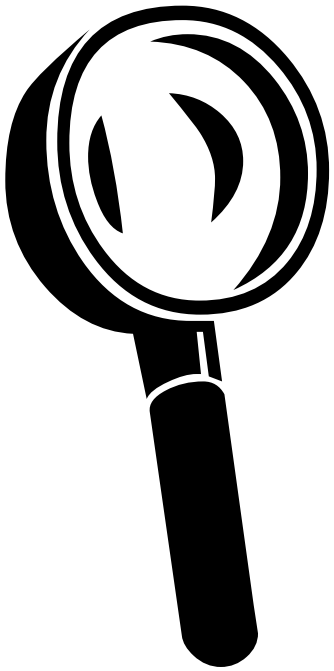

Institute for Microbiology shows

TRACING THE PATHOGEN

Part eleven:

Cooperation at investigation or Clinical Microbiology II

Survey of topics

Respiratory infections – introduction

Indications for examination in respiratory infections

Sampling and examination in respiratory infections

Processing and interpretation of respiratory specimens

Importance and classification of digestive tract infections

Sampling and examination in intestinal infections

Respiratory infections - introduction

Importance of respiratory infections

- The **most common infection** in general practitioner's (microbes multiply well in respiratory ways)
- Big **economy impact** (inability to work, necessity for parents to stay at home with ill children)
- Often seen in **collectives** and sometimes causing outbreaks
- ¾ of respiratory infections (even more in children) caused by **viruses**

Localization of infection in respiratory infections

- **It is not the same, which part of respiratory ways is affected by the infection (examination, treatment and seriousness is different).**
  - Symptoms of infections of different parts of respiratory tract are different (sneezing in rhinitis, cough in lower respiratory ways infections)
  - Causative agents are different, too
- **It is necessary do differentiate infections of:**
  - Upper respiratory ways (+ anatomically also middle ear)
  - Lower respiratory ways including lungs ***(lungs are often put aside as it is not a „way“)***

On the other hand the infection often affects more parts of respiratory ways simultaneously

Classification of respiratory infections

Upper respiratory ways and connected organs

- Infections of nose and nasopharynx
- Infections of oropharynx and tonsils
- Infections of paranasal cavities

Sometimes middle ear infections are also counted here (for anatomical reasons)

Lower respiratory ways and lungs

- Infections of epiglottis
- Infections of larynx and trachea
- Infections of bronchi
- Infections of bronchioles
- Lung infections

Flu is not „flu“

- Majority of common acute respiratory infections are rhinitis, pharyngitis of rhinopharyngitis. Epidemiologists would use abbreviation „**ARI“ – acute respiratory illness** People often speak about a „flu“, but it is no flu
- True influenza (flu) attacks rather lower respiratory ways, there is a dry cough and general symptoms (tiredness, fever). Nevertheless, parainfluenza and many other diseases are similar. Epidemiologists would call it **„ILI“ (influenza-like illness).**

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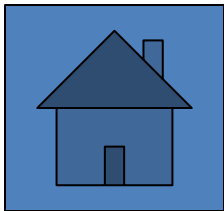
Normal inhabitants of respiratory ways

- **Nasal cavity** has no specific microbiom, there is skin microbiom (frontal part) and pharyngeal microbiom (back part)
- **In pharynx** (and also oral cavity) we can find oral streptococci, Neisserias, non-virulent strains of haemophili etc. Many other strains are also present, but we cannot culture them.
- **Lungs and lower respiratory ways** use to be nearly microbes-free in a healthy person
- **Other sites** (larynx) have transient microbes (larynx – like pharynx, but less microbes)



Indications for examination in respiratory ways

Examination and treatment in infections of nose and nasopharynx

- **Examination is useless.** Even mucopurulent secretion is not a reason for bacteriology examination, if it does not persist too long.
- **Therapy is symptomatic** (drops in congested nose; otherwise liquids, e. g. tea; antipyretics are not too useful, as elevated temperature helps against viruses). Antibiotic treatment is not indicated. Sometimes topic treatment by framycoin may be used.
- **Only in case of infection continuing more than 10–14 days** it is useful to examine nasal swab (to avoid skin contamination!) and to use targeted antibiotic treatment according to susceptibility

What do the experts say

„More than 80 % of rhinitis is accompanied by changes on paranasal cavity mucosa, therefore the disease is sometimes also called rhinosinusitis. The cough is present in 60–80 % rhinosinusitis cases. Mucous secretion is in three days after infection oncome changed into a mucopurulent one, containing desquamated epithelial cells and colonizing bacteria commonly found in nasal cavity. This qualitative change of secretion, in practice commonly misinterpreted for „bacterial superinfection“, especially in case of cultivation examination of mucus of nasal swab; but it is a part of a normal rhinitis course.“

