Appropriate Collection and Handling of Respiratory Specimens
Specimen Collection

Who collects specimens?
Who will be collecting or has collected in the past?
Who does not collect specimens?
Objectives

PPE

Specimen Collection (Timing)

Specimen types

Labeling

Storage

Package and shipping

Examples
Specimen Collection
Overview: Points to Consider

The time of specimen collection relative to symptom onset and clinical presentation are very important when considering what specimens to collect.

Maintain proper infection control when collecting specimens.

Use approved collection methods and equipment when collecting specimens.

Handle, store and ship specimens appropriately.
Specimen Collection
Personal Protective Equipment (PPE)

Gloves

Mask (fitted N-95)

Protective clothing

Eye protection

Sharps protection
Specimen Collection

Timing

Respiratory specimens

Collect as soon as possible after symptoms begin (ideally within 7 days)
Ideally before antiviral medications are administered
Collect multiple specimens on multiple days
Specimen Collection

Timing

Serum for serology
- Paired serum samples are most useful
  Acute sample (within 7 days after symptom onset)
  Convalescent sample (2 weeks or more after acute sample)
Specimen Collection

Timing

Days After Onset of Illness

- Incubation
- PCR
- Antigen detection / Culture
- Antibody detection
- Virus shedding
- Antibody response
Specimen Collection  
Upper and Lower Respiratory Tract

Conducting Passages

Upper respiratory tract
- Nasal cavity
- Pharynx
- Larynx

Lower respiratory tract
- Trachea
- Primary bronchi
- Lungs

Rhinitis (common cold)
Pharyngitis/Tonsilitis
Laryngitis

Tracheitis
Laryngotraceobronchistis (croup)
Bronchitis
Bronchioliitis
Pneumonia
Specimen Collection
Types of Respiratory Tract Specimens

URT specimens
nasal swab (N)
nasopharyngeal swab (NP)
nasopharyngeal wash or aspirate (NW/NA)
NW/NA > NP > N
oropharyngeal (throat) swab or wash

Specimen Collection
Swab Specimen Collection

Inferior nasal turbinate
Path of Swab
Nasopharynx

Swab here in posterior oral pharynx

Nasopharyngeal Swab
Oropharyngeal (Throat) Swab
LRT specimens
sputum
tracheal aspirates
bronchoalveolar lavage (BAL)
pleural fluid (sterile)
# Specimen Collection

## Clinical Presentation

LISTS OF RECOMMENDED CLINICAL SPECIMENS TO COLLECT FROM OUTPATIENTS, INPATIENTS, AND FATAL CASES IN THE SETTING OF AN UNEXPLAINED RESPIRATORY DISEASE

The specimens are listed in order of priority; those listed first are those most useful for testing for the greatest number of different pathogens with a single clinical specimen.

<table>
<thead>
<tr>
<th>OUTPATIENTS</th>
<th>INPATIENTS</th>
<th>FATAL CASES</th>
</tr>
</thead>
</table>
| **Upper Respiratory**
  - Nasopharyngeal (NP) and oropharyngeal (OP)
  - Nasopharyngeal wash/aspirate
| **Lower Respiratory**
  - Bronchoalveolar lavage, tracheal aspirate, pleural fluid
  - Sputum
| **All available premortem specimens**
  - Tissue
    - Fixed tissue from all major organs (e.g., lung, heart, spleen, liver, brain, kidney, adrenals)
    - Non-fixed tissue from lung and upper airways (e.g., trachea, bronchus)
  - Lower Respiratory
    - Bronchoalveolar lavage, tracheal aspirate, pleural fluid
    - Sputum
| **Blood**
  - Serum: Acute (at onset) and convalescent (3-6 weeks post onset)
  - Blood (plasma)
| **Blood**
  - Serum: Acute (at onset) and convalescent (3-6 weeks post onset)
  - Whole blood (plasma)
| **Blood**
  - Serum
  - Blood (plasma)
| **Tissue (e.g., lung)**
  - Urine
  - Stool
| **Tissue**
  - Deep lung swab for bacterial culture

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www.aphl.org
Specimen Collection
Swab Specimens

Should be drayon, rayon, or polyester fiber swabs
Select swabs with serrated shafts

*Do not* use calcium alginate or cotton swabs nor ones with wooden sticks; they may inhibit PCR
Specimen Collection
Swab Specimens

OP swab
NP swab

Inferior nasal turbinate
Path of Swab
Palate
Nasopharynx

Nasopharyngeal Swab
Oropharyngeal (Throat) Swab

Swab here in posterior oral pharynx
Specimen Collection
Flocked Swabs

Flocked swab
Rayon swab

Specimen Collection
Viral Transport Media

Annex 8. Viral transport media (VTM)

Specimens from humans

WHO HQ in Geneva has stocks of commercially prepared viral transport media (COPAN Universal Transport Medium).

Another suitable commercially available medium is Eagle Minimum Essential Medium (E-MEM).

