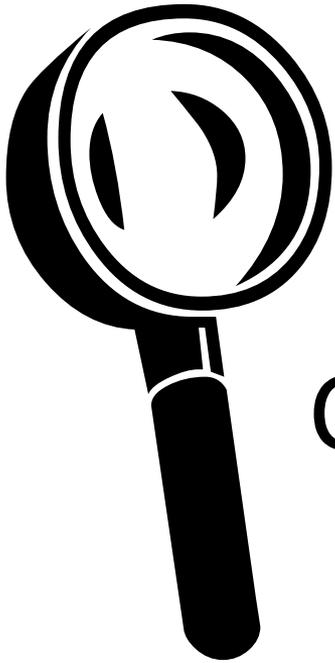


Institute for microbiology shows

TRACING THE CRIMINAL



Part ten:

Cooperation at investigation or
Clinical Microbiology I

Introduction (material comes on Christmas, too 😊)



Survey of topics

Introduction to clinical microbiology

1 Indications

2 Sampling (including request form)

3 Sample transport

4 Decision, how to elaborate

5 Proper elaboration

6 Result sending

7 Interpretation



Introduction to clinical microbiology

Story One – 1

- Peter was coughing all the time, without expectoration (dry cough), so he visited a doctor. The doctor wanted to prescribe antibiotics directly, but then he remembered, that microbiologists told him to perform examinations. So he performed a throat swab. In the swab, *Haemophilus influenzae* was found, susceptible to cefuroxime. Peter started to use ZINNAT (a drug that contains cefuroxime).

Story One – 2

- Peter, though, was no better. He became angry and visited a doctor on a pulmonary clinic. Here, serology of respiratory viruses was performed and high titres of antibodies against *Mycoplasma pneumoniae* found. Peter started to use SUMAMED (Azithromycin) and soon his status became much better.

The problem was caused not only by *Mycoplasma pneumoniae*, but

- also by GP X. Y., because:
 - he was right in remembering, that it is mostly useful to know the pathogen and antibiotic susceptibility before treatment
 - nevertheless, he made a mistake in decision what microbiological method is indicated in this case
 - a specimen of sputum had to be sent, and at negativity of culture (or at indicia showing rather an atypical pneumonia than a classic one) eventually clotted blood for serology of respiratory viruses (this examination contains also some non-viral agents, namely *Mycoplasma* and *Chlamydia*)

Remark, that sometimes macrolides are the good solution (although usually I am rather fighter against their abuse)

Story Two

- Nicol felt sore throat, and so she visited a doctor. Throat swab was performed, but only common flora was found. Doctor was surprised, elevated polymorphonuclears and CRP showed that it should be a bacterial pyogene infection.
- Doctor knew Nicol and knew that she had more sexual partners. After a direct question, she admitted that she had performed oral sex with a risky partner. A new swab was performed, now with notice „gonorrhoea examination“. And he was not mistaken.

Who was guilty? Only *Neisseria gonorrhoeae*

- General practitioner worked very good; gonococcal pharyngitis is not so common that it would be routinely examined. But he was good, that he found it after the primary negative examination.
- Doctor was clever – he knew that each specimen type has its routine laboratory schedule. This schedule is used always when there are no special requests. Special requests should be written on laboratory request.

Clinical microbiology – what is it?

- Clinical microbiology „sensu lato“ is medical microbiology – so the part of microbiology, that describes microbial flora of human and human pathogens – we can say, all that you have to study for your exam
- Clinical microbiology „sensu stricto“ describes proper processes between clinical workplace and the laboratory, including organization of proper laboratory examination. It is rather organ system oriented, and also oriented for the various types of specimens sent for the examination

Process of clinical examination – everything matters!!!

CLINICIAN

LABORATORY

Indication: to do it? what type?

