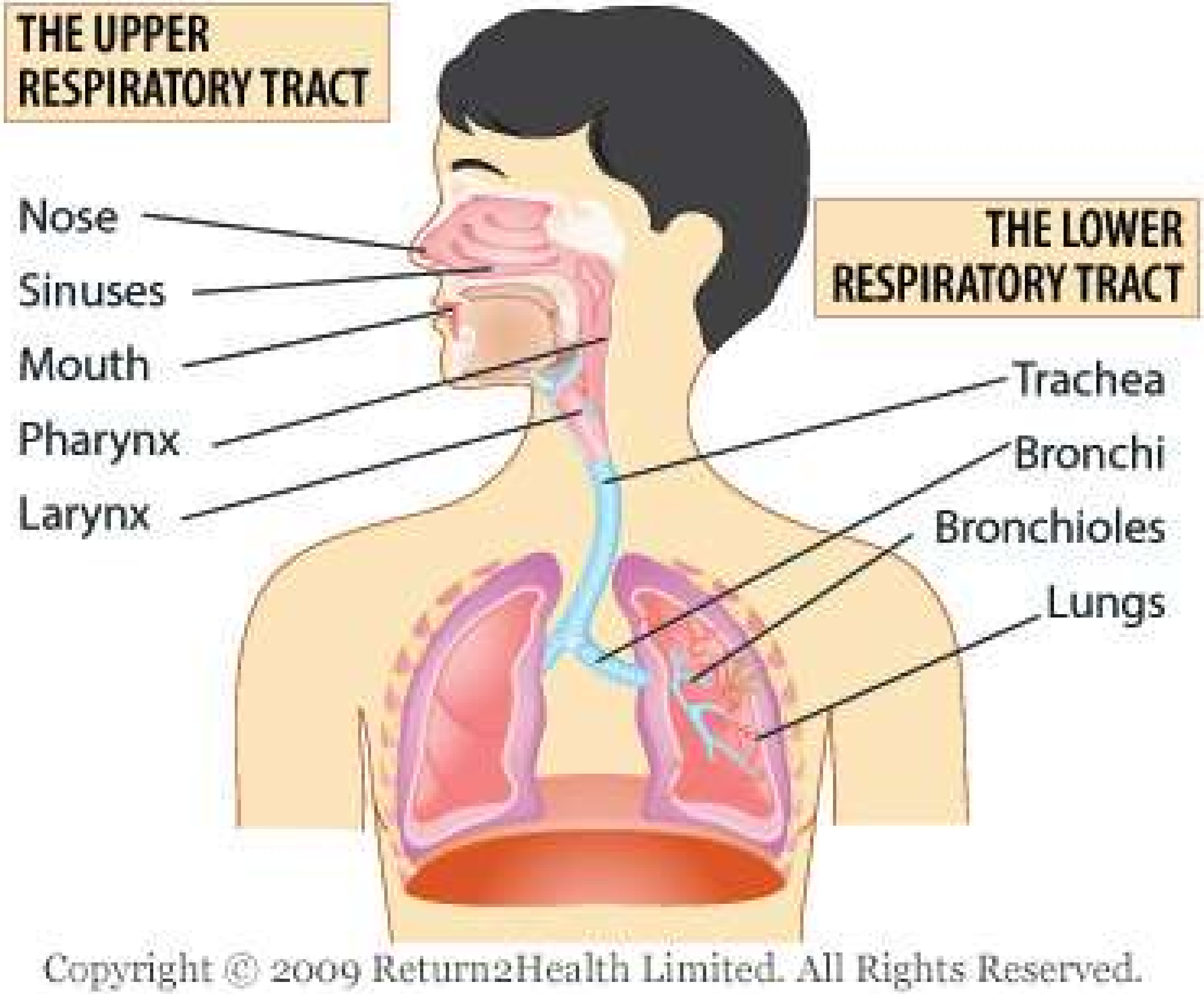
Respiratory infections

Respiratory tract anatomy



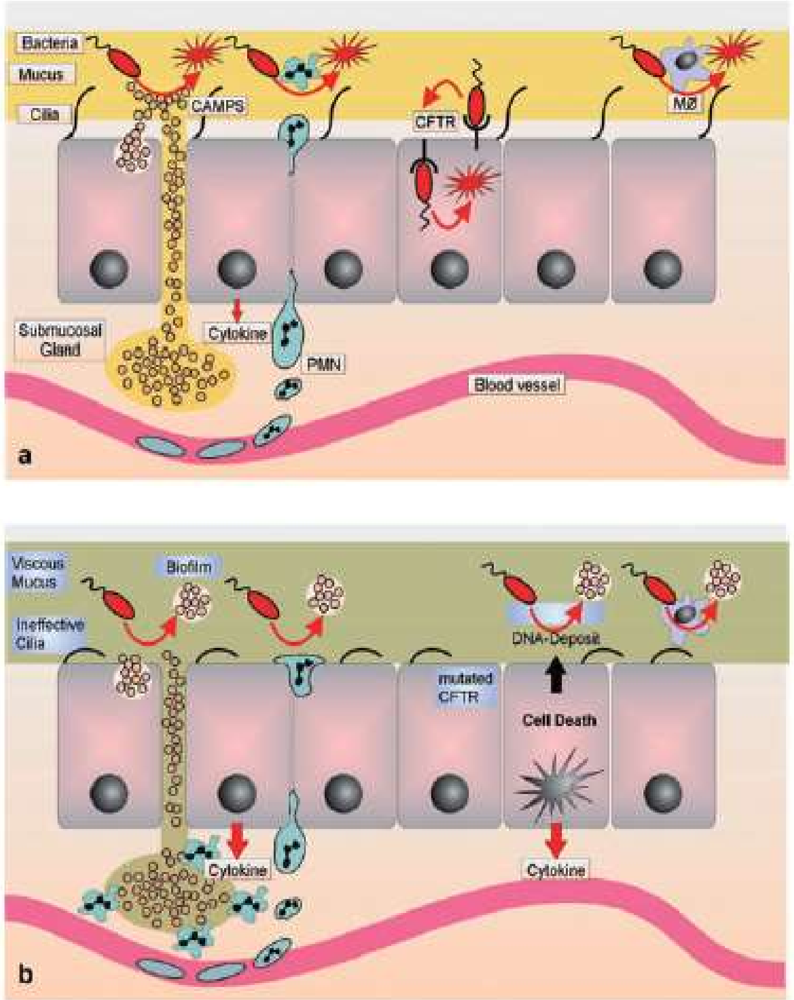
RELEVANCE, ETIOLOGY, EPIDEMIOLOGY

- **RELEVANCE** – highest morbidity
- seniors, chronic infections – frequent hospitalization, significant mortality
- **ETIOLOGY** – viruses, bacteria, fungi, parasites
- **EPIDEMIOLOGY** – etiology, symptomatology and significance are age depended
- transmission – most frequently by droplets or direct contact
- seasonal epidemics (cold periods)
- repeatedly occur – many pathogens and serotypes (short immunity answer)
- transmission is potentiated – high mobility and people concentration, stress factors, emerging and re-emerging pathogens, e.g. coronavirus (SARS 2002-2003, SARS Cov 2) or antigenic shift of flu virus

