

Pathology of viral disease

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Topics for the first lecture....

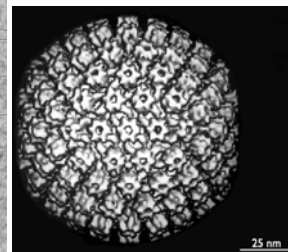
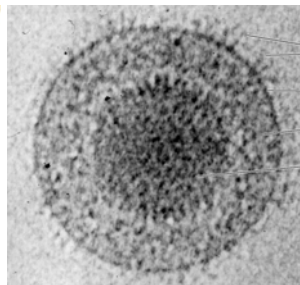
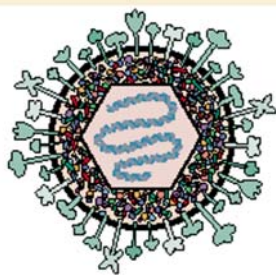
General virology
Viral lifecycle
Viral pathogenesis
Laboratory diagnosis

Virus size

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

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

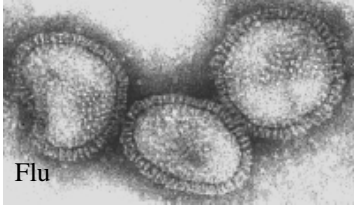
Viral Structure Herpes virus




- Envelope
- Tegument
- Spikes
- Nucleocapsid
- Genome

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Viral Structure



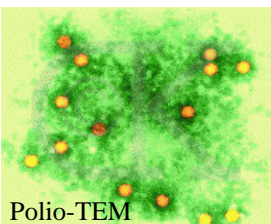
Flu



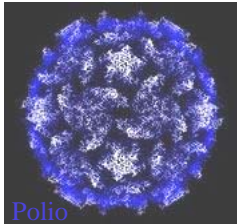
Ebola



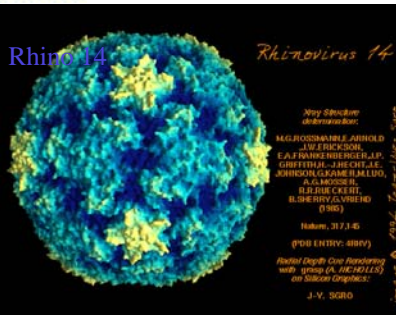
Vaccinia



Polio-TEM



Polio



Rhino 14


Rhinovirus 14

3D Structure Determination:
M.G. ROOSMANS, E. AHMED,
J.W. BICKSON, E.A. BARNES, B. BODILUP,
G. BRITTON, J. CHEN, J.E.
JOHNSON, G. LAM, I. LALLO,
A.G. LÖNNER, B. J. M. CHEN,
B. SHERRY, A. WISSE
(1992)

Nature, 357, 33-36
© 1992 Nature Group
Revised Depth Color Representation
with Gray (0.0021116)
on Colored Grayscale
J.-Y. SORO

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

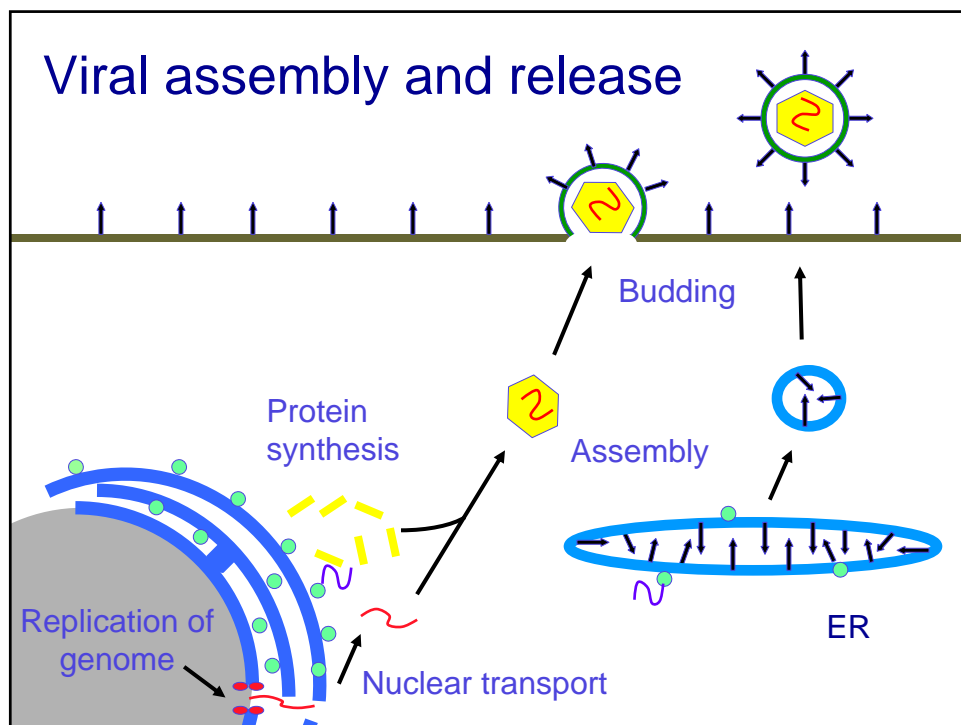
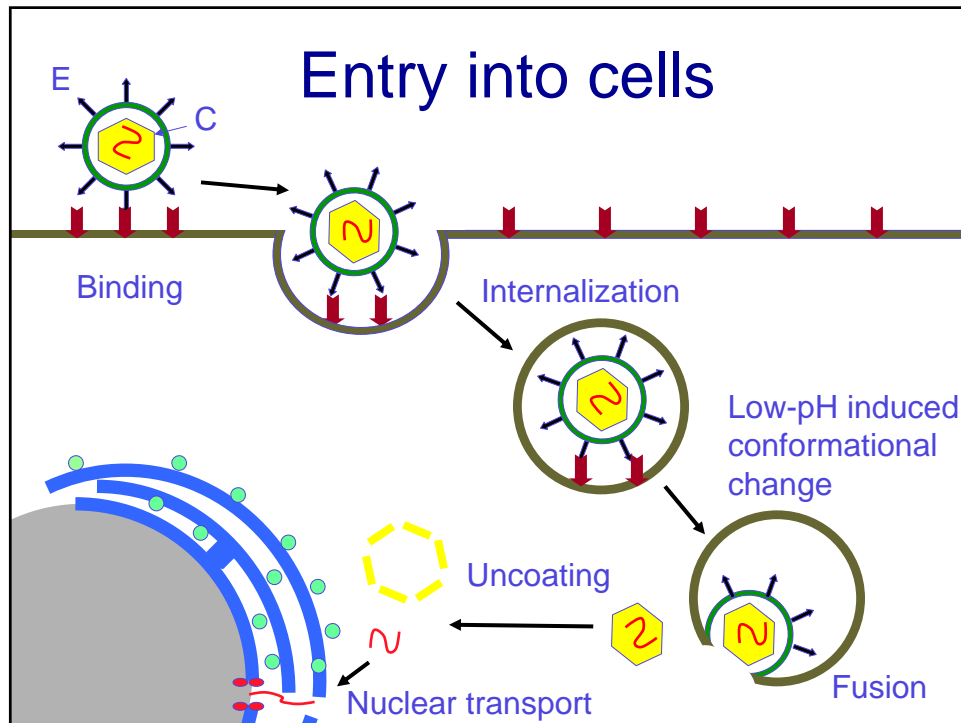
Classification schemes for animal RNA viruses

