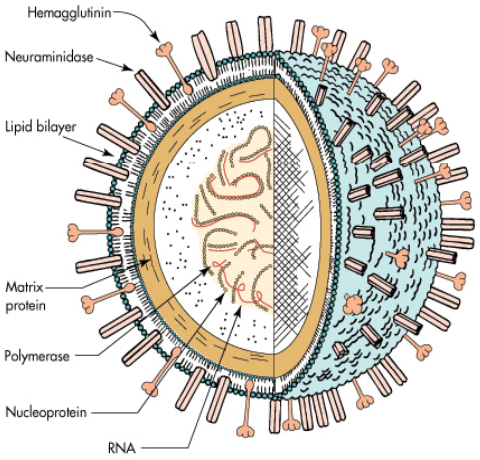
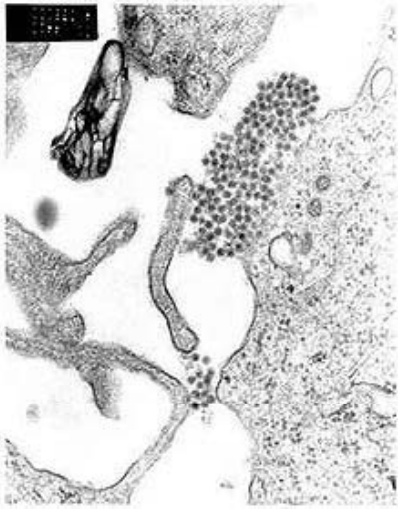
Principles of clinical virology
Structure and pathogenesis





Bill Rawlinson

Virology Division, SEALS Microbiology

May 2010

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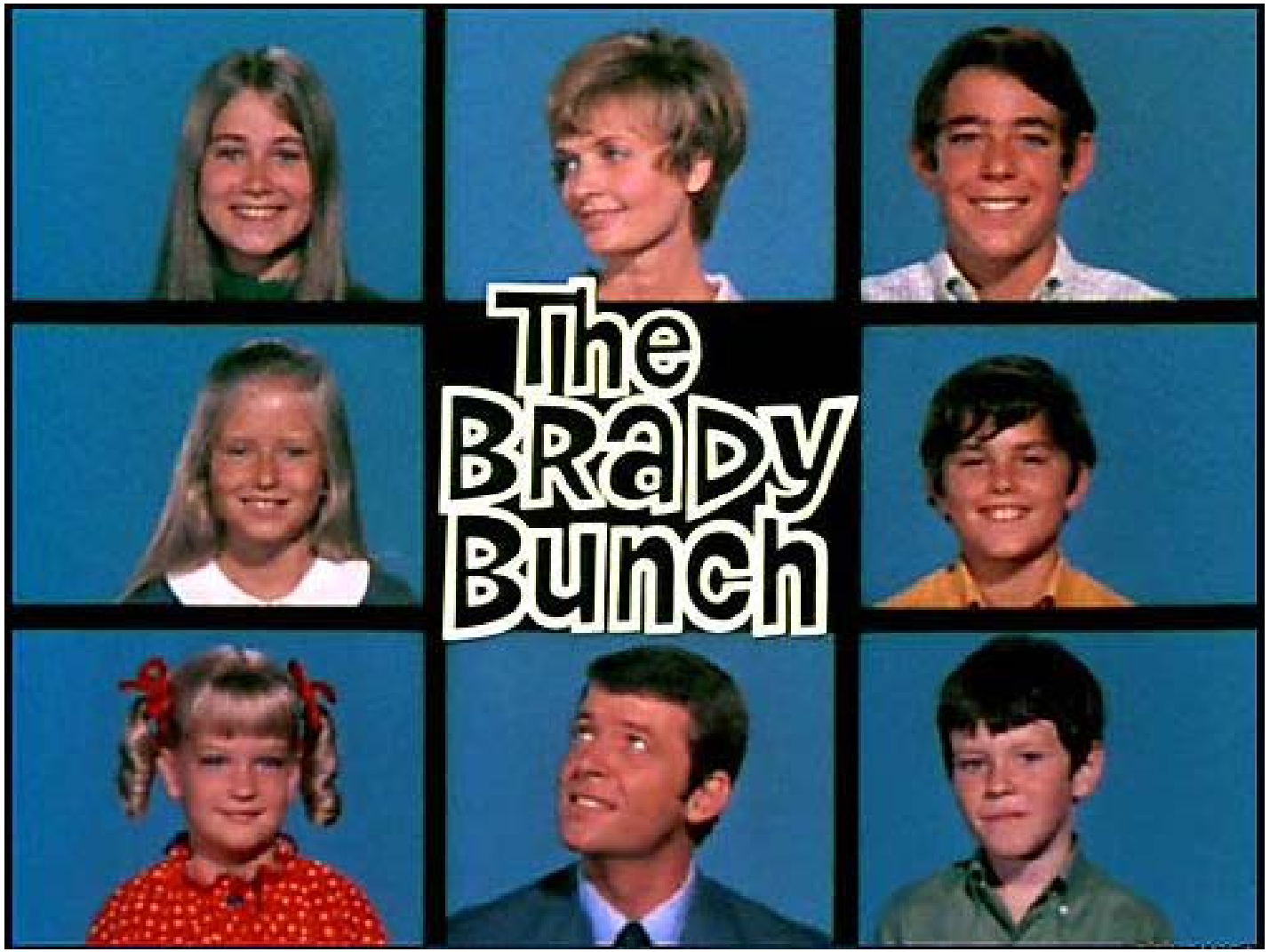

What were the names of the Brady Bunch?
Who died of an infectious disease?

Viral Disease

- Oldest recorded disease (Rabies, polio)
- Modern epidemics/pandemics – HIV-AIDS, HCV, SARS, Avian Influenza
- Impact on - humans
  - animals
  - plants
  - evolution