PHYSIOLOGY AND PATHOPHYSIOLOGY

- Upper respiratory tract (URT) - large number of commensal bacteria
- Lower respiratory tract (LRT), middle ear, paranasal cavity – nearly sterile, if inflammation – large number of polymorfonulear cells (PMN) with majority of one bacterial morphology
- defence mechanisms – cilliary epithelia of URT and LRT, lysozyme, mucosal IgA
- bacterial infection – non-specific (innate) mechanisms – e.g. C-reactive protein (CRP), complement, PMN
- viral infection – interferon (bloking of NA metabolism in neighbour cells – inhibition of virus replication)
- NALT – in URT, *nasopharyngeal-associated lymphoid tissue*
- BALT – in LRT, *bronchus -associated lymphoid tissue*
- Lymphatic tissue (part of Waldeyer's ring) – without capsule, directly penetrating epithelia (lymphoepitelium – dendritic cells, Langerhans cells, intraepithelial location – B lymfocytes, plasmatic cells) – antigen monitoring

Innate immunity and microbes elimination



Ref. Goring, Gulbins, Cellular Microbiology (2009) 11(2), 208–216

Bacterial killing mechanism of innate immunity in the respiratory tract of healthy individuals and cystic fibrosis (CF) individuals.

A. In healthy individuals, bacteria, entering the mucus layer overlaying the respiratory epithelium, are effectively removed from the airways by a functional mucociliary clearance system and killed by CAMPs derived from submucosal glands or epithelial cells, by functional neutrophils or macrophages or within epithelial cells after uptake via functional CFTR. PMN, polymorphonuclear leukocytes.

B**. In individuals with CF** defective CFTR leads to a highly viscous mucus layer which impairs mucociliary clearance, the migration of CAMPs, neutrophils and macrophages towards the bacterial targets, the uptake of bacteria by CFTR itself, induces a pH shift in epithelial lysosomes which due to ceramide accumulation results in DNA deposits which in turn serve as adhesion matrices for bacteria. A similar pH shift in the phagolysosomes of macrophages impairs bacterial killing. Ceramide accumulation also triggers cytokine release which induces further neutrophil influx.

Bacterial agents & respiratory infections

* **Staphylococci** (*S. aureus* – **sinusitis, pneumonia**)

* **Streptococci** (*S. pyogenes* – **tonsilitis, pharyngitis** – strep throat, scarlet fever, autoimmune complication – rheumatic fever, glomerulonephritis, *S. pneumoniae* – **pneumonia** but also meningitis and otitis media)

* **Hemophilus** (H. influenza b – e.g.**epiglottitis, pneumonia**, non-typable – e.g. **sinusitis, chronic bronchitis**)

* ***Bordetella pertussis*** – **whooping cough, upper respiratory tract (affected also ciliated epithelium trachea bronchi)**

* ***Corynebacterium diphteriae*** – **pseudomembranes** – tonsills, pharynx, larynx

* ***Chlamydia pneumoniae, Mycoplama pneumoniae, Legionella pneumophila*** – **atypical pneumonia**

Fungal agents & respiratory infections

• e.g. *Cryptococcus neoformans*, *Pneumocystis jirovecii* – pneumonia in AIDS patients *(more in the presentation of fungal infections*)

• treatment – *Cryptococcus neoformans -* fluconazole, amphotericin B, Pneumocystis *jirovecii* – co-trimoxazole

DIAGNOSTICS OF UPPER RESPIRATORY TRACT INFECTION (1)

RHINITIS – around 100 serotypes of rhinoviruses, **microbiological dg is not significant**, waterish nasal secretion, afebrile, secondary bacterial colonisation – purulent character – without antibiotics, dif.dg – alergic rhinitis, symptomatic therapy

TONSILITIS AND PHARYNGITIS – most frequent patients in ordination, most often tonsilopharyngitis, 80% viruses, affected – often children and young adults, children up 3 years – mainly adenoviruses. **Viral pharyngitis** – swallowing disorders, fever, conjunctivitis, etiology – herpangina – various serotypes of Coxsackie A (isolation – pharyngeal swab and stool, RT-PCR, neutralisation antibodies, antibodies IF). **Streptococcal tonsillopharingitis** – *S. pyogenes* (beta hemolytic streptococcus of group A, pharyngeal swab – cultivation, direct detection using immunochromatographic test, complication – reumatic fever and acute glomerulonephritis (ASO – antibodies to streptolysin O), rarely also betahemolytic streptococci of group C and G

Infectious mononucleosis – EBV virus, heterophilic antibodies (Paul Bunnelova reaction), specific antibodies to EBV, elevated liver enzymes **Arcanobacter tonzilopharyngitis** – *Arcanobacterium haemolyticum*, young adults, culture, gram-positive rods (reverse CAMP test), **Angina Plaut-Vincent** – necrotic ulcerative tonzilitis, *Fusobacterium* and *Borrelia vincentii*

DIAGNOSTICS OF UPPER RESPIRATORY TRACT INFECTION (2)

SINUSITIS – **when nasopharyngitis - always inflammation in paranasal cavities**, viruses - rhinoviruses, adenoviruses, influenza, parainfluenza, bacteria – *H. influenzae, S. pneumoniae, M. catarrhalis, S. pyogenes, S. aureus,* gram-negative and anaerobic bacteria, in immunosuprosupressed – fungi *(Aspergillus)* secondary to infection of URT, dg – microscopy, culture of aspirate, dif.dg – alergic sinusitis, tumors, adenoid vegetation

OTITIS MEDIA– **inflammation of middle ear**, complication of URT, up to 3 years – 80% of children, complication – spontaneous perforation of eardrum, mastoitis, meningitis, pathogens - *S. pneumoniae,* non-capsulated *H. influenzae, S. pyogenes, M. catarrhalis, S. aureus,* dg – culture, elevated CRP

EPIGLOTITIS – peracute, life-threatening, agents – *H. influenzae* typ b (vaccination available today), dg – leukocytoses, elevated CRP, laryngeal swab (after providing respiratory passage) and from hemoculture

LARYNGITIS AND TRACHEITIS (trachea – LRT) – 90% flu viruses, parainfluenza, adenoviruses, RS viruses, *C. diphteriae* – diphteria, complication - bacter. superinfection, e.g. *S. aureus*, dg – antigen in sputum, virus isolation from nasopharyngeal wash, laryng. swab, serology – seroconversion of specific titres, therapy – symtomatic (antitusics, antipyretics…)

TREATMENT & PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS

ACUTE TONSILOPHARYNGITIS

- caused by *S. pyogenes* – **penicillin** V (prevention of reumatic fever, glomerulonephriis) or penicillin depot (in non-compliant patients)
- Peritonsilar abscess – **klindamycin**
- **Diphteria – caused by toxigenic strains of C. diphteria – PNC G + antitoxin**
- not to be administered an aminopenicillin – EBV infection can result in exanthema

SINUSITIS, RHINOSINUSITIS, OTITIS

- **amoxicilin**, amoxicilin + inhibitors of betalactamases

ACUTE EPIGLOTITIS

- **cephalosporins of II or III generation**

PREVENTION

- Conjugated vaccines ***H. influenzae*** **b** (hexavaccine), ***S. pneumoniae***

