Pathology of viral disease
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Topics for the first lecture....
General virology
Viral lifecycle
Viral pathogenesis
Laboratory diagnosis

Virus size

Viral Structure

Herpes virus

Envelope
Tegument
Spikes
Nucleocapsid
Genome

Classification schemes for animal RNA viruses

Principles of Virology: Molecular Biology, Pathogenesis, and Control,
S. J. Flint, L. W. Enquist, V. R. Racaniello, A. M. Skalka
Some useful terms

- Plaque
- pfu
- MOI
- Particle to infectivity ratio
- Neutralizing Abs
- Cytopathic effect

Viral life cycle

Methods of diagnosis for viral diseases

- Serology
- Cytology or Histology
- Viral growth in cell culture
- Detection of viral genome

I. Serology

- Look for viral antigens or anti-viral antibodies
- A four fold or greater rise in titer between two serum specimens provides a positive diagnosis. Paired sera, the first taken as early as possible in the illness and the second 10 to 14 days after the onset of symptoms.
Serology Methods

- ELISA
  - Rapid tests for Flu, RSV
  - Hep B, Hep C etc etc
- Western Blots

Serology: ELISA

EIA for RSV

- 93-97% sensitivity and 90-97% specificity when compared to tissue culture
- results in about 6 minutes
- room temperature storage of kit

ELISA

- HIV antigens - from virus or recombinant proteins or synthetic peptides are immobilized on microtitre plates
- Incubate test serum. Wash
- Enzyme-labeled antibody specific for hu-IgG. Wash
- Substrate changes color

Serology: Western blot

Principles of Virology: Molecular Biology, Pathogenesis, and Control,
S. J. Flint, L. W. Enquist, V. R. Racaniello, A. M. Shakha
II. Histology and cytology

- Inclusion bodies
- Syncytia
- Tzanck test for VZV and HSV
- Negri bodies in rabies

Inclusion bodies

CMV: owl-eyed nucleus

Inclusion bodies

Adenovirus: cytomegaly, multinucleate cells, inclusions

Inclusion bodies

Adenovirus: cytomegaly, multinucleate cells, inclusions

Inclusion bodies

Hamster Adenovirus

Adenovirus: cytomegaly, multinucleate cells, inclusions

Tzanck smear

HSV

HSV, VZV: multinucleated giant cells
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Negri bodies

Electron microscopy

HSV

Rota

Adenovirus: cells, inclusions

Corona

III. Grow virus in culture

- Look for cytopathic effects (CPE) in culture
- Detect viral antigens by Shell vial culture

Cell culture in virology

Primary human fibroblasts

NIH3T3

HeLa

Cytopathic effect

Syncitia

- Identify virus by type of cell it grows in, time to detection of CPE and morphology of CPE
- Rounding, syncitia, vacuoles etc
- Confirm with fluorescent-labeled antibodies
- Results in days to weeks

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Cytopathic effect

Cell and Tissue-types for culture
Screening cells
- Rhesus Monkey Kidney (1°)
  - Myxo-, Paramyxoviruses etc
- Human Embryonic Kidney (1°)
  - Very sensitive for adenovirus and important for lung transplants
- MRC-5 (human embryonic lungs)
  - CMV, VZV, HSV

Cell-types for culture
- African Green Monkey Kidney
  - Rubella grows only on these
- Hep-2
  - RSV
- Vero
  - HSV
- Primary rabbit kidney
  - HSV, enteroviruses

Cell culture plus IF
- Grow virus in culture
  - Detect viral antigens by Shell vial culture
    - Inoculate specimen into many vials (one for each virus to be tested)
    - Stain with specific antibody
    - Results in 1-2 days

Monoclonal antibodies
(commercially available and FDA approved)
- HSV 1 and 2
- VZV
- CMV
- Flu A and B
- Parainfluenza 1, 2 & 3
- RSV
- Adeno
- Mumps
- Measles
- Some enteroviruses
- Chlamydia

Detect and analyze viral genomes
- PCR
- RT-PCR
- Quantitative PCR to detect viral load
- Branched DNA
- Hybridization, using microarrays
- Genotyping
- Phenotyping?
Polymerase chain reaction

Detect and analyze viral genomes

- PCR
- RT-PCR
- Quantitative PCR to detect viral load
- Branched DNA
- Southern blots
- Hybridization, using microarrays
- Genotyping
- Phenotyping?

Southern blot

Hybridization with microarrays

Sensitivity of NAT

- Combination of PCR/Southern blot: 95% confidence intervals
  - HAV, 5-9 copies/ml
  - HBV, 1-2 copies/ml
  - HCV, 3-5 copies/ml

Reduce risk of HCV transmission
From 1:100,000 to 1:500,000-1:1,000,000

Data from National Genetics Institute, Labcorp
Pool testing

$8 \times 8 \times 8 = 512$ samples

Other labs

- State Department of Health lab
- Centers for Disease Control
- Other commercial labs

NYDOH lab for viral encephalitis

- Herpes Simplex
- Varicella Zoster
- Cytomegalovirus
- Epstein-Barr Virus
- Enteroviruses
- St. Louis Encephalitis (SLE)
- Eastern Equine Encephalitis (EEE)
- California Encephalitis
- Powassan (POW)
- Rabies
- West Nile Virus

Tests include: 1) PCR, and 2) ELISA.
Freeze leftover CSF at -70°C in the event that PCR testing becomes necessary.

CDC

- Small pox, Hantavirus, Ebola etc
- Usually via the State labs

Viremia

What specimen to collect? When?
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### What specimen to collect?

#### When?

- **Throat**
  - First presentation with fever (measles, mumps, rubella, also viral meningitis caused by enteroviruses and neonatal HSV). Vigorous swab, because you need cells.
- **Nasopharyngeal swab or wash**
  - Flu, RSV, Rhino-, CMV (if lots of virus)
- **Rectal**
  - Entero- and adenoviruses (meningitis), rotavirus
- **Urine**
  - Adenovirus (hemorrhagic cystitis)
  - MMR, after cleared from throat or sometimes concomitant
  - CMV and HSV (rare)

### What specimen to collect?

#### When?

- **CSF**
  - PCR for HSV, VZV, CMV, adeno or flu
  - Rarely can grow coxsackie or echo
- **Lesion**
  - VZV, CMV, measles (scrape for cells)
  - HSV, Tzanck smear
- **Conjunctival**

### Transport to lab

- Since we still depend on viral growth for diagnosis, rapid transport to lab is essential
- Specimen on ice
- Refrigerate if delay inevitable, DO NOT FREEZE
- If need to store for more than 6 days, freeze at -70°C
- Transport and store in viral transport medium
- Enteroviruses more stable and will tolerate some delay
- Hand delivery encouraged (also for better communication: viruses suspected, source of material)

### Web resources

- [www.cdc.gov](http://www.cdc.gov), get a free electronic MMWR subscription
- [www.wadsworth.org](http://www.wadsworth.org)
- HIV database: hiv-web.lanl.gov
- All the Virology on the WWW: [www.virology.net/garryfavwebindex.html](http://www.virology.net/garryfavwebindex.html)
- Pan-American Society for Clinical Virology: [www.virology.org/](http://www.virology.org/)
- [www.specialty.com](http://www.specialty.com)