

Influenza, RSV, and SARS: What Every Laboratory Should Know

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Objectives

At the end of the session, the participant will be able to:

- Describe the epidemiology and clinical presentations of influenza, respiratory syncytial virus, and the SARS-coronavirus.
- Discuss the importance of disease prevalence on test predictive values and test result interpretation.
- Summarize the current CDC recommendations for specimen testing and laboratory safety.

Overview

- Clinical presentations of influenza, respiratory syncytial virus (RSV) and SARS.
- Epidemiology of influenza, RSV and SARS.
- Laboratory diagnostic methods for influenza, RSV and SARS-coronavirus.
- Predictive values, test performance characteristics, disease prevalence, and result interpretation.
- CDC recommendations for specimen collection, testing and laboratory safety for SARS
- Closing comments.

Human Respiratory Viruses

Orthomyxoviridae

INFLUENZA TYPE A (2 subtypes, new strains each year)

INFLUENZA TYPE B (new strains each year)

Paramyxoviridae

RESPIRATORY SYNCYTIAL VIRUS (2 subgroups)

PARAINFLUENZA VIRUS (4 serotypes)

HUMAN METAPNEUMOVIRUS

Picornaviridae

RHINOVIRUSES (at least 113 serotypes)

ENTEROVIRUSES (68 serotypes)

Adenoviridae

ADENOVIRUSES (49 serotypes)

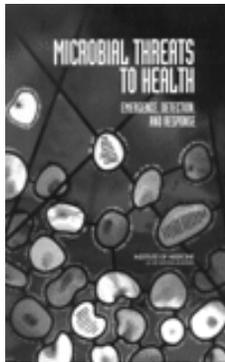
Coronaviridae

CORONAVIRUSES (2 subgroups, unknown # of strains)

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Resource:

<http://www.nap.edu/books/030908864X/html/>



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Epidemiology, Clinical
Presentations, and Public Health
Importance

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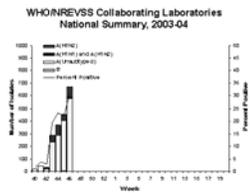
Influenza: Resources (I)

- CDC home page for influenza
<http://www.cdc.gov/ncidod/diseases/flu/overview.htm>
- ACIP recommendations
<http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5208a1.htm>
- National Immunization Program
<http://www.cdc.gov/nip/Flu/default.htm>

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Influenza: Resources (II)

- National Influenza Summary – Weekly Update
<http://www.cdc.gov/ncidod/diseases/flu/weekly.htm>



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Influenza

- Family: Orthomyxoviridae
- segmented (8), ssRNA genome
 - lipid envelop
- Genera: Influenzavirus Types A, B and C
- Subtypes: Human - H_1N_1 , H_2N_2 , H_3N_2 , H_5N_1 , H_9N_2 , H_1N_2 , H_7N_7
Animals - H_1 to H_{15} , N_1 to N_9
- Strains: A/Moscow/10/99 (H_3N_2) - like
(2003) A/New Caledonia/20/99 (H_1N_1) - like
B/Hong Kong/330/2001- like

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Influenza: Clinical Syndrome

- Incubation period 1 to 3 days
- Abrupt onset of high fever and chills
- Associated symptoms
 - headache, malaise, myalgia, cough, sore throat, nasal congestion
- Duration of intense symptoms 3 to 5 days
- Prolonged recovery
- Note: CDC definition of *influenza-like illness*
 - Temp > 100°F orally and either cough or sore throat

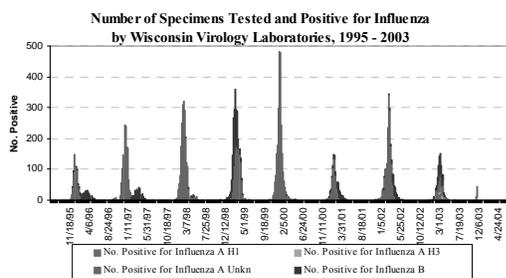
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Influenza: Potential Complications

- Pneumonia: primary viral
secondary bacterial
- Exacerbations of asthma, chronic bronchitis
- Myocarditis and pericarditis
- Meningitis/encephalitis
- Reye's syndrome
- Guillain-barre syndrome
- Myositis, myoglobinuria

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Influenza – Seasonality/Epidemicity Wisconsin, 1995-2003



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Influenza: Public Health Importance

- Pandemic potential
- Significant annual (inter-pandemic) morbidity and mortality
- Prophylactic and therapeutic measures available
 - Advent of rapid diagnostic methods

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Influenza: Epidemic (Inter-Pandemic) Influenza

Disease caused by a new strain of influenza virus that has evolved gradually by point mutations within the hemagglutinin or neuraminidase or both such that preexisting antibody cannot completely neutralize the new strain.