		RNA													
		Icosahedral							Helical						
Classification criteria	Symmetry of capsid	Naked							Enveloped						
	Naked or enveloped	Naked							Enveloped						
	Genome architecture	ds 10-18 seg.	ds 2 seg.	(+) ss cont.	(+) ss cont.	(+) ss cont.	(+) ss cont.	(+) ss 2 copies	(+) ss cont.	(-) ss cont.	(-) ss cont.	(-) ss 3 seg.	(-) ss 8 seg.	(-) ss cont.	(-) ss 2 seg.
	Baltimore class	III	III	IV	IV	IV	IV	VI	IV	V	V	V	V	V	V
Properties															
	Family name	Reo	Birna	Calici	Picorna	Flavi	Toga	Retro	Corona	Filo	Rhabdo	Bunya	Orthomyxo	Paramyxo	Arena
	Virion polymerase	(+)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
	Virion diameter (nm)	60-80	60	35-40	28-30	40-50	60-70	80-130	80-160	80 X 790-14,000	70- 85 X 130-380	90-120	90-120	150-300	50-300
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

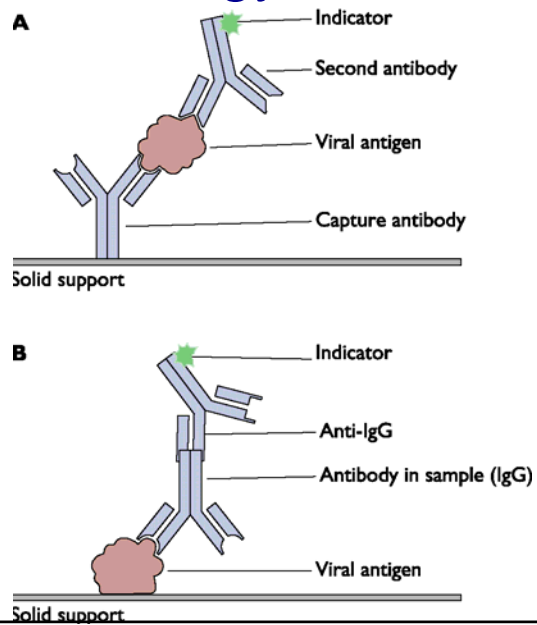
I. Serology

- 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

Serology: ELISA



EIA for RSV

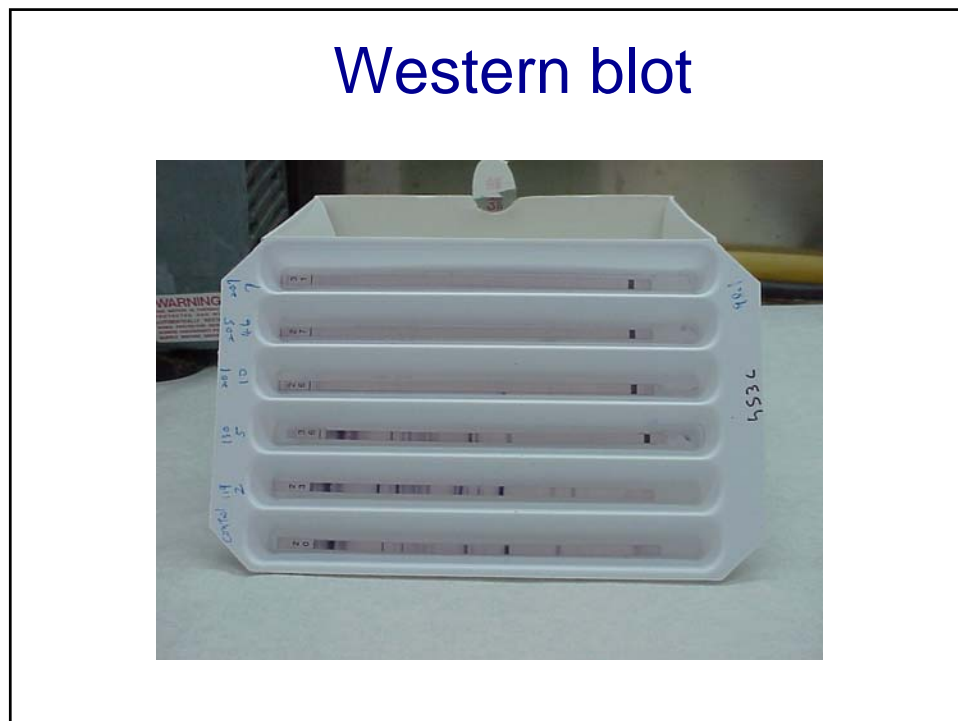
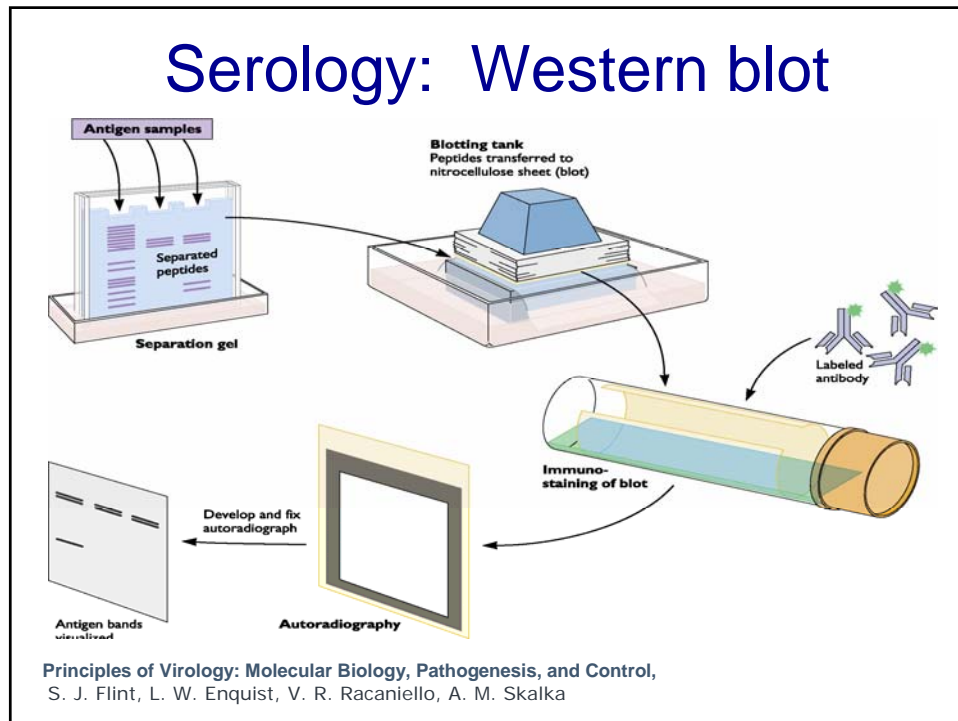


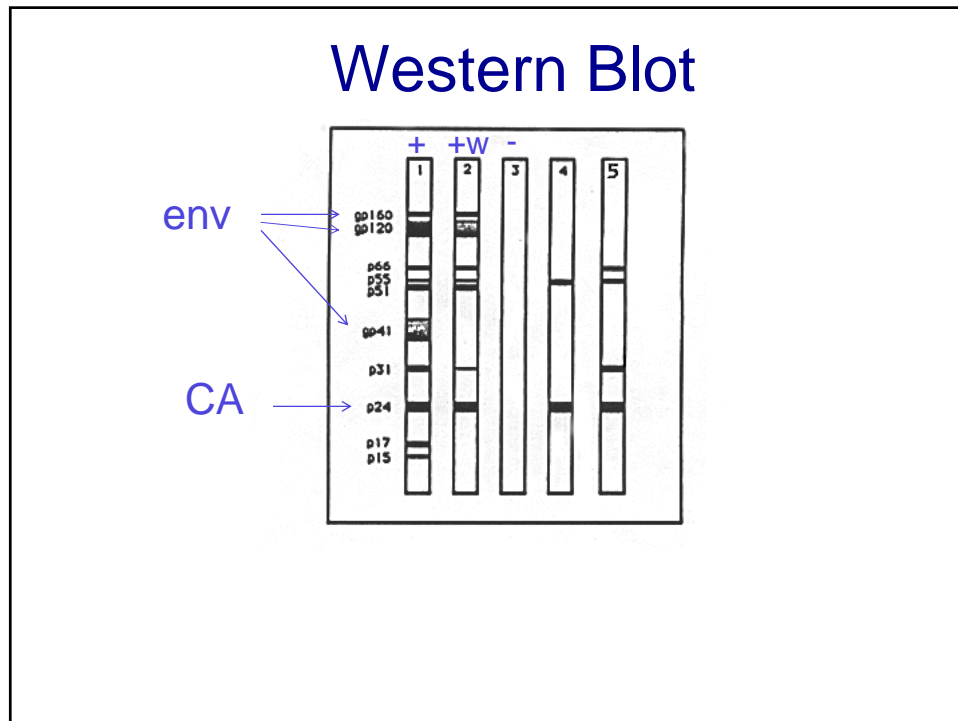
- 93-97% sensitivity and 90-97% specificity when compared to tissue culture
- results in about 6 minutes
- room temperature storage of kit