(Respiratory infection – recommendation guidelines by Czech Medical Association of John Evangelist Purkyně)

Examination and treatment of sinusitis

- **Treatment** of sinusitis of probable bacterial origin should be done immediately, even without examination.
- **Drug of choice** is amoxicillin (e. g. AMOCLEN) or amoxicillin-clavulanate (AUGMENTIN, AMOKSIKLAV), alternative might be doxycycline (DOXYBENE), in children co-trimoxazole (e. g. BISEPTOL)
- Examination of **nasal swab or throat swab is useless**.
- If we are in doubts about treatment and we want to use targeted treatment, the only possibility is **properly performed punction or washing on oto-rhino-laryngology,** of course, if it would be washing, then no boric acid!! **The request form should include an information**, whether it is a pure pus or washing with physiological saline

Examination and treatment of otitis media

- **Treatment** is only meaningful if it is a real inflammation (pain, redness, fever) and it does not react to anti-inflammatory treatment
- **Drug of choice** is amoxicillin (e. g. AMOCLEN), as alternative, co-trimoxazole can be used
- It has only sense to examine **external ear examination** after paracenthesis
- Otherwise it is useful to send **pus containing fluid**, sampled during paracenthesis

Throat infections – dg. and treatment

- Always **throat** (tonsillar) **swab** should be performed to check bacterial origin and pathogen determination. ***(The mere fact that it is not performed usually does not mean that it is correct.)***
- As it is usually not possible to wait for cultivation result, we perform **PCR examination** (elevated in bacterial infections, typically over 60 mg/l – in viral infection less than 40 mg/l), the result is available much sooner
- **The treatment should be targeted**. In tonsillitis caused by *Streptococcus pyogenes* (and that is the majority) the first choice drug is **V-penicillin.** Macrolids (RULID, KLACID, SUMAMED, AZITROX) should be only used in allergic patients.
- Eventually also EB virus (infection mononucleosis virus) and cytomegalovirus serology

Examination and treatment of laryngeal (and tracheal) inflammations

- It is nothing to examine. It is useless to perform throat swab, as there are completely different bacteria in throat. So, microbiological examination is not performed, except specific situations (chronic disease)
- **Treatment is symptomatic**. Antibiotics are not indicated, not regarding the circumstances.

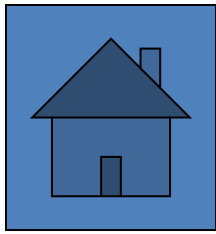
Examination and treatment of inflammations of bronchi and bronchioles

- Basic is **clinical examination** that shows development of cough with expectoration, without findings on the lung tissue (according to X-rays and clinical examination)
- **Microbiology examination** is almost useless. In case of pus expectoration sputum may be sent because of the risk of secondary infection. In this case, also CRP could be measured. It is also possible to sent blood for serology of respiratory pathogens
- **Antibiotic treatment is almost useless**, in macrolids and tetracyclins might be used

Special situation: acute deterioration of chronic bronchitis

- Characterized by
  - Cough deterioration
  - Elevated expectoration, change of character and colour of sputum
  - Often deterioration of breathlessness
- **Causative agents: less than 40 % viruses**
- Among bacteria, the most common agents are *Haemophilus influenzae, Streptococcus pneumoniae* or *Moraxella catarrhalis.*
- Routine antibiotic treatment of patients is not recommended
- **Antibiotics have only effect in cases with presence of all three disease symptoms**

Microbiology examination: lung infections



- **In classic community pneumonias**
  - blood for blood culture (haemoculture)
  - sputum – microscopic and basic culture examination
  - sputum – cultivation of *Legionella pneumophila**
  - urine – detection of antigen of *Legionella pneumophila**
- **In atypical pneumonias**
  - blood – serology examination (antibody detection
  - blood culture and sputum for bacteriology (for sure)
  - virology examination (serology, direct detection)
  - sputum – direct detection of agent (EIA, PCR)
- Special cases: TB (sputum for TB), lung aspergillosis (BAL culture, detection of antigens in blood, detection of antibodies)

Pneumonias caused by Legionella pneumophila are borderline between classic and atypical pneumonias.