The mechanism: **ANTIGENIC DRIFT**

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Influenza: Annual (Inter - pandemic) Morbidity and Mortality

- Greater than 35,000 deaths in US each year
 - 90% of mortality among elderly
 - Increased mortality in other risk groups
- From 15,000 to >200,000 flu-associated hospitalizations per epidemic
- Nursing home attack rate of 60%
- Attack rates of 5-20% in general population
- A significant childhood pathogen

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Pandemic Influenza

Disease caused by a **reassortment of Influenza A subtypes** that results in the emergence of a new virus containing a novel hemagglutinin and/or neuraminidase that is immunologically distinct from previously circulating strains.

The mechanism: **ANTIGENIC SHIFT**

Resource: <http://www.cdc.gov/od/nvpo/pandemics/>

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Influenza: Noteworthy events

1997: “Avian flu” (H5N1)
 1997: “A/Sydney” (H3N2)
 1999: Influenza A (H9N2)
 2003: H5N1 strikes again
 2003: Avian influenza A (H7N7)
 2003: “A/Fujian” (H3N2)
 2001-03: H1N2

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hRSV: Resources

- National Respiratory and Enteric Virus Surveillance System (NREVSS) home page
<http://www.cdc.gov/ncidod/dvrd/revb/nrevss/index.htm>

National RSV Data

Percent Positive Respiratory Syncytial Virus Tests
in the United States, by Week of Report
3-Week Running Average

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hRSV: Clinical Syndrome (I)

- Incubation period of 2-8 days
- Acute respiratory illness
 - Fever
 - Cough
 - Copious nasal discharge
 - Malaise
 - Respiratory distress and decreased feeding
- Leading cause of severe lower respiratory tract illness (bronchiolitis/pneumonia) in infants
 - Infants with underlying cardiopulmonary disease are at particularly high risk

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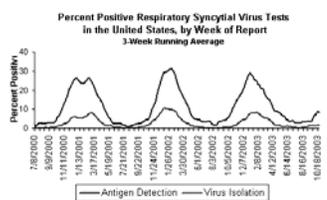
hRSV: Clinical Syndrome(II)

- Otitis media is a major complication
- **Highly contagious**-a leading cause of nosocomial infections in infants and the elderly
- Symptomatic re-infections occur throughout life:
 - Upper respiratory tract illness (URI)/bronchitis in adults
 - Severe lower respiratory illness in the elderly
- May be important precursor to asthma in later life

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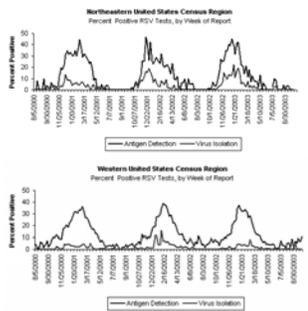
hRSV: Epidemicity/Seasonality

NREVSS National RSV Data



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hRSV NREVSS Regional RSV Data Examples



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hRSV: Public Health Importance

- Virtually all children are infected with RSV during the first 3 years
- The major viral cause of serious life-threatening lower respiratory tract illness (LRI) in infants worldwide
- Significant morbidity and mortality in U.S.
 - 1-2% hospitalization rate of infants
 - many others with LRI not hospitalized
 - up to 5,000 deaths/yr in US

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Metapneumovirus: *“The new kid on the block”*

References
www.cdc.gov
 ↓
 Homepage
 ↓
 Click on search
 ↓
 Enter: metapneumovirus

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Metapneumovirus: Clinical Syndrome

Similarities To and Differences From RSV

- Presumed similar incubation period
- Similar clinical spectrum and risk groups
 - Wide range of illness severity
- Lower incidence of clinically significant disease than hRSV
 - 1.5-7% RTI; year-to year variability; similar to PIV
- Asymptomatic and mild illness much more common, especially in older children and adults
- Reinfections???
- Age extremes and immunocompromised may experience severe LRI with hospitalization
- Similar relationship to asthma: initiation & exacerbation