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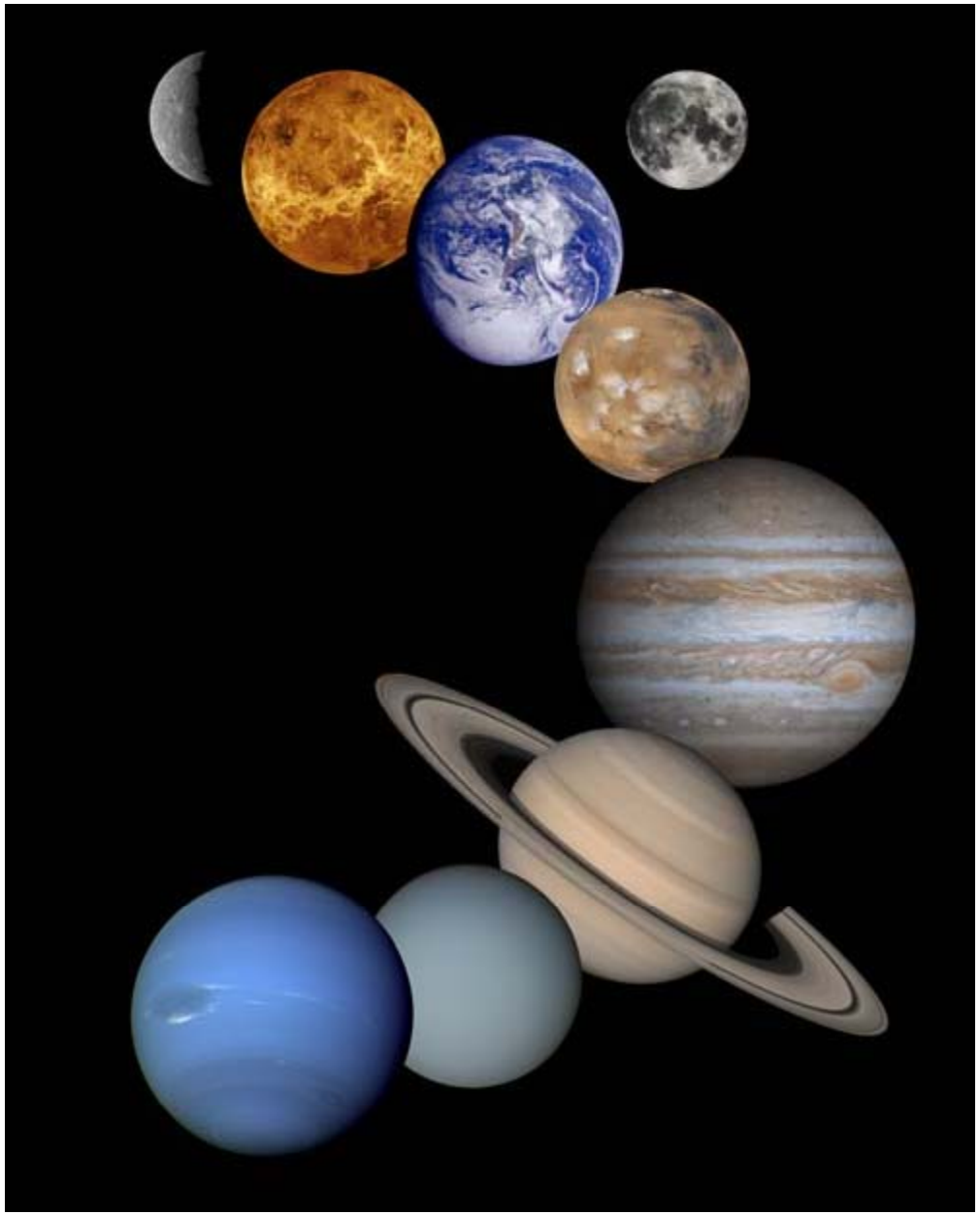

OUTLINE

1. What is a virus
   - Characteristics
   - Structure
   - Replication
   - Definitions
2. How viruses cause disease
   - Molecular principles
   - Disease pathogenesis
3. Diagnosis of viral illness
   - Principles
   - Methods
4. Clinical virology
   - principles

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A virus is a molecular genetic parasite that uses cellular systems for its own replication

Filtrable agents

Delivery system surrounding a payload

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Viruses

- This intimate relationship between the virus and the cell causes several important effects:
  - Viruses are not killed by antibiotics
  - Antivirals often damage the cell
  - Viruses can persist in cells either replicating (HIV) or resting – latent (HSV)

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Viruses

- Viruses are the simplest organisms, containing DNA or RNA, but not both
- RNA viruses are more diverse and replication often error prone

- Enveloped (environmentally unstable) and non-enveloped (environmentally stable)

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Viruses have life

- Can be killed
- Can become extinct
- Undergo Darwinian selection
- Subject to evolutionary biology

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But viruses

- Have no sexual exchange process
- Species is defined by its lineage
- Species is a class that occupies a replicating lineage and occupies an ecological niche

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Day 1

Day 2

Day 5

Day 6

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Herpes zoster

a

b

Herpes zoster

a

b

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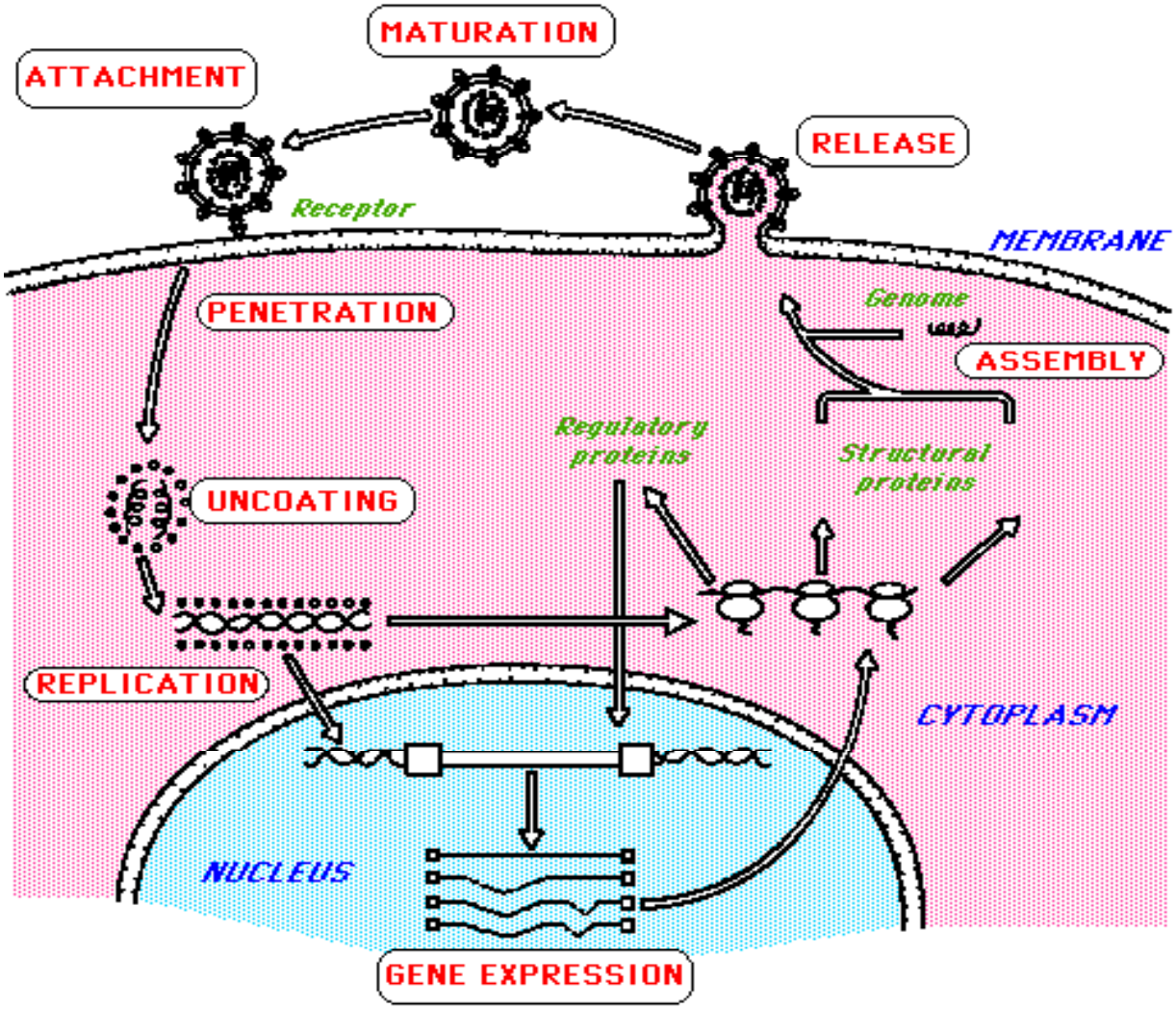
Structure