Proper sampling of material

Material transport

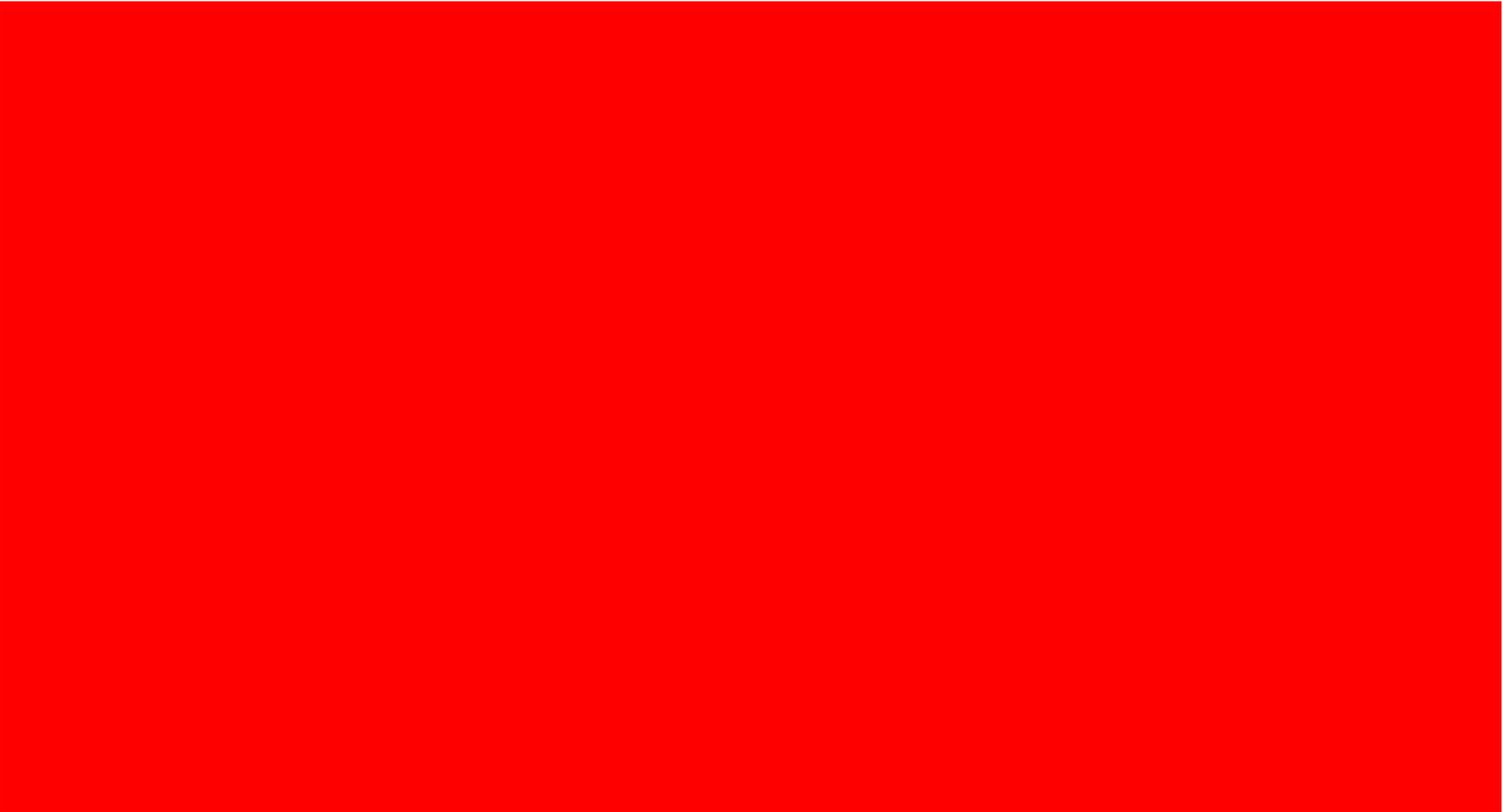
Decision how to elaborate

Proper material elaboration

Result sending

Interpretation in context of other results and patient status (to treat **patient**, not **lab finding**)





1 Indication

1A Indication – WHETHER to do anything

- The main key to success is to ask **how will be my action changed in relation with examination result.**
- When I see that not regarding the result **my further relation to the patient will be the same**, the examination is probably **useless**
- This is not valid in **epidemiologic indications** and in **prophylactic indications** (like screening of microbial colonization in serious patients)