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Metapneumovirus:

Epidemiological Characteristics

- 2 genetic lineages with 2 subgroups each
- World-wide distribution
- Similar age/infection profile
 - Seroprevalence: 70-80% - < 5 yrs
100% in adults
- Similar seasonality; however, summer infections may occur
- Serologic studies indicate hMPV circulation at least 50 years ago

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SARS-Coronavirus: Resources

- CDC's Home Page on Severe Acute Respiratory Syndrome (SARS)
<http://www.cdc.gov/ncidod/sars/>
- CDC's Draft Plan for Preparedness and Response to Severe Acute Respiratory Syndrome (SARS)
<http://www.cdc.gov/ncidod/sars/sarsprepplan.htm>
- CDC's archived webcasts (September 23 & 30, 2003 - Parts 1 & 2) "Preparing for the Return of SARS: Are we ready?"
<http://www.phppo.cdc.gov/PHTN/webcast/sars-return/>
- WHO
<http://www.who.int/csr/sars/en/index.html>
http://www.who.int/csr/sarsarchive/2003_04_11/en/print.html

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SARS – What is it?

- Severe Acute Respiratory Syndrome
- Often severe influenza-like illness
 - Fever, chills, headache, malaise, myalgia, respiratory symptoms
 - Development of dry cough, 2-7 days
 - Diarrhea (10-20%)
 - Most develop pneumonia
 - Mechanical ventilation required (10-20%)
- Mortality rate ~15%; >50% in elderly
- Etiology: Novel, previously unrecognized coronavirus
 - SARS-CoV

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SARS – Key Epidemiologic Features

- Incubation period: 2 - 7 days; as long as 10d
- Transmission: Close person - to - person contact
 - Droplet spread, fomite
 - Mechanism of community spread?
- Infectivity: Maximum with symptoms (fever, cough)
 - Asymptomatic virus shedding?
- Duration and scope of immunity?
 - Good antibody response; often delayed
 - Re - infection possible?

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The SARS Outbreak: 2002-03

- First cases: 11/16/02, Quangdong Province, China
- **First cases reported: 02/11/03, China**
 - Singapore
 - ↑
- Initial spread: **China → Hong Kong → Vietnam**
 - ↓
 - Toronto
- Initial “hot zones” with rapid increase in cases
 - 1° Tx in healthcare settings
 - 2° community chains of Tx
- Final totals - World: 8422 cases; 916 deaths; 30 countries

U.S.: 33 cases; 0 deaths

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- SARS -

“ A particular threat to international health”

- Pathogenicity; high mortality
- Human - to - human spread; no vector
- Initial sxs non-specific, common
- Targets healthcare workers
- Relatively long incubation period
- High proportion of patients requiring intensive care
- Possible zoonotic source
- No vaccine or treatment

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Laboratory Diagnostic Methods

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Influenza: Laboratory Diagnosis

- Culture
 - 1-5 days
 - “Gold standard”
- Direct Specimen Immunofluorescence
 - Dependent on reader expertise
 - Limited to laboratories with IF capability
 - Variable sensitivity & specificity
- Molecular
 - Not yet widely available/used
- Serology
 - Retrospective
- Rapid EIA and “EIA-Like” Tests
- Note: The nasal mist vaccine may replicate in cell cultures and produce a positive result in rapid tests up to 3 weeks post-immunization.

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Influenza: Rapid EIA and “EIA-Like” Tests

Test	CLIA Status	Antigen Detected
Directigen Flu A	Moderate	A
Directigen Flu A & B	Moderate	A & B
Flu OIA	Moderate	A / B
Flu OIA A/B	Moderate	A & B
NOW Flu A	Waived	A
NOW Flu A/B	Waived	A & B
QuickVue Influenza	Waived	A / B
Xpect Flu A/B	Pending (interim moderate)	A & B
ZstatFlu	Waived	A / B

Influenza: Rapid EIA and “EIA-Like”
Test - Specimens & Storage

Test	Specimen Type	Specimen Storage
Directigen Flu A	NP wash/asp, NP Sw, Throat Sw	2-8°C/72 hr.
Directigen Flu A+B	NP wash/asp, NP Sw, Nasal wash, Th Sw, BAL	2-8°C/72 hr.
Flu OIA	Nasal asp, NP Sw, Th Sw, Sputum	2-8°C/24 hr.
Flu OIA A/B	Nasal aspirate, nasopharyngeal swab, throat swab, or sputum	2-8°C/24 hr.
NOW Flu A	Nasal wash, NP Sw	2-8°C/24 hr. (elute swabs)
NOW Flu A/B	Nasal wash, NP Sw	2-8°C/24 hr. (elute swabs)
QuickVue Influenza	Nasal Sw, wash, asp	2-30°C/8 hr.
Xpect Flu A/B	Nasal wash, swab, Th Sw	2-8°C/72 hr (in transport medium) or -20°C/6 mo.
ZstatFlu	Th Sw	0-40°C/24 hr.