Upper respiratory tract samples

*** Bacteriological diagnosis**

Pharyngeal and tonsilar swab
Puncture of paranasal sinuses
Nasal swab (exceptionally)

*** Virological diagnosis**

Nasopharyngeal swab (more often swab – pharynx + nose, not from tonsils)

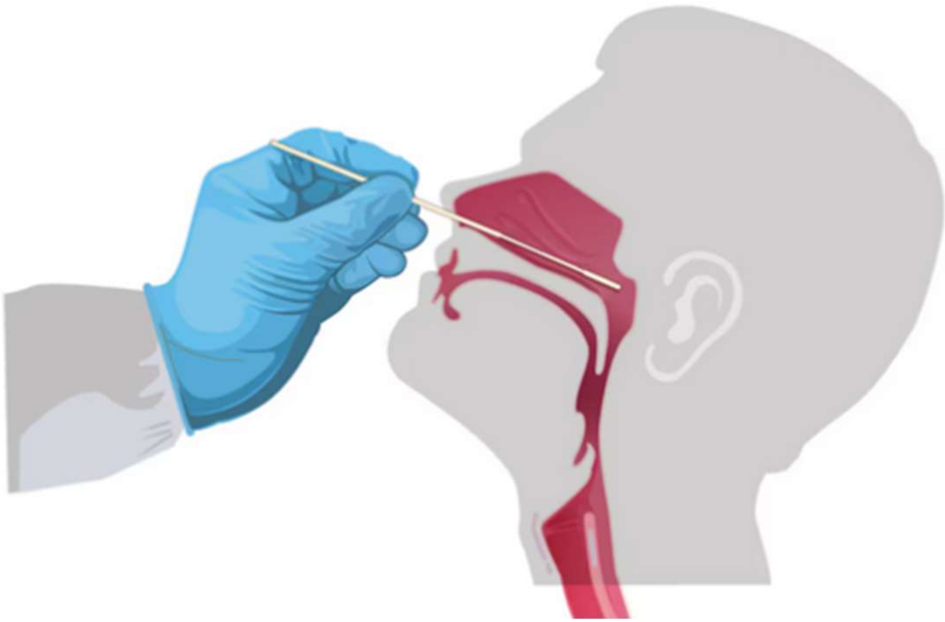
*** Serology**

Coagulated blood (yields serum)

Bacteriology – swabs, spatula, container

Specimen collection & transport
Respiratory tract infection

Technic to collect (deep) nasopharyngeal swab



Next methodology: trnasport media, diagnosis of viral and atypical pneumonia agent – isolation of DNA and PCR amplification, diagnosis of pertusis – culture on Bordetella diagnostic media and/or isolation of DNA and PCR amplification

Note: see also https://www.youtube.com/watch?v=aTcGckOrU88

DIAGNOSTICS OF LOWER RESPIRATORY TRACT INFECTION (1)

TRACHEITIS (LOOK LARYNGITIS A TRACHEITIS)

BRONCHITIS – also very fequent in ordination, **Acute** – 90% viruses, adenoviruses, flu viruses parainfluenza, rhinoviruses, metapneumovirus, *M. pneumoniae*, other bacteria – rarely, occurence – spring and winter season, pertussis (*B. pertussis*) and parapertussis (*B. parapertussis*) – catharal to necrotic inflammation, dg – clinical – auscultation, bacterial superinfection – larynx swab or sputum, diagnostic culture or serology **Chronic** – persistant cough at least 3 months during the year more than 2 years, <u>acute exacerbation of chron. bronchitis</u> – relapsed cough, increased expectoration of purulent sputum,dyspnoea, mainly viruses (rhinoviruses, influenza a parainfluenza, adenoviruses) and bacteria *S. pneumoniae*, non-capsulated *H. influenzae, M. pneumoniae,* CF patients – *P. aeruginosa, B. cepacia* complex, complication – emphysema, chronic obstructive pulmonary disease, dg – sputum culture, dispensarization by pneumologist

BRONCHIOLITIS – in infants – obstruction of bronchi and bronchioles, pathogens – often RSV, also influenza and parainfluenza viruses, *M. pneumoniae*, dg – direct detection, nasopharyngeal secretion – undirect detection – increased antibodies – ELISA, CFR

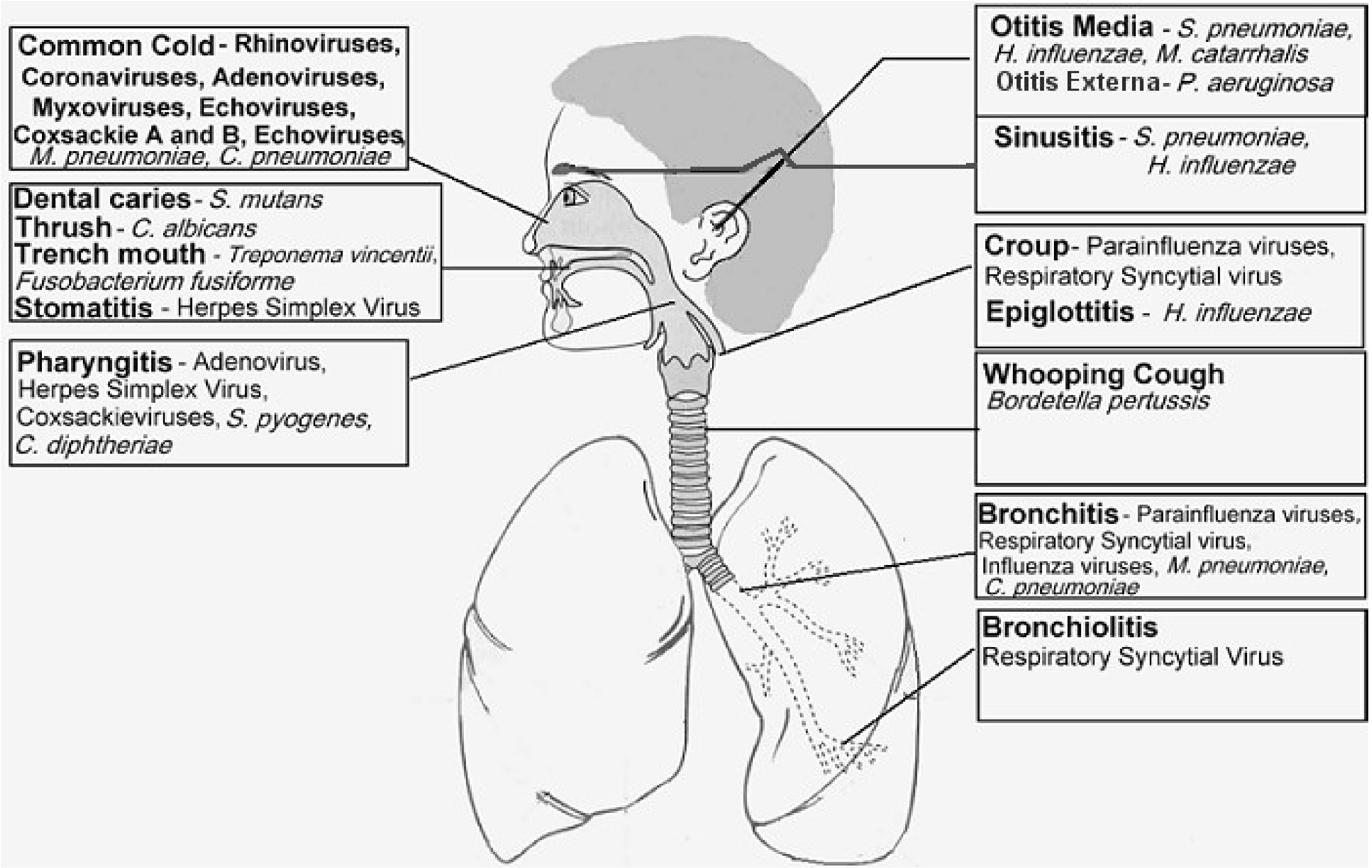
DIAGNOSTICS OF LOWER RESPIRATORY TRACT INFECTION (2)

