Microbiology I

Preanalytical phase of microbiological diagnostics

Jakub Hurych

Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University and Motol University Hospital

What do you need medical microbiology for?

- Knowledge of infectious agents, their diagnosis and treatment

- Your first encounter with clinical medicine - we will already be teaching you to think like a doctor

- The knowledge of microbiology is needed in all branches of medicine
  - Pathology
  - Infectious medicine
  - Internal medicine, paediatrics, surgery
  - And all the others

Medical Microbiology at the our faculty

- One of the biggest, most subjectively difficult tests
- Can't be done in a week or a month
- Requires continuous learning in both semesters
  - In the summer term you have to learn microbes and methods
- = make your own bricks
  - In the winter term you learn clinical procedures
- = you build a house out of bricks

If you don't have finished bricks, you can't build, you don't understand, you don't keep up, etc.

Aims

- **The aim is to prepare you for a stay in the world of real medicine**
- **Microbiology I and II** - examination in the 3rd year of the ZS
- **Practical part - 1 question**
  - knowledge of sampling material in a given situation
  - indication of a portfolio of tests for the diagnosis of infectious agents
  - examination methods,interpretation of findings
- **Theoretical part - 2 questions**
  - Special microbiology (viruses, bacteria, fungi, parasites)
  - Antimicrobial agents
  - Clinical microbiology
- **Organisation**
  - You must make your first attempt during the winter exam period
  - Make-up dates are announced during the summer term, summer exam period and in September
- **Clinical Microbiology- exam in 4th year**
  - Differential diagnosis of findings, their interpretation and resolution
  - Correct indication of ATB in individual clinical situations

Conditions for credit

- **Presence** - you have two allowed absences
  - Weeks 2 and 3 are mandatory (see Dean's Provision 4/2022)
- **Credit test** in weeks 6 and 13, 60% limit (12/20 bb)
  - If you fail to do any of these, you will be tested by the group assistant

- You can substitute with another group after a discussion with the group assistant
- If you want to attend the training at another time, the exchange is ideally "piece for the piece" or by agreement with the Deputy Head of Teaching

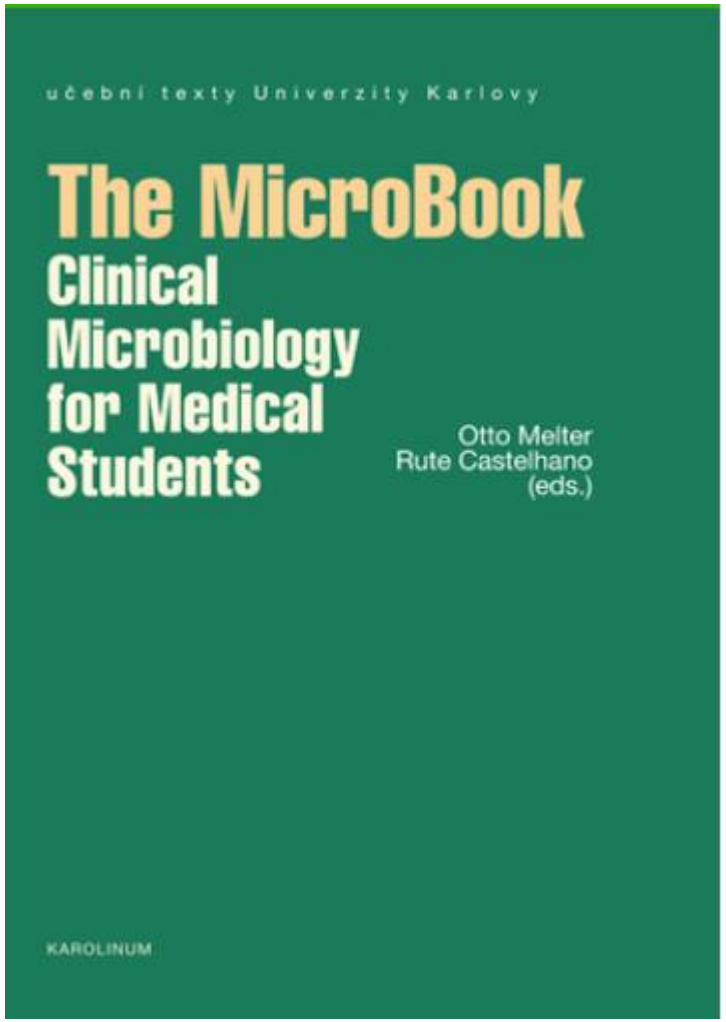
How will the lessons be conducted?

How to learn it?

- **Lectures**
  - We take it for granted that you attend
  - Based on the content of the lectures and practices, we test you
  - Who doesn't want to, has to get it out of the book
- **Practical training**
  - Application of knowledge from lectures - it won't be repeated here