Influenza: Rapid EIA and “EIA-Like” Test
Performance Characteristics*

Test	Sensitivity	Specificity
Directigen Flu A	91% (A)	95% (A)
Directigen Flu A+B	86% (A) 81% (B)	91% (A) 99.5% (B)
FLU OIA	62-88% (A/B)	52-80% (A/B)
FLU OIA A/B	62-88% (A/B)	52-80% (A/B)
NOW Flu A	78-82 (A)	92-94 (A)
NOW Flu A/B	78-82% (A) 58-71% (B)	92-94% (A) 97% (B)
QuickVue Influenza	73-81% (A/B)	96-99% (A/B)
Xpect Flu A/B	89-100% (A) 83-100% (B)	100% (A) 100% (B)
ZstatFlu	58-65% (A/B)	98-100% (A/B)

* Per Manufacturer, without discrepant analysis 36

hRSV: Laboratory Diagnosis

- Culture
 - 1-10 days
- Direct Specimen Immunofluorescence
 - Dependent on reader expertise
 - Limited to laboratories with IF capability
 - More sensitive than culture
- Molecular
 - Not yet widely available/used
- Serology
 - Retrospective
- Rapid EIA and “EIA-Like” Tests

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hRSV: Rapid EIA and “EIA-Like” Tests

Test	CLIA Status
Directigen RSV	Moderate
Directigen EZ RSV	Moderate
RSV OIA	Moderate
NOW RSV	Waived
Xpect RSV	Pending (interim moderate)

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hRSV: Rapid EIA and “EIA-Like” Tests - Specimens & Storage

Test	Specimen Type	Specimen Storage
Directigen RSV	NP wash/asp, NP Sw, Throat Sw	2-8°C/48 hr.
Directigen EZ RSV	NP wash/asp, NP Sw, Throat Sw	2-8°C/72 hr.
RSV OIA	Nasal wash, NP Sw	Room temp/2 hr 2-8°C/24 hr. Frozen
NOW RSV	Nasal wash	Room temp/4 hr 2-8°C/24 hr.
Xpect RSV	NP wash, asp., swab	2-8°C/48 hr -20°C/1 week

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hRSV: Rapid EIA and “EIA-Like” Test Performance Characteristics*

Test	Sensitivity	Specificity
Directigen RSV	93-97%	90-97%
Directigen EZ RSV	67% (NPS) 87% (NPW)	92% (NPS) 86% (NPW)
RSV OIA	67% (NPS) 87% (NW)	96% (NPS) 83% (NW)
NOW RSV	89%	98-100%
Xpect RSV	96%	94%

* Per Manufacturer, without discrepant analysis 40

SARS-Coronavirus: Laboratory Diagnosis

- All methods developmental
- Culture
 - Not recommended
 - Test for other respiratory viruses
 - Strong evidence
- Direct Specimen Immunofluorescence
 - Not available
- Molecular
 - Real-time PCR
 - Available at CDC & state public health laboratories
 - Available as “home-brews”??, non-FDA approved kits??
- Serology
 - “Gold standard”
 - ELISA for total antibody (IgG, IgM, and IgA)
 - Retrospective

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SARS-CoV Laboratory Testing:

- Real-time RT-PCR
 - Good sensitivity (1-10 copies).
 - Highly specific for SARS-CoV, but <50% sensitive during 1st week of infection.
 - Low viral titers early in infection
 - Pathogenesis of disease may not allow a definitive diagnosis early in illness.
 - Changes in type, quality, and quantity of specimens and in specimen processing procedures may improve the detection of SARS-CoV infection in patients.
 - Potential for false negatives and false positives.
 - PPV low when prevalence is low.
 - Positive result considered presumptive until confirmed.
 - Negative result does not rule out SARS & should not affect patient management.
 - Must be used and interpreted carefully.