Specimens and examination in respiratory infections

Specimens for respiratory infection diagnostics – globally (1)

- For **bacteriology** we send
  - **swabs** – (throat, tonsillar, nasal etc.), always with **transport medium** (e. g. Amies medium), describe the localisation
  - **sputum, tracheal aspirate or bronchoalveolar lavage,** eventually also endotracheal canulas and similar specimens – for bronchitis and pneumonia *(eventual request for TB should be written on the form!)*
  - **blood culture** in pneumonias
  - **urine** for Legionella antigen
- For **mycology examination** swab in FungiQuick (but also common Amies) is sent

Specimens for respiratory infection diagnostics – globally (2)

- **Viral** agents are usually not examined
- In rare need for viral agent determination we use **nasopharyngeal or bronchoalveolar lavages** with special medium, or **blood for serology** of **respiratory viruses** (i. e. for antibodies; we have to count that antibodies are only formed one or two weeks after start of the disease)
- **For influenzavirus** we use swab from rear face of pharynx using special transport medium

Throat swab – technique

- **Sampling material:** Swab with plastic stick in Amies transport medium.
- **Way of sampling:**
  - The swab is placed **behind the palatal arcs** with help of a spatula without contacting the oral mucosa.
  - By rolling movement **the surface of both tonsils and palatal arcs** is swabbed so that sufficient amount of mucosal secretion would be sucked in the swab.
  - Simultaneously **back side of pharynx is swabbed**.
  - The swab is **pulled out carefully** to avoid its contamination, and placed to a special test tube with transport medium
- **Storage:** Maximum 24 h at room temperature *(for gonorrhoea do not store it and send it immediately)*
- **Transport:** Maximum 2 h at room temperature

Nasopharyngeal swab („pertussoid“ syndrome, suspicion for pertussis)

- **Specimen: A wired** swab; for Bordetella, inoculate immediately to a special culture medium, for Haemophilus it is sufficient to send in a transport medium
- **Sampling:** The end part (approx. 3 to 4 cm) of the swab on wire is flexed using the edge of test tube to the 90°, lead through oral cavity behind palatal arcs to the back side of the nasopharynx without touching the mucosa of oral cavity or tonsils. By circulating, punka-like movement the swab from pharyngeal mucosa is done (cotton-up)
- **Storage:** Immediate transport to the laboratory
- **Transport:** Maximum two hours at the room temperature.

Sputum sampling

- **Specimen:** Sterile transparent plastic container with a screw cap.
- **Sampling:**
  - **Sampling is always performed at supervision of a nurse or a doctor.**
  - Patient washes the oral cavity and gurgles with water (decrease of oral bacteria contamination)
  - **After that, the patien should deeply cough so that to press out the secretion from lower respiratory ways, not saliva or nasopharynx secretion**.
  - So gained sputum is kept in a sterile container in volume of minimally 1ml.
- **Storage:** Maximum 24 h at room temperature
- **Transport:** Maximum 2 h at room temperature

Possible examinations in lung infections

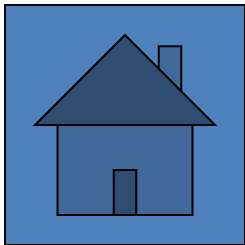
- The basis is **clinical examination and X-rays**, important differentiation classic × atypical pneumonia (different spectrum of causing agents)
- **In classic pneumonias** properly taken sputum has sense, eventually (especially in septic course) also blood for blood culture
- **In atypical pneumonias** serology of Mycoplasma and Chlamydia (eventually in frame of „serology of respiratory viruses“
- **In hospital pneumonias** it also might be useful to perform **examination for Legionella**. Besides culture examination it is also possible to examine urine for Legionella antigen, eventually serology

What to write on a request form

- In addition to filling in the usual fields (name, number of the patient...) is an important field of the request, what is to be examined.
- **Examples of formulation on the request form:**
  - throat swab for bacteriology
  - fluid gained during paracentesis of frontal sinuses for bacteriology + yeast examination
  - blood for serology of agents of atypical pneumonias
  - sputum for bacteriology
  - sputum for TB (culture + PCR)
  - blood culture No. II from a venipuncture
  - bronchoalveolar lavage (BAL) for *Pneumocystis jirovecii*

What to know

- **The request form** should contain an information, what type of specimen is it, what testing is required, and, where appropriate, other relevant information
- Microbiologist has the right to **reject the wrong sample of sputum (non-pyogene**, does not contain leucocytes, only epithelia → it is saliva!!!)
- **TB culture** takes several weeks, similarly also culture of some fungi
- For **virology and detection of various antigens** the speed of examination depends mainly on the organization of work



