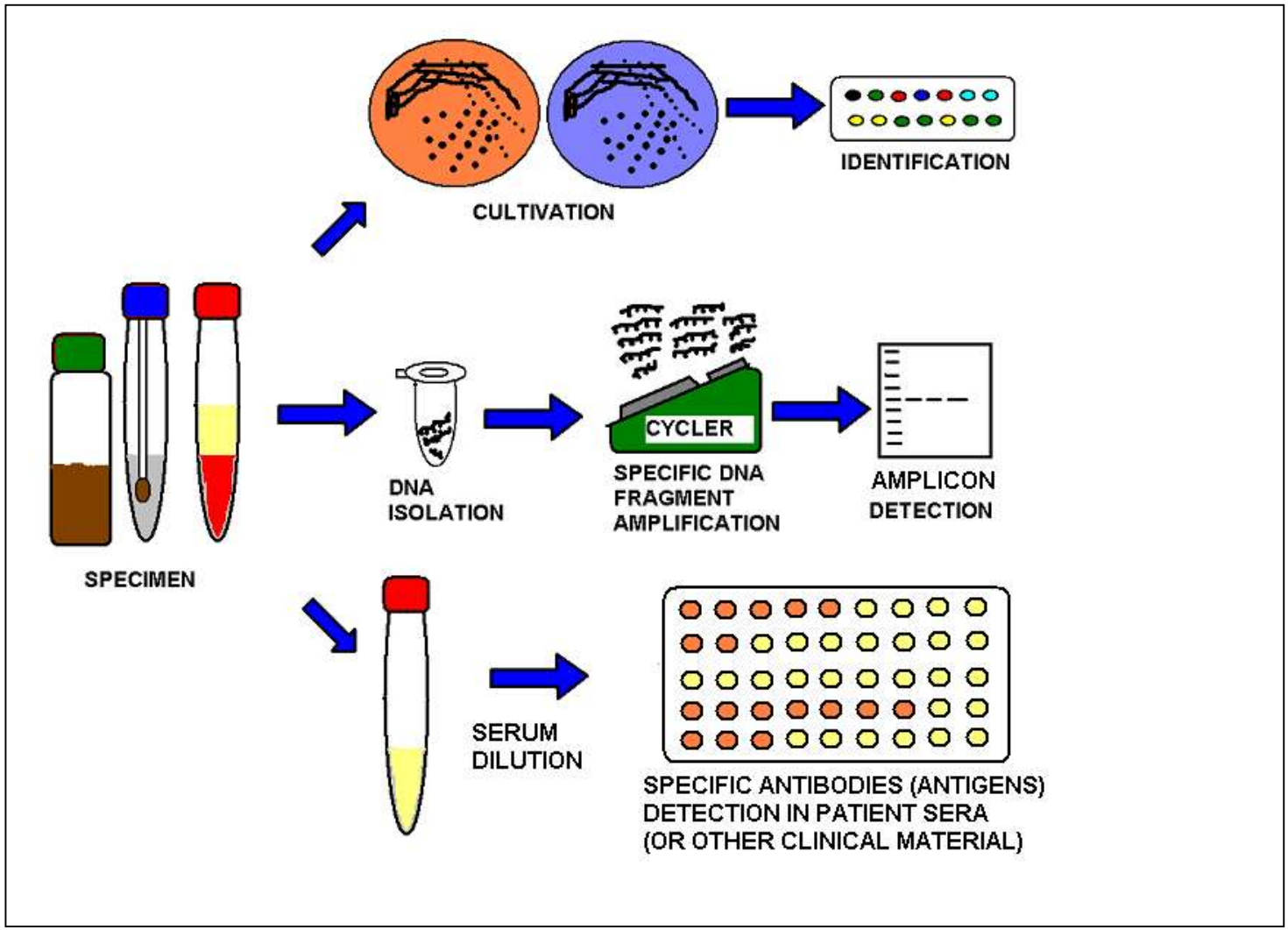
Specimen collection
&
transport

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)

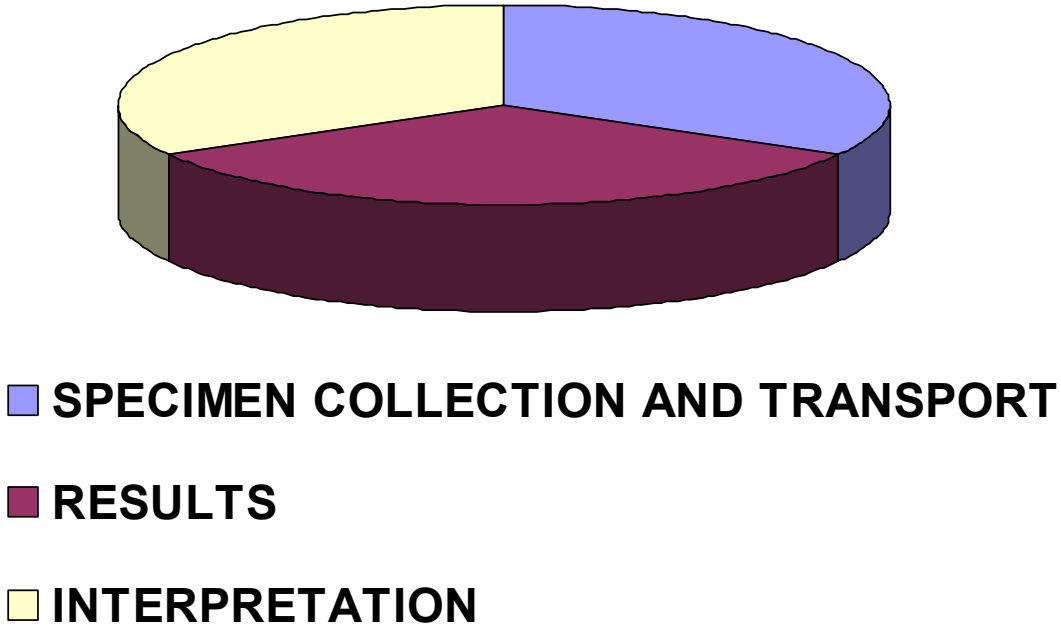
C) syringe tip capped to minimize exposure to air (PUS & ANAEROBIC BACTERIA)

