SARI CLINICAL CARE TRAINING

DIFFERENTIAL DIAGNOSIS, SPECIMEN COLLECTION & DIAGNOSTIC TESTS

World Health Organization

HEALTH EMERGENCIES programme

Learning objectives

At the end of this lecture, you will be able to:

- Develop a differential diagnosis for patients with severe pneumonia.
- Recognize patients with SARI that may have respiratory virus with pandemic potential.
- Describe when and what specimens to collect for laboratory diagnosis.
- Describe the characteristics of diagnostic tests for respiratory virus infections.

Differential diagnosis severe pneumonia

- Respiratory virus, including those with pandemic potential

- Bacterial causes:
  - Community acquired pathogens (CAP): according to local epidemiologic patterns and patient factors.
  - Hospital associated pathogens (HAP): if SARI onset occurred after hospital admission for another illness or working as a health care worker. According to local epidemiology and patient factors.

Respiratory viruses with pandemic potential

- Seasonal influenza A or B:
  - when influenza viruses are known or suspected to be circulating in the community.
- Zoonotic influenza A infection (H5N1, H5N6, H7N9):
  - if exposure risk factor present.
- MERS infection, SARS infection:
  - if exposure risk factor present.
- **Emerging respiratory viruses: COVID-19**
  - **if clinical and epidemiologic clues are present.**

Other respiratory viruses

Common pathogens:
•Respiratory syncytial virus (RSV), parainfluenza virus, rhinoviruses, adenovirus, enterovirus (EVD68), human metapneumovirus, bocavirus.

Less common, unless at risk:
•Varicella zoster, measles, human coronavirus, hantavirus.

If immunosuppressed (i.e. PL-HIV):
•Cytomegalovirus, herpes simplex viruses in addition to above.

Community-acquired: bacterial pathogens

Most common pathogens:

•*Streptococcus pneumoniae, Hemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila, non-pneumophila Legionella, Chlamydia pneumonia, Mycoplasma pneumoniae, Klebsiella pneumonia, Staphylococcus aureus*

Less common, unless at risk or in high prevalence country:

•*Mycobacterium tuberculosis, Burkholderia pseudomallei, Rickettsial infections, Coxiella burnetti (Q fever), Leptospira spp, Chlamydia psittaci, Bortedella pertussis. Salmonella sp.*

Health care-associated: bacterial pathogens

Risk factors for multi-drug resistant pathogens*:

- intravenous antimicrobial therapy within < 90 days
- admission from nursing home

Resistant pathogens include:

•methicillin-resistant *S. aureus* (MRSA).

•non-fermenters such as *Pseudomonas aeruginosa, Acinetobacter baumannii.*

•extended spectrum beta-lactamase (ESBL) producers such as *E. coli, Klebsiella, Enterobacter.*

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* Aliberti S et al. Clinical Infect Dose. 2012;54(4):470-478

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Pneumonia due to fungal pathogens

In PL-HIV or with other immunosuppressed conditions:

•*Pneumocystis jirovecii, Penicilliosis, Aspergillosis, cryptococcosis, Mucormycosis, Fusarium.*

Endemic infections:

•*Histoplasmosis, Coccidiodomycosis, Blastomycosis, Paracoccidiomycosis, Sporotrichosis*

If suspect an emerging infection of international public health concern:

- isolate patients and apply appropriate IPC
- collect specimens
- start supportive management
- start empiric treatments based on broader differential, as soon as possible.
- notify health officials

Specimen collection

Collect correct biological specimens

- Guided by differential diagnosis and laboratory capacity:

  – collect samples before antimicrobial therapy provided it does **not** delay the administration of antimicrobial therapy by > 45 minutes

  – notify laboratory and public health authorities if concerns regarding emerging or high risk pathogens

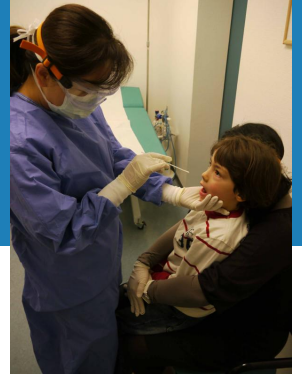
  – use results for better and focused clinical management

  – use results to influence public health interventions.