Alternatively, VTM can be prepared locally. A suitable VTM for use in collecting throat and nasal swabs from human patients is prepared as follows:

- Add 10g veal infusion broth and 2g bovine albumin fraction V to sterile distilled water (to 400 ml).
- Add 0.8 ml gentamicin sulfate solution (50 mg/ml) and 3.2 ml amphotericin B (250 µg/ml).
- Sterilize by filtration.
Comparison of Nasopharyngeal and Oropharyngeal Swabs for the Diagnosis of Eight Respiratory Viruses by Real-Time Reverse Transcription-PCR Assays

Curi Kim1, Jamal A. Ahmed2, Rachel B. Eidex2, Raymond Nyoka2, Lilian W. Waiboci2, Dean Erdman1, Adan Tepo2, Abdirahman S. Mahamud2, Wamburu Kabura3, Margaret Nguhi4, Philip Muthoka5, Wagacha Burton6, Robert F. Breiman2, M. Kariuki Njenga2, Mark A. Katz2*

1 U.S. Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, 2 Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya, 3 Kenya Medical Research Institute, Nairobi, Kenya, 4 International Rescue Committee, Nairobi, Kenya, 5 Ministry of Public Health and Sanitation, Nairobi, Kenya, 6 United Nations High Commissioner for Refugees, Nairobi, Kenya

Abstract

Background: Many acute respiratory illness surveillance systems collect and test nasopharyngeal (NP) and/or oropharyngeal (OP) swab specimens, yet there are few studies assessing the relative measures of performance for NP versus OP specimens.

Methods: We collected paired NP and OP swabs separately from pediatric and adult patients with influenza-like illness or severe acute respiratory illness at two respiratory surveillance sites in Kenya. The specimens were tested for eight respiratory viruses by real-time reverse transcription-polymerase chain reaction (qRT-PCR). Positivity for a specific virus was defined as detection of viral nucleic acid in either swab.

Results: Of 2,331 paired NP/OP specimens, 1,402 (60.1%) were positive for at least one virus, and 393 (16.9%) were positive for more than one virus. Overall, OP swabs were significantly more sensitive than NP swabs for adenovirus (72.4% vs. 57.6%, p<0.01) and 2009 pandemic influenza A (H1N1) virus (91.2% vs. 70.4%, p<0.01). NP specimens were more sensitive for influenza B virus (83.3% vs. 61.5%, p=0.02), parainfluenza virus 2 (85.7% vs. 39.3%, p<0.01), and parainfluenza virus 3 (83.9% vs. 67.4%, p<0.01). The two methods did not differ significantly for human metapneumovirus, influenza A (H3N2) virus, parainfluenza virus 1, or respiratory syncytial virus.

Conclusions: The sensitivities were variable among the eight viruses tested; neither specimen was consistently more effective than the other. For respiratory disease surveillance programs using qRT-PCR that aim to maximize sensitivity for a large number of viruses, collecting combined NP and OP specimens would be the most effective approach.
Added Value of an Oropharyngeal Swab in Detection of Viruses in Children Hospitalized with Lower Respiratory Tract Infection

Laura L. Hammitt, Sidi Kazungu, Steve Welch, Anne Bett, Clayton O. Onyango, Rory N. Gunson, J. Anthony G. Scott and D. James Nokes

KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya; Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom; Health Protection Agency, London, United Kingdom; West of Scotland Specialist Virology Centre, Gartnavel General Hospital, Glasgow, Scotland; and School of Life Sciences, University of Warwick, Coventry, United Kingdom

Received 23 December 2010/Returned for modification 28 February 2011/Accepted 21 March 2011

Paired nasopharyngeal and oropharyngeal swabs collected from 533 children hospitalized with lower respiratory tract infection were assessed by multiplex reverse transcription-PCR. Oropharyngeal swabs increased the number of viral infections detected by 15%, compared to collection of a nasopharyngeal swab alone. This advantage was most pronounced for detection of influenza, parainfluenza, and adenovirus.
Specimen Labeling, Storage, and Transport

Specimen labeling
Specimen documentation
Specimen storage
Specimen packaging/Shipping
Specimen Labeling

Label all specimen containers with:
Patient name
Patient ID number Specimen type(s)
Date collected.
Specimen Documentation

Each package with specimen(s) should include line list:

- Patient name
- Patient ID number
- Specimen type(s)
- Date collected
- Clinical contact name, phone #, email
- Clinical submitter, affiliation, phone #, email
- Relevant clinical information (symptoms, date of onset, etc.)
- For deaths: preliminary autopsy report

http://www.who.int/csr/emc97_3.pdf
Specimen Packaging

The shipper – not the transport company – is responsible for the shipment until the package reaches its destination.