Resources

- Books
- Moodle etc.
- Me ☺ **jakub.hurych@lfmotol.cuni.cz**



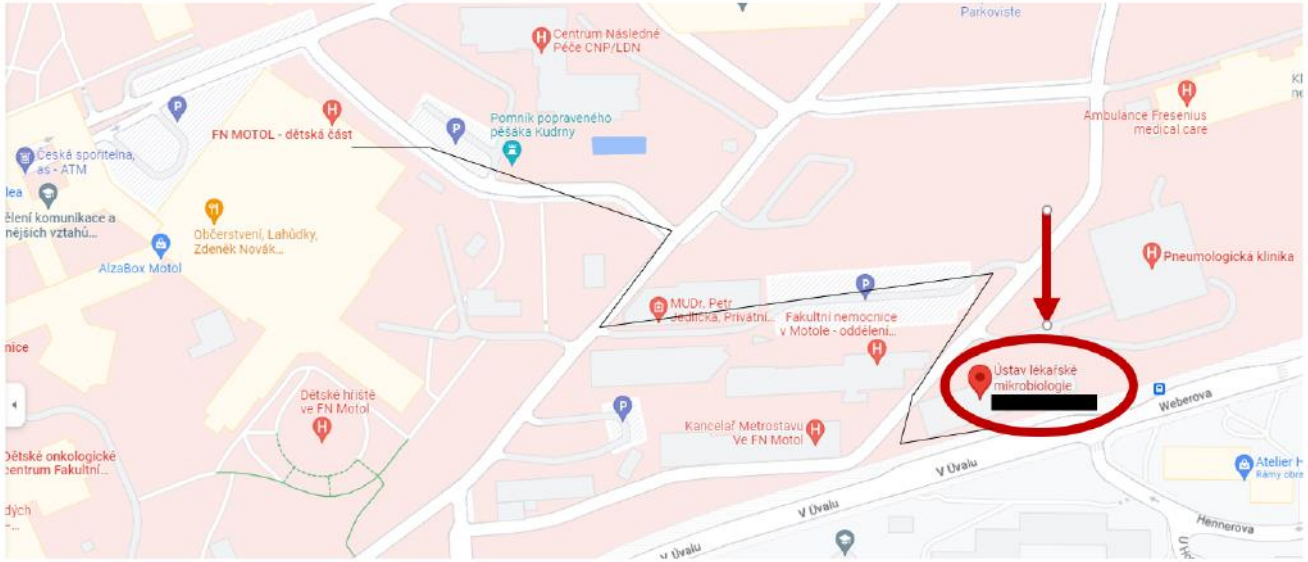
Week	Date	Lecture (Wednesday 8:00-9:40)	Lecturer	Practical training	Date	Lecturer
1	22.02.2023	A broader introduction to the field of medical microbiology, basics of bacterial cell genetics	Prof. MUDr. Pavel Dřevínek, Ph.D.	Preanalytical phase of microbiological diagnostics	21.02.2023	MUDr. Jakub Hurych
2	01.03.2023	Bacterial cell. Pathogenicity of bacteria.	Mgr. Jan Tkadlec, Ph.D.	Microscopy in bacteriology	28.02.2023	MUDr. Jakub Hurych
3	08.03.2023	Introduction to antibiotics. Systematics of antimicrobials I.	Prim. MUDr. Otakar Nyč, Ph.D.	Cultivation of bacteria and procedures leading to the identification of bacteria	07.03.2023	MUDr. Jakub Hurych
4	15.03.2023	Systematics of antimicrobials II.	Prim. MUDr. Otakar Nyč, Ph.D.	Antimicrobials susceptibility testing (AST)	14.03.2023	MUDr. Jakub Hurych
5	22.03.2023	Significant G+ cocci I (staphylococci)	Mgr. Jan Tkadlec, Ph.D.	Serological methods	21.03.2023	MUDr. Jakub Hurych
6	29.03.2023	Significant G+ cocci II (streptococci and enterococci) **- CREDIT TEST I.**	Mgr. Jan Tkadlec, Ph.D.	Diagnostics of major G+ cocci I (staphylococci)	28.03.2023	MUDr. Jakub Hurych
7	05.04.2023	Significant G+ rods (corynebacteria, listeria, clostridia)	Doc. MVDr. Otto Melter, Ph.D.	Diagnostics of major G+ cocci II (streptococci and enterococci)	04.04.2023	Doc. MVDr. Otto Melter, Ph.D.
8	12.04.2023	G- bacteria I (non-fermenting rods, enterobacteria)	Prof. RNDr. Alexander Nemec, Ph.D. et Ph.D.	Diagnostics of important G+ rods (corynebacteria, listeria, clostridia and bacilli)	11.04.2023	Doc. MVDr. Otto Melter, Ph.D.
9	19.04.2023	G- bacteria II (culture challenging: bordetella, Legionella, haemophilus, meningococcus)	Doc. MVDr. Otto Melter, Ph.D.	Diagnostics of G-bacteria I. (non-fermenting rods, enterobacteria)	18.04.2023	Doc. MVDr. Otto Melter, Ph.D.

10	26.04.2023	G-bacteria III (Campylobacter, Helicobacter). G+ and G- anaerobic bacteria (except clostridia).	Doc. MVDr. Otto Melter, Ph.D.	Diagnostics of G-bacteria II - culturally challenging (bordetella, Legionella, haemophilus, meningococcus)	25.04.2023	MUDr. Jakub Hurych
11	03.05.2023	Medical mycology	MUDr. Daniela Lžičařová	Diagnostics of G-bacteria III - culture challenging bacteria (*Campylobacter* , *Helicobacter*) and anaerobic bacteria	02.05.2023	MUDr. Jakub Hurych
12	10.05.2023	The Rector's day - NO LECTURE	NA	Diagnostic methods in mycology	09.05.2023	MUDr. Daniela Lžičařová
13	17.05.2023	Atypical bacteria I (rickettsia, coxiella, bartonella, chlamydia, mycoplasma) **- CREDIT TEST II.**	Doc. MVDr. Oto Melter, Ph.D.	Diagnostic methods in parasitology	16.05.2023	Doc. MVDr. Oto Melter, Ph.D.
14	24.05.2023	Atypical bacteria II (mycobacteria, spirochetes)	Doc. MUDr. Pavel Čermák, CSc.	**Credits**	23.05.2023	MUDr. Jakub Hurych

How to get to us?