COMMUNITY ACQUIRED PNEUMONIA – pyogenic bacteria, mycoplasma and chlamydia, viruses, legionela, *M. tuberculosis*, *Ch. pneumoniae, Pneumocystis jiroveci*, microb. dg – sputum culture, tracheal or aspiration culture (intubated patient), BAL, hemoculture (20% sensitivity), exudate

NOSOCOMIAL PNEUMONIA – 48h after admission, often *S. aureus*, *P. aeruginosa,* VENTILATORY – 48h after intubation, pseudomonads, acinetobacters, MRSA, microb. dg – as indicated above

ASPIRATION PNEUMONIA – polymicrobial etiology, **LUNG ABSCESS** – often anaerobes, *S. aureus, K. pneumoniae*, **PLEURITIS** AND **EMPYEMA**, **ATELECTASIS**

Respiratory infections



Dr. Neal R. Chamberlain

TREATMENT & PREVENTION OF LOWER RESPIRATORY TRACT INFECTIONS

Pathogen	**antibiotics, first-line antibiotics**	**antibiotics, second-line antibiotics**
Streptococcus pneumoniae	penicillin G, aminopenicillins	macrolides, moxifloxacin
Staphylococcus aureus (MSSA)	oxacillin, flucloxacillin, cephalosporins I., II.gen., aminopenicillins + inhibitors	vancomycin, clindamycin, linezolid
Staphylococcus aureus (MRSA)	vancomycin, linezolid	tigecycline
Chlamydophila pneumoniae, C. psittaci, Mycoplasma pneumoniae	macrolides, doxycycline	fluoroquinolones
Haemophilus influenzae, Moraxella catarrhalis	aminopenicillins or aminopenicillins + inhibitors, cephalosporins I., II.gen.	macrolides
Klebsiella pneumoniae	cephalosporins I., II.gen.	aminopenicillins + inhibitors, fluoroquinolones
Pseudomonas aeruginosa	piperacillin + tazobactam, ciprofloxacin, ceftazidime, cefoperazone	aminoglycosides, carbapenems
Legionella sp.	macrolides	moxifloxacin
Bordetella pertussis	macrolides	

Lower respiratory tract samples

*** Sputum** – simple collected expectorated sputum. Validity of the sputum should be evaluated (number of leukocytes, epithelia, contaminating nasopharyngeal flora)

Nonvalid sputum

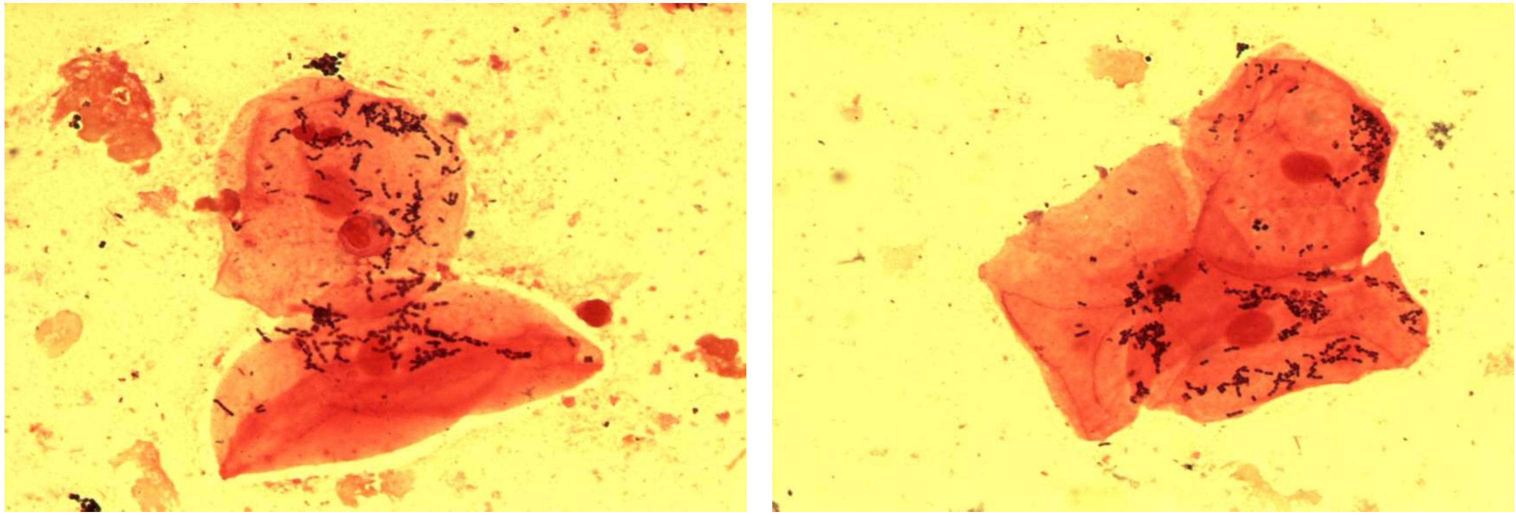


Fig. Large epithelia of upper respiratory tract (saliva in the sample) covered with adhered grampositive cooci in chains (usually comensal Viridans streptococci)(photo Melter)