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

Time of collection

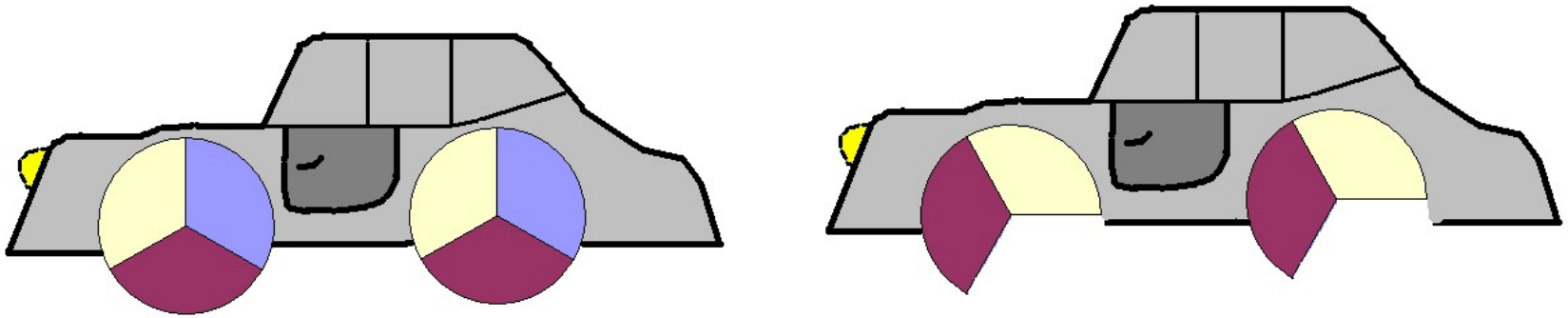
• collect the appropriate specimen at the appropriate time (acute or convalescent phase) and if possible before the patient receives antimicrobials

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.

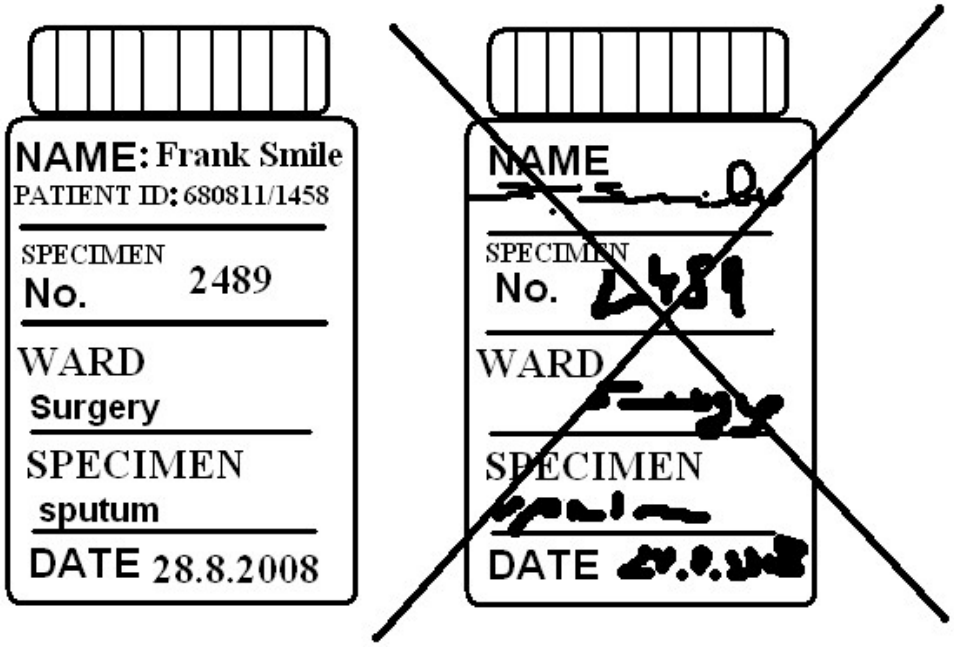
Microbiology diagnosis is only as reliable as the quality of the appropriate specimen on which is based !!!

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, antigen) 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	**throat swab** , ear, nasal, eye	„cough“ swab
Central nervous system	**cerebrospinal fluid**	brain biopsy		
Genital tract		endometrial biopsy	urethral,vaginal, **cervical swab**	direct microscopy
Skin and soft tissue	vesicle fluid, pus	skin biopsy, scrapings	skin, **Wound aspirate/swab**	impression plate
Bone and joint	pus**, joint aspirate**	bone		
Septicaemia Pyrexia of Endocarditis	**blood hemoculcure**	heart valve		blood smear

Blood culture = hemoculture

- **symptoms or infection**: pyrexia, bacteremia, 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 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 from first urine portion. 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 changes 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