- Come all at once.

- The main entrance from the road - ring for the Secretariat



Entering the infectious zone

- Class is held in an infectious environment.
- **Keep all your belongings in the lockers** (you will be given a key on entry).
- Please don't bring anything with you to the practical training (this applies to all books, notebooks, etc.). Always leave food and drink in the lockers, you will be able to get to them if necessary.
- Always carry out **thorough hand hygiene** (soap and alcohol disinfection) when entering and leaving the practice room.

Wash your hands

Steps to wash your hands with soap and water

Scrub your hands for at least 20-40 seconds

Lather hands with soap and water and rub hands palm to palm

Right palm over back of left hand with interlaced fingers and vice versa

Palm to palm with fingers interlaced

Backs of fingers to opposing palm with fingers interlaced

Rotational rubbing of left thumb clasped in right palm and vice versa

Rotational rubbing backwards and forwards with clasped fingers of right hand in left palm and vice versa

Rinse hands with water

Dry hands on single use towel or air dry and your hands are safe

MINISTRY OF HEALTH & MEDICAL SERVICES

PHD Public Health Division

Pacific Community Communauté du Pacifique

Training room = laboratory

- **Follow the safety rules**
  - Sharp objects
  - Chemicals
  - Fire
  - Electricity
  - Turn off microscopes
  - Stroke broken slides before you cut yourself
- The toilet is across the hall. Watch your heads.

Sign our H&S awareness form

Questions?

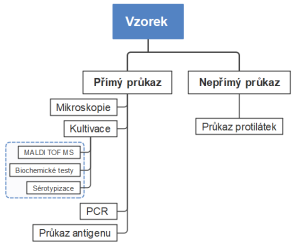
Ask.

- nagano semifinále

Who are you, actually?

General principles of microbiological diagnostics

General principles



Symptom
Cough, fever

→

Working diagnosis
Pneumonia?

→

Sampling
Sputum

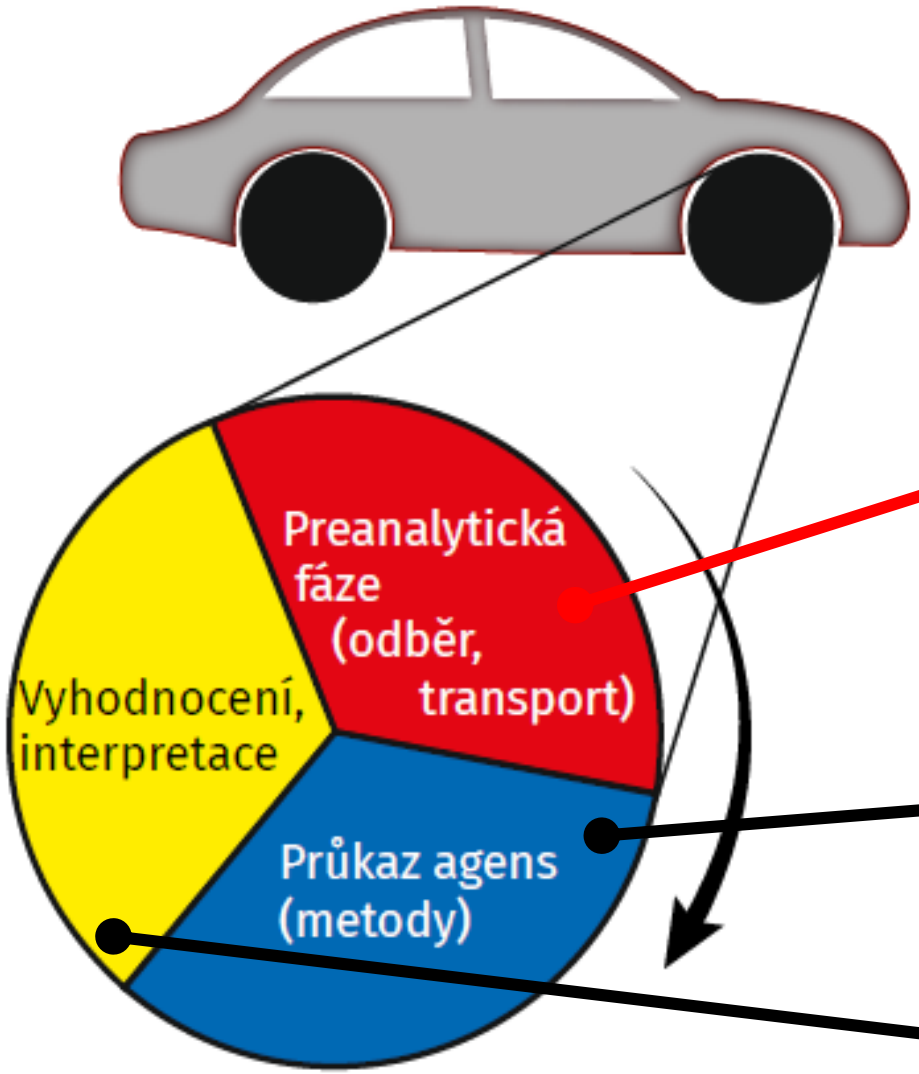
→

Microbiology examination

- Direct methods
  - Microscopy
  - Cultures
  - PCR
  - Antigen test
- Indirect methods
  - Serology (Antibodies)