Processing and evaluation of respiratory specimens

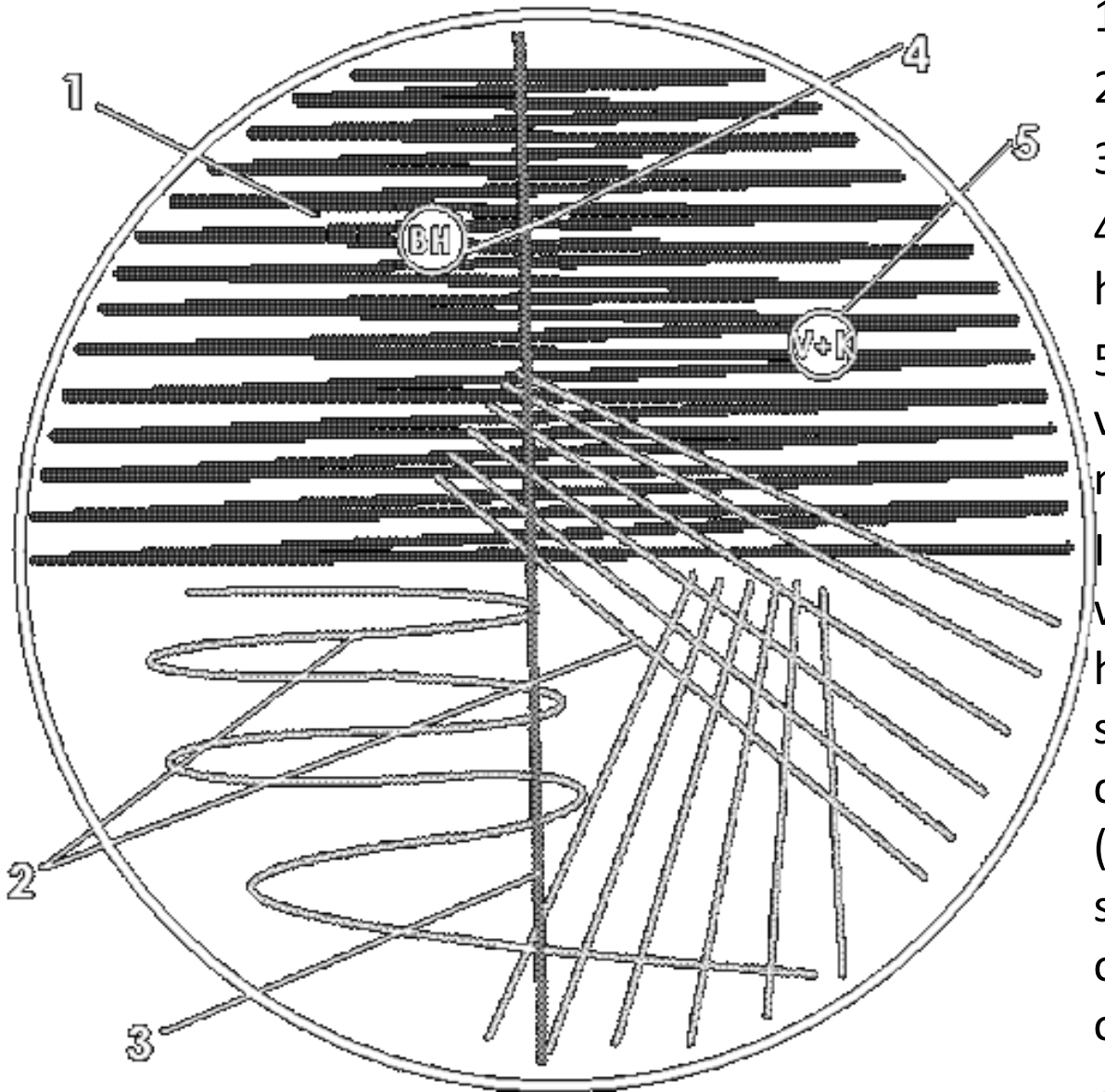
What happens with the samples in the laboratory

- Most swabs are cultured on **blood agar**. On the agar we place disks, whose aim is to suppress the normal flora and to allow detection of pathogens. Because of *Haemophilus*, which would be only able to grow there in presence of *S. aureus*, we inoculate a *S. aureus* line on the agar. (Note: Some laboratories do not search for *Haemophilus* as they consider it to be normal in pharynx.)
- In sputum and similar samples, **microscopy is used**
- Besides blood agar, **more media** (Endo) are used
- **Virology samples** are isolated on the eggs or tissue cultures, antigen detection is performed
- In **serology specimens** we search for antibodies

How to find a possible pathogen among common oropharyngeal flora

- Normal flora consists of greyish, viridating colonies (oral streptococci) and yellowish, usually a-haemolytic colonies (oral Neisserias). They use to make a dense „carpet“ on the surface of agar medium and they make search for pathogens quite difficult, nevertheless possible:
  - Haemolytic streptococci (and also *Staphylococcus aureus*) are visible by a strong haemolysis on blood agar
  - For Haemophilus detection we use antibiotic disc with bacitracin – higher concentrations than in bacitracin test (to decline the normal microflora)
  - For meningococcal detection we use another disk, with mixture of vancomycin and colistine

Detection of pathogen in throat/sputum



1 swab inoculation

2 loop inoculation

3 staphylococcus line

4 bacitracin disc (for hemophili)

