Laboratory diagnosis of viral infections

Specimen collection and transport
Steps in Pathogenesis of Viral Diseases

1. Entry
   • through skin
   • through mucosa (respiratory, gastrointestinal or genital)
   • through conjunctive
   • directly in the bloodstream by animal
     or insect bite or by needles

2. Spread
   • to bloodstream (first viremia)
   • to the cells of RES
   • to the bloodstream again (second viremia)
   • to the target organ

3. Excretion (Shedding)
   • from the same site as entry (skin, mucosa)
   • from site different than entry
     (by urine, mother’s milk…)
Laboratory Diagnosis of Viral Infections

Most viral infections have typical clinical manifestations and not need to be confirmed by laboratory diagnosis.
Laboratory diagnosis is necessary in cases:

- **When treatment and prognosis depend entirely on correct diagnosis**
  (teratogenic viruses, differential diagnosis of encephalitis, viral diseases with atypical clinical manifestations)

- **When there is an epidemic spread of viral disease**
  (influenza, HAV, variola)

- **When viral disease can be treated with antiviral drugs**
  (HSV, CMV, HIV)
Laboratory Diagnosis of Viral Infections

**Viral (direct) diagnosis**

- isolation and identification of viruses in the systems of living cells
- by electron microscopy
- detection of viral antigenes
- detection of viral nucleic acids

**Serological (indirect) diagnosis**

- detection of anti-viral antibodies in serum
Laboratory Diagnosis of Viral Infections

Includes:

- Taking specimen
- Transport specimen
- Speciment processing and inoculation in system of living cell
- Virus identification

Important:
- Correctly interpretate the results, especially in serology!
- Normal viral microflora doesn’t exist!
Rules of Specimen Collection

1. At the correct time

2. From the correct site

3. In the correct way

4. With active (urine, feces, sputum) or passive (swabs, blood, aspirates) participation of the patient

5. In the adequate volume (for all tests needed)

6. In the proper containers (sterile and chemically clean)

7. Correctly labeled (name, date, type of specimen) and with additional information (age, sex, clinical diagnosis, epidemiological data – vaccinations, recent trips, animal bites etc.)
VTM is used to:

• preserve viral infectivity within the specimen
• prevent specimen from drying
• stop the growth of bacteria and fungi
VTM contains:

- saline (adequate ion concentration)
- proteins (albumine or gelatine)
- buffer (adequate pH)
- antibiotics and fungicides

* MEM, Hank’s solution, Stuart’s
SPECIMEN COLLECTION

- SWAB/SCRAPING VESICLE ASPIRATE SMALL TISSUE SAMPLES
  - PLACE IN STERILE TUBE WITH 1-2 mL VTM

- FLUIDS: URINE, CSF, BLOOD IN EDTA OR CITRATE, BRONCHOALVEOLAR LAVAGE, NASOPHARYNGEAL ASPIRATE
  - PLACE INTO STERILE CONTAINER: VTM IS NOT ADDED

- SOLID SPECIMEN: STOOL, TISSUES
  - PLACE INTO STERILE CONTAINER WITH 5-10 mL VTM

TRANSPORT TO LABORATORY DURING TRANSPORT STORAGE AT 2-8°C
SWABS

For diagnosis of viral infections, swabs should be:
• made of reyon

✓ Should not be made of cotton or calcium alginate

Swab’s shaft should be:
• made of plastics or metal

✓ Should not be made of wood
**COLLECTION OF SPECIMEN**

**Blood**
- by venepuncture or through venal catheter
- Blood is taken for viral or serology diagnosis

**Bone marrow**
- by puncture - about 2 ml
  (with EDTA, sodium-citrate or heparin)
**Blood**

For viral diagnosis
- **whole blood** with EDTA, sodium-citrate or heparin
- “buffy coat” (Leukocytes or Thrombocytes) or
- **plasma**
- heparin should not be used for PCR!

For serology - **serum**
**Fluid Specimen**

**Saliva**
1 swab from the bottom of the mouth
1 swab from the area around Stenson’s ductus
place in tube with VTM

**Urine**
10-20 ml, middle stream (sterile container)

**CSF**
lumbal puncture (2-5 ml)

**Pericardial fluid**
pericardial puncture (2 ml)
Specimen from upper respiratory tract

**Nasopharyngeal swab:**

through nostrile to nasopharincs place in VTM
Nasopharyngeal wash:

pouring of saline by syringe through nostrile to nasopharingings
aspirating of saline with respiratory secretions
place in VTM
Specimen from lower respiratory tract

**Bronchoalveolar lavage (BAL):**
patient in anestesia, placement of bronchoscope
8-10 mL in sterile container
no VTM

**Sputum:**
Not usefull for viral detection
Specimen from gastrointestinal tract

**Feces**
2-4 g of feces (sterile container) place in VTM (8-10 ml)

**Rectal swab**
place in VTM
Specimen from genital tract

**Endocervical swab**
Thin swab – 1 cm in cervix
place in VTM

**Urethral swab**
The patient should not urinate 1 h before this swab.
Thin swab - 2-4 cm in uretra
place in VTM

**Sperm**
Place in sterile container
Specimen from skin

Swab from the lesion
imbue with sterile physiologic solution
place in the test-tube in VTM

Vesicle swab
cleaning with sterile saline; piercing with sterile instrument
1 swab for the fluid
1 swab for the material from the bottom of the vesicle
both swabs in VTM

Vesicle aspirate
piercing with sterile needle
aspiration of fluid with syringe
washing the syringe with VTM
Specimen from mucosae lesion (oral and anogenital)

- Swab from the lesion with rotation
  place in test-tube with VTM

Tissue samples from biopsy or autopsy

Place with VTM
Swab from conjunctiva
imbue with sterile physiologic solution
take with rotation
in VTM

Swab from cornea/scraping
Ophthalmologist only!
in VTM
Effect of physical and chemical factors on viruses

Inactivation of virus represents permanent loss of contamination.

- **Temperature**: - viruses are stable on low temperature (keeping) - viruses with envelope are heat sensitive

- **Drying**: most of the viruses are sensitive

- **Irradiation (UV, X-ray...)**: inactivation of viruses

- **Chemical agents**: organic solvent (chloroform) – inactivation of the viruses with envelope oxido-reduct. agents (formaldehyde, chlorine, iodine) – inactivation

- **pH**: low pH - viruses without envelope are mostly stable
SPECIMEN TRANSPORT

During transport specimen should be:

→ protected from breaking

→ protected from light

→ At adequate temperature:

48 h at $+4^\circ\text{C}$ (refrigerator, wet ice)
more than 48 h at $-70^\circ\text{C}$ (dry ice)
must not be frozen at $-20^\circ\text{C}$!