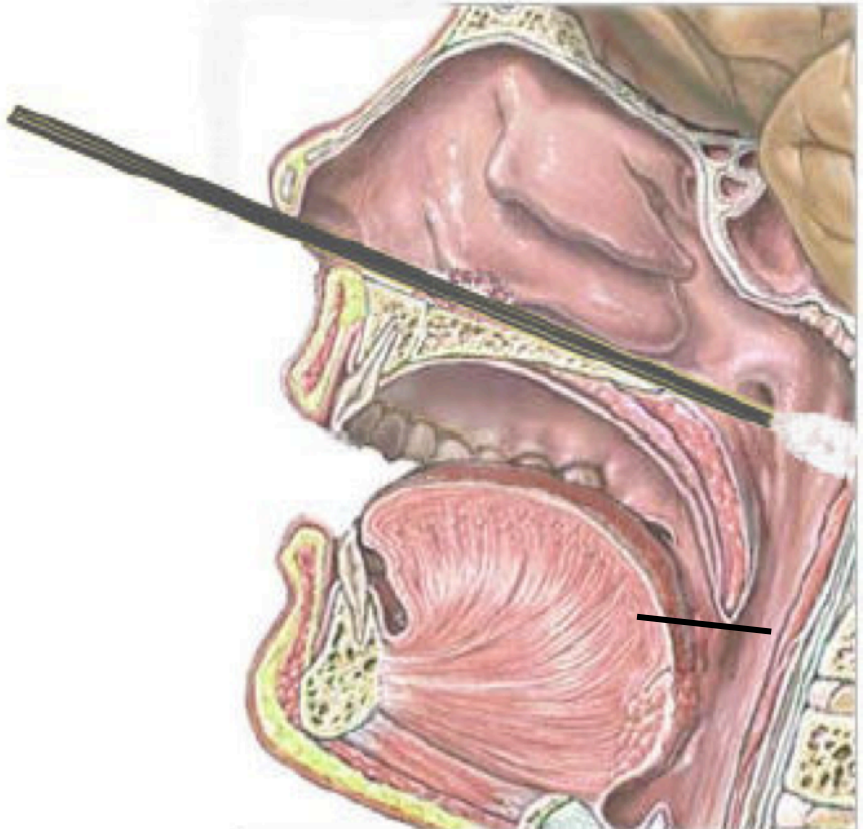
Upper respiratory tract samples

- Use appropriate PPE during collection procedure (gown, mask, gloves and eye protection).
- Nasal or nasopharyngeal samples have highest yield for detection of seasonal influenza A or B viruses.
- **Also collect throat swabs** to improve the yield for suspected emerging or zoonotic viruses **(i.e. nCoV).**
- Collect samples as soon as possible.

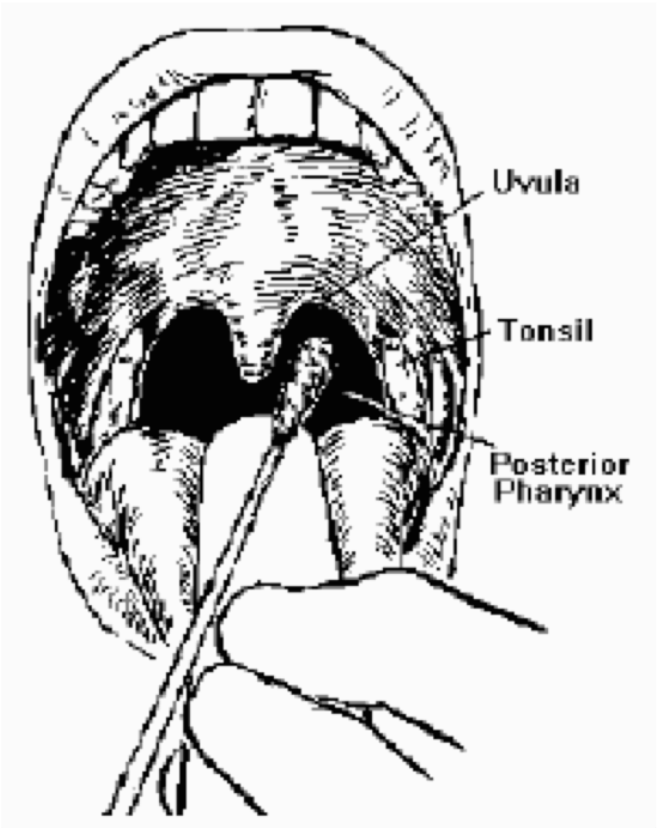
✓ Use sterile dacron or rayon swabs. Do not use cotton swabs or wood shafts as can interfere with RT-PCR assays