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

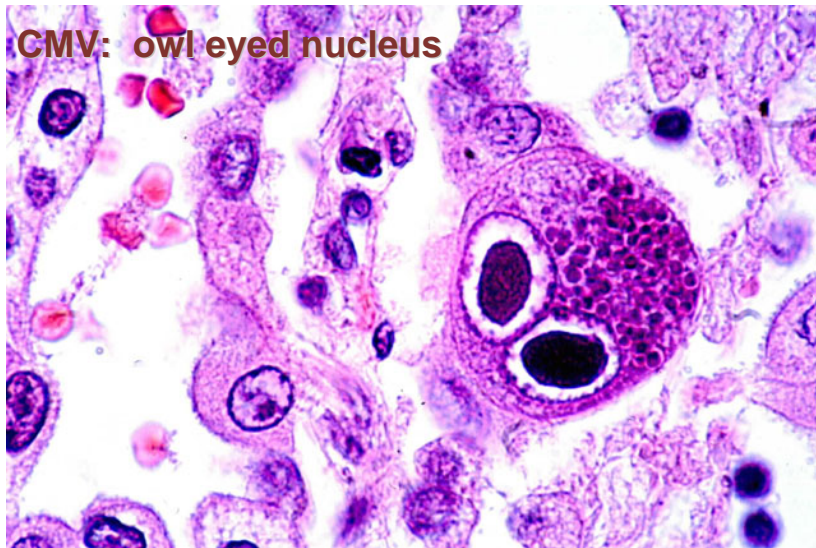




II. Histology and cytology

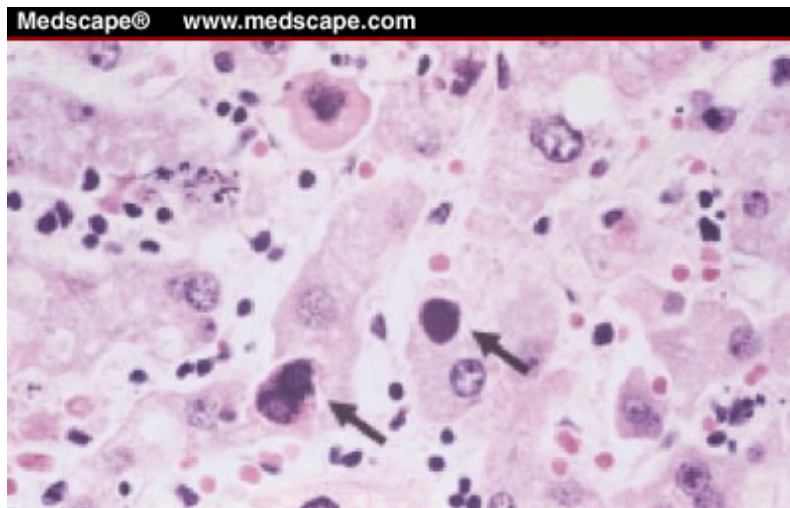
- ◆ Inclusion bodies
- ◆ Syncytia
- ◆ Tzanck test for VZV and HSV
- ◆ Negri bodies in rabies

Inclusion bodies



www.asmtusa.org

Inclusion bodies



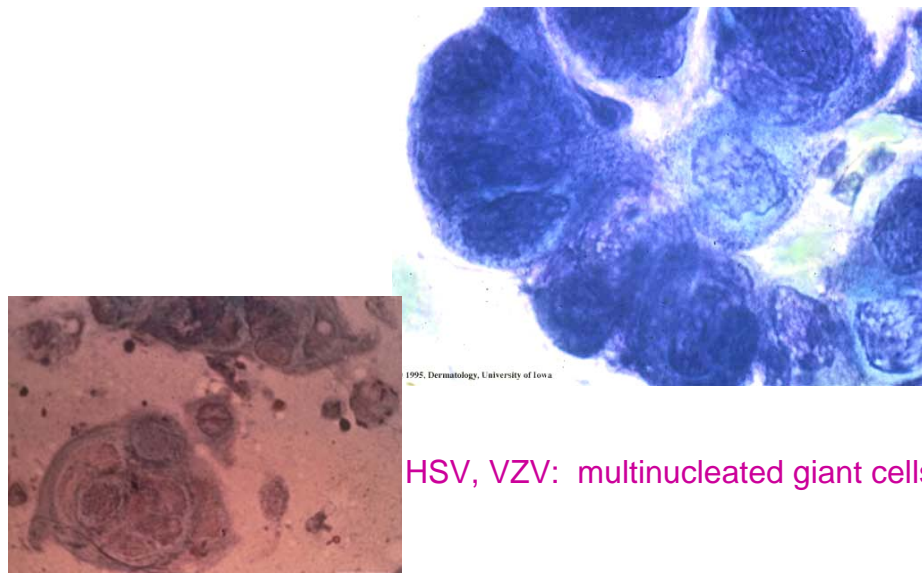
Adenovirus: cytomegaly, multinucleate cells, inclusions

