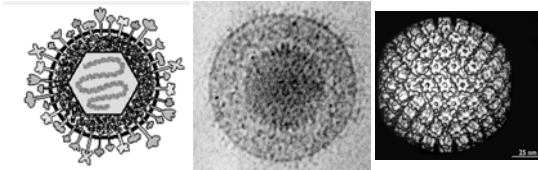


Pathology of viral disease

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Viral Structure
 Herpes virus



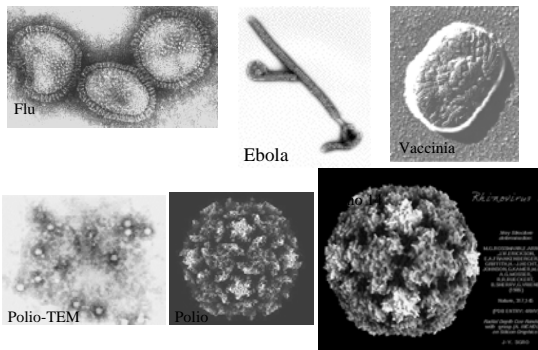
- Envelope
- Tegument
- Spikes
- Nucleocapsid
- Genome

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

Topics for the first lecture....

General virology
 Viral lifecycle
 Viral pathogenesis
 Laboratory diagnosis

Viral Structure




www.tulane.edu/~dmsander/Big_Virology/BVHomePage.html

Virus size

QuickTime™ and a
 TIFF (LZW) decompressor
 are needed to see this picture.

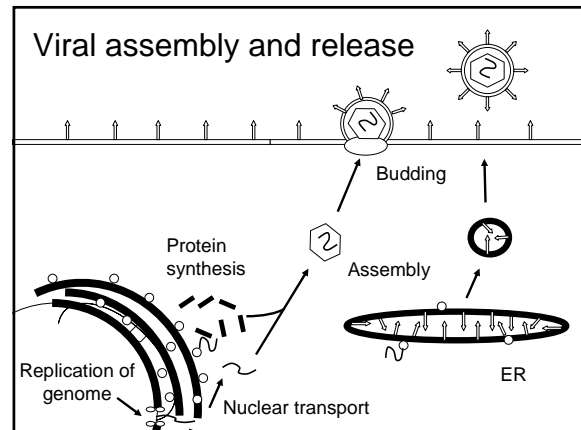
Principles of Virology: Molecular Biology, Pathogenesis, and Control,
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**Classification schemes for
 animal RNA viruses**

Classification criteria	RNA													
	Icosahedral						Helical							
Symmetry of capsid	Naked			Enveloped			Enveloped							
Naked or enveloped	Naked			Enveloped			Enveloped			Enveloped				
Genome architecture	ds 10-18 seg	ds 2 seg	(+) ss cont.	(+) ss cont.	(+) ss cont.	(+) ss 2 coplat	(+) ss cont.	(-) ss cont.	(-) ss cont.	(-) ss 3 seg	(-) ss 8 seg	(-) ss cont.	(-) ss 2 seg	
Baltimore class	III	III	IV	IV	IV	VI	IV	V	V	V	V	V	V	
														
Family name	Reo	Birna	Calci	Picorna	Flavi	Toga	Retro	Corona	Filo	Rhabdo	Bunya	Ortho-myxo	Para-myxo	Arena
Virion polymerase	(+)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
Virion diameter (nm)	60-80	80	35-40	28-30	40-50	60-70	80-120	80-160	70-1,000	80 x 130-380	70-120	90-120	100-300	10-14
Genome size (total in kb)	22-27	7	8	7.2-8.4	10	12	3.5-9	16-21	12.7	13-16	13.5-21	13.6	16-20	10-14