1B Indication – WHAT to do

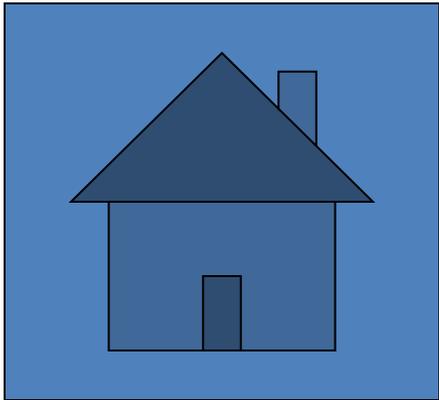
- Decision that „I want to perform an examination“ is not the end of everything. I have to think about **what examination should be done.**
- I have to know **pathogens spectre and methods of their examination**
- One part of that is also **decision about how to perform sampling technically** (including: what vessel or sampling kit should be used)

Three types of pathogens (1)

- Pathogen type *Streptococcus pyogenes*. It is not necessary to know that I mean just THIS pathogen, but I have to know pretty well where it is supposed to be localised (throat, lungs...)
- Pathogen type *Mycobacterium tuberculosis*. I have to know where the pathogen is localised, but also to know what group of pathogen is searched – so I have to write it to the request form
- Pathogen type *Toxoplasma gondii*. It is not necessary to know where in the body the pathogen is placed, but I have to know that I search for THIS pathogen.

Three types of pathogens(2)

- Pathogen type *Streptococcus pyogenes*. Bacteria and yeasts that can be cultivated, so majority of microbes from P01 to P06 and partially also J12
- Pathogen type *Mycobacterium tuberculosis*. It is still direct diagnosis, but special methods, the agent cannot be caught at normal culture. Mostly microbes from P07, P08, J13, part of P06 (gonorrhoea).
- Pathogen type *Toxoplasma gondii*. Indirect diagnostics, eventually direct diagnostics of viral antigen. Spirochetes of P09, viruses from J10 + J11, but also many others (for example just *Toxoplasma*)





2 Sampling

(including order form)

3 Transportation

2 Proper sampling

3 Sample transport to the laboratory

- These phases cannot be divided – sampling should be performed with regard to material transport to the laboratory
- There are three types of samples:
 - Cotton swabs on a plastic stick or wire
 - Liquid and solid specimens sent in vessels (mostly sterile vessels)
 - Other and special cases, see later
- Proper filling of the request, sent together with the sample, is very important too!



Other types of material than „swabs“ and „vessels“

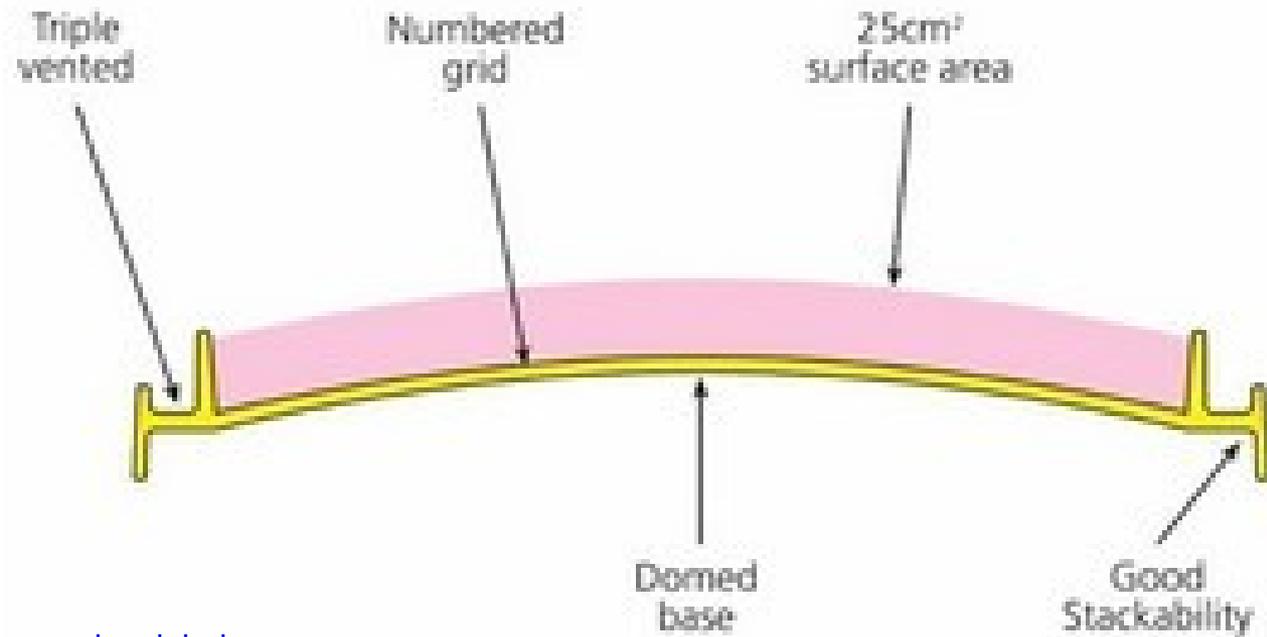
- **smear on a slide:** gonorrhoea, actinomyces, directly sent thick drop and thin smear etc.
- **in dermatology and epidemiology** just the culture medium that is filled to the margin of the dish; **in surgery moulage** with a filtration paper
- **uricult** – special way of urine sending just cultured on a medium; for many reasons, it is not very common.
- **quick diagnostic sets**, mostly based on direct antigen detection; simple manipulation, available even for non-microbiological personnel. In case of doubts about the result it is necessary to use classical sending to the lab.