Inclusion bodies



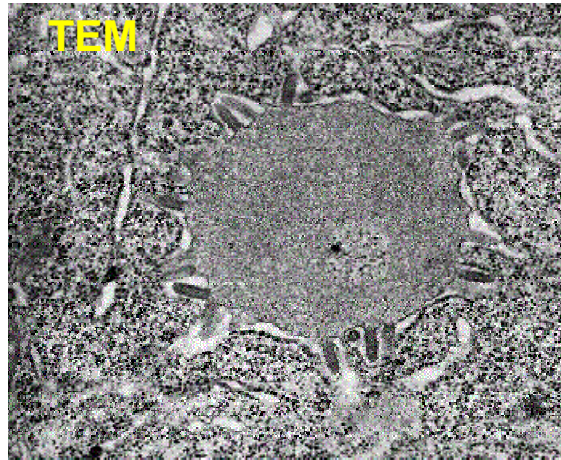
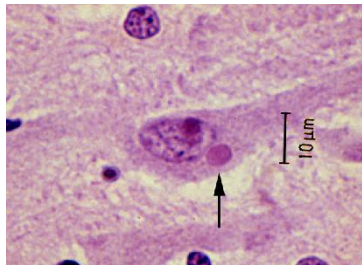
Adenovirus: cytomegaly, multinucleate cells, inclusions

Tzanck smear

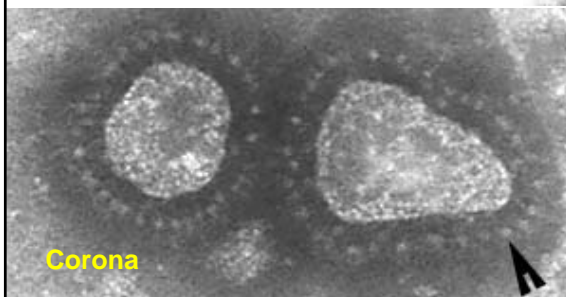
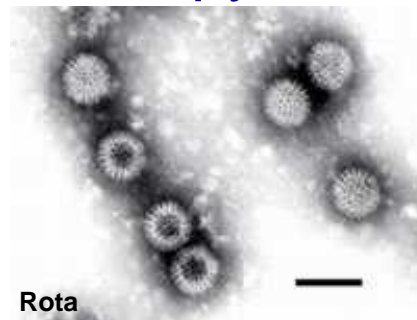