Some useful terms

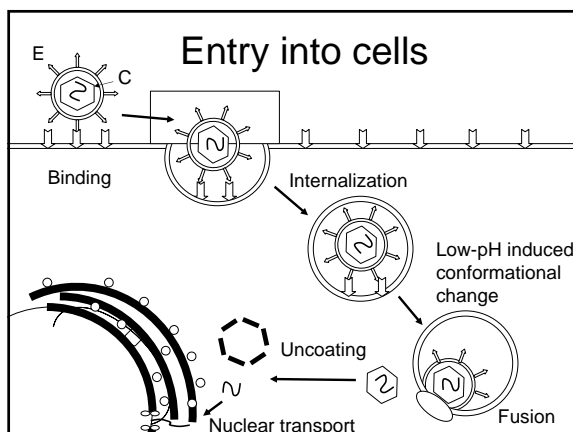
- Plaque
- pfu
- MOI
- Particle to infectivity ratio
- Neutralizing Abs
- Cytopathic effect



Viral life cycle

Methods of diagnosis for viral diseases

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



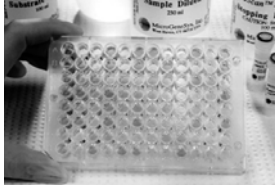
I. Serology

- 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.

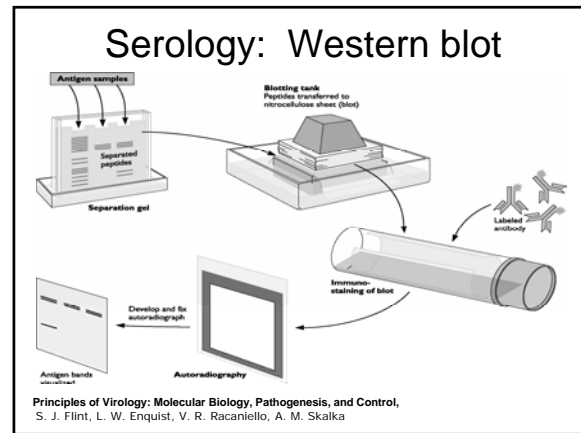
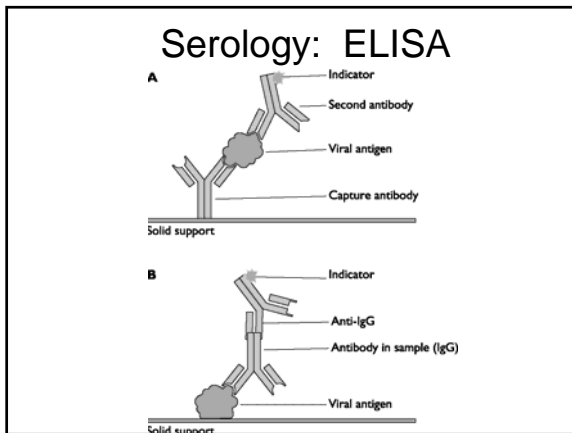
Serology Methods

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


ELISA



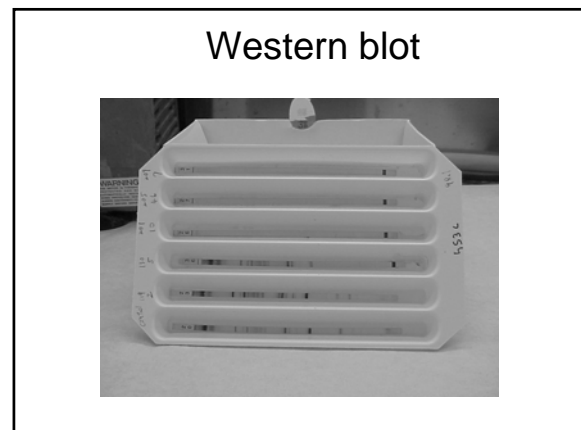
- HIV antigens - from virus or recombinant proteins or synthetic peptides are immobilized on microtitre plates
- Incubate test serum. Wash
- Enzyme-labeled antibody specific for hu- IgG. Wash.
- Substrate changes color