Choice: How to sample?

Liquid sample, or swab?

- Usually, sending solid/liquid sample is preferred in comparison with sending swab
- Though, there are many exceptions, e. g.
 - in bacteriology usually rectal swab is sent and not stool (although it is not a mistake to send stool)
 - urethral swab in gonorrhoea is recommended rather than urine sending

Contact plate



Uricult

www.mediost.com



Some types of swabs



Plain (dry) swab www.calgarylabservices.com

Today its use is for PCR and antigen detection only, not for culture!



Amies medium with charcoal www.herenz.de

Universal transport medium for bacteriology (all types of swabs). The wire variant important, if we want to go „behind the corner“ (nasopharyngeal swab for influenza, swab of a wound with recesses)

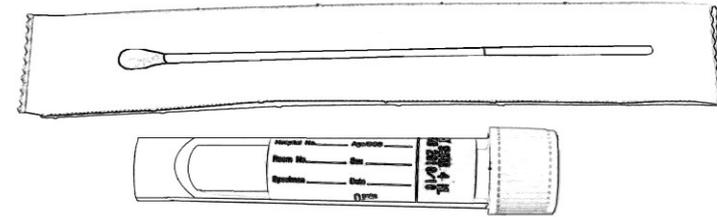
More swabs

Fungi Quick (for yeast and molds)

www.copanswabs.com

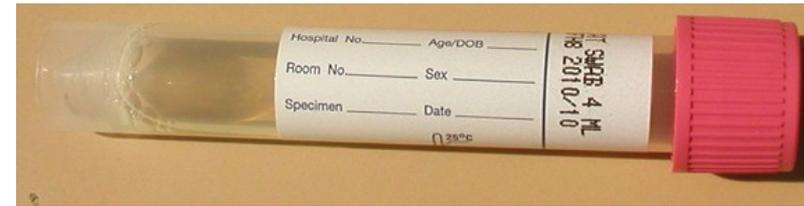
Notes:

- 1) It has a light-blue cap, otherwise it is similar to C. A. T. swab
- 2) Actually its use is minimal (even for yeast examination they mostly use Amies swab)



C. A. T. swab (for Candida And Trichomonas, from genitals only)

www.copanswabs.com



Virus swab

www.copanswabs.com

Chlamydia swab

www.copanswabs.com



Survey of swabs

Dry swab on a stick: search for antigen and DNA

Dry swab on a wire: the same, if I need to get to an inaccessible place

Swab in Amies medium on a stick: universal for bacteriological culture (incl. anaerobes, gonorrhoea, campylob.)