- Patient has symptoms - choose the most appropriate type of sample
- Correct collection technique, instrumentation and container
- Correct transport and admission to the laboratory
- Agent identification (select method)

Detection of the agents



Today

This semester

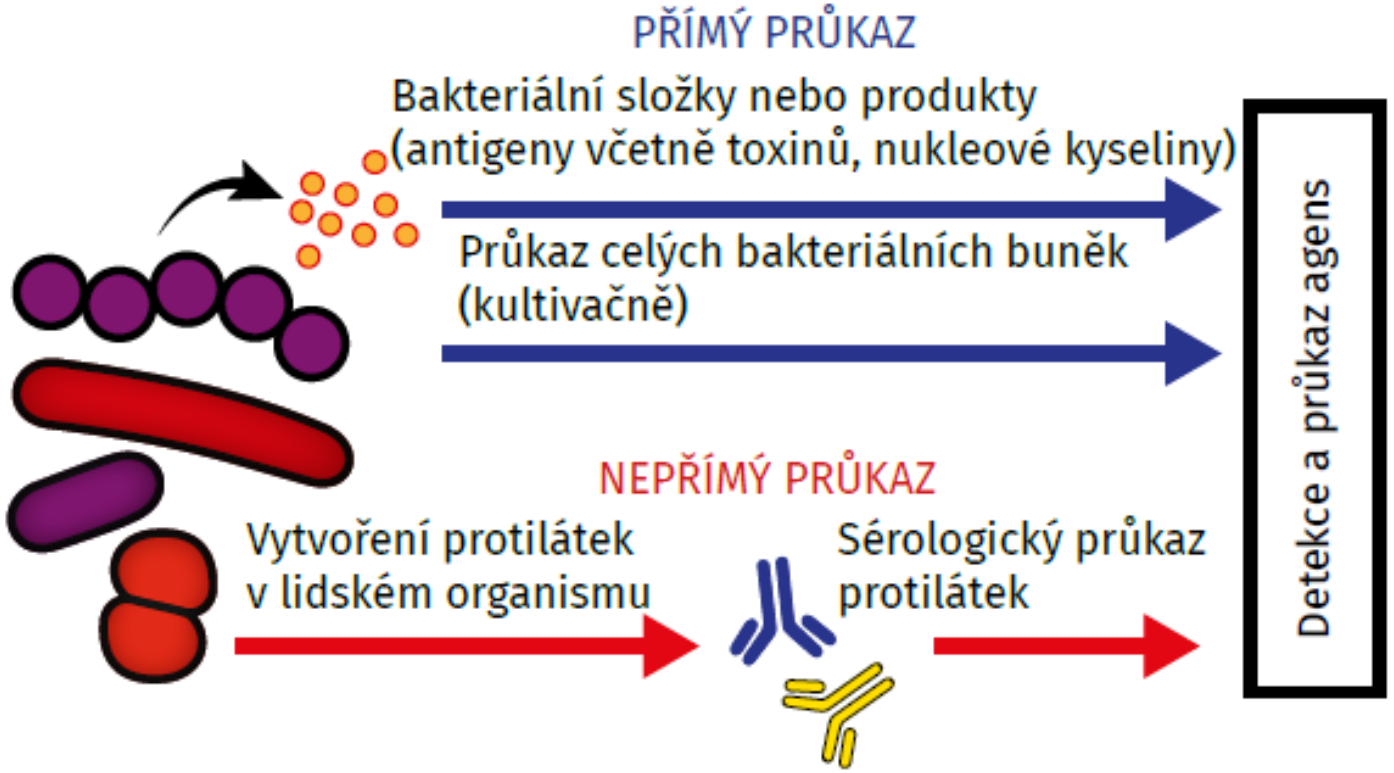
Next semester

DETECTION OF MICROBIAL AGENTS

DETECTION + IDENTIFICATION

