DIAGNOSTIC MICROBIOLOGY

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Which factors should precipitate testing?

- CLINICAL SYMPTOMS
- CONTACT WITH INFECTED INDIVIDUALS
- TRAVEL HISTORY
- IMMUNE STATUS OF THE PATIENT (e.g. compromised patient - increase in the number of patients whose immune systems are compromised through underlying illness, chemotherapy, transplantation)
- DOCUMENTED PREVIOUS INFECTION
- SCREENING (e.g., outbreak situation)
How determine causative agent of the disease?

- DIRECT OR INDIRECT METHODS

- **Direct methods** (e.g., microscopy, cultivation of specific nucleic acids, detection of specific antigens) = highly specific and unambiguously recommendable, however, in some cases: either low sensitivity (microscopy) or expensive, but important - the possibility of testing the sensitivity to ATB.

- **Indirect methods** (e.g. serological methods = sometimes can be of low sensitivity and specificity)
How determine causative agent of the disease?

- Examination of **exact sample** (dependence on clinical symptoms and signs!!!) **isolated**:
  - from **exact site**;
  - at the **exact time interval**;
  - **transport** to laboratory examination **under adequate conditions** (standards)
  - examined by **adequate methods** (standards)
Clinical symptoms = specific material in which the causative agent can be detected = isolation at exact time

e.g.,
- stool
- urine
- blood
- cerebrospinal fluid
- sputum
- organ biopsies, aspirates, smears, etc.
<table>
<thead>
<tr>
<th>Body site</th>
<th>Specimen (examples)</th>
<th>Test options (examples)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
<td>whole blood, serum, anticoagulated blood, etc.</td>
<td>culture, QBC microhematocrit centrifugation, Buffy coat films, Knott concentration, membrane filtr. techniq. immunoassays, animal inoculation</td>
</tr>
<tr>
<td><strong>Bone narrow</strong></td>
<td>aspirate</td>
<td>culture, histopathology, thick and thin smears, PCR</td>
</tr>
<tr>
<td><strong>CNS</strong></td>
<td>spinal fluid, brain biopsy specimen</td>
<td>culture, wet examination, stained smears, immunoassays, PCR</td>
</tr>
<tr>
<td><strong>Eye</strong></td>
<td>aspirates from below surface, biopsy specimen</td>
<td>culture, wet preparation, stained smears,</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td>smears, scrapings, aspirates from below surface, biopsy specimen</td>
<td>culture, histopathologic testing, squash preps (stained smears),</td>
</tr>
<tr>
<td><strong>Intestinal tract</strong></td>
<td>fresh stool</td>
<td>culture, direct wet smear, concentr., permanent stained smear, ag. det.</td>
</tr>
<tr>
<td></td>
<td>anal smear</td>
<td>culture, direct wet smear</td>
</tr>
<tr>
<td></td>
<td>preserved stool</td>
<td>concentration, permanent stained smear</td>
</tr>
<tr>
<td></td>
<td>sigmoidoscopy material</td>
<td>direct wet smear, stained smear</td>
</tr>
<tr>
<td></td>
<td>duodenal contents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>anal impression smear</td>
<td>exam. of tapes for pinworm eggs</td>
</tr>
<tr>
<td><strong>Liver and spleen</strong></td>
<td>sputum, induced sputum, broncholaveolar lavage fluid, transbronchial aspirate, brush biopsy specimen, aspirate, open-lung biopsy specimen</td>
<td>wet preparation, stained smear, immunoassays, histopathologic testing, PCR</td>
</tr>
<tr>
<td><strong>Lymph node</strong></td>
<td>biopsy specimen</td>
<td>culture, stained smear, histopathol. test., PCR</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td>biopsy specimen</td>
<td>histopathologic testing, PCR</td>
</tr>
<tr>
<td><strong>Urogenital system</strong></td>
<td>vaginal discharge, urethral discharge, prostatic secretions, urine, biopsy specimen</td>
<td>culture, wet preparation, stain.smears, histopathol.test.</td>
</tr>
</tbody>
</table>
DETECTION OF THE AGENT

1) **DIRECT** – macroscopically or microscopically

- **culture**: predermined culture media or tussue cultures under controlled laboratory conditions
- **non-concentration methods**: nativ fresh mounts stained smears
- **concentration methods**: flotation sedimentation filtration
- **specific methods**: detection of DNA, circulating antigens