HSV, VZV: multinucleated giant cells

Negri bodies

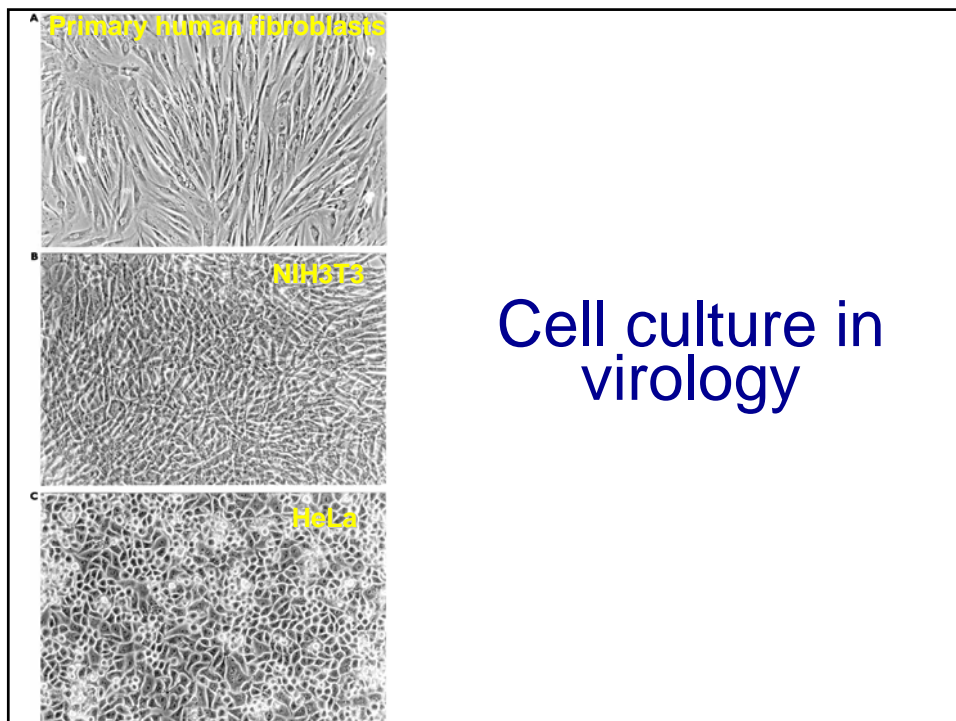


Electron microscopy

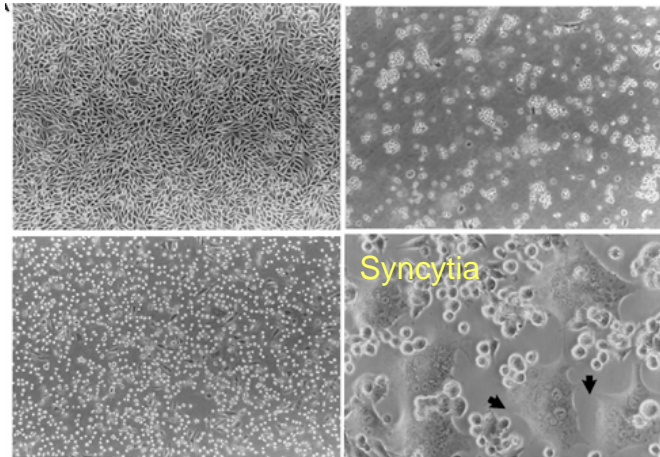


III. Grow virus in culture

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



Cytopathic effect

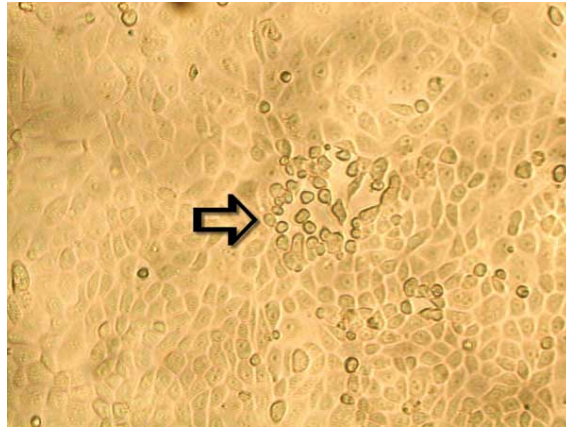


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Cytopathic effect

- 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 and Tissue-types for culture Screening cells

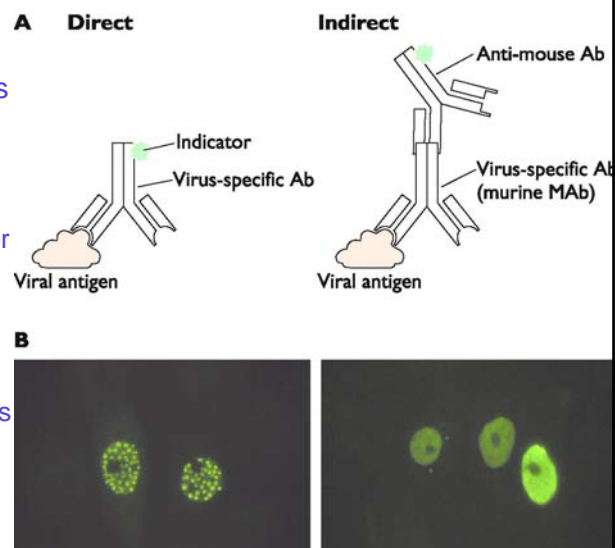
- Rhesus Monkey Kidney (1°)
 - ◆ Myxo-, Paramyxoviruses etc
- Human Embryonic Kidney (1°)
 - ◆ Very sensitive for adenovirus and important for lung transplants
- MRC-5 (human embryonic lungs)
 - ◆ CMV, VZV, HSV

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

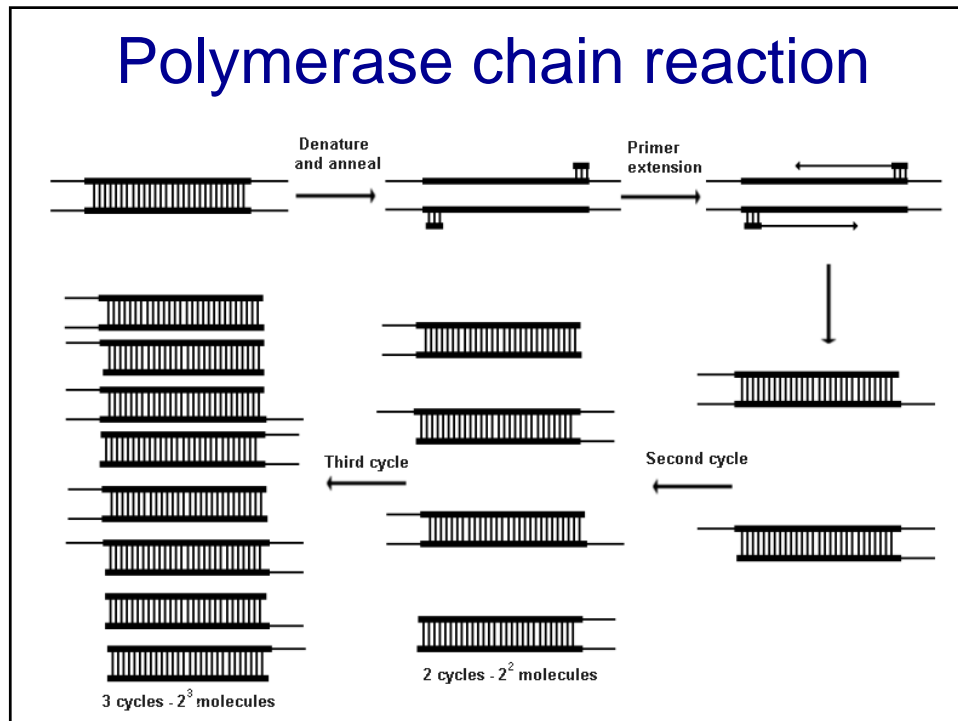


Monoclonal antibodies (commercially available and FDA approved)