Swab in Amies medium on a wire: the same, if I need to get to an inaccessible place

Fungiquick – fungi

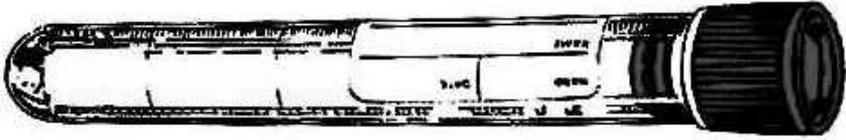
C. A. T. – fungi and trichomonas (genital swabs)

Samples with medium for viruses, event. chlamydiae

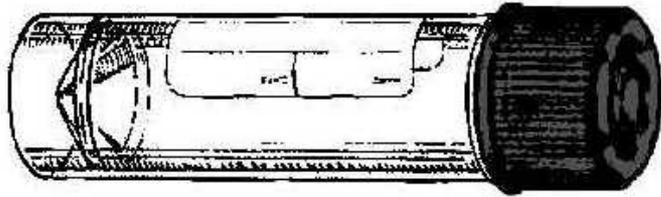
Vessels

- Sampling vessels are used for **solid and liquid samples**. In fact, size is not so important. Also colour of the cap is only important in context of provider. If you provider offers sterile tubes with red cap and non-sterile ones with white cap, it is necessary to keep it. But pay attention at provider changes!
- In anaerobic culture it is better to send just a **syringe with special cap (not with a needle!)**
- Specimens should be transported to the laboratory **as soon as possible**, but the most important situation is in urine sampling; here, 2 h is maximum transport time!

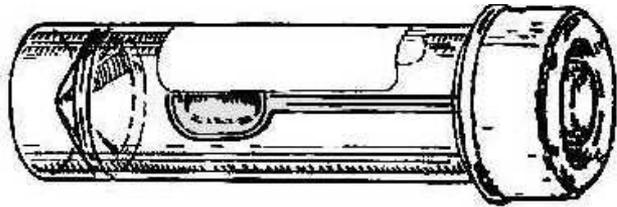
Vessels



Common test tube. Universal use: clotted blood (serology), urine, CSF, pus, punctate etc.; blood and urinary cathethers, parts of tissue...



Sputum vessel. Not only for sputum, but also larger parts of tissue etc.



Stool vessel, for parasitology. Only this one does not have to be sterile!



Vessel for urine sampling. It is better, if the patient urinates just into a test-tube, but especially for women this is difficult (except if they are in shower). So they can urinate into this vessel, and then a nurse removes the urine into a test-tube.

Various vessels



Order form 1

- Properly filled order form is very important!
- Identification data: for identification, payment, for knowing, whom to send the result etc.
- Precise description of material and requested examination
 - do not write only „swab“ without adding more
 - even „wound swab“ is not enough (what type of wound, where is its localisation)
 - Catheterized urine × urine from permanent catheter
 - write, whether e. g. anaerobes are requested
 - not to request examination that is not available or is useless (e. g. cultivation examination of syphilis)

Order form 2 – what to write in

- **real diagnosis**, in case of more diagnoses, write all of them, or the one related with examination /e. g. (1) diabetes mellitus, (2) vaginal discharge/
- **acute / chronic status / control after treatment**
- to add present or planned **antibiotic therapy**, eventually allergy to antibiotics

Order form 3 – what to add more

- traveller anamnesis – tropical countries etc.
- professional anamnesis – job in agriculture etc.
- in serological examination date of first symptoms, first / second specimen
- in gynaecological materials phase of menstruation cycle (and rather not to sample during menses)
- in case of irregular samples to consult it telephonically

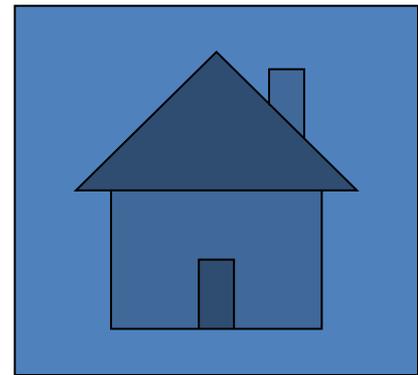
Order form filling – conclusion

We should not forget to fill in all important parts of the order form:

- fields describing the patient (name, date of birth/birth number, insurance, ward, diagnosis...)
- fields describing the sample (type of specimen, localisation, important circumstances)
- and all other important parts (especially anamnesis)

Mistakes in order form filling

- A common type of mistake is an insufficient description of sample type
- It is also bad to order examinations that are not suitable for the given situation (for example, search for antibodies in a pathogen, where cellular immunity is leading and antibody search does not have any importance)





4 Decision how to
process

5 Proper
processing

4 Decision, how to process the specimen

- It is described in operation standards (OS). For each sample type the OS says, what methods should be used for it, and what methods should be applied.
- Nevertheless, everything is not in OS. Especially in extraordinary cases it is on decision of experienced microbiologist, how to process the specimen
- In important cases is no mistake to phone to the laboratory and ask for an advice.