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SARS-CoV Laboratory Testing:

- Serology
 - Specific; no cross reactions with other coronavirus infections.
 - Serology can be positive 8-10 days after onset.
 - Serology cannot be considered negative until >28 days post onset
 - Must be used and interpreted carefully.

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Test Performance Characteristics

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Appropriate use of the rapid tests requires the integration of knowledge of *epidemiology* with knowledge of *test performance characteristics*.

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Test Performance Characteristics: Definitions

Sensitivity

- The probability of a positive test result given the presence of disease
- How good is the test at detecting infection in those who have the disease?

Specificity

- The probability of a negative test result given the absence of disease.
- How good is the test at calling uninfected people negative?

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Sensitivity and Specificity

		DISEASE	
		Present	Absent
TEST	Positive	True Positive (TP)	False Positive (FP)
	Negative	False Negative (FN)	True Negative (TN)

$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{TN}{TN+FP}$$

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Predictive Value

- The probability of the presence or absence of disease given the results of a test
 - PVP is the probability of disease in a patient with a positive test result.
 - PVN is the probability of not having disease when the test result is negative.

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Predictive Value

		DISEASE	
		Present	Absent
TEST	Positive	True Positive (TP)	False Positive (FP)
	Negative	False Negative (FN)	True Negative (TN)

Predictive Value Positive (PVP) = $TP / (TP + FP)$

Predictive Value Negative (PVN) = $TN / (TN + FN)$ ₄₉

Predictive Value

- How predictive is this test result for this particular patient?
- Determined by the sensitivity and specificity of the test, **and** the prevalence rate of disease in the population being tested.

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Prevalence Rate

Number of cases of illness existing at a given time divided by the population at risk

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Hypothetical Influenza Test Performance

Prevalence = 20.0%

Disease

		+	-
Test	+	380	64
	-	20	1536

Sensitivity = $380/400 = 95.0\%$

Specificity = $1536/1600 = 96.0\%$

Predictive Value Positive (PVP) = $380/444 = 85.6\%$

Predictive Value Negative (PVN) = $1536/1556 = 98.7\%$

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Hypothetical Influenza Test Performance

Prevalence = 1.0%

Disease

		+	-
Test	+	19	80
	-	1	1900

Sensitivity = $19/20 = 95.0\%$

Specificity = $1900/1980 = 96.0\%$

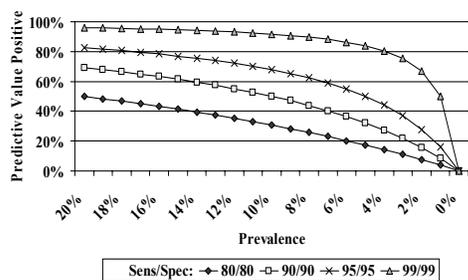
Predictive Value Positive (PVP) = $19/99 = 19.2\%$

Predictive Value Negative (PVN) = $1900/1901 = 99.9\%$

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Predictive Value Positive

Dependence on Sensitivity, Specificity and Prevalence



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Recommendations for
Use of Rapid Tests for Influenza or RSV

- Use prevalence indicators to decide:
 - When to test
 - Whether to qualify result
 - Whether to confirm results
 - Potential prevalence indicators:
 - Laboratory Detections
 - CDC
 - Statewide data
 - Your laboratory data
 - “Sister” laboratory’s data
- Culture confirm your first influenza positives, others as needed
- Educate clinicians on predictive values & limitations of tests

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CDC Recommendations for
Specimen Collection, Testing and
Laboratory Safety for SARS

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SARS-CoV Laboratory Testing:
Specimen Submission

- Consult with your state health department for evaluation of case
 - If possible, include informed consent form for serology testing
 - Testing will be performed if consent not obtained
 - FDA regulation for use of non-licensed test
 - **Allows use of patient specimen for future studies**
- http://www.cdc.gov/ncidod/sars/specimen_collection_sars2.htm

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SARS-CoV Laboratory Testing: Specimens to collect during the course of illness

SPECIMEN	<1 week post symptom onset	1-3 weeks post symptom onset	>3 weeks post symptom onset
Serum	++	++	++
Blood (EDTA/purple top)	++		-
Respiratory (sputum, BAL, nasal aspirate/wash, NP swab, throat swab)	++	++	
Stool		++	++