Detection - capture of the n

Identification - accurate determination of genus/spe

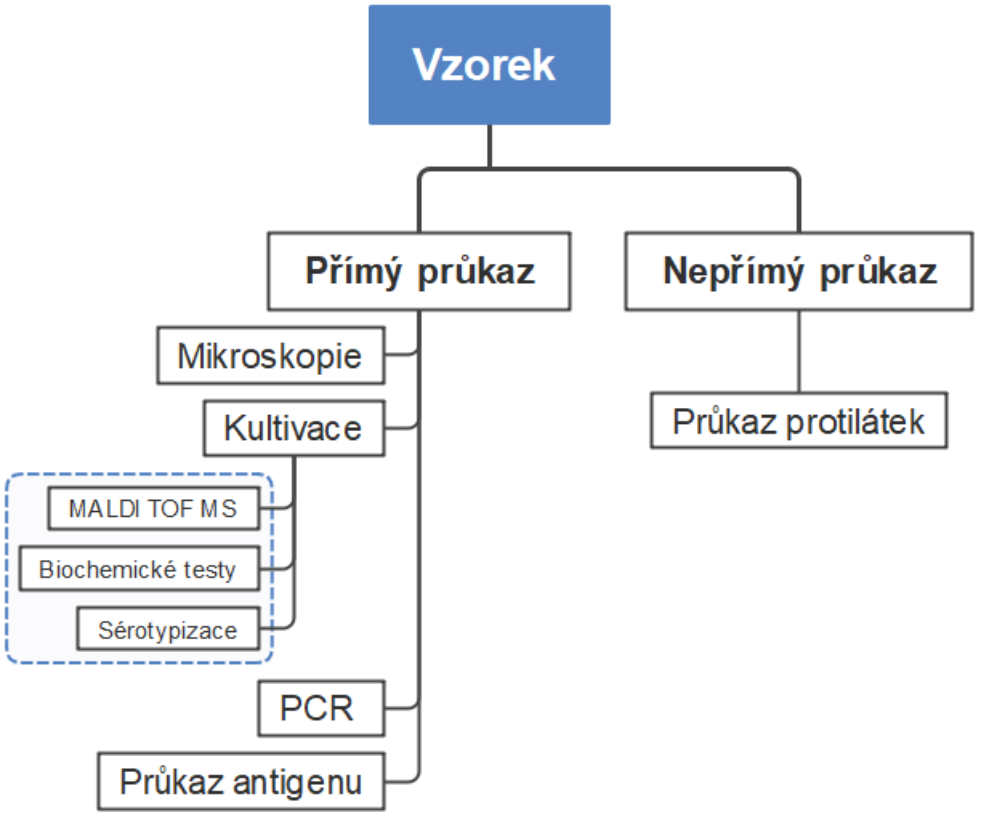


DETECTION OF MICROBIAL AGENTS

DETECTION + IDENTIFICATION

Detection - capture of the microbe

Identification - accurate determination of genus/species

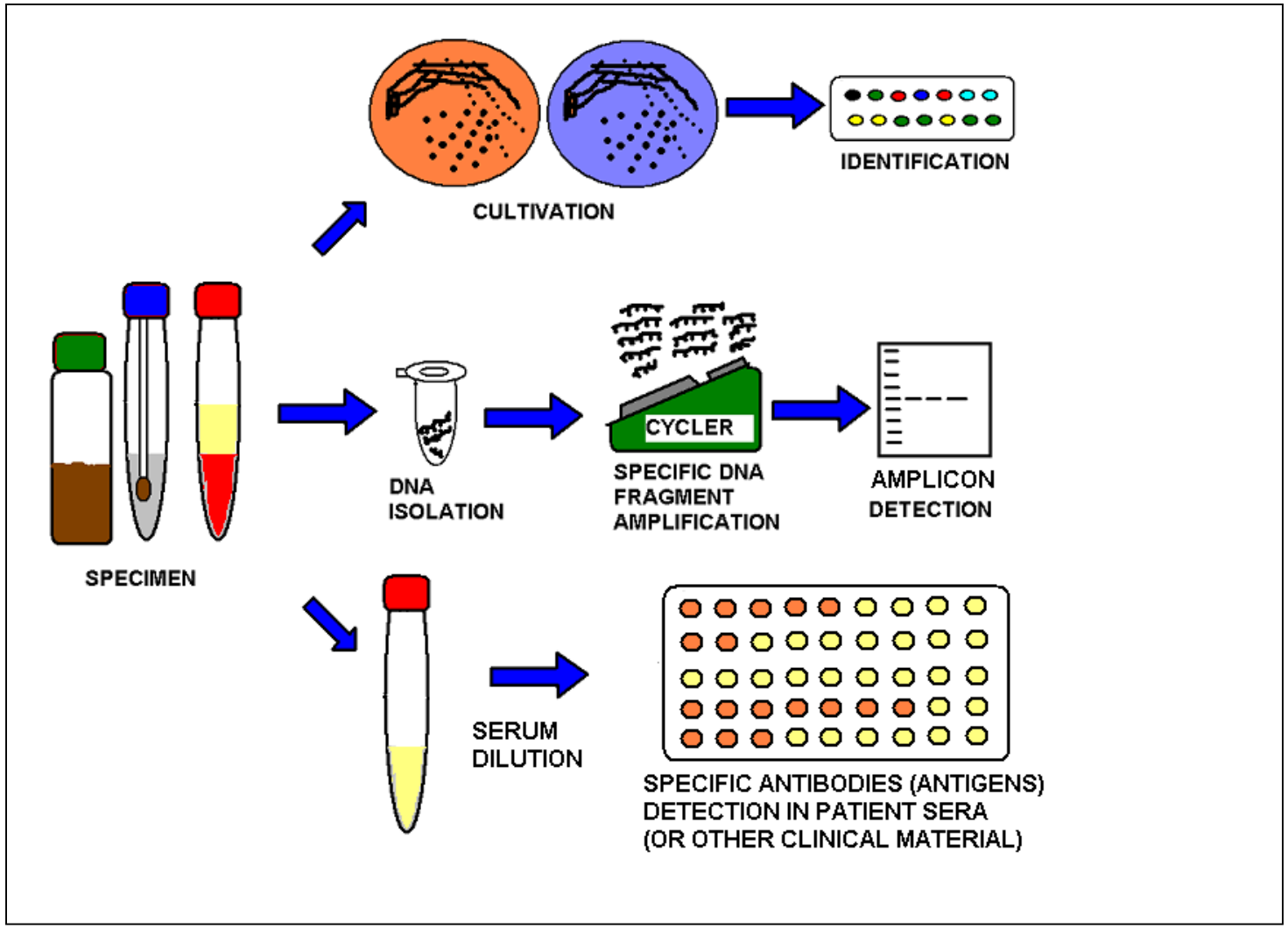


Specimen collection & transport

Credits: Oto Melter

Why collect specimens?