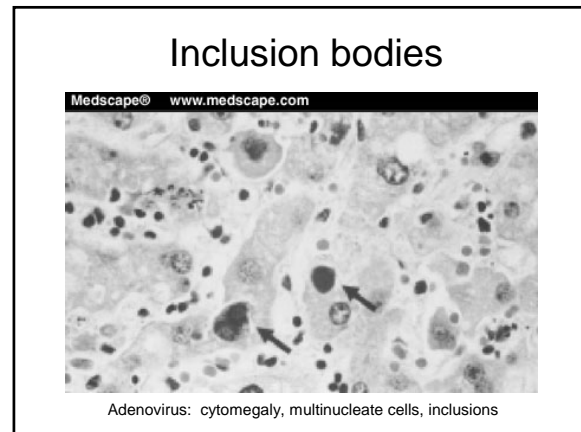
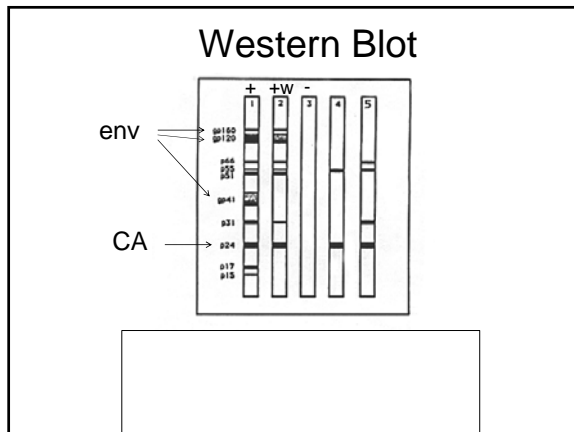


EIA for RSV



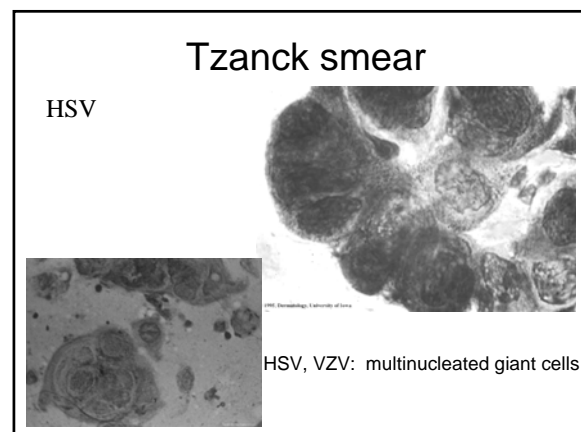
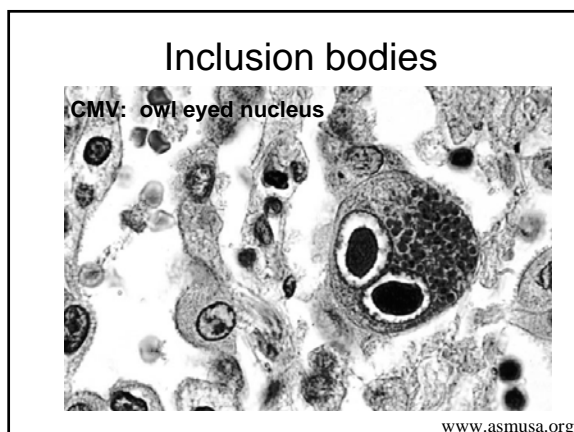
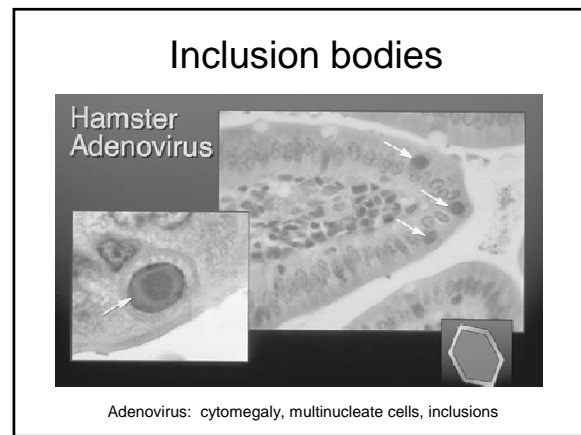
- 93-97% sensitivity and 90-97% specificity when compared to tissue culture
- results in about 6 minutes
- room temperature storage of kit