++ Preferred specimen
 - Not recommended

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SARS-CoV Laboratory Testing: Specimens for PCR

- Lower respiratory tract specimens
 - Sputum, bronchoalveolar lavage, tracheal aspirate, pleural tap
- Upper respiratory tract specimens
 - Nasopharyngeal aspirate/wash
 - Nasopharyngeal/oropharyngeal swab
 - Place in dry sterile container
 - Dacron or rayon with plastic shaft
 - NOT calcium alginate or wooden shafted swab
- Blood
 - 5-10ml in EDTA tube (Purple top)
- Stool
 - 10 - 50cc in stool cup or urine container, no preservatives
- Store all specimens at 4°C prior to shipping

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SARS-CoV Laboratory Testing: Specimens for Serology

- Acute and convalescent sera
 - Acute serum
 - Collect ASAP
 - approx. 30% positive within 6-10 days post-onset
 - Convalescent serum
 - Collect >28 days post-onset
 - Most patients positive at 2 weeks

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SARS-CoV Laboratory Testing: Specimen Shipping and Transport

- Resource: <http://www.cdc.gov/ncidod/sars/packingspecimens-sars.htm>
 - Check with your state health department or laboratory for in-state guidance.
 - Proper packaging is the responsibility of the shipper.
- In brief: Package as “Diagnostic Specimens”
 - Primary receptacle: watertight; screw-capped closures wrapped with tape or parafilm; individually wrapped;
 - Secondary receptacle: watertight; include absorbent.
 - Outer packaging: dry ice or coolant outside secondary packaging; allow release of CO₂ gas if dry ice used.
 - At least 4 inches on smallest side & large enough for documents
 - Additional: Volume limits, container specifications, itemized list of contents, & labelling requirements based on mode of transport (ground, air, USPS)

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Biosafety in the Laboratory: Resources

- Biosafety in Microbiological and Biomedical Laboratories (BMBL),
4th edition
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention
and
National Institutes of Health
Fourth Edition, May 1999.
US Government Printing Office
Washington: 1999
<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>



- Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 2nd Edition
U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control and Prevention
And
National Institutes of Health
September 2000
<http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>



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Biosafety in the Laboratory with SARS (I)

- Establish process to identify SARS specimens.
- If recommended safety equipment not available, reduce risk by other means.
 - Perform risk assessment
 - Consider referral to another laboratory
- Viral culture of suspect SARS specimens must only be performed in BSL-3 facility.

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Biosafety in the Laboratory with SARS (II)

- Blood and urine
 - Biosafety Level 2 (BSL-2)
 - PPE: gloves, laboratory coats, face shield or eye protection/surgical mask
 - BSC for aerosol potential
 - Sealed centrifuge rotors or sample cups, if available.
- Other untreated specimens
 - Biosafety Level 2 (BSL-2) facilities using BSL-3 practices
 - Use biosafety cabinet (BSC) with PPE: gloves, solid front gown, full face protection
 - If BSC not available/feasible, N-95 respirator, full face protection, other containment devices
 - Centrifuge using sealed rotors or sample cups
 - Unload in the BSC

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2003-04 – What can we expect?

- The first SARS case of the season: Singapore, 9/9/03
- Developments to watch for:
 - Enhanced national and international surveillance
 - Stages/levels of activity to guide response
 - Enhanced public health laboratory testing capability and capacity
 - Enhanced public health response strategies
 - Isolation, quarantine, travel advisories
 - Updated specimen collection, lab testing, results interpretation and biosafety guidelines
 - Identification of natural reservoir

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Closing Comments (I)

- CDC and other response partners have developed a plan for SARS preparedness & response.
- The potential for global spread of SARS requires collaboration and communication between healthcare and public health communities.
- Early case detection and diagnosis of SARS cases is critical to prevent transmission and limit the outbreak.
- Because the signs and symptoms are similar, we may not be able to differentiate influenza and SARS clinically if they occur simultaneously.

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Closing Comments (II)

- Results of SARS laboratory tests in the clinical setting are only relevant IF SARS cases have been documented.
- Inappropriate laboratory testing that produces false positive or negative results will have adverse consequences.
- Identification of another respiratory pathogen in SARS-like illnesses can be significant.
- Current specimen collection and test procedures for SARS are interim guidelines that may change as we learn more.
- A new model for biosafety in the laboratory?
 - Universal precautions for blood in the 80's;
 - respiratory precautions in the 2000's?

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