile containers containing small amounts of viral transport media or saline. Label as outlined in section A.

Store and ship fresh tissue frozen at -70°C and shipping on dry ice is preferable.

SPECIMEN PROCESSING

(Excerpted from " WHO post-outbreak biosafety guidelines for handling of SARS-CoV specimens and culture. WHO Geneva web document, 18 December 2003")

The following activities may be performed in **BSL2 facilities** with appropriate basic laboratories – **Biosafety Level 2 (BSL2)** work practices, as described in the WHO Laboratory Biosafety Manual, 2nd revised edition.

- Routine diagnostic testing of serum and blood samples (including haematology and clinical chemistry)
- Manipulations involving neutralized or inactivated (lysed, fixed or otherwise treated) virus particles and/or incomplete, non-infectious portions of the viral genome
- Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.

The following activities may be performed in **BSL2 facilities with additional BSL3 work practices:**

Examples of activities that require BSL3 working practices for work with SARS-CoV in BSL2 facilities include:

- Aliquoting and/or diluting specimens
- Inoculation of bacterial or mycological culture media
- Performance of diagnostic tests that do not involve propagation of viral agents in vitro or in vivo
- Nucleic acid extraction procedures involving untreated specimens
- Preparation and chemical- or heat-fixing of smears for microscopic analysis

BSL3 practices include:

- Any procedure that may generate aerosols or droplets should be performed in a biological safety cabinet (e.g., sonication, vortexing).
- Laboratory workers should wear protective equipment, including disposable gloves, solid-front or wrap-around gowns, scrub suits, or coveralls with sleeves that fully cover the forearms, head covering and, where appropriate, shoe covers or dedicated shoes, eye protection and a surgical mask, or full-face shield, because of the risk of creating aerosols or droplets exposure when performing specific manipulations.
- Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be unloaded in a biological safety cabinet.
- Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents that are effective against enveloped viruses should be sufficient if used according to the manufacturer's recommendations. Generally, 5% bleach solutions are appropriate for dealing with biohazardous spillage. More information on disinfection and sterilization is provided in the WHO Laboratory Biosafety Manual, 2nd revised edition .
- Biological waste contaminated with suspect or confirmed SARS specimens, or with SARS-CoV, should be treated as outlined in the WHO Laboratory Biosafety Manual, 2nd revised edition before disposal .

WHO strongly recommends that the BSL3 precautions described above are adopted and followed for work in BSL2 laboratories with SARS specimens.

When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g. respirators, face shields) and physical containment devices (e.g. centrifuge safety cups or sealed rotors) must be used.

The following activities should be performed in containment laboratories - **Biosafety Level 3 (BSL3)**, by personnel trained in the use of appropriate **BSL3 work practices.**

- Performance of diagnostic tests that involve propagation of viral agents in vitro or in vivo
- Work involving the replication of SARS-CoV in cell culture and/or storage of cell culture isolates
- Recovery of viral agents from cultures of SARS-CoV specimens
- Manipulations involving growth or concentration of SARS-CoV

The following activities require **Animal BSL3 facilities and Animal BSL3 work** practices:

- Animal studies with live SARS-CoV or with closely related viruses from wildlife sources
- Any protocol involving animal inoculation for confirmation and/or characterization of putative SARS agents

SPECIMEN STORAGE

Key points:

- If the local laboratory cannot reliably freeze and store specimens, arrange to ship specimens to the reference laboratory as soon as possible.
- Arrange with the receiving reference laboratory ahead of time, whether acute illness serum samples will be stored at the local laboratory until the convalescent sample is obtained, or will be sent and stored at the receiving reference laboratory.

1. *Respiratory specimens-*

- Refrigerate immediately (4°C).
- If transport/shipping will be within national boundaries and will take place within 5 days, then keep specimens in refrigerator for planned shipping on cold packs or ice packs.
- If transport/shipping will be internationally, or will occur > 5 days after collection of last specimen, freeze specimens at lowest temperature possible (-70°C or -20°C) for planned shipping with dry ice if available (cold packs/ice packs otherwise).

Exception: formalin fixed cell pellets or tissue can be stored and eventually shipped at room temperature.

2. *Stool specimens-*

- Refrigerate immediately (4°C).
- Keep in refrigerator in anticipation of shipping on cold packs

3. *Blood/serum specimens*

- If transport/shipping will be within national boundaries and will take place within 5 days, then serum specimens can be placed in refrigerator for planned shipping on cold packs/ice packs.
- If transport/shipping will be internationally, or will occur > 5 days after collection of last specimen, freeze the serum specimens at -20°C or lower for planned shipping with dry ice if available (cold packs/ice packs otherwise).
- Keep the acute illness serum specimens frozen at -20°C or lower while awaiting collection of the convalescent sample

.

- If at a local laboratory, arrange with the reference laboratory who will store acute illness serum specimens while awaiting collection of the convalescent ones.