Nasopharyngeal swabs



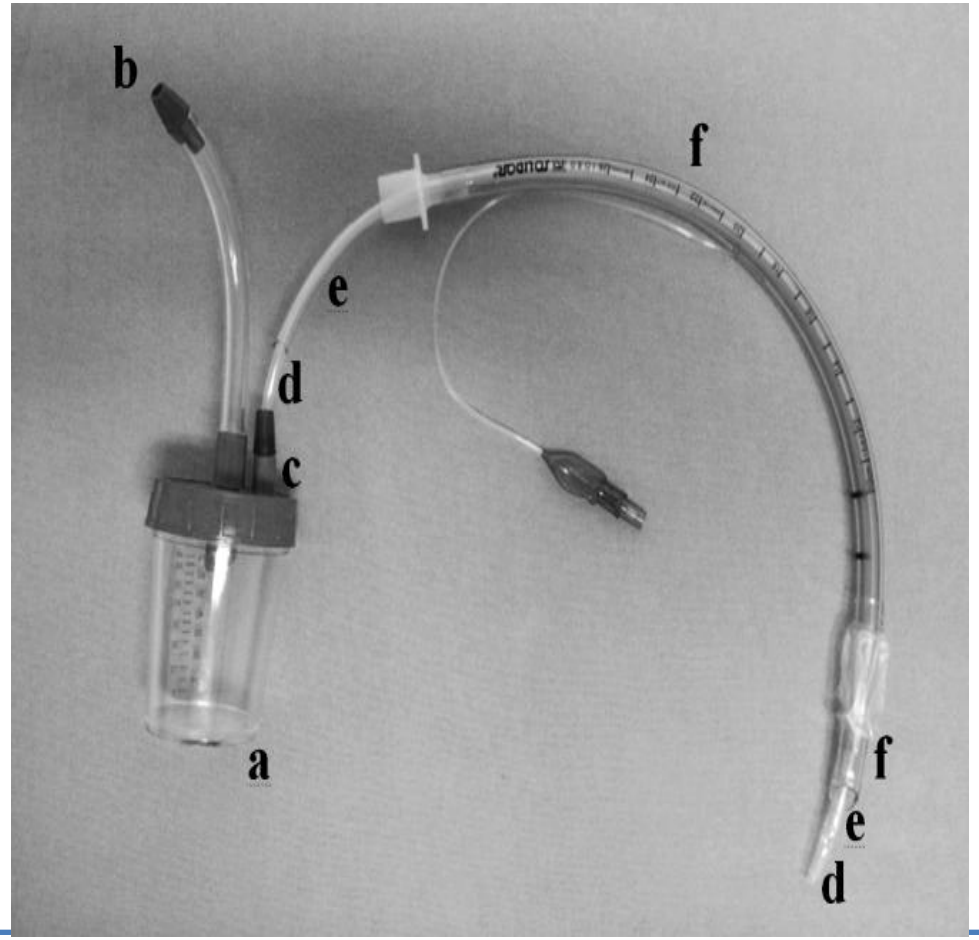
Throat swabs



Lower respiratory tract samples

- Also collect **lower respiratory tract** samples in patients with radiographic evidence or clinical diagnosis of lower respiratory tract disease, in certain situations, if results will impact clinical interventions:
  - expectorated sputum
  - tracheal aspirates
  - bronchoalveolar lavage.
- Can generate aerosols, thus **use airborne precautions** during procedure.

In intubated patient, can collect tracheal aspirate



- Collection can generate aerosols, thus use airborne precautions.
- Using sterile collection trap.
- Do not send suction catheter tip to laboratory.

Benefits of lower respiratory tract samples

- Higher sensitivity than upper respiratory specimens for zoonotic influenza virus, MERS-CoV and other emerging respiratory viruses.
- Increases diagnostic yield for seasonal influenza if upper samples are negative or tested late.
- Can also be tested for bacterial, fungal and parasitic infections
  - e.g. M. tuberculosis, PjP.

Collection time and site matter

- Collect samples as soon as possible:
  - Ideally less than 4 days of illness onset for seasonal influenza A or B, as yield goes down as viral shedding decreases.
  - In patients with respiratory failure diagnosis may still be made by sampling the lower respiratory tract at any time.
  - In children, oropharyngeal swabs may be alternative.*
- Collect lower tract samples for zoonotic influenza and MERS:
  - If you sample the upper respiratory tract at illness day 6 you might miss detection of these viruses, and still make the diagnosis by testing endotracheal aspirate.