Detection & identification of infectious agent



Role and constitution of transport media

- As quickly as possible

A) hardy species may overgrow the fastidious one

B) fastidious organisms (e.g. *Neisseria*, viruses) – survive poorly

- Tools

A) sterile containers without addition of any preservative (FLUIDS AND TISSUE SPECIMENS)

B) swabs & transport media (e.g. Amies or Stuart transport medium)– prevent multiplying, drying (agar without any nutrition) and remove toxic agents such as fatty acids (charcoal) (OUTER OR INNER BODY SURFACES)

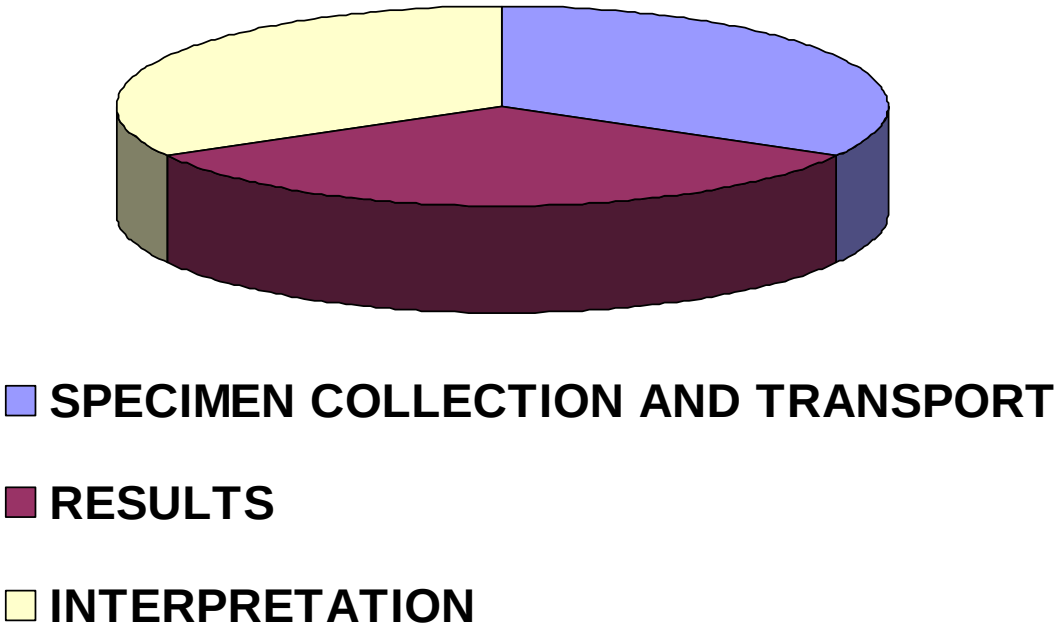
C) syringe tip capped to minimize exposure to air (PUS & ANAEROBIC BACTERIA)

D) virus transport media prevent drying and bacterial and fungal contamination, non-toxic to cell culture (FLUIDS, TISSUE SPECIMENS)

Time of collection

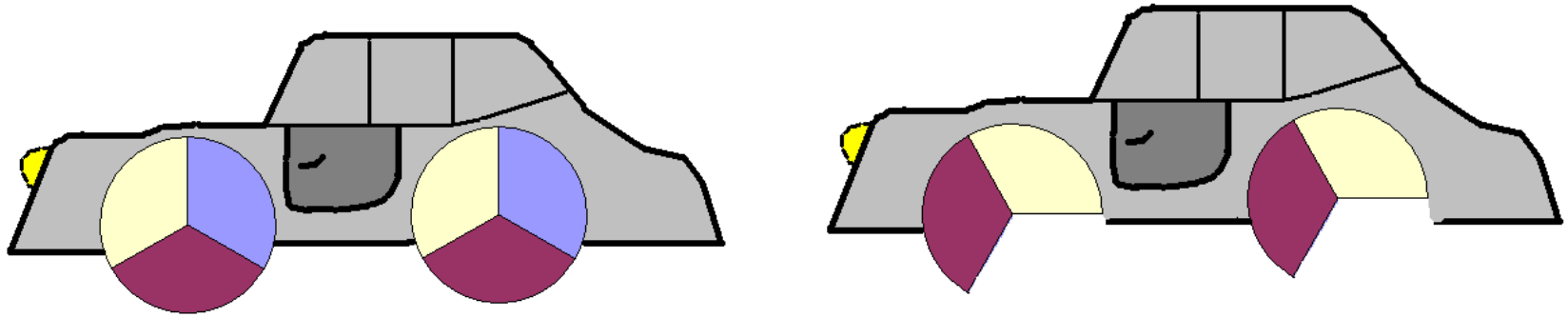
- collect the appropriate specimen at the appropriate time (acute or convalescent phase) and if possible before the patient receives antimicrobials

Three equal parts of microbiological examination



Fundamental steps of clinical microbiology analysis

A) choice of appropriate specimen
B) high quality specimen



If one third (specimen collection and transport) of your car wheel is missing you will be not able to use the car.