5 Proper specimen processing (1)

- Proper processing is usually done by laboratory assistants, formerly with secondary education, today with bachelor's degree or equivalent
- The procedure should be aseptic, to avoid risk of laboratory contamination. Work in a biohazard box is also a good prevention of hospital infections

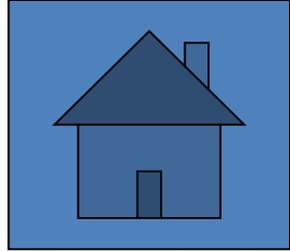
www.medmicro.info



5 Proper specimen processing (2)

- Processing of bacteriological specimens usually contains following:
 - before proper processing, some specimens are homogenized, centrifuged etc.
 - in some specimen types quick methods – microscopy, eventually direct antigen examination
 - nearly always it is based on culture on several solid media
 - sometimes also multiplication in liquid media (in conjunctiva swab: ONLY this point – subcultivation in positivity only)
- Processing of other specimens (serology, PCR, mycology, parasitology) is special and related with examination type and specimen type

Laboratory of clinical bacteriology (example of arrangement – St. Ann's hospital)



Assistant 2 „does repeatings“: in positive specimens susceptibility test and identification tests are prepared

Microbiologist (master degree) „reads the laboratory“ – observes the results of the culture

Assistant 1 (secondary school or bachelor degree) writes the results



6 Result sending

7 Interpretation

6 Result sending

- Result is **sent after finishing of the diagnostic process**. Sometimes a preliminary result is sent after finishing the basic aerobic culture, and the remaining part (yeast culture, anaerobic culture etc.) is sent later
- Result contains **a partial interpretation**: a microbiologist comments clear contaminations, accident findings, common flora, comments the findings in a note

7 Interpretation

- Definitive interpretation of finding should be done by the clinician. Only the clinician, not the microbiologist, has the microbiology result together with the biochemical, x-rays, ultrasound result. And only he has done the anamnesis and clinical examination of the patient.
- Of course, consultation of a clinician and a microbiologist is very useful in serious cases. On the other hand, it is not possible to consult each case.

Direct diagnostics interpretation

- **A pathogen.** Of course, a microbe found in a direct method might be a pathogen. Not always.
- **A common flora member.** Reason, why to know common flora in different sites
- **An accidental finding (= component of transient common flora).** Example: small amounts of Enterobacteriaceae in throat swab, especially in case of an out-patient, is normal
- **Colonizing microbe.** Especially in wound infections. Many microbes only colonize the wound, especially Gram-negative (and above all, genus *Pseudomonas*)
- **A contaminant** during sampling, transport etc.

Survey of common flora



Skin, nose, external ear, skin and axilla	Staphylococci (incl. <i>Staph. aureus</i>), coryneforms, yeasts
Pharynx & oral cavity	Oral streptococci and neisseriae. Hemophili, small amounts of pneumococci, meningococci, anaerobes, non pathogenic treponema
Large (and small) bowel	Anaerobes, enterobacteria, enterococci, <i>Entamoeba coli</i>
Vagina	Lactobacilli, small amounts of other bacteria
Margins (lips etc.)	Mixture from both sides

Indirect diagnostics interpretation

- **Acute infection.** Characterized by IgM positivity, fourfold titre elevation, low-avidity antibodies. But sometimes even this is not enough – it might be e. g. cross-reactivity.
- **Disease in history.** Usually only IgG positivity and low, not changing titre. Unfortunately, here, too, not always the rules are valid.
- **Chronic disease.** Sometimes difficult interpretation. In hepatitis B big difference between individual forms.

A picture of a laboratory

www.medmicro.info



The End