4. Urine specimens

- Refrigerate immediately (4°C).
- Keep in refrigerator in anticipation of shipping on cold packs.
- Best for exclusion of a Legionella infection (urinary antigen test).

SPECIMEN TRANSPORT/SHIPPING

Key points:

- Use a basic triple packaging system (http://www.who.int/emc/pdfs/emc97_3.pdf)
- Contact receiving laboratory ahead of time to make them aware of shipment. The sending laboratory is responsible for the proper packing of the shipment and assuring that the shipment reaches its final destination.

Transport of specimens within all National borders should comply with the procedure detailed within national regulations.
International air transport of human specimens from suspect or probable SARS cases must follow the current 44th edition (2003) of the International Transport Association (IATA) Dangerous Goods Regulation and may be transported as UN3373 "Diagnostic Specimens" when they are transported for "diagnostic or investigational purposes".

1. *Primary packaging*

- Make sure all specimen vials and containers are labeled, capped, and sealed with parafilm (so that they are water tight)
- Wrap each specimen vial or container individually with absorbent material or enough tissue/paper to prevent breakage and absorb contents in case of leakage.
- Determine the total volume of all specimen vials and containers being shipped.
- This primary receptacle must be leak proof and cannot contain more than 500ml of liquid or 500gm of solids.

2. *Secondary packaging*

- Secondary packaging must be watertight. For international transport, secondary packaging must be IATA-compliant for shipping of diagnostic specimens.
- Examples of secondary packaging for primary plastic specimen vials and containers are zip-locked, water tight, plastic bags, 50 ml or larger centrifuge tubes with screw caps, and Bio-Bottles.
- More than one individually wrapped primary container can be placed in the secondary container.
- Place primary containers and enough absorbent material in the secondary container to absorb the entire contents of all primary specimen vials/containers in case of leakage or damage.

3. *Outer packaging (cushioning material)*

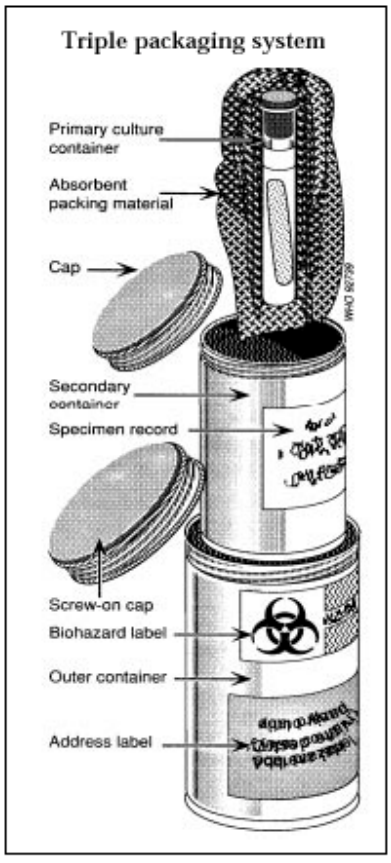
- Pack secondary container in a transport box (*e.g.* Styropor, Coleman). This outer packaging cannot contain more than 4 L or 4 kg.

- If transporting with wet ice, surround secondary container with ice in sealed plastic bags (to prevent leakage, contamination of ice). Outer packaging must be leak-proof.
- If transporting with cold packs, surround secondary container with cold packs.
- If transporting with dry ice, this should be placed between the secondary packaging and outer packaging, which must permit the release of carbon dioxide gas and not allow build-up of pressure that could rupture the packaging.
- Place an itemized list of contents in a sealed plastic bag between the secondary container and the outer packaging.
- Place the SARS Case Laboratory Referral forms in a sealed plastic bag between the secondary container and the outer packaging.
- If shipping internationally, the package and airway bill must be marked with the label

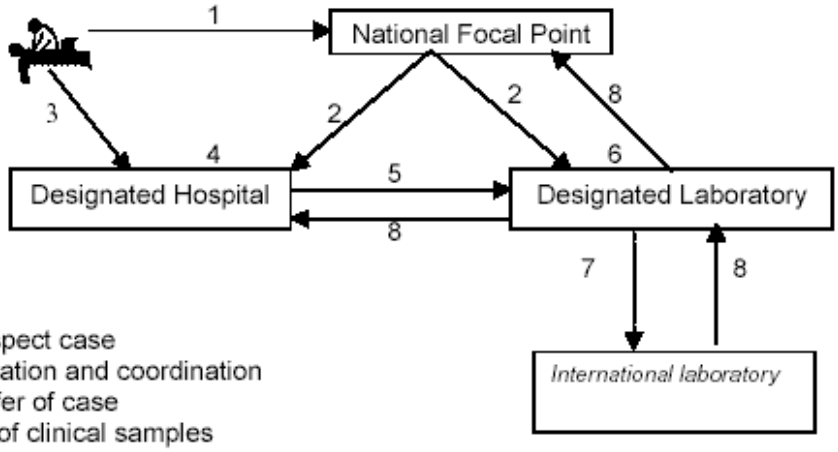
UN 3373 DIAGNOSTIC SPECIMEN
PACKED IN COMPLIANCE WITH
IATA PACKING INSTRUCTION 650