- Viruses are:
  - not cells
  - dependent upon the cell they infect. Inside cells they can replicate, outside cells they can be transmitted, but cannot replicate (grow)
  - sometimes viruses integrate their nucleic acid into the host cell genome

Virus replication
The delivery system and the payload



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Virion Architecture
The delivery system

Architecture of virions regardless of host is based on two simple themes:

Sphere – normally in the form icosahedron (cubical)
- Best way of producing a shell of equivalently bonded identical structures
- Minimum free energy state
- Strong structure that can enclose a maximal volume

Helix – cylindrical shape (spiral staircase)

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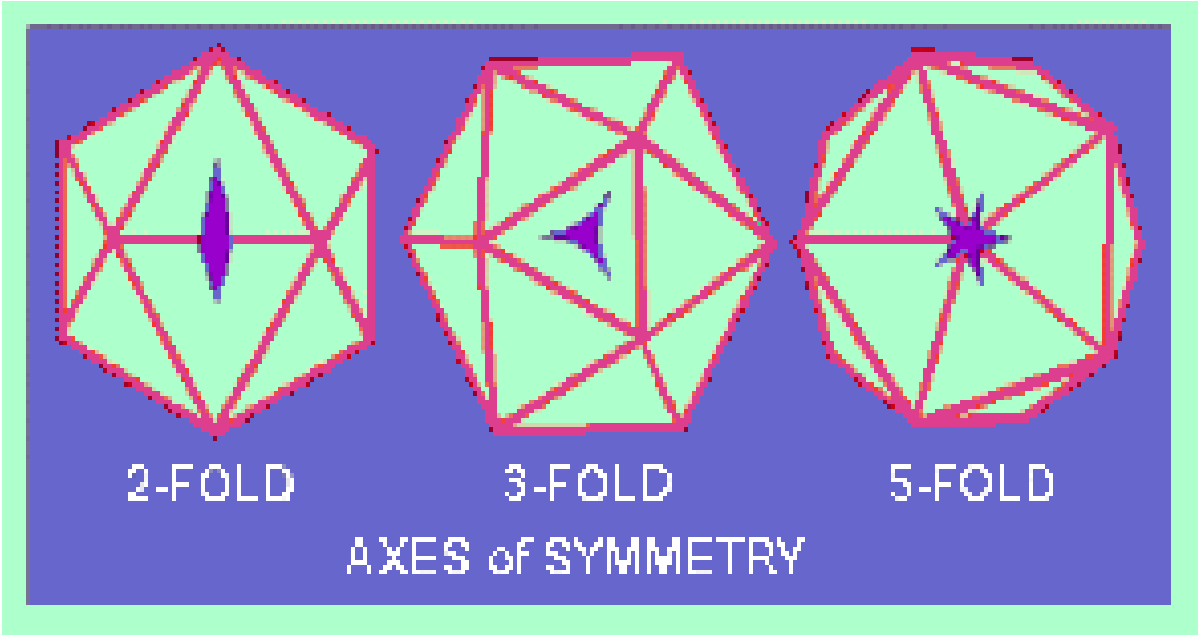
Virion Architecture – icosahedron

An ICOSAHEDRON is composed of 20 facets, each an equilateral triangle, and 12 vertices (corners)



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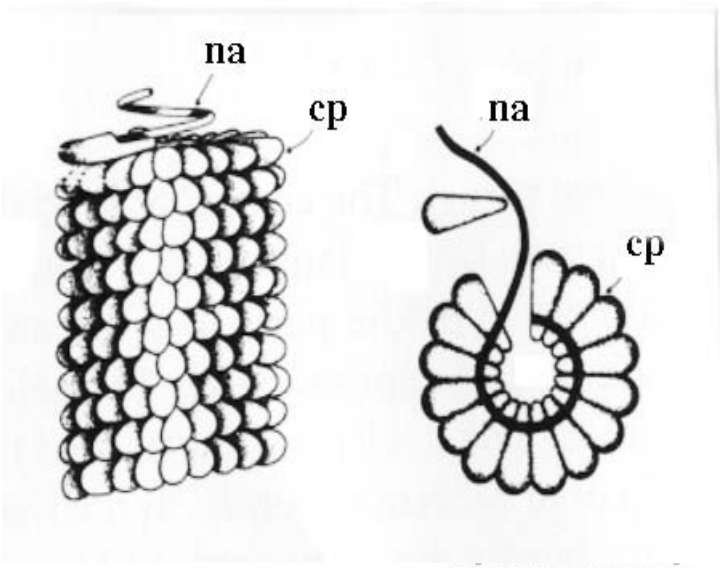
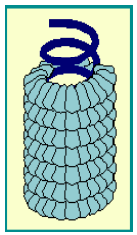
Helical viral structure

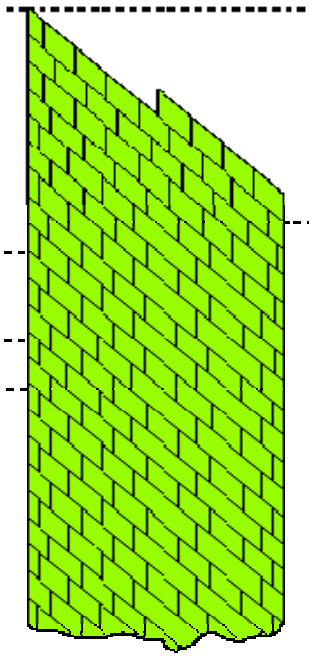
Several RNA viruses undergo self assembly as a cylindrical nucleocapsid. *(hollow tube)*