*Le Wang, Shuo Yang, Xiaotong Yan, Teng Liu, Zhishan Feng & Guixia Li. Comparing the yield of oropharyngeal swabs and sputum for detection of 11 common pathogens in hospitalized children with lower respiratory tract infection. Virology Journal 2019:16, Article number: 88

Collect additional specimens for laboratory diagnosis

- Complete blood cell count for white blood cells.
- Sputum for bacteriology:
  - including TB if in high prevalence country or fungus if immunosuppressed, etc.
- Specimens from other sites that may be infected and can yield pathogens, as clinically indicated:
  - urine, cerebrospinal fluid, stool, pleural fluid, peritoneal fluid, etc.
- Two sets of blood cultures for bacteriology from two different sites (where possible) for patients with sepsis.

Additional specimens for public health and research purposes

- Discuss with local public health officials need for additional samples and interval of repeat testing, if suspect emerging infection:
  - collection of blood for virus detection may aid in prognosis and IPC implementation
  - repeated specimens can enhance understanding of viral replication patterns and response to experimental treatments for research purposes (use standard protocol)
  - serial collection should be part of standardized protocol (e.g. ISARIC protocol).

Laboratory testing

Diagnostic tests for COVID-19 (1/3)

- This is a rapidly evolving area of work. Real time (RT-PCR) is currently recommended for diagnosis of patients with suspected COVID-19.
  - As sequence information from the COVID-19 has recently been made available, PCR assays can be designed to detect these sequences.

- For latest information refer to your national laboratory and health ministry recommendations and to the WHO COVID-19 website.

- https://www.who.int/emergencies/diseases/novel-coronavirus-2019

- https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance

Diagnostic tests for COVID-19

- Laboratories may desire to use a pan-coronavirus assay for amplification followed by sequencing of amplicons from non-conserved regions for characterization and confirmation.

- The importance of the need for confirmation of results of testing with pan-coronavirus primers is underscored by the fact that four human coronaviruses (HcoVs) are endemic globally: HCoV-229E, HCoV-NL63, HCoV-HKU1 as well as HCoV-OC43.
  - The latter two are. betacoronaviruses. Two other betacoronaviruses that cause zoonotic infection in humans are MERS-CoV, acquired by. contact with dromedary camels and SARS arising from civets and cave-dwelling horseshoe bats

Diagnostic tests for COVID-19

- Alternatively, amplification and detection of COVID-19 specific sequences can be diagnostic without the necessity for further sequencing.

- If testing does not occur in an expert/reference laboratory it is encouraged to send the sample for confirmation to a regional, national or international. reference laboratory with pan-coronavirus or specific COVID-19 detection capacity.
  - WHO can assist Member States to identify laboratories able to provide this support

- If case management requires, screen also for other common causes of respiratory illness according to local guidelines, as co-infections can occur.

RT-PCR for influenza and other respiratory virus detection

- Real time (RT-PCR) are the **recommended** diagnostic tests for accurate and timely diagnosis of influenza virus:
  - detects presence of virus RNA in respiratory tract specimens (or other clinical specimens)
  - high sensitivity (86–100%) and high specificity
  - can identify influenza A virus infection
  - requires specific primers and probes to specifically identify viruses.

Limits

- Requires specialized laboratory.
- Usually tested in batches
- Can take 6–8 hours to perform test; but results may be delayed due to transport and laboratory batching.

Rapid tests for influenza

- **Digital immunoassays (DIAs)** and **rapid nucleic acid amplification tests (NAATs)** are RIDTs with analyser devices.
  - Available in clinical settings and can provide results within 30 minutes.
  - A recent systematic review reported that the newer rapid antigen detection tests (NAATs, DIAs) have higher sensitivities for detecting influenza A and B than RIDTs that do not utilize analyzer devices in adults and children (91.6% vs 80% vs 54.4%, respectively) [16].

- **Rapid molecular assays with higher sensitivity** than DIAs to detect influenza viruses in respiratory tract specimens are commercially available for point of care use in clinical settings, and a recent systematic review reported a pooled sensitivity of 90.9%[17]

RIDT for influenza virus detection

- **Rapid, antigen based,** influenza tests are practical but have variable sensitivity:
  - rapid, point-of-care
  - result available in 15–30 min
  - give indication of presence of influenza in the community during potential outbreak situation.

If negative, respiratory specimens should be collected for influenza testing by RT-PCR assay.

Limits

- Variable sensitivity (10–70%), thus miss many infections.
- False negatives are common.
- Cannot differentiate specific influenza virus from another (some differentiate A and B).
- False positives common outside of influenza season. Test multiple patient samples.