Detection of the parasite DNA: limited use

**Material**: e.g. incondesable blood, stool, urine (fresh, frozen, fixed in pure **100% alcohol**)
MACROSCOPICAL examination of samples

Cestoda: proglotides

Ascaris lumbricoides
Very important - e.g., due to sepsis, pneumonia, fever of unknown origin, puerperal sepsis, pelvic inflammation, neonatal epiglottitis...
Principles for Collection

- Gloves will be worn in accordance with standard precautions.
- A physician’s order must be obtained for specimen collection.
- Appropriate verification of the patient's identity, by means of an armband or area specific procedure, will occur before the specimen collection.
- Cultures should be drawn before administration of antibiotics, if possible.
- If at all possible, blood cultures should not be drawn from lines, but should be drawn via venipuncture.
Materials

- Chlorhexidine swabs (1-2 packages)
- Alcohol swabs
- Blood culture bottles (2 bottles per set)
- 2 syringes (adult: 20 cc, pediatric: 5 cc)
- 2 needles (adult: 22 gauge or preferably larger butterfly or standard needle; pediatric: 25 or 23 gauge butterfly or standard needle)
- Gloves (sterile & nonsterile)
- Tourniquet
- Sterile gauze pad
- Adhesive strip or tape
- Self-sticking patient labels
- Plastic zip lock specimen bags
Step 7 – Draw Blood

7. Draw blood. Note the appropriate volume to obtain:

<table>
<thead>
<tr>
<th></th>
<th>Syringe needed</th>
<th>Aerobic bottle</th>
<th>Anaerobic bottle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>20 ml</td>
<td>10 ml</td>
<td>10 ml</td>
</tr>
<tr>
<td>Pediatric</td>
<td>20 ml</td>
<td>2.5 - 10 ml</td>
<td>2.5 - 10 ml</td>
</tr>
<tr>
<td>Infant</td>
<td>3 ml</td>
<td>0.5 -1 ml</td>
<td>0.5-1 ml</td>
</tr>
<tr>
<td>Adult (low volume)*</td>
<td></td>
<td>All</td>
<td>None</td>
</tr>
</tbody>
</table>

Do not overfill bottles (do not add more than 10 ml of blood to each bottle)

*In some cases, it may not be possible to obtain 20 ml blood from an adult. If 10 ml or less is obtained, place all of the blood in the aerobic bottle.
Quality Problem
Contamination of blood cultures produces false positive test results for blood stream infections leading to adverse patient outcomes and wasted healthcare resources.

Preventability/Improvement
Blood culture contamination rates
Range: 0.6% - 12.5%
Target: ≤ 3% (ASM, CLSI)

Practices/Interventions
- Venipuncture (versus IV catheter) collection
- Phlebotomy team (versus other staff)
- Prepackaged prep kit (versus no prep kit)

Intermediate Outcomes
- Blood culture contamination rates
- Additional testing/follow-up associated with re-evaluation
- Costs associated with above

Healthcare/Health Outcomes
- Diagnostic and treatment delays
- Diagnostic and treatment errors
- Unnecessary and increased hospital stays
- Unnecessary antibiotic use
- Hospital acquired infection
- Costs associated with above
- Morbidity
- Mortality

Harms
- Needle-stick injury risk
- Patient collection site infection
1-3 ml of fluid transported to the laboratory as soon as possible

CEREBROSPINAL FLUID CULTURE + other normally sterile fluids – e.g., peritoneal, pleural, synovial

Source: Wikimedia Commons
DEFINITION OF SIGNIFICANT BACTERIURIJA IN PREGNANCY

• in an asymptomatic pregnant woman, bacteriuria is considered significant if two consecutive voided urine specimens grow > 10^5 cfu/mL of the same bacterial species on quantitative culture; or a single catheterised specimen grows > 10^5 cfu/mL of a uropathogen

• in a pregnant woman with symptoms compatible with UTI, bacteriuria is considered significant if a voided or catheterised urine specimen grows > 10^3 cfu/mL of a uropathogen
• $> 10^3$ cfu/mL of uropathogens in a mid-stream sample of urine (MSU) in acute uncomplicated cystitis in women

• $> 10^4$ cfu/mL of uropathogens in an MSU in acute uncomplicated pyelonephritis in women.