The viral RNA forms a spiral within the capsid structure

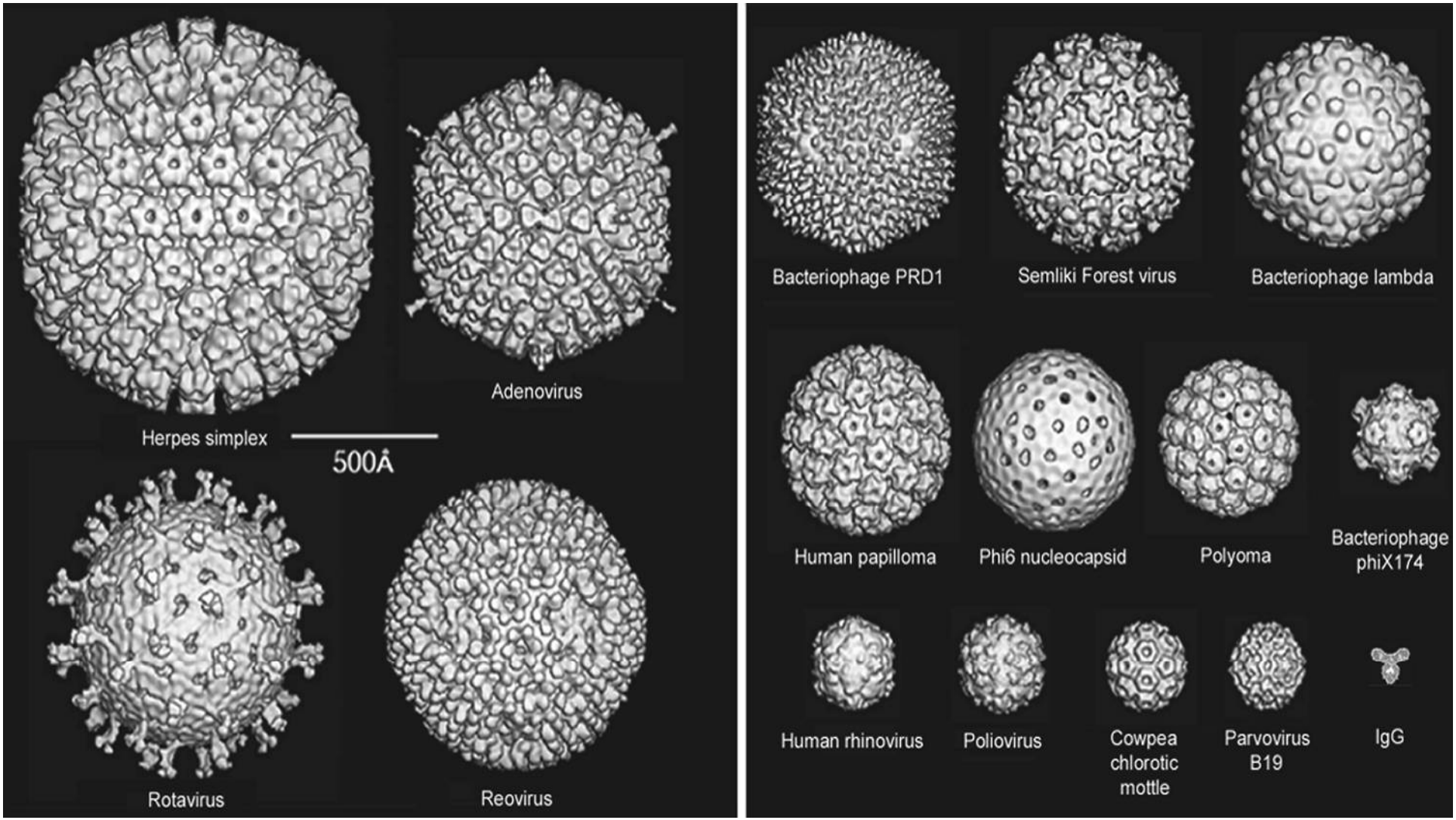
Each capsomer consists of a single protein

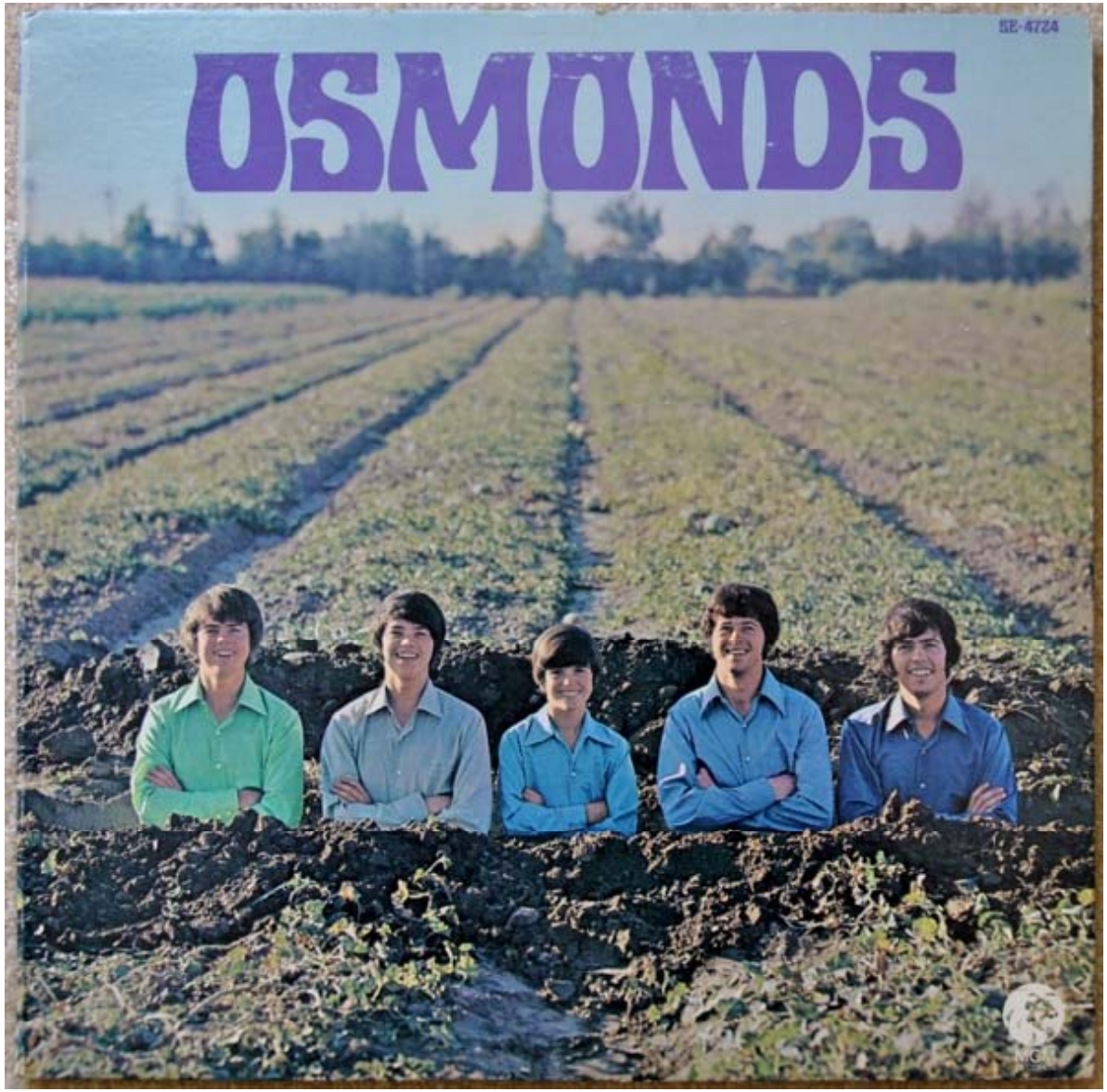






Examples of virus structure




SE-4724
OSMONDS

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Viruses as a molecule
The payload