IATA NOTE: If dry ice is used, the shipment must comply with the provisions of the IATA Dangerous Goods regulations applicable it i.e. a "Miscellaneous Dangerous Goods" symbol is required on the outside of the package along with the wording "UN1845 Dry Ice x kg. (where x is the weight of the dry ice included in the package).

- See diagram of triple packaging system below from WHO Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens (http://www.who.int/emc/pdfs/emc97_3.pdf):



Overall summary plan



1 Report suspect case
2 Communication and coordination
3 Safe transfer of case
4 Collection of clinical samples
5 Domestic transport of the samples
6 Storage and laboratory diagnosis
7 International Shipment (if necessary)
8 Reporting

SUMMARY TABLE

TYPE OF SPECIMEN	DAYS POST ONSET	TEST / AGENT
NPA (or combined N/T/S), naso- or oro- pharyngeal swabs, Sputum	Presentation, day 4 & 7	Isolation, PCR, detection
Tracheal aspirate, bronchoalveolar samples or tissue	When available	Isolation, PCR, detection
Clotted blood; serum,	At presentation, days 7, 14, 28, 3 months	Serology, PCR, detection
EDTA blood; plasma	At presentation, days 7, 14, 28, 3 months	PCR, detection
Stool	Day 7, 10, 14 or when diarrhoea begins	Isolation, PCR, detection
Urine	At presentation, day 7, 14	Legionella urinary antigen

It is important to note that each facility may have available varying numbers of tests for the differentiation of other causes of the infection. These tests may comprise isolation, nucleic acid detection, culture or Serology. Other causes may include human metapneumovirus, enterovirus, human coronavirus, rhinovirus, adenovirus, parainfluenza viruses, influenza A or B virus, Chlamydia psittaci, Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, Legionella longbeachae or fungal.

Detection of another respiratory pathogen DOES NOT NECESSARILY EXCLUDE SARS, as SARS-CoV infection may be accompanied by another infection. The results need to be assessed in combination with the clinical findings.

References

1. WHO Interim Guidelines- Sampling for Severe Acute Respiratory Syndrome (SARS) Diagnostic Tests (www.who.int/entity/csr/sars/sampling).

2. WHO Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens (http://www.who.int/emc/pdfs/emc97_3.pdf).

3. U.S. CDC SARS Laboratory and Specimen Information (http://www.cdc.gov/ncidod/sars/lab.htm).

4. For complete packing instruction see: http://www.iata.org/dangerousgoods/index

5. Guidelines for collection of clinical specimens during field investigation of outbreaks: http://www.who.int/emc-documents/surveillance/whocdscsredc2004c.html

6. Laboratory Biosafety Guidelines. Second Edition (revised). Interim guideline: http://www.who.int/csr/resources/publications/biosafety/Labbiosafety.pdf

7. Biosafety in Microbiological and Biomedical Laboratories, 4th Ed (CDC/NIH): www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

8. Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, (WHO): www.who.int/emc/biosafety.html

9. Specimen Collection Protocol for Severe Acute Respiratory Syndrome (SARS) PHLN (An Australian Collaborative Microbiology Group) 20 May 2003 PHLN@health.gov.au

10. WHO post-outbreak biosafety guidelines for handling of SARS-CoV specimens and culture. WHO Geneva web document, 18 December 2003.

PATIENT INFORMATION – LABORATORY FORM

As soon as SARS is suspected, CONTACT: (Name of National Focal Point)						
General Patient Information				**Tracking Record Number**		
Name:				Date of Birth:		
Address:				Sex: M [ ] F [ ]		
				Nationality:		
				Occupation:		
Does suspected case have:	**Date of onset** dd/mm/yy			**History of travel,** within 10 days of onset of symptoms, to an area where SARS has ever been reported: No [ ] Yes [ ]		
Fever >38°C				**List names of areas patient visited during last 2 weeks:**		
Cough						
Shortness of breath						
Difficulty breathing						
Had close contact, within 10 days of onset of symptoms, with person who has been diagnosed with SARS: No [ ] Yes [ ] Date: ____/____/____						
Clinical Specimens						
Unique ID No.	**Type**	**Date of Collection**	**Type of Laboratory Test**	**Results**	**Name of Laboratory**	**Remarks**
Postmortem Specimens Date of death: ___/___/___						

Name of person completing form:_____
Institutional Affiliation:_____
Contact details:_____ Date:____/____/____