Other laboratory methods

Test	Method	Time	Comments
IFA	Antigen detection	2–4 hours	• Moderate sensitivity, high specificity.
Viral isolation	Virus isolation	days	• Moderate sensitivity, high specificity. • Genetic characterization. • Antigenic characterization. • Drug susceptibility.
Serology	Antibody detection	days	• Time consuming, generally not clinically relevant unless RT-PCR is non-diagnostic or late in the course > 14 days). • Requires paired sera, 14–21 days apart. • Need specialized laboratory.

IFA - Immunoflourescence

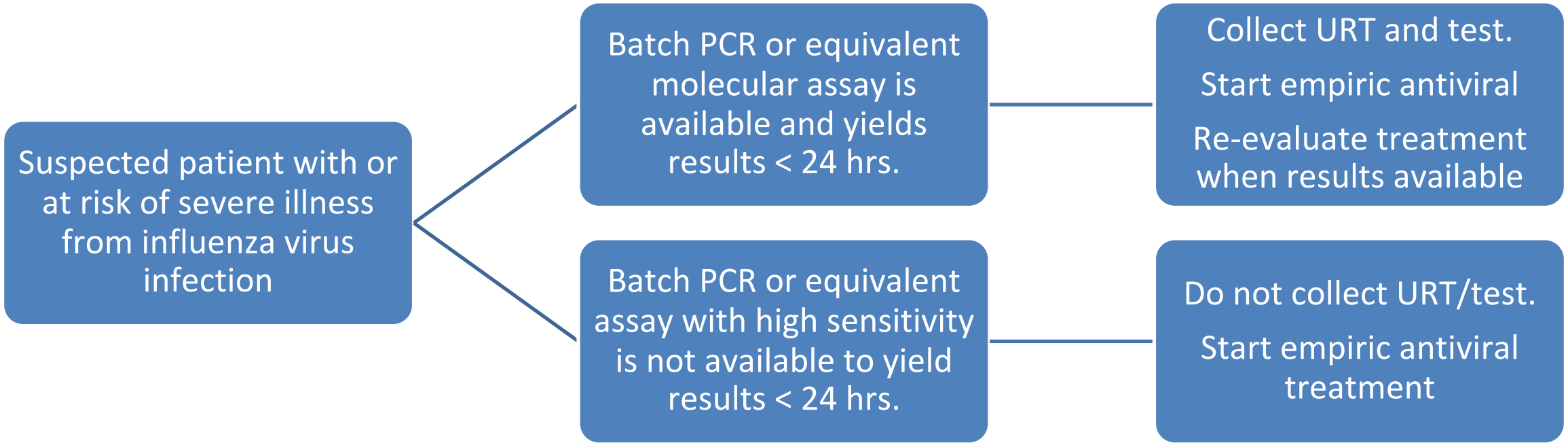
Empiric treatment of patients with SARI (1/2)

- Patients with SARI can be clinically diagnosed with seasonal influenza based upon clinical findings in the context of seasonal influenza A or B virus activity in the community.
  - Use diagnostic algorithm on next slides for use of diagnostics.

- Patients with SARI can be suspected with avian influenza A virus infection (e.g. H5N1, H7N9) if there is recent exposure to poultry in an endemic area; but confirmatory diagnosis is necessary.

- **Patients with SARI can be suspected COVID-19 if there is recent travel to affected area (case definition) but confirmatory diagnostic test is necessary.**

Test and treat: emergency department during times when influenza is known or suspect to be circulating



Empiric treatment of patients with SARI (2/2)

- **Do not delay** empiric antiviral treatment for seasonal influenza or zoonotic influenza A (e.g. avian influenza A virus) **and** antimicrobials for possible community-acquired pneumonia, while results of diagnostic tests are pending.

Summary

- In patients with SARI and pneumonia/sepsis, the differential diagnosis includes community- or hospital-acquired pathogens (i.e. bacterial, fungal and viral pathogens) and should be guided by local epidemiology and patient factors.
- Suspect respiratory viruses with pandemic potential, such as seasonal influenza A and B viruses, if there is seasonal influenza activity in the community. Suspect avian influenza A viruses, MERS-CoV, **COVID-19** or another emerging virus if exposure risk factor is present.
- Do not delay IPC interventions and standard treatments (empiric antimicrobials) while waiting for diagnostic test results.
- Collect upper respiratory tract specimens for viral testing with RT-PCR. Lower tract samples can be useful when upper samples are not diagnostic or if suspect zoonotic or emerging respiratory virus.

Acknowledgements

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