- Most viruses
  - 10 – 20 genes
  - Genomes 5,000 – 25,000 bp
- ICTVdb
  - 3,600 species
  - 30,000 strains + subtypes

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Viruses as a molecule

- ssRNA – most diverse Noro, HCV, HIV
- dsDNA – Adeno, CMV, HSV, Variola
- dsRNA – Rota
- ssDNA – least diverse PVB19

Acute and persistent virus life strategies

- No persistence in individual host
- Often disease associated
- High mutation rates (RNA viruses)
- Virus replicates in more than one species
- Little coevolution with host
- Horizontal transmission
- Highly dependent on host population structure
- Seldom evolves to persistence

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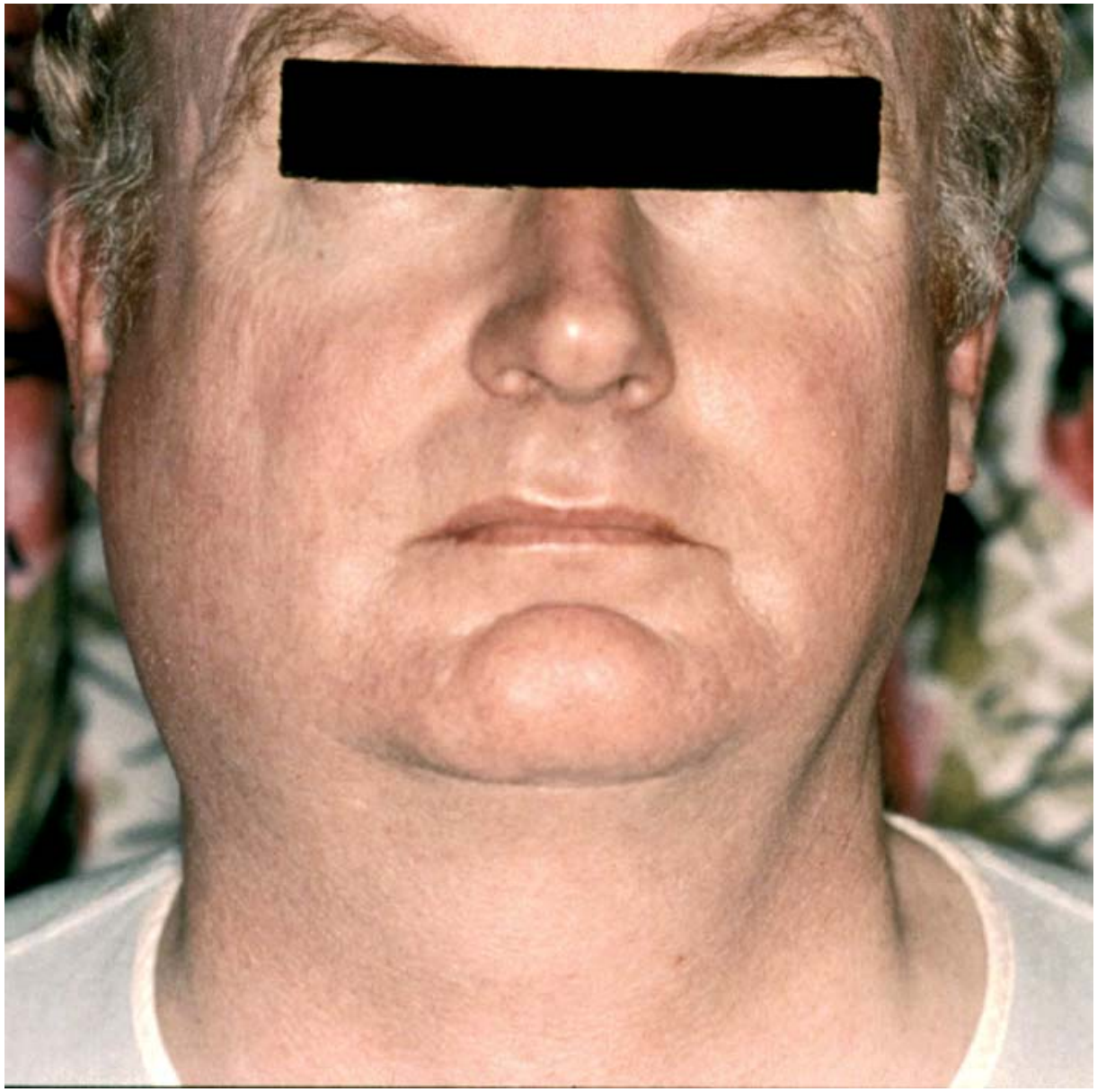

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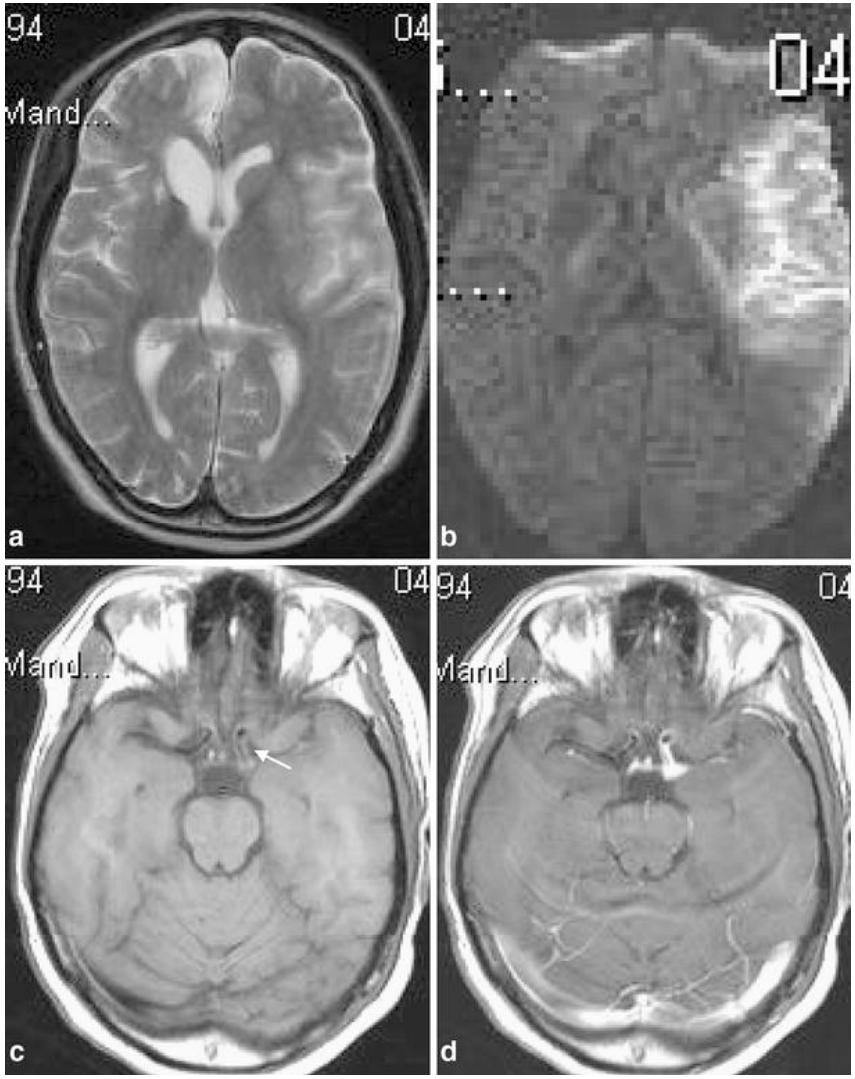

