Pathology of viral disease

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Topics for the first lecture....

General virology
Viral lifecycle
Viral pathogenesis
Laboratory diagnosis

Classification schemes for animal RNA viruses
Some useful terms

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

Viral assembly and release

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

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: ELISA

B

Solid support

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


Western blot

II. Histology and cytology

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

Inclusion bodies

Inclusion bodies

Inclusion bodies
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III. Grow virus in culture

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

Cytopathic effect

- 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

Cell culture in virology

Electron microscopy

Corona


Negri bodies

Electron microscopy

HSV
Adenovirus: cells, inclusions

Rota

Corona

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Cytopathic effect

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

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

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 x 8 x 8 = 512 samples

CDC

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

Other labs

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

What specimen to collect?
When?

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.

Viremia

Principles of Virology: Molecular Biology, Pathogenesis, and Control,
S. J. Flint, L. W. Enquist, V. R. Racaniello, A. M. Strauss
HIV infection

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)
- 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

What specimen to collect? When?
- Genital
  - HSV, vulvar swab (not endocervical) in last month of pregnancy
- Buffy coats
  - CMV (fresh specimen, <1hr)
- Bronchial and BAL wash
  - RSV, Flu, Adeno-, CMV etc
- Other
  - Biopsy, autopsy specimens

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