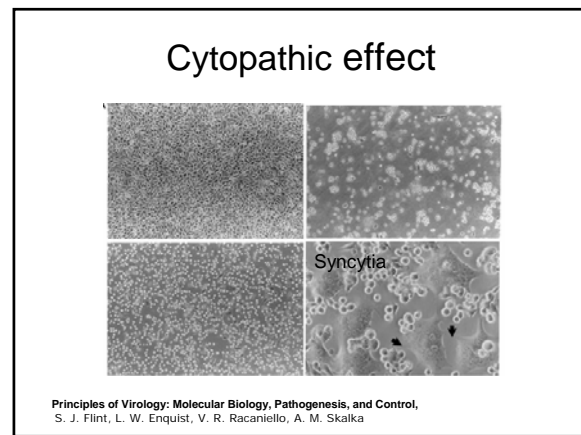
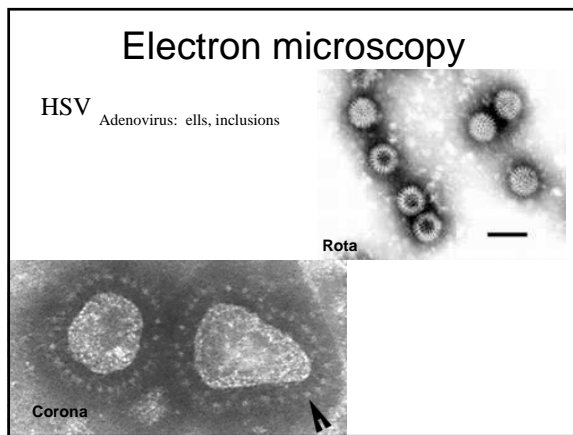
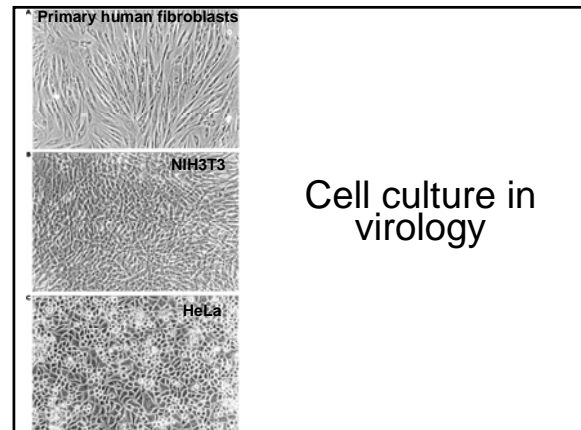
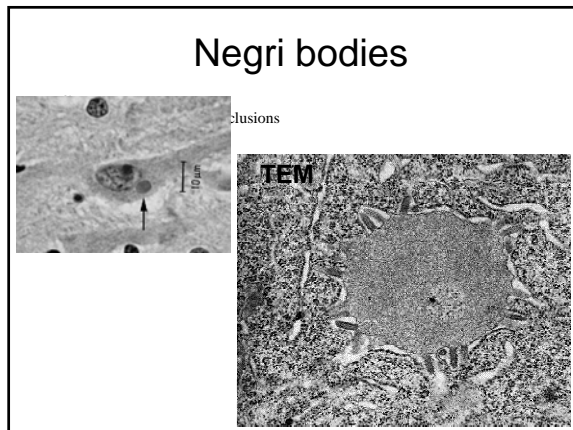