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Acute and persistent virus life strategies

- Persistent in individual host
- Acute disease often inapparent
- Genetically stable
- Highly species specific
- Coevolution with host
- Transmission is often from parent to offspring (vertical) or through sexual contact
- Less dependent on host population structure
- Often the source of emerging acute disease in new host species

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Classification

- Viral nucleic acid + virus capsid + envelope
- Other characteristics:
  - Genomic makeup e.g: Caliciviruses
  - Virion structure – EM appearance e.g: herpes
  - Replication strategy
  - Virion antigenicity e.g: adenoviruses, serological distinction MVE / JE / WNV
  - Virion chemical characteristics, stability
  - Diseases caused in the host e.g: hepatitis

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Diagnostic Methods

- Serology - retrospective
- Ag
  - Protein based
  - IFA (Respiratory)
  - WB (HIV)
  - Protein function (HIV-RT)
- Culture – some viruses non-cultivable
- Molecular
  - Virion nucleic acid
  - HIV RNA, HCV RNA
  - CMV DNA
- Emerging Microarray different formats, HPLC, Protein amplification, MALDI-TOF

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Serology

- ELISA, IFA, CFT
- Total (EV, HAV, HCV, HW, Influenza)
- Igm (Adeno, HSV)
- IgG (CMV, Mumps, Measles, PVB19 Rubella)
- Complex (HBV, EBV)
- No use (Rota, Noro, Variola)

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Molecular testing

- Rapid, sensitive, costly (initially)
- Amplified
  - Target (PCR, LCR, NASBA, TMA)
  - Signal (bDNA)
- Non amplified
  - Probe based (ISH, Hybrid Capture)

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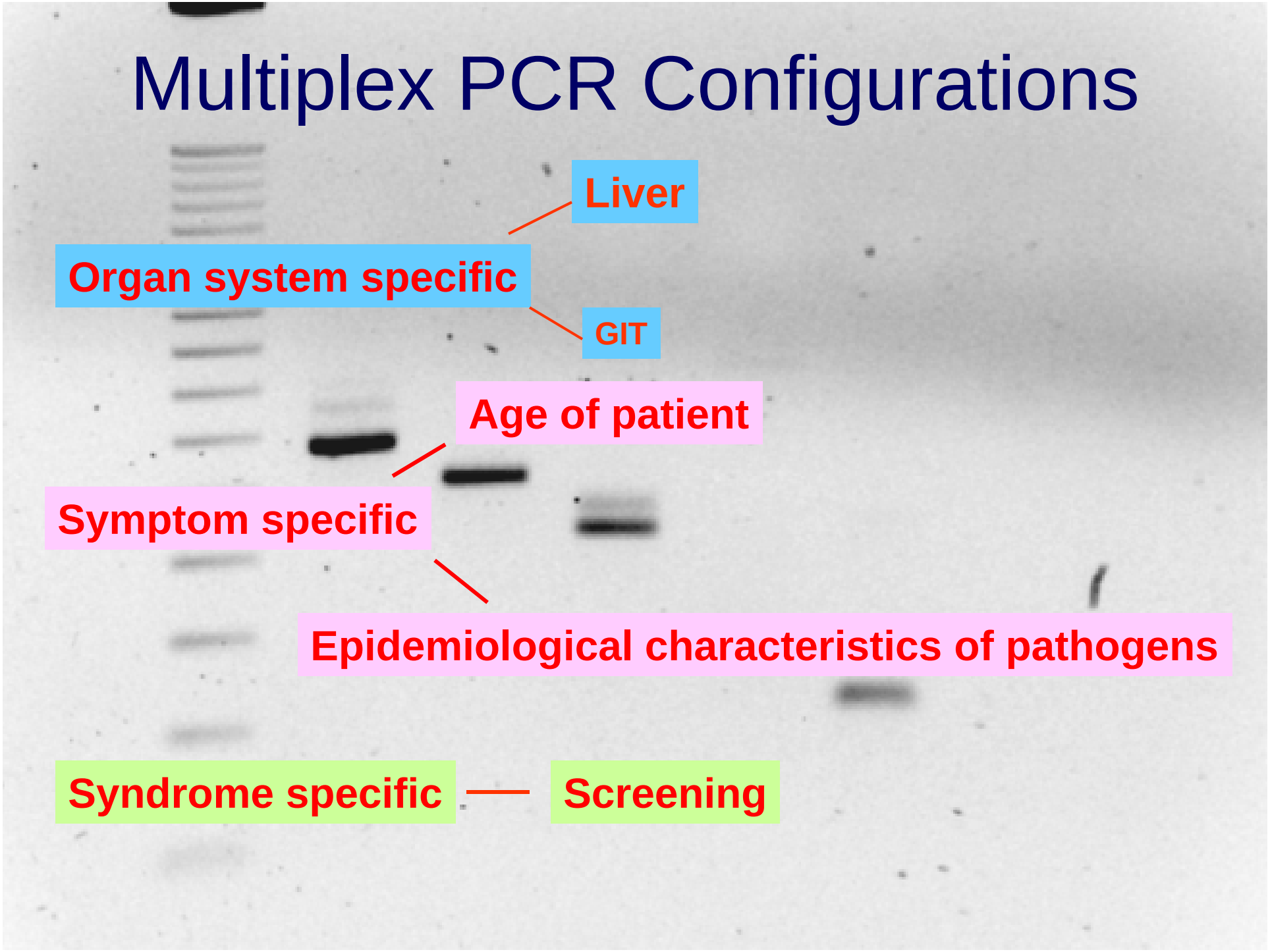
Multiplex PCR Configurations



VDL01
DNA agents
Toxoplasma gondii
HSV-1 and -2
CMV
Parvovirus
Varicella-zoster virus

VDL05
Common pathogens
HSV-1 and -2
CMV
Varicella-zoster virus
Epstein-Barr virus
Enterovirus

VDL STI
U. parvum
U. urealyticum
M. hominis
M. Genitalum

VDL STI
C. trachomatis
T. vaginalis
T. pallidum
GBS

VDL STI
HSV-1
HSV-2
VZV

Ag testing

- Existing technique (Respiratory)
- Initial testing (p24)
- Only available (Prion)
- Dependent upon
  - ➢ many host cells in specimen
  - ➢ operator