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

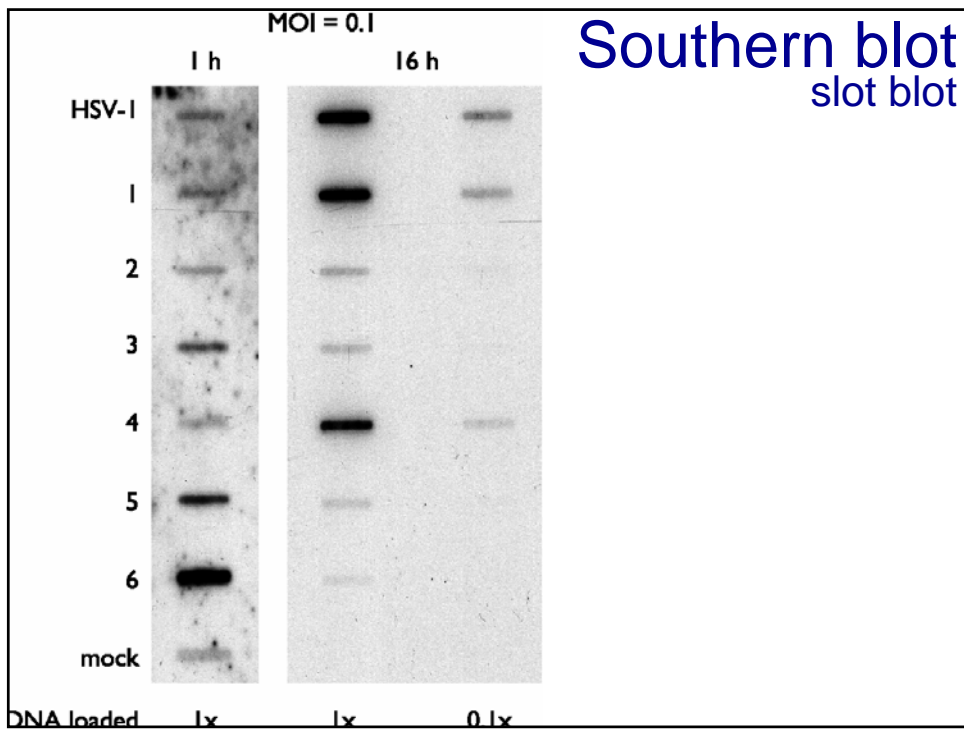
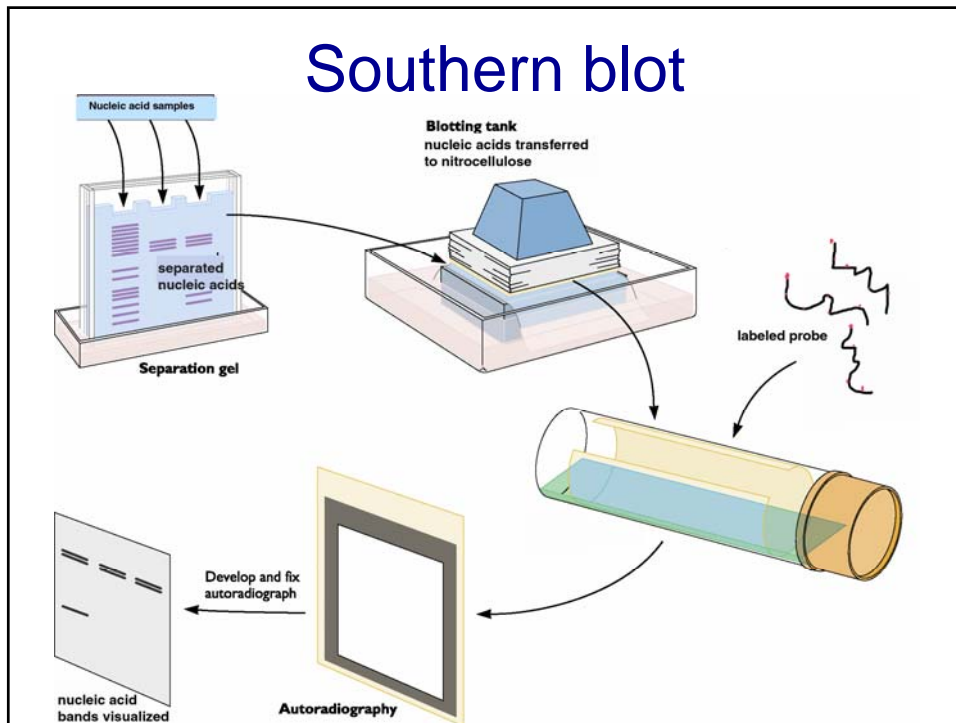
Detect and analyze viral genomes