5 V + K disc (colistine and vancomycine) for meningococci

In all parts of inoculated area we search for colonies with haemolysis. They could be streptococci (rather colourless) or *S. aureus* (rather goldish). We also search for pneumococci (like oral streps, but coin-shaped, or large, mucous

Cultivation result of throat swab with common flora and a pathogen

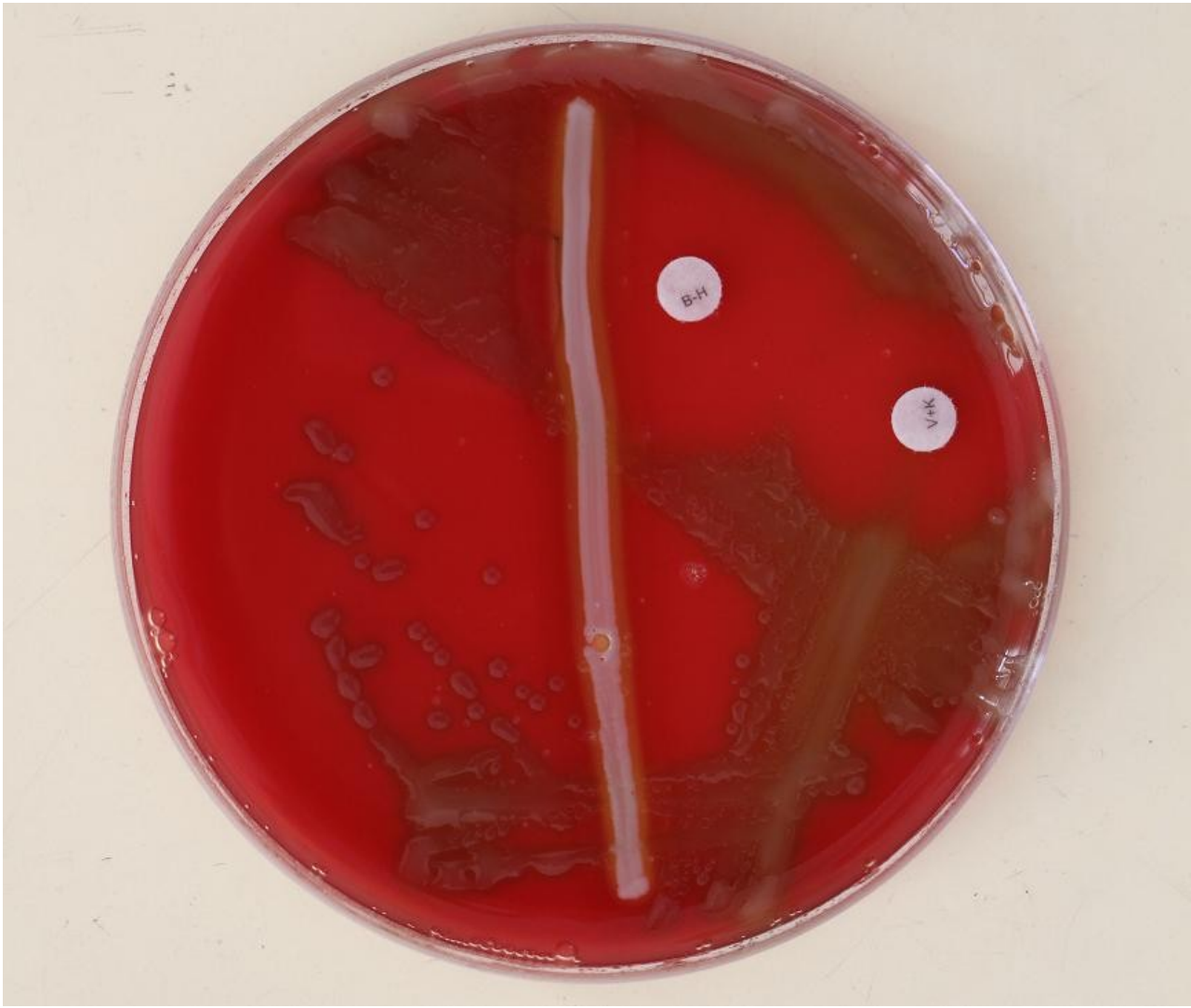


Photo: Filip Daněk

Explanations to following screens

- BA – blood agar
- EA – Endo agar; usually, McConkey agar may be used as an alternative
- BA+AMIK – blood agar with amikacin, selective for streptococci a enterococci
- NaCl – BA with 10 % NaCl, selective for stafylococci
- B – broth

Sputum examination
Diagnostic scheme (1)