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Dark grey
Really bad star
Nasty star
Death star

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Viral Developments

- New pathogens identified
  - Coronaviruses SARS, NL63, HKU1
  - Parvoviruses bocavirus, PARV4
  - Retroviruses XRMV in CFS
  - Arenavirus in transplantation
  - Mimivirus *Acanthamoeba polyphaga mimivirus* (APMV)
  - Adenovirus 14 in pneumonia

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Viral Developments

- New intraspecies transmission
  - H5N1
  - Sin Nombre/hantaviruses
  - Nipah
  - Ebola
  - Arenavirus
  - nvBSE
  - HIV
  - PERV
- Transmission without clear diseases
  - Spumaretorviruses
  - Reovriuses
  - Adeno associated parvoviru ses
  - TT virus

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Viral Developments

- New diseases of old pathogens
  - hMPV
  - HRV
- Extended spread of known diseases
  - Chikungunya
  - Dengue
- Documentation of chronic diseases
  - HIV
  - HCV occult
  - HBV occult

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Viral Syndromes

- Adenopathy and glandular fever
- Arthritis
- Carditis
- Chronic Fatigue Syndrome
- Congenital and perinatal disease
- Exanthemata and skin disease
- Eye disease
- Gastroenteritis

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Viral Syndromes

- Haemorrhagic fevers
- Hepatitis
- Immunocompromised infections
- Neurological disease
  - encephalitis and meningitis
- Pancreatitis and diabetes
- Respiratory disease
- Sexually Transmitted Infections (STD, STI)

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Agents of risk
Known / tested

- HB_sAg - Sept 1970
  - Abbott PRISM
- HCVAb - Feb 1990
  - Abbott PRISM
- HIV 1/2 Ab - April 1985
  - Abbott PRISM
- HTLV I/II Ab - Jan 1993
  - Abbott PRISM
- HCV RNA - June 2000
  - Pooled 24, TMA Chiron
  - Pooled 16, April 2005
- HIV 1 RNA - June 2000
  - Pooled 24, TMA Chiron
  - Pooled 16, April 2005
- CMV - selected

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Agents of risk
Known / not tested

- CMV
- GB-C virus - unknown
- HHV8
  - few donors
  - theoretical KS
- Prions vCJD
  - few donors
  - transmissible encephalopathy
- SARS coronavirus - few donors
- TT virus – 80-95% of donors
- SEN-V
  - 2% of donors
  - types A-H
  - hepatitis
- WNV
  - few donors
  - 3 week deferral process
  - encephalitis

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Agents of risk
Known / emerging risk

- Dengue - deferral procedures for at-risk
- Prions - vCJD [Llewellyn, 2004]
  - exclusion
- SARS coronavirus
- WNV
- Unknown

[Chamberland, 2001]

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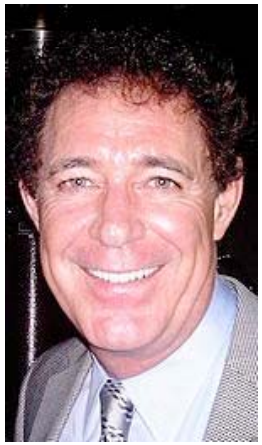
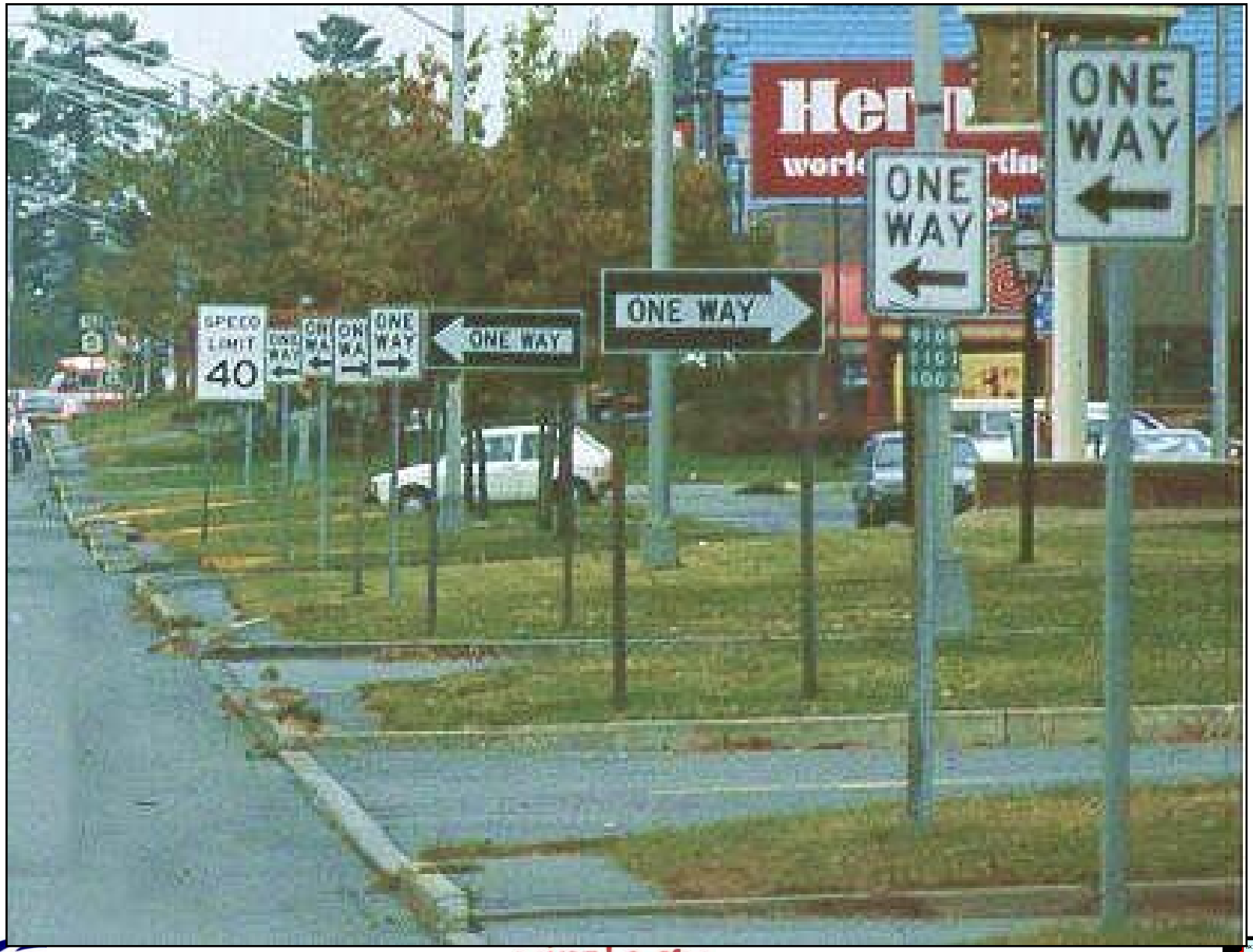
Carol Brady
Mike Brady
Greg Brady
Marcia Brady-Logan
Peter Brady
Jan Brady
Cindy Brady