Valid sputum

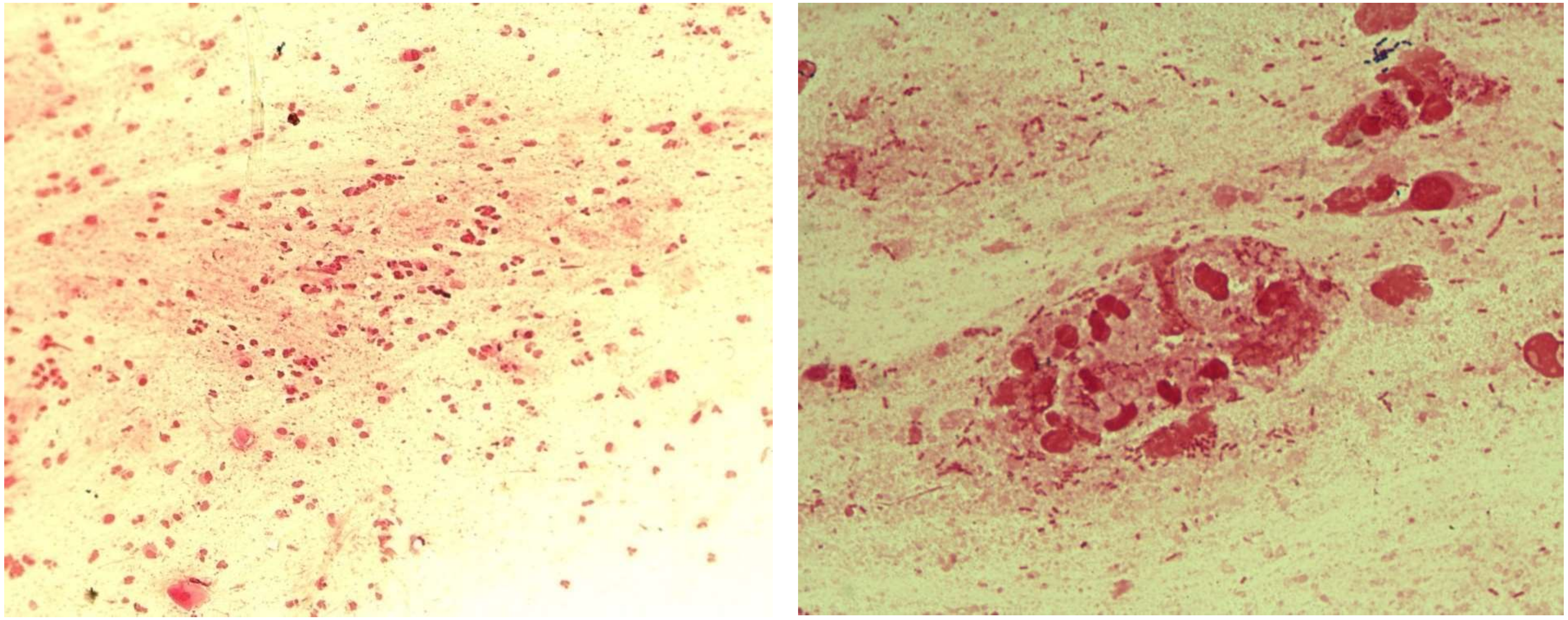


Fig. Large number of leucocytes with lower respiratory tract origin (small magnification, on the left) and uniform morphology of the bacterial cells (large magnification, gram-negative rods, on the left)(photo Melter)

Lower respiratory tract samples

* **Bronchoalveolar lavage** – from bronchoscopy or intubated patient
* **Other invasive samples**
* **Blood for hemocultivation** – in case of bacterial pneumonia
* **Serum** – serology (detection of specific IgM and IgG antibodies)
* **Nasopharyngeal swab for viral detection**
* **Urine – antigen detection** (pneumonia - *S. pneumoniae, L. pneumophila*)

Viral agents & respiratory infections (1)

Orthomyxoviruses (ss RNA)
• **influenza viruses** – ½ acute respiratory illnes, major determinant of morbidity and mortatility caused by respiratory infections

Paramyxoviruses – ss RNA, acute resp.inf. & pneumonia 4 mil.children yearly (WHO)
• **parainfluenza** virus - ubiquitous in all ages, nose throat – **common cold, croup-laryngotracheobronchitis, bronchiolitis, pneumonia)**
• **respiratory syncicial virus** – **bronchiolitis and pneumonia** in infants under 1 year
• **metapneumovirus** – described 2001, wide range of disease – **from mild upper resp.inf. to severe lower resp.tract inf.**

Viral agents & respiratory infections (2)

Adenoviruses
DNA virus, icosahedral symetry, naked
typical symptoms - cough, nasal congestion, fever, sore throut, pneumonia

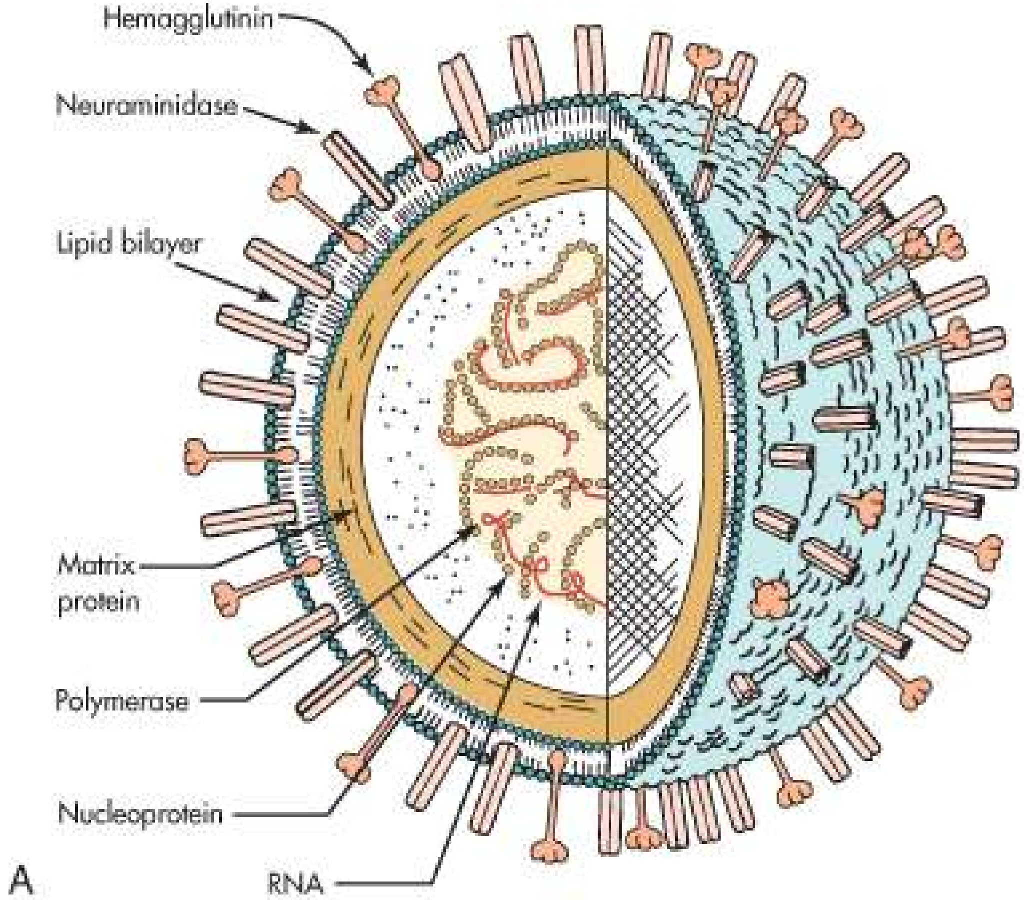
Coronaviruses
ssRNA,helical, enveloped, high frequency of mutations and recombinations, tropism to respiratory and GIT tract
SARS (Severe Acute Respiratory Syndrome) – acute respiratory distress requiring ventilation support (death 10%),
SARS-CoV-2 pandemic (2020)