- Day 0: microscopy (Gram staining)
- Day 1: result of primary culture on BA and EA. If only common flora is present, EA is discarded and BA is prolonged to another day. An eventual pathogen is identified and its antimicrobial susceptibility assessed. If there is a small amount of a pathogen, isolation is performed (colony is carefully picked by a loop and reinoculated to a new agar plate to obtain a pure culture)
- ***NaCl*** *is not used in sputum specimens, but is used in some other specimens (tracheal aspirations, bronchoalveolar lavage) it is used.*

Sputum examination
Diagnostic scheme (2)

- Day 2: expedition of negative results (observation of prolonged BA cultivation). Expedition of majority of positive results, if identification is finished antibiotic test result is OK. If not (too many resistances, more atb needed), or if only isolation is done, it is necessary to continue.
- Day 3: expedition of majority of remaining positive results (resistant, difficult detection…)
- Day 4: extraordinarily expedition of remaining results (combination of several problems)

Sputum – possible findings

- Common flora: There is no flora in LRT, but always a contamination from URT is present: oral streptococci and Neisserias
- Pathogens: pneumococci, pyogenous streptococci, haemophili (typical pneumoniae). Causative agents of atypical pneumoniae are mostly non-culturable.
- One of typical findings is *Staphylococcus aureus*, you can use treatment using oxacilin, eventually, if oral oxacillin would not be available, to use Ist generation cephalosporins.

Practical note

- Small, greyish, nearly colourless, viridating, are oral streptococci.
- Small, yellowish, without viridation, without haemolysis (or a slight partial haemolysis), oxidase positive are oral Neisserias
- If there is something more on our plate, and especially if this „something“ has a strong haemolysis, it is probably the expected pathogen.

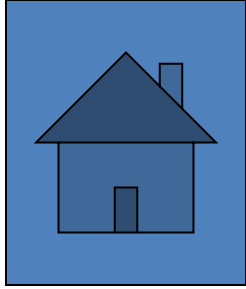
„Reading“ of bacteriology

Throat swab
Diagnostic schedule

- Day 0: only start of the cultures
- Day 1: result of primary culture of specimen on BA and EA. NaCl is not used here. Here, too, BA cultures with common flora are prolonged
- Day 2: expedition of all negative and majority positive results
- Day 3: expedition of mostly all remaining results

Throat swab – common flora and pyogenous streptococci

Pharynx – possible findings



- Common flora: Oral streptococci a Neisserias; haemophili (mostly *H. parainfluenzae*), but normal are also small amounts of *S. aureus*, pneumococci, meningococci, Moraxellas etc. More components of common flora (anaerobes, spirochetes) are not found in normal culture
- Pathogens: pyogene streptococci, arcanobacteria; often nothing is found and it is viral origin (EB viruses and others)
- Treatment: In case of *Streptococcus pyogenes* found to be a pathogen, V-penicillin is used.

Importance and classification of digestive tract infections

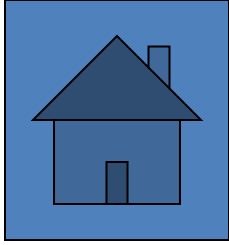
Importance of digestive tract infections

- Many of them are transmitted by **contaminated food and water**
- Unpleasant **economic loses** not only for infected people, but also for their contacts
- For prevention, **hygiene** in **food industry** and food stores and protection of **water sources** is basic
- Important is also **personal hygiene** including oral cavity hygiene
- In therapy **use of antibiotics is only exceptional**

Classification of digestive infections

- **We speak about**
  - Infections in the **oral cavity**
  - Infections of **pharynx** – see respiratory infections
  - Infections of **oesophagus** – very rare, usually secondary after originally non-infectious disease
  - Infections of **stomach** (or rather cooperation of gastric microbes in some diseases)
  - Infections of **small intestine** (enteritis)
  - Infections of **large intestine** (colitis)
  - Often infections of both parts (enterocolitis)

Normal microflora of GIT



- **Lips** are transition between skin and oral flora
- **Oral cavity** (as in pharynx) we find oral streptococci, Neisseria, avirulent Haemophilus strains etc. Many others are present, but cannot be cultured.
- **Oesophagus and stomach** are normally microbe-free
- **In small and large intestine** we usually find approx. 1 kg anaerobes, also Enterobacteriaceae, enterococci, yeasts, sometimes even non-pathogenic amoeba
- **Anus** is again transition intestine-skin

Sampling and examination in intestinal infections

Sampling and stool transport for individual examinations

- **Bacteria** – in Amies transport medium
- **Yeasts** – better in FungiQuick medium, but substantially Amies medium is also sufficient
- **Viruses** – hazelnut-sized specimen; for isolation of a virus cooling is necessary
- **Parasites** – hazelnut-sized again, not necessarily sterile. Traveller anamnesis necessary. Usually three specimens (one negative does not mean complete positivity)
- **Toxin of *Clostridiodides (Clostridium) difficile*** – liquid stool in a test tube
- **Pinworms** – Graham method – perianal moulage on a special tape, for microscopy
- ***Intoxications by bacterial toxins*** – *vomit, food remainders*