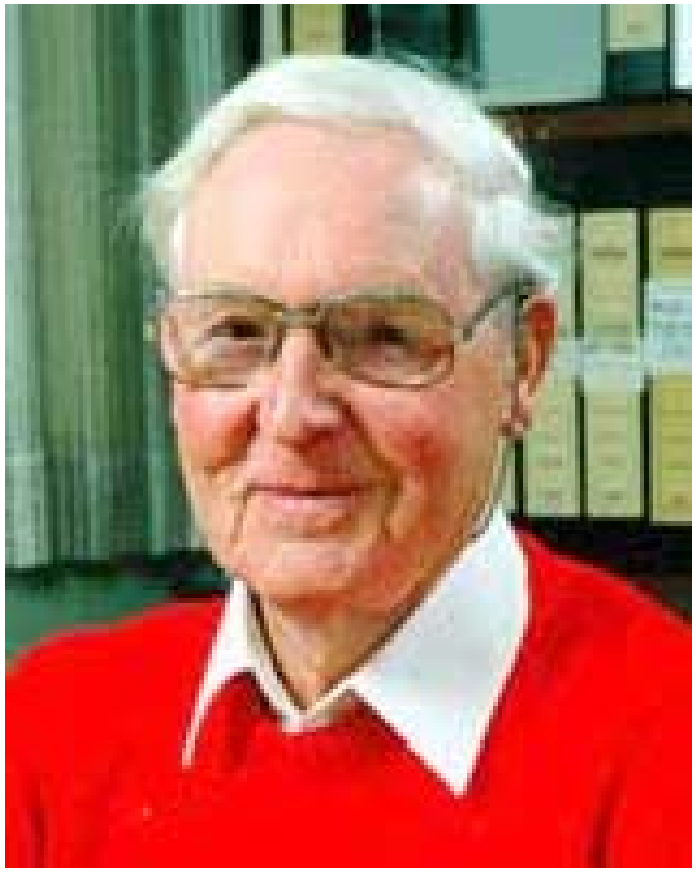
Alice Nelson

Tiger the dog
Fluffy the cat



and Mike Brady (Robert Reed) died of AIDS in 1992
and Marcia Brady (Maureen McCormick) grandmother died of syphilis


ONE WAY
ONE WAY
ONE WAY
ONE WAY
SPEED LIMIT 40



- Poxviruses
  - Variola (smallpox)
  - Myxoma
  - Ectromelia (mousepox)
- Infectious diseases
  - Malaria
  - TB
- Viral taxonomy
- Microbiology history

- Prime Minister's Prize (2002)
- Royal Society Copley medal (1996)
- Japan Prize for Preventive Medicine (1988)

Epidemics

- Measles
  - Plague of Athens (436 B.C.) described a distemper-like epidemic with high mortality
  - Epidemics - Rome, China AD165, AD251
  - Considered a normal process of development
  - All adults survivors of childhood infection in Europe

Epidemics

- Smallpox
  - Earliest accounts from India, in Sanskrit medical text, China, 1122 B.C.
  - Entered Europe via Islamic North African expansion to Spain; epidemics in Syria (A.D.302) and Mecca (A.D. 569)
  - Reintroduced to Europe via crusaders
  - Disease milder limited to children (Spain in the 1400s)

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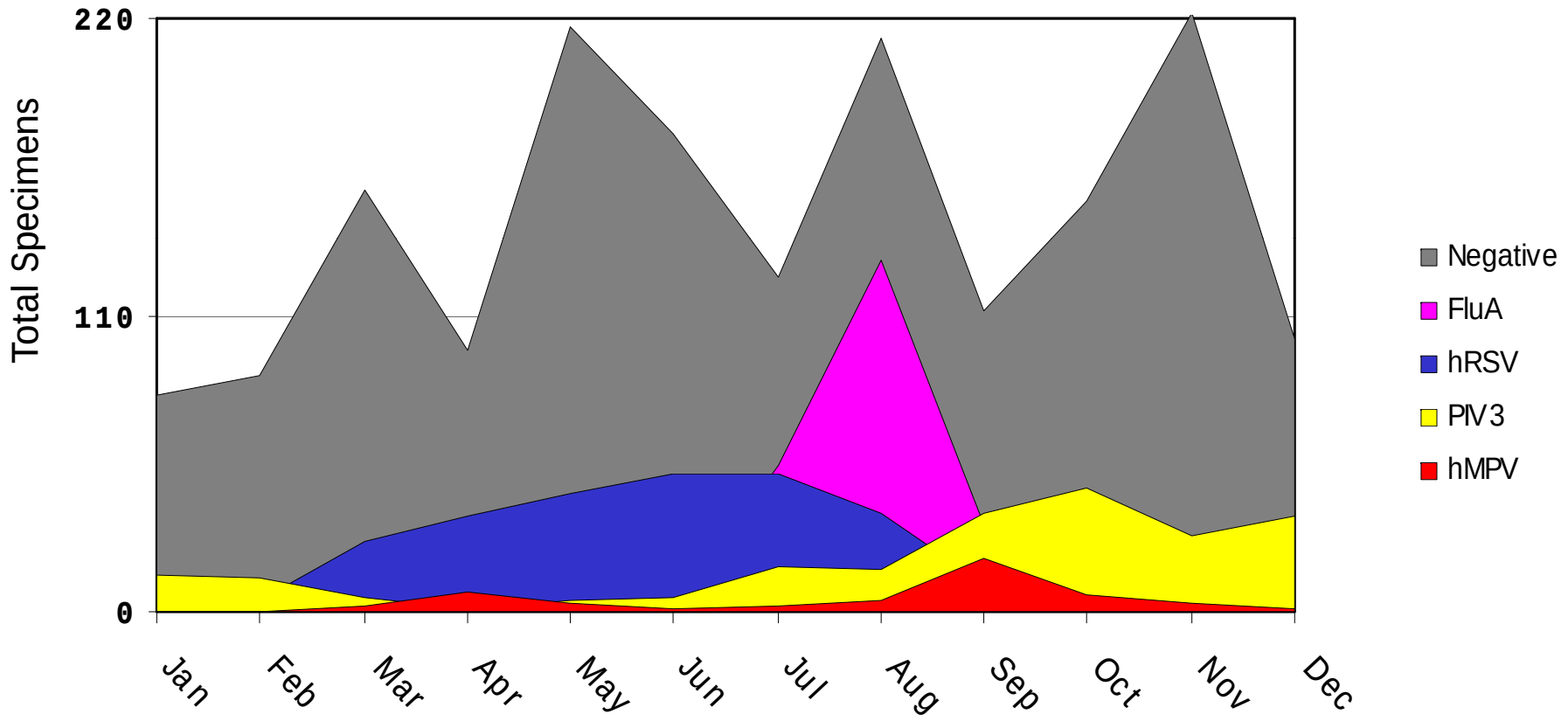
VIRAL INFECTIONS CHILDREN



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