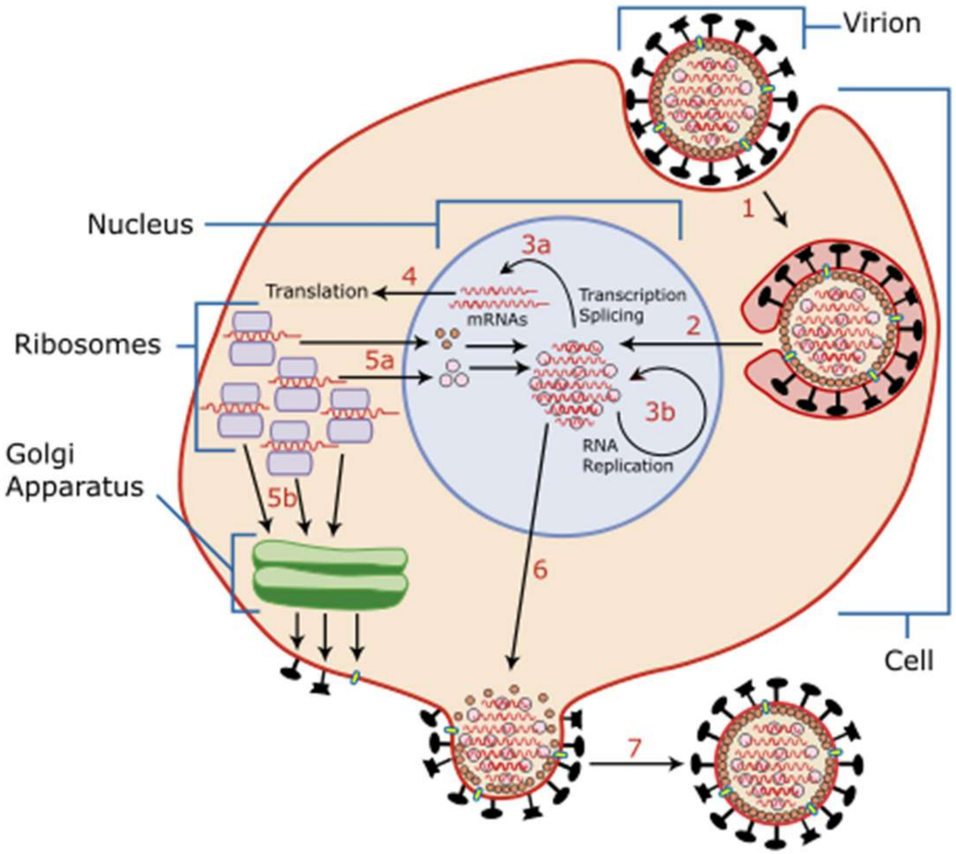
Picornaviruses
ssRNA, icosahedral symmetry
rhinovirus group (100 species) – **common cold viruses**
coxsackieviruses – herpangina - severe febrile pharyngitis

Orthomyxoviruses – structure & function

- ssRNA, spherical, 100 nm
- the envelope contains two **glycoproteins**, **hemagglutinin (HA)** and **neuraminidase (NA),** and is internally lined by the matrix (M1) and membrane (M2) proteins.
- **genome** occurs as **8 separate segments of RNA !!!**
- most of the segments code for a single protein
- eight different helical nucleocapsid segments, each of which contains a negative-sense RNA associated with the nucleoprotein (NP) and the transcriptase (RNA polymerase components: PB1, PB2, PA)

Structure of influenza A





Scheme of Influenza A virus replication (NCBI): "A virion attaches to the host cell membrane via HA and **enters the cytoplasm** by receptor-mediated endocytosis (**STEP 1**), thereby forming an endosome. A cellular **trypsin-like enzyme cleaves HA** into products HA1 and HA2 (not shown). HA2 **promotes fusion of the virus envelope and the endosome membranes**. A minor virus envelope protein M2 acts as a ion channel thereby making the inside of the virion more acidic. As a result, the major envelope protein M1 dissociates from the nucleocapsid and **vRNPs are translocated into the nucleus** (**STEP 2**) via interaction between NP and cellular transport machinery. In the nucleus, the viral polymerase complexes transcribe (**STEP 3a**) and **replicate** (**STEP 3b**) the vRNAs. Newly **synthesized mRNAs migrate to cytoplasm** (**STEP 4**) where they are **translated**. Posttranslational processing of HA, NA, and M2 includes **transportation via Golgi apparatus to the cell membrane** (**STEP 5b**). NP, M1, NS1 (nonstructural regulatory protein - not shown) and NEP (nuclear export protein, a minor virion component - not shown) move to the nucleus (STEP 5a) where bind freshly synthesized copies of vRNAs. The newly formed **nucleocapsids migrate into the cytoplasm** in a NEP-dependent process and eventually interact via M1 with a region of the cell membrane where HA, NA and M2 have been inserted (**STEP 6**). Then the **newly synthesized virions bud from infected cell** (**STEP 7**). **NA destroys the sialic acid moiety** of cellular receptors, thereby **releasing the progeny virions**."

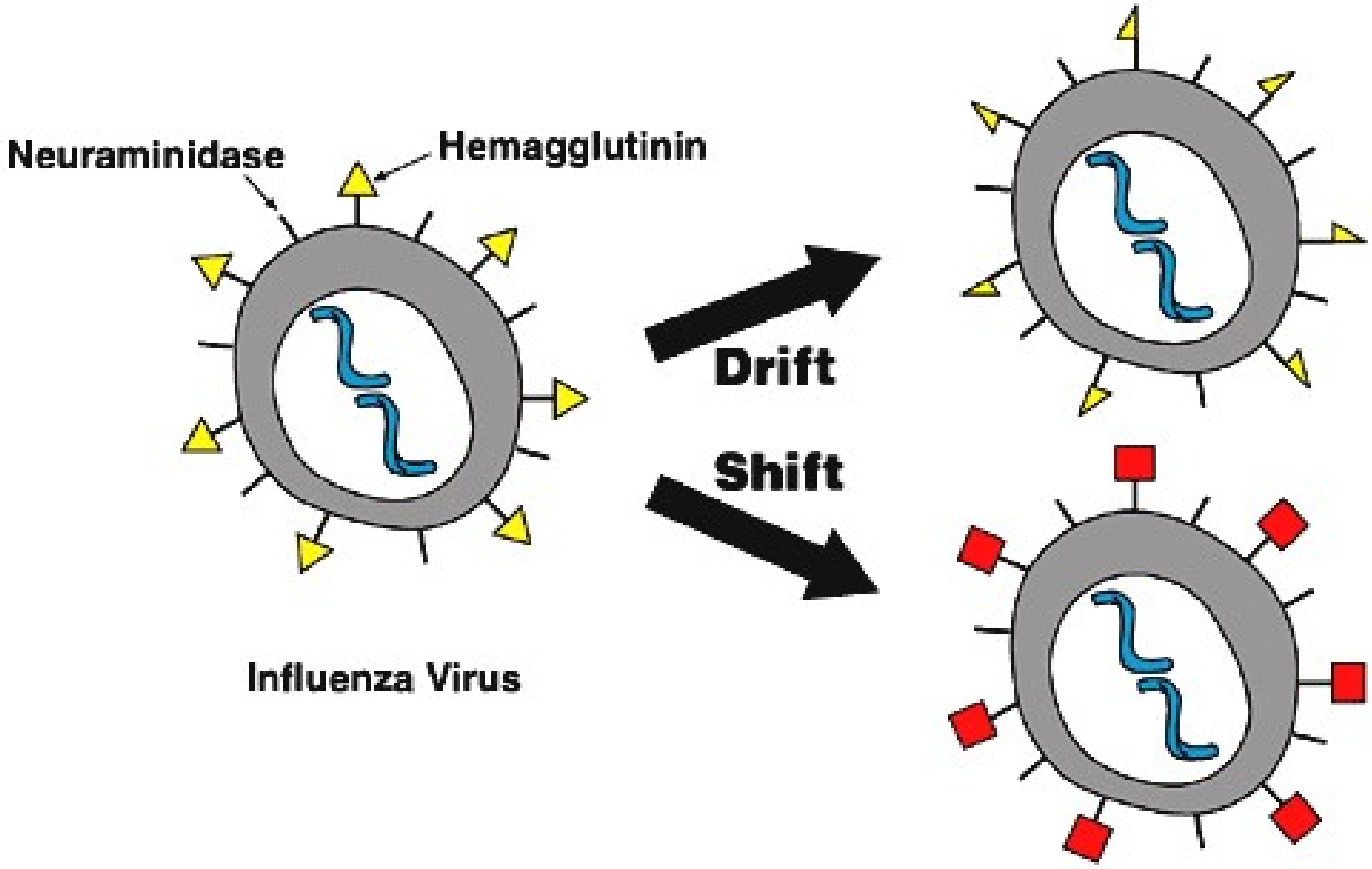
Structure & function of hemagglutinin (HA)