Primary packaging

Primary container must be water tight. Wrap multiple containers individually to prevent breakage. Everything in the primary container, including transport media, is considered the infectious substance.
Specimen Packaging

Packing and Labeling of Clinical Specimens

http://www.who.int/csr/emc97_3.pdf
Specimen Storage

Specimens must be kept cool
  if transported ≤72 h, keep at 4°C
  if transported >72 h, keep at -70°C (ship on dry ice)
Avoid repeated freeze/thaw cycles; do not use frost-free freezers
Sera may be stored at 4°C for up to one week; long term storage at -20°C in non-frost-free freezer acceptable
Prepare multiple aliquots of specimens

http://www.who.int/csr/emc97_3.pdf
Specimen Collection

Unacceptable Specimens

- Improperly identified specimens (specimen container info and form do not match)
- No identification on specimen container or form
- Specimens with insufficient quantity for testing
- Improper specimen type sent
- Spillage or possibility of cross contamination
- Specimens shipped at improper temperature
- Excessive specimen transport time
Does the length of specimen storage affect influenza testing results by real-time reverse transcription-polymerase chain reaction? An analysis of influenza surveillance specimens, 2008 to 2010
### Table 2

Association between duration of storage of respiratory samples and percentage of influenza A and B-positive using zero to one storage days as a reference, Kenya, 2008–2010

<table>
<thead>
<tr>
<th>Storage time in days</th>
<th>n/N (% positive)</th>
<th>Bivariate analysis</th>
<th>Multivariable analysis&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>aOR (95% CI)</td>
</tr>
<tr>
<td>0–1</td>
<td>462/3,969 (12)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>2</td>
<td>240/1,899 (13)</td>
<td>1.10 (0.93–1.30)</td>
<td>0.98 (0.83–1.17)</td>
</tr>
<tr>
<td>3</td>
<td>79/617 (13)</td>
<td>1.12 (0.86–1.44)</td>
<td>0.96 (0.74–1.25)</td>
</tr>
<tr>
<td>4</td>
<td>103/673 (15)</td>
<td>1.37 (1.09–1.73)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.26 (1.00–1.61)</td>
</tr>
<tr>
<td>5</td>
<td>26/222 (12)</td>
<td>1.01 (0.66–1.53)</td>
<td>0.98 (0.66–1.54)</td>
</tr>
<tr>
<td>6</td>
<td>11/180 (6)</td>
<td>0.49 (0.27–0.92)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.49 (0.27–0.93)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>8/119 (7)</td>
<td>0.55 (0.27–1.13)</td>
<td>0.50 (0.25–1.07)</td>
</tr>
<tr>
<td>8</td>
<td>7/67 (10)</td>
<td>0.89 (0.40–1.95)</td>
<td>0.88 (0.40–1.94)</td>
</tr>
<tr>
<td>9</td>
<td>2/47 (4)</td>
<td>0.34 (0.08–1.40)</td>
<td>0.33 (0.08–1.35)</td>
</tr>
<tr>
<td>10</td>
<td>2/40 (5)</td>
<td>0.4 (0.10–1.66)</td>
<td>0.36 (0.09–1.53)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Logistic regression model controlling for patient age, days since illness onset, surveillance site, and syndrome classification (influenza-like illness vs severe acute respiratory illness); 7,792 samples were used in the multivariate analysis; eight samples had missing syndrome classification and 33 had missing data for days since illness onset.

<sup>b</sup> Statistically significant.

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**aOR**: adjusted odds ratio; **CI**: confidence interval; **OR**: odds ratio; **REF**: reference category.
MERS-CoV

Who collects MERS samples?
Correct sample types for MERS-CoV
Specimen Collection

MERS-CoV: Changes in Interim Guidelines

Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2.1

Summary of Changes in Version 2.1
This is an updated version of the interim guidance document issued by the Centers for Disease Control and Prevention (CDC) January 2014. CDC has revised the interim guidance based on comments received from public health partners, healthcare providers, professional organizations, and others. CDC will continue to update the document as necessary to incorporate new information that increases our understanding of MERS-CoV.

Updates:
Minor changes were made to clarify specimen type and collection procedures.

1. Emphasized the recommendation to collect all 3 specimen types (lower respiratory, upper respiratory, and serum) if possible and not just one or two of the three specimen types for testing using the CDC MERS rRT-PCR assay
2. Deleted the recommendation to collect a stool specimen for MERS-CoV testing
3. Provided additional information for collection and processing serum specimens

Before collecting and handling specimens for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) testing, determine whether the person meets the current definition for a “patient under investigation” (PUI) for MERS-CoV infection prepared by the Centers for Disease Control and Prevention (CDC). See case definitions (http://www.cdc.gov/coronavirus/mers/case-def.html).
Specimen Collection
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Specimen Collection
MERS-CoV: Preferred Specimens

Lower respiratory tract specimens (sputum, trach aspirate, BAL)
Nasopharyngeal/Oropharyngeal (NP/OP) swabs combined
Serum and/or whole blood (PCR and serology)
Use approved collection methods and equipment when collecting specimens (BSL2 or BSL2+)
Handle, store and ship specimens as per protocol
Proper infection control when collecting specimens
Influenza (HPAI/LPAI)

Who collects HPAI/LPAI samples?
Correct sample types for HPAI/LPAI
Specimen Collection
HPAI: Preferred Specimens

URTI
- a nasopharyngeal swab, or
- a nasal aspirate or
- two swabs combined (ex. NPS/OPS)

LRTI
- endotracheal aspirate
- bronchoalveolar lavage fluid (BAL)
Conclusions

Use proper PPE

Collected at the appropriate time

Collect the appropriate specimen

Use the proper collection equipment

Label the specimen

Proper storage

Package and ship
Questions