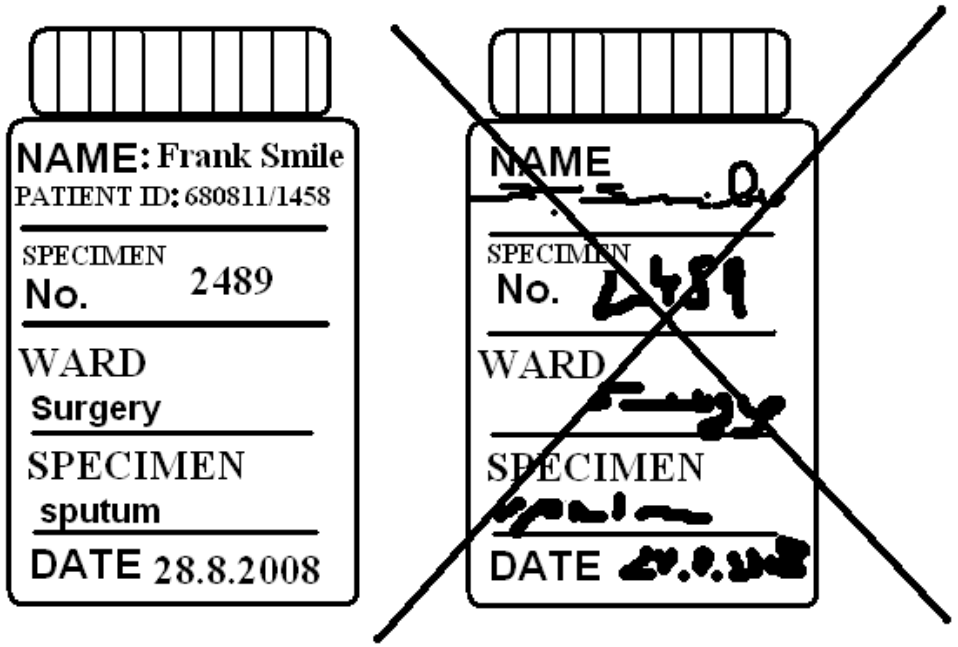
Microbiology diagnosis is only as reliable as the quality of the appropriate specimen on which is based !!!

Request form

- all specimens should be acoppanied by a request form with information about:

- patient (name, date of birth, sex)
- clinical diagnosis
- current antimicrobial therapy

Specimen labeling and request form



*** all specimens must be properly labeled by person collecting the specimen**

*** errors in specimen identification can have disastrous consequences**

Example of request form

- record number: 2489
- date of specimen collection: 28.8.2008
- surname: Smile, first name: Frank, patient ID: 680811/1458
- ward: Surgery
- physician: Dr. Smith
- specimen: sputum
- clinical diagnosis: bronchopneumonia
- investigation required: microscopy, culture, ATB sensitivity

Specimen handling

* don´t contaminate specimen from sterile sites with commensal flora, non-sterile equipment or aerobic sporulating bacteria

* all clinical specimens are potentially infectious – handle with them with suitable precautions

Laboratory tests are carried out and specimen collected

A) to detect microorganisms or their products in specimens collected from the patient

- culture of microorganisms
- detection of their parts (DNA) or products (enzymes)
- detection of susceptibility to antibiotics, chemoterapeutics and fungicidal drugs

Laboratory tests are carried out and specimen collected

B) to detect evidence of the patient´s immune response to infection

- serum (cerebrospinal fluid)
- acute and convalescent phase (ideally 10-14 days apart)

site/infection	type of infection			
	fluid	**tissue**	**swab**	**other**
Urinary tract	urine	renal biopsy		
Gastrointestinal infection	bile,pus,peritoneal aspirate, ascitic fluid	liver biopsy	rectal	faeces
Respiratory tract	washings,sputum,alveolar lavage,pleural fluid	lung biopsy	nasal, throat, ear, eye	„cough“ plate
Central nervous system	cerebrospinal fluid	brain biopsy		
Genital tract		endometrial biopsy	urethral,vaginal, cervical	direct microscopy
Skin and soft tissue	vesicle fluid, pus	skin biopsy, scrapings	skin, wound	Impression plate
Bone and joint	pus,aspirate	bone		
Septicaemia Pyrexia of Endocarditis	blood	heart valve		blood smear

Blood culture

- symptoms or infection: pyrexia, septicemia, endocarditis
- volume – directly limit success of the method (40% increasing positive results) adults 20 ml of venous blood, children and neonates 10 ml (after skin desinfection with alcohol and 2% iodine)
- bacteremia – presence of bacteria in blood stream (e.g. dectable amount of bacteria in blood stream after a dental procedure, bacteria are unable replicate in the blood of most people)
- septicemia – which is a condition where bacteremia is associated with an inflammatory response from the body (causing systemic inflammatory response syndrome, characterised by rapid breathing, low blood pressure, fever, etc.) continuous septicemia: intravascular inections (e.g. endocarditis, septic trombophlebitis, intravascular catheter infections or overwhelming sepsis (e.g.septic shock) intermittent septicemia: other infections – the focus is at a distal site (e.g. lungs, urinary tract, soft tissue), 2-3x blood samples/24h h, not when developed clinical signs of sepsis (chills, hypotension) because they occur only 1h after elimination of the agent from the blood stream
- bottles with enriched nutrient broths (aerobic, anaerobic): 2 bottles incubated at 37°C (up 5-7 d), inspected regularly, subcultered, identification of organisms and ATB susceptibility testing

Cerebrospinal fluid

• symptoms or infection: meningitis

• specimen -
aseptically collected (1-5 ml, lumbar puncture) and immediately delivered to lab - should not exposed to heat or refrigeration (lability *N.meningitis*, *S.pneumoniae*)
- transport system – sterile screw capped tube

• the specimen is concentrated and the sediment inoculated on culture media and Gram stained

Other normally sterile sites and fluids

- site of infection: e.g. abdominal, chest, synovial, pericardial

- specimen - aseptically collected as large volume as possible by needle and syringe (air should not be injected into culture bottles - inhibit growth of anaerobes) - transport system – sterile screew capped tube (for Gram staining) or blood culture bottle with medium