- glycoprotein - name from its ability to agglutinate erytrocytes
- the protein **binds virus particles to susceptible cells**
- **major Ag for neutralizing Ab** - **responsible for continual evolution of the HA**
- the HA spike on the virus is a trimer – cleavage by protease is necesary to be virus particle infectious (because the proteases is common in only respiratory tract – the site of infection)

Structure & function of neuraminidase (NA)

- glycoprotein, the spike is a tetramer
- its a sialidase enzyme – **releasing infected viral particules by budding from host cells**

Unusual antigenic variation of influenza A virus



Consequences of the unusual properties

- **Antigenic drift -** accumulation of point mutations resulting in amino acid changes in protein, a variant must sustain 2 or more mutations before a new, epidemiologically significant strains emerges

- **Antigenic shift** – reflect drastic changes in the sequence of viral surface protein, **genome segments** are **reasorted** readily **in doubly infected cells – result in epidemics !!!**

- **every 10-40 years when a new type of influenza A appears a PANDEMIC RESULTS**

- **1918 (H1N1, Spanish flu), 1957 (H2N2), 1968 (H3N2), 977 reemerged H1N1 (Russian flu)**

- **Since 1977 virus A (H1N1) and H3N2 and influenza B have been in global circulation**

- **new PANDEMIC with the 2009 H1N1 type (swine/mexican flu)**

Pathogenesis and pathology

• spread: air-borne droplets, direct (hands) or undirect (surfaces) contact

• if deposited viral particles avoid removal by cough reflex and neutralization by preexisting IgA – virions spread to cells

• viral NA lowers viscosity of mucous film – bare cell surface – promoting the spread

• incubation period 1-4 d

• viral shedding 1 d before symptoms appear, duration 5 days seasonal but 10 days mexican flu

• cellular destruction – reparation 1 month (could also secondary bacterial infection – staphylococci, streptococci, *Hemophilus influenzae*)

Clinical findings

- attacks mainly – upper respiratory tract
- seasonal flu – very young, elderly people, mexican flu – mediate age

a) **uncomplicated influenza** – appear abruptly, headache, dry cough, high fever, muscular aches. Many of the classic "flu" symptoms (e.g., fever, malaise, headache, and myalgia) are associated with interferon induction.

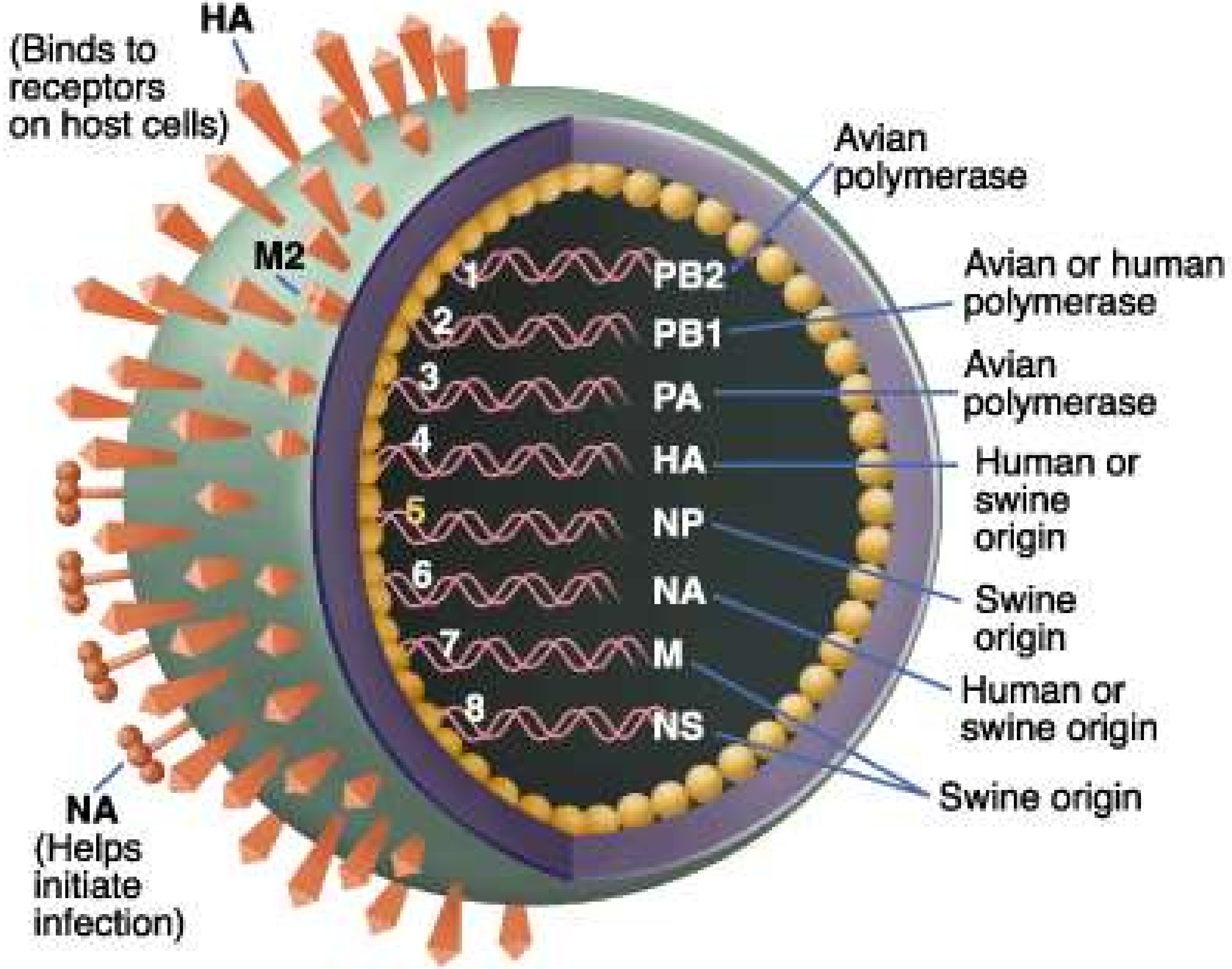
b) **pneumonia** – elderly and debilitated and underlying chronic diseases, the pneumonia could be **viral**, secondary **bacterial** or **combination**