• $> 10^5$ cfu/mL of uropathogens in an MSU in women, or $> 10^4$ cfu/mL uropathogens in an MSU in men,

• or in straight catheter urine in women, in a complicated UTI.

In a suprapubic bladder puncture specimen, any count of bacteria is relevant.
Escherichia coli – $10^8$ / ml
ASYMPTOMATIC BACTERIURIA

• diagnosed if two cultures of the same bacterial strain (in most cases the species only is available), taken > 24 h apart, show bacteriuria of > $10^5$ cfu/mL of uropathogens
MICROSCOPICAL examination of samples

Entamoeba histolytica cysts

Plasmodium falciparum
CULTURE & MICROSCOPY

Material: **Smears**

e.g., of vaginal mucosa *Trichomonas vaginalis*

1. MICROSCOPY

EXAMINATION of smear - *in vivo*, staining by Giemsa - MOP)

2. CULTURE → MIKROSCOPY
Enterobius vermicularis
MICROSCOPY
WET FRESH STAINED MOUNT

Staining e.g. by Lugol's iodine (e.g., amoebae)
MICROSCOPY
WET FRESH MOUNTS OF ORGAN BIOPSIES

Quantitative compressed biopsy technique (QCTB)

e.g. Schistosoma eggs
**MICROSCOPY**

**STAINED DRY SMEARS**

**Thick smear** stained by Giemsa *(no fixation by methanol)*

- e.g. blood: malaria, filariases
- material: peripheral blood

**Thin smear**: following fixation by methanol, staining by Giemsa

*Source: Wikimedia commons*
PERIPHERAL BLOOD: THICK SMEARS

PERIPHERAL BLOOD: THIN SMEARS

MICROSCOPY

EXAMINATION OF FAECAL SMEARS

smear → fixation → staining

e.g. eggs of intestinal protozoa
MICROSCOPY
EXAMINATION OF FAECAL THICK SMEARS

Kato-Katz Technique – celophane faecal thick smears
(glycerol-malachite green or glycerol-methylene blue solution; solutions are poured into the celophane strips and soaked in this solution in a jar)

e.g. eggs of intestinal helminths
MICROSCOPY
EXAMINATION OF CONCENTRATED MOUNTS

Fecal concentration procedures
various layers seen in the tubes after centrifugation

A) Formalin-ether
B) Zinc sulfate

(the surface film should be within 2 to 3 mm of the tube rim)
MICROSCOPY
EXAMINATION OF CONCENTRATED MOUNTS

FLOTATION
Zinc Sulfate (33% aqueous solution)

e.g. protozoan cysts, helminthic eggs
DETECTION OF THE AGENT

2) **INDIRECT** – using specific methods, detection of specific **antibodies in the serum, vitreous humour, CTF** (when the agent is losalised in the organ/tissue)

methods: e.g., ELISA, IHA, IFAT, WB
material: condensable blood
**Trichomonas vaginalis**

Trophozites: vaginal cavity and urethra  
Transmission: venereal contacts  
Diagnose: examination of discharge, vaginal smears (staining mounts, culturing)

*fa:* anterior flagella, *fr:* posterior flagella,  
*n:* nucleus, *ax:* axostyle, *um:* undulating membrane  

Size: 10-30 µm x 6-20 µm
STAINED DRY SMEARS

10 μm
Plasmodium

Disease: malaria
Transmission: vector
Diagnose: examination of peripheral blood smears and other techniques such as PCR

3) STAINED BLOOD SMEARS

Thick smear

Thin smear
a) *Entamoeba histolytica*
b) *Giardia intestinalis*

**Disease:**
- a), b) intestinal and a) extraintestinal infections

**Transmission:**
- per os (food born infection)

**Diagnose:**
- intestinal: examination of stool,
- extraintestinal: detection of antibodies, imaging

4,5) STAINED FAECAL SMEARS

- Giardia
- *Entamoeba histolytica*
Ascaris lumbricoides

Disease: mainly intestinal infection
Transmission: per os (food born infection)
Diagnose: examination of stool

6) MOUNTS PREPARED BY FLOTATION METHOD

- Size: 60 x 45 μm
- Size: 80 x 45 μm
Schistosoma mansoni

Disease: intestinal and organ infection
Transmission: by cercariae (water-born infection)
Laboratory dg.: examination of stool, detection of antibodies, imaging

7) Quantitative compressed biopsy technique

Size: 130-180 x 60-76 µm
Thank you for attention