Stool sampling for bacteriology

- Patient stands (kneels) and is supported by hands (elbows), or is in lying position
- Sampling swab is **carefully pushed behind the anal sphincter**, by careful rotation the surface of anal mucosa and crypts is taken
- At normal sampling **stool is macroscopically visible** on swab surface
- The swab is placed into a test-tube for transport. It should be merged **deep in the medium**. The test-tube should be well re-capped
- Storage and transport **at room temperature**, preferred soon delivery without storage
- Request form should contain **patients address**

Why address?

- In case of obligatory pathogen (Salmonella, Shigella, Campylobacter, Yersinia) finding, the laboratory (in Czechia) is **obliged to send a report to regional public health office** that contacts the ill person for depistage (to find the source of infection, and also to know possible risks for other people)
- In case of missing address a **telephonic question** is addressed to the doctor that has sent the specimen

Piece of stool sampling (parasites, viruses)

- For sampling we use a **container with a scoop, sterility is not required,** especially for parasites
- After defecation a **hazelnut sized bit of stool** (not smaller) is taken, not from surface, to avoid contamination. If the stool is liquid (for *C. difficile* typically) the volume should be about the same.
- Examination for parasites requires examination **several times, usually three following days**
- Material can be **stored in refrigerator**, but not frozen
- In case of examination for lamblia, **fresh** material is recommended; it is better to arrange the timing of sampling with the laboratory. At viral isolation storage at 0 °C is necessary
- If the stool is liquid, it is possible to use any container for sampling. This is also valid for examination for the toxin of *Clostridioides difficile*

More about stool for parasites

- Traveller anamnesis is necessary, not only „was abroad“, but also what countries he/she visited
- In case of **macroscopic finding of a whole parasite** (e. g. roundworm), it is possible to send the complete organism in a test-tube
- Be careful – patients often use to insist on saying that a worm was present in their stool, but in fact the organism (e. g. earthworm) just fell from the window parapet
- Sometimes the „being sure“ about presence of a parasite is a part of psychiatric diagnosis of the patient

Sampling for pinworms (Graham method)

- The sampling is done **in the morning without washing** (the female pinworms lay eggs to perianal region during the night)
- Prior to sampling, the **transparent (!)** tape is carefully removed from the slide, placed to perianal region, the thighs are pressed one against the other, released and the tape is placed back to the slide
- In adults (it would be painful because of hairs) we rather use **stool sampling** (with lower effectiveness), of we use **Schüffner stick**