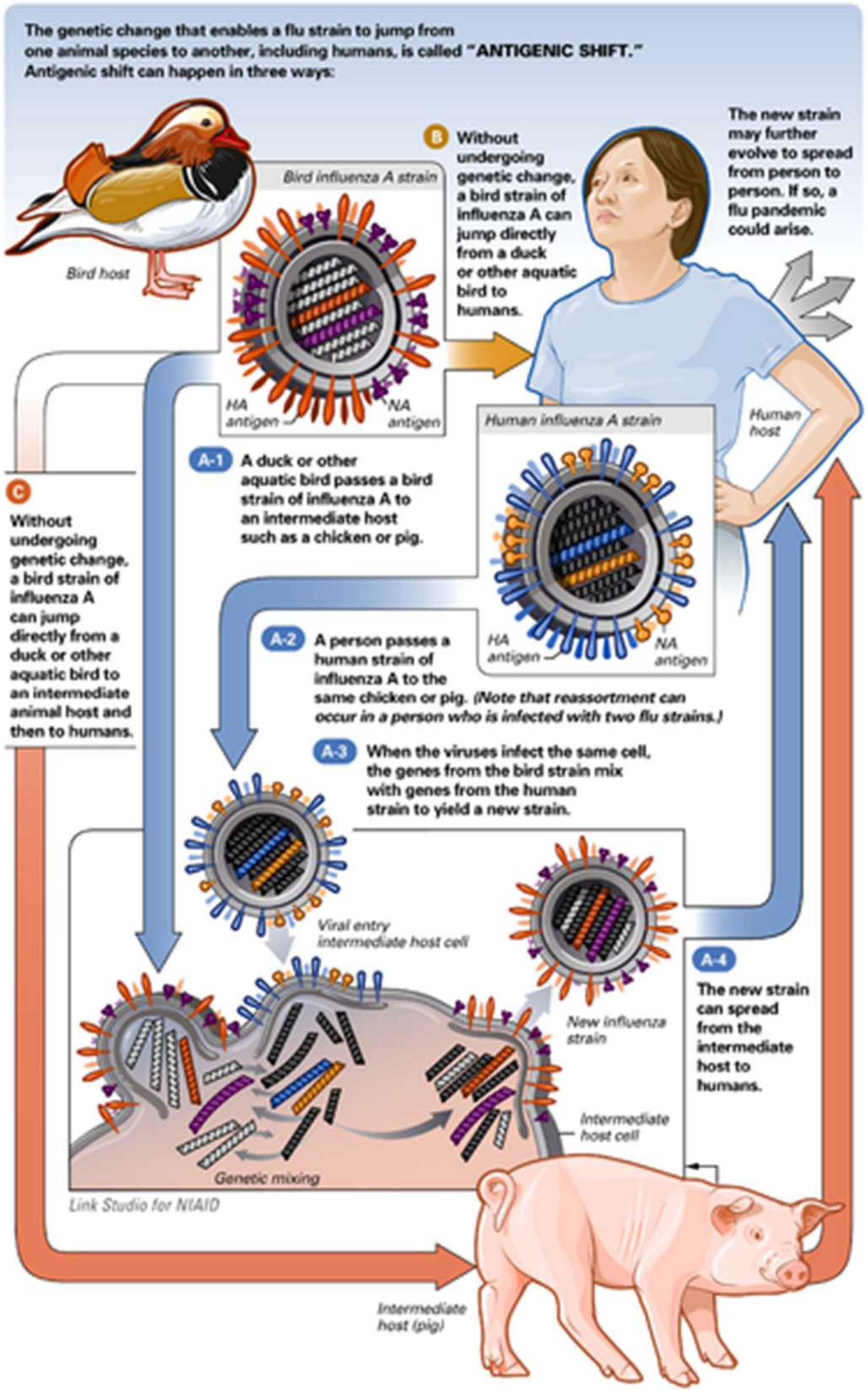
c) **Reye's syndrome – acute encephalopathy** of **children** and a**dolescents**, usually between 2-16 years of age (high mortality 10-40%), posible relation between salicylate use and development of the syndrome

Ecology of the swine flu

- most of the genome segment appear fro swine but some also from birds and humans
- it originated/evolved in pig but probably because of more appropriate receptors the virus can cause clinical disease in human
- it is not agent of pig infection!!!

The 2009 H1N1 influenza virus A





The genetic change that enables a flu strain to jump from one animal species to another, including humans, is called "ANTIGENIC SHIFT." Antigenic shift can happen in three ways:
Bird host
Bird influenza A strain
HA antigen
NA antigen
B Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to humans.
The new strain may further evolve to spread from person to person. If so, a flu pandemic could arise.
Human host
Human influenza A strain
HA antigen
NA antigen
A-1 A duck or other aquatic bird passes a bird strain of influenza A to an intermediate host such as a chicken or pig.
C Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to an intermediate animal host and then to humans.
A-2 A person passes a human strain of influenza A to the same chicken or pig. (Note that reassortment can occur in a person who is infected with two flu strains.)
A-3 When the viruses infect the same cell, the genes from the bird strain mix with genes from the human strain to yield a new strain.
Viral entry intermediate host cell
New influenza strain
A-4 The new strain can spread from the intermediate host to humans.
Intermediate host cell
Genetic mixing
Link Studio for NIAID
Intermediate host (pig)

Treatment

Hundreds of millions of dollars are spent on acetaminophen, antihistamines, and similar drugs to relieve the symptoms of influenza. The antiviral drug **amantadine** and its analogue **rimantadine** inhibit an uncoating step of the influenza A virus but do not affect the influenza B and C viruses. The target for their action is the M2 protein. **Zanamivir** and **oseltamivir** inhibit both influenza A and B as enzyme inhibitors of the neuraminidase. Without the neuraminidase, the hemagglutinin of the virus binds to sialic acid on other viral particles to form clumps, thereby preventing virus release. Zanamivir is inhaled, whereas oseltamivir is taken orally as a pill. These drugs are effective for prophylaxis and for treatment during the first 24 to 48 hours after the onset of influenza A illness. Treatment cannot prevent the later host-induced immunopathogenic stages of the disease.

Prevention

Natural immunization, which results from prior exposure, is **protective for long periods**. **A killed-virus vaccine** representing the "strains of the year" influenza vaccine is **available every year**. Killed whole-virus vaccines are prepared from **virus grown in embryonated** eggs and then **chemically inactivated**. Ideally the vaccine incorporates antigens of the A and B influenza strains that will be prevalent in the community during the upcoming winter. Persons with allergies to eggs should not get the vaccine. A **live vaccine** is also available for administration as a nasal spray. The trivalent vaccine consists of reassortants for the HA and NA gene segments of different influenza strains with a master donor virus that is **cold adapted** to optimum growth at 25°C. **This vaccine will elicit a more natural protection**, including cell-mediated, antibody and mucosal-secretory immunoglobulin (Ig)A antibody.