II. Histology and cytology

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





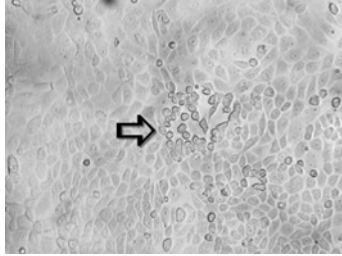
III. Grow virus in culture

- ◆ 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, syncytia, vacuoles etc
- Confirm with fluorescent-labeled antibodies
- Results in days to weeks

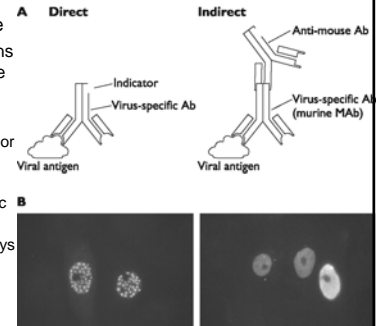
Cytopathic effect



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

Monoclonal antibodies (commercially available and FDA approved)

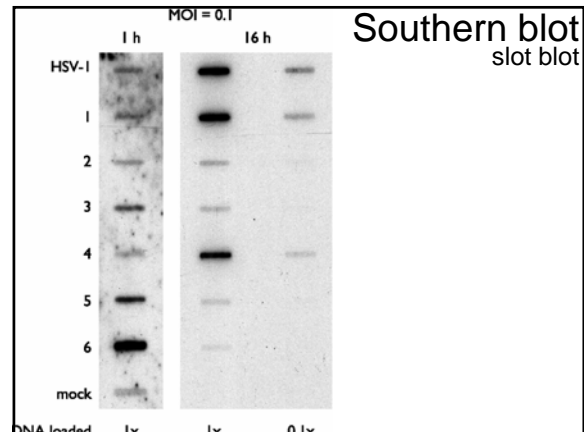
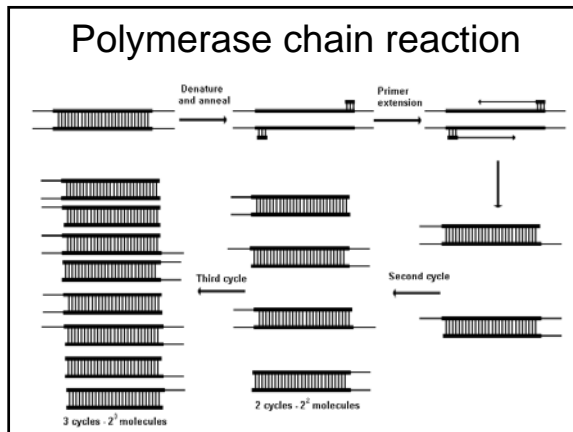
- | | |
|--------------------------|----------------------|
| ▪ HSV 1 and 2 | ▪ Adeno |
| ▪ VZV | ▪ Mumps |
| ▪ CMV | ▪ Measles |
| ▪ Flu A and B | ▪ Some enteroviruses |
| ▪ Parainfluenza 1, 2 & 3 | |
| ▪ RSV | ▪ Chlamydia |