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

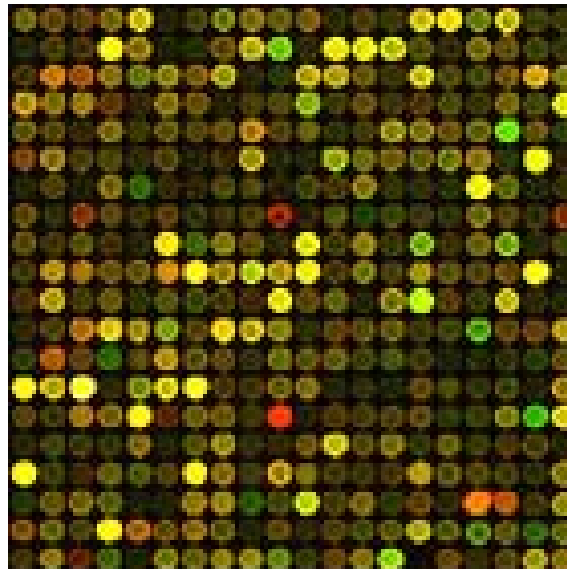


Detect and analyze viral genomes

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



Hybridization with microarrays



Sensitivity of NAT

- Combination of PCR/Southern blot:
95% confidence intervals

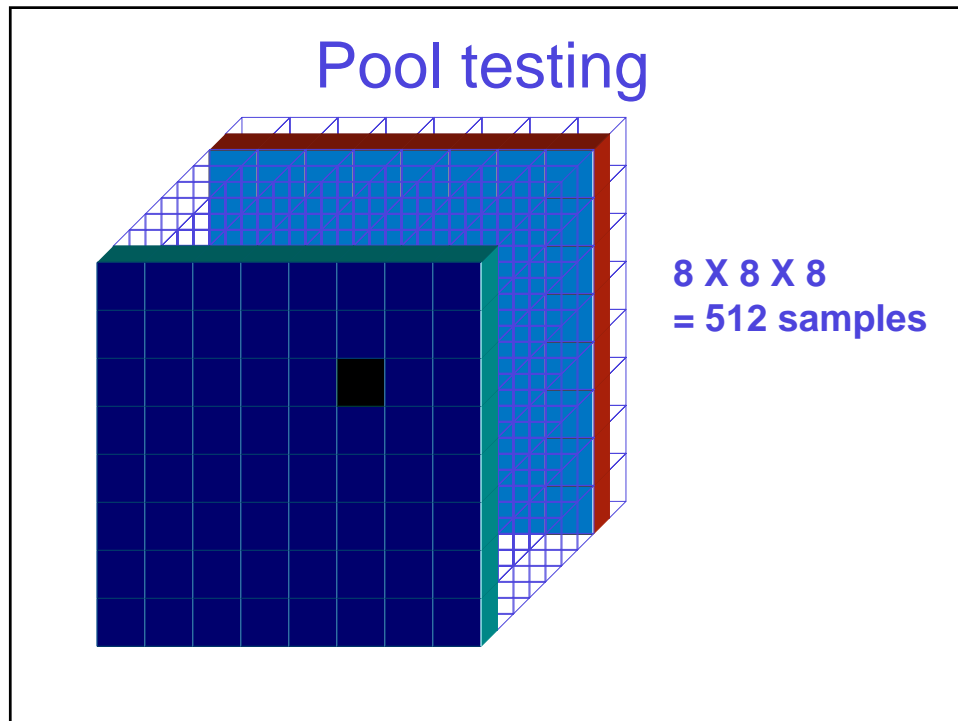
- ◆ HAV, 5-9 copies/ml

- ◆ 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



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

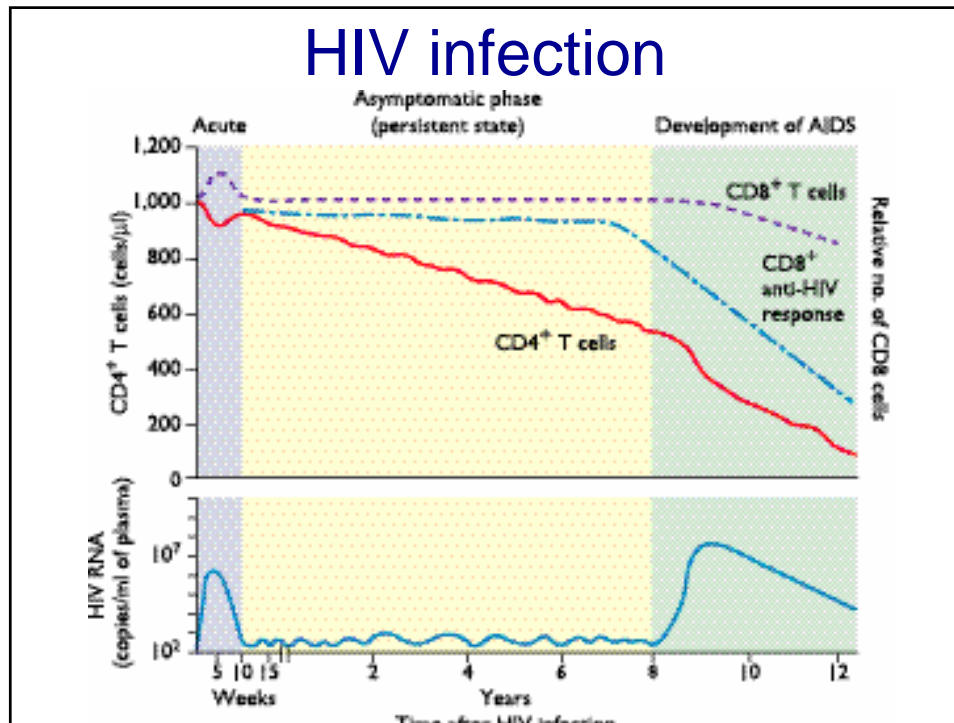
- Small pox, Hantavirus, Ebola etc
- Usually via the State labs

What specimen to collect?
When?

Viremia

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

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Skalka



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**

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