Respiratory tract specimens

swab – pharynx, tonsills, posterior pharynx, exudative or ulcerative area (contamination with saliva should be avoided – inhibit recovery of group A streptococci), pseudomemranes – submitted for culture

• blood culture – epiglotitis (swabbing can precipitate complete airway closure)

• needle and syringe (& vials) – sinusitis (aerobic and anaerobic culture), most common pathogens – *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S.aureus* and anaerobes)

• transport & transport media – *B.pertussis* and *N.meningitis* inoculated onto culture media and immediately sent to the lab – *Chlamydophila* spp. and *Mycoplasma* spp. are fastious and should be transported in a special transport medium – *C.diphteriae* and *S.pyogenes* are resistant to drying, useful classical transport medium

• nasopharynx, oropharynx – bacterial culture is not useful and should not be performed (viral etiology in 75 procent of the infections)

• lower respiratory tract specimen - expectorated sputum, bronchoscopy specimen, transtracheal or lung aspirate (sterile screew capped bottle, also AN avoiding upper tract flora)

Ear infections

- middle ear infection – for specific diagnosis required tympanocentesis
- most common pathogenes – *S. pneumoniae, H. influenzae, M. catarrhalis*
- outer ear infection – swab, common pathogens *P.aeruginosa*, *S. aureus*

Eye infections

- swab – surface, before application topical anesthetics, follow by corneal swab
- deep-seated infections – aspiration of aqueous or or vitrous fluid (special dept.)
- common pathogens – grow rapidly: *S. aureus, S. pneumoniae, H. influenzae, P. aeruginosa*, coagulase-negative staphylococci – need special culture media: *Chlamydia trachomatis*, *N. gonorrhoeae*

Wound, abscesses and tissue

- collect samples from deep in the wound by aspiration after the surface has been cleaned (open wounds can frequently be colonized with potentially pathogenic organism unrelated to the specific infectious process (swab shold be avoided), if fluctuance is not obtained saline can be infused and witdraw for culture
- abscess - aspirates from the center and the wall (organisms replicate at the base of the abscess)
- tissue – into screew capped container, sterile container shoul be added to prevent drying

Urine

midstream urine – first portion is discarded (contamination with bacteria from urethra or vagina) and urine (usually 1 ml) is collected into sterile container

• transport of the specimens without any delay to the laboratory (urinary tract pathogen can grow in urine) if cannot be cultured immediately it should be refrigerated

• cultivation on selective and non-selective media and quantitated bacteria (significance), although small numbers can be in a patient with pyuria also significant

• numerous urine screening procedures cannot be recommended (insensitive in detecting low-grade bacteriuria)

Genital specimens

* bacterial and fungal infection

- cultivation method focused on detection of *N. gonorrhoeae, C. trachomatis*, endocervix and urethra (specially designed swabs, inoculated directly on/in culture media and trasported immediately to laboratory), also amplfication based method are useful. Fluid or swab is appropriate material if candidiasis is suspected (microscopy and serology if *Treponema pallidum* infection occur)

* mycoplasmas (*Mycoplasma* spp., *Ureaplasma*) and viruses (herpesvirus HSV1, papillomavirus)

-specimen are collected into special cultivation media or swabs and transported immediately to the laboratory for detection by cultivation, amplification or antigen detection based method (e.g. ELISA)

* parasitic infection – concentrated on *T. vaginalis* detection (swab, smear, cultivation and amplification methods)

Fecal infections and specimens

- bacterial – rectal swab inserted usually into transport medium (e.g. Amies medium), or stool collected into sterile container is transported to laboratory without any delay to prevent acidic chnages in the stool caused by bacterial metabolism (toxic for some organisms e.g. *Shigella* spp.)
- some bacteria need special transport (Carry Blair medium & *Campylobater*) or culture conditions (*Vibrio*, *Clostridium difficile*)
- stool specimens for detection of enterotoxicosis (e.g. enterotoxins of *S. aureus*)
- parasitic and viral – appropriate amount of stool in sterile container is usually useful for direct detection of the agents

General conditions for specimen collection & transport

	conditions	temperature*
bacteria	swab & transport medium	RT
viruses	swab, fluid, tissue transport m.= culture m.	4 - 8°C
parasites/eggs	collection 3x (every other day) container / tube	RT (storage 4 - 8°C up to week)
anaerobes	fluid, tissue (avoid contact with oxygen)	RT
fastidious bacteria	special conditions (e.g. *Neisseria* spp.)	various (*Neisseria* – if delay store and transport them frozen)

Note: * RT room temperature

Summary

- **collect the appropriate specimen at the appropritate time (acute or convalescent phase) and if possible before the patient receives antimicrobials**
- **collect enough material and an adequate number of samples**
- **avoid contamination of the specimen (from normal flora – e.g.mid stream urine; from non-sterile equipment or aerobic sporulating bacteria) and laboratory workers (all specimens are potencially infectious)**
- **use the correct containers and transport media**
- **label specimens properly**
- **add completed request form (clinical data, possible aetiology, special test required)**
- **transport specimens rapidly to the laboratory**

References and additional references

*Mims CA et al, Medical Microbiology, 1993

*Murray PR et al, Medical Microbiology, 4th (2002) or 5th edition (2005)

*http://www.infectioncontrolservices.co.uk/lab_specimens_collection.htm

* Murray et al, Manual of medical microbiology, 2003

* Isenberg HD, Clinical Microbiology procedures Handbook, 2004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