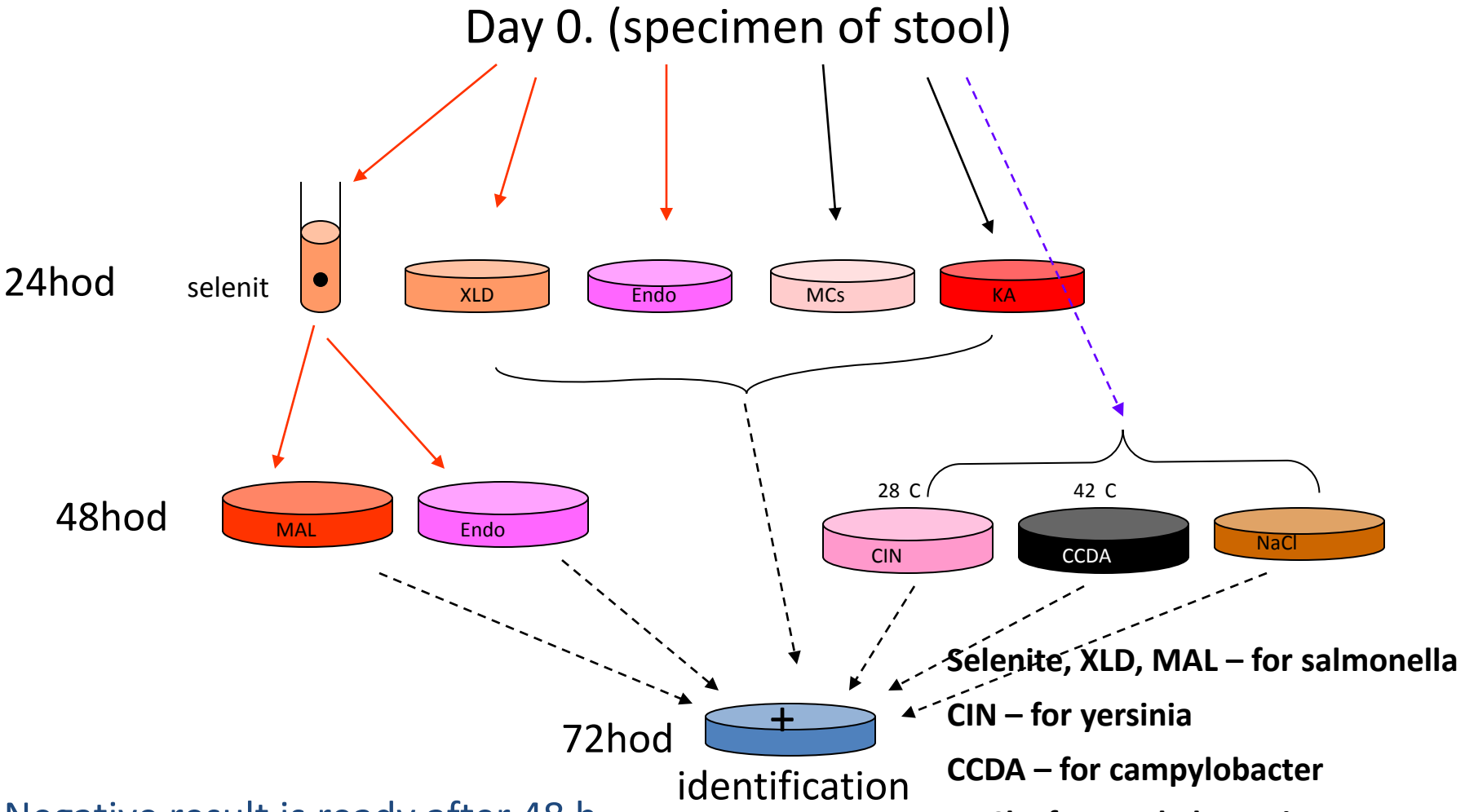
Diagnostics of bacterial pathogens

- **Microscopy** has low practical importance only
- **Cultivation** is performed on various media (choice depends on the patient‘s age and diagnosis, in travellers eventually more rare media are added), found pathogens are identified – see further
- **Direct detection of A and B toxin (*Clostridioides difficile*)** as antigen. Toxin detection is more important than mere finding of clostridium or its structural antigen – the antigen may be even present in healthy persons, but positive toxin means serious problem

Diagnostics of viral agents: usually antigen detection, eventually nucleic acid detection

Diagnostics of parasitic and fungal agents: see more in mycology and parasitology lessons

Stool cultivation



Selenite, XLD, MAL – for salmonella

CIN – for yersinia

CCDA – for campylobacter

NaCl – for staphylococci

MCS – for some STEC strains

Endo – for various enterobacteria

KA – for some more bacteria

Negative result is ready after 48 h

Positive in 72 h or more

*If not written differently, culture runs at 37 °C

Identification of a bacterium

Bacteria are **cultured on various media**; after the culture we „read“ the result so that

- On Endo agar we try to identify normal flora (mostly *Escherichia coli*) and possible pathogens
- On other media it is just
  - **„suspicious“** = a microbe was found that resembles the positive control (more diagnostic procedures needed)
  - **„negative“** = it does not always mean „no growth“, but also „bacteria different from the control“

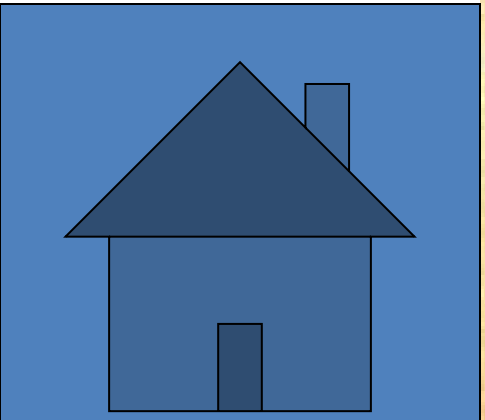
Bacteria are further diagnosed by **biochemical tests or other diagnostic approaches (MALDI-TOF)**

In some cases (*Salmonella, Escherichia* in small babies) it is necessary to perform **antigen analysis of the strain**

Interpretation of stool examination

- In results of stool examination it is necessary to differentiate whether they are **primary pathogens** (Salmonella, Shigella, Yersinia, Campylobacter) or **secondary pathogens**; in some secondary pathogens (especially *Escherichia coli*) further determination is needed (EPEC, STEC, EAggEC etc.)
- Interpretation should be done in **context of clinical signs** (in high quantity of „non-pathogenic amoeba“ and serious symptoms the treatment might be useful)
- In case of *Clostridioides difficile* infection it is important to know whether **clostridium toxin** is positive. Nevertheless, also positive antigen and negative toxin is considered important, if there are relevant clinical signs.

The end



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