Cell-types for culture

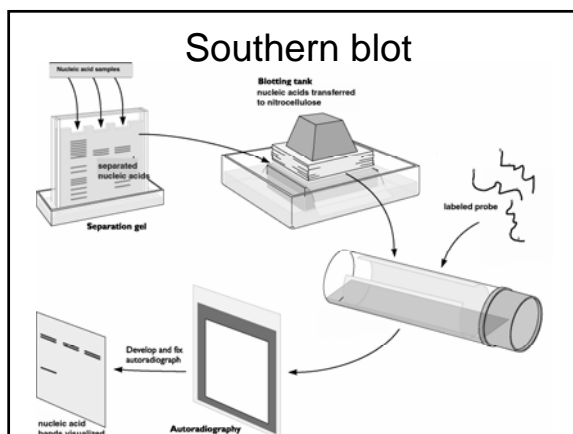
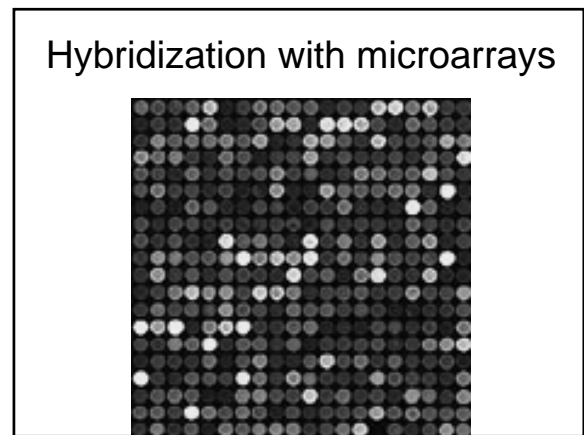
- African Green Monkey Kidney
 - ◆ Rubella grows only on these
- Hep-2
 - ◆ RSV
- Vero
 - ◆ HSV
- Primary rabbit kidney
 - ◆ HSV, enteroviruses

Detect and analyze viral genomes

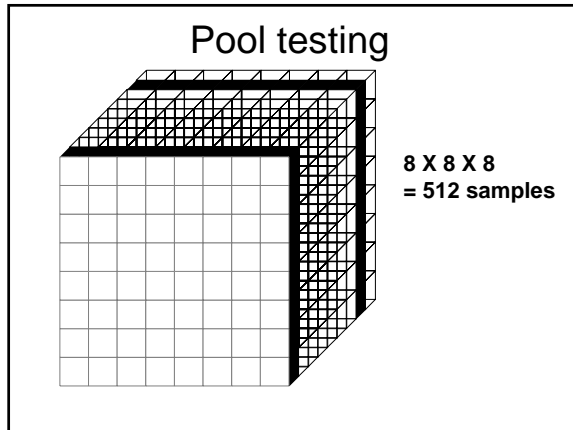
- ◆ PCR
- ◆ RT-PCR
- ◆ Quantitative PCR to detect viral load
- ◆ Branched DNA
- ◆ Hybridization, using microarrays
- ◆ Genotyping
- ◆ Phenotyping?



- ### Detect and analyze viral genomes
- ◆ PCR
 - ◆ RT-PCR
 - ◆ Quantitative PCR to detect viral load
 - ◆ Branched DNA
 - ◆ Southern blots
 - ◆ Hybridization, using microarrays
 - ◆ Genotyping
 - ◆ Phenotyping?



- ### 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



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

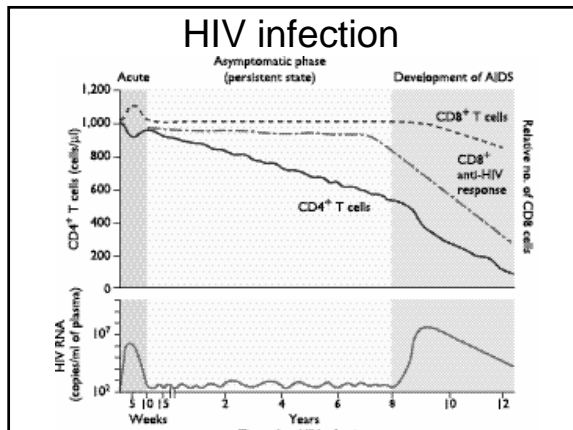
- Herpes Simplex
- Varicella Zoster
- Cytomegalovirus
- Epstein-Barr Virus
- Enteroviruses
- St. Louis Encephalitis (SLE)
- Eastern Equine Encephalitis (EEE)
- California Encephalitis
- Powassan (POW)
- Rabies
- West Nile Virus

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

Viremia

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

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Skalka



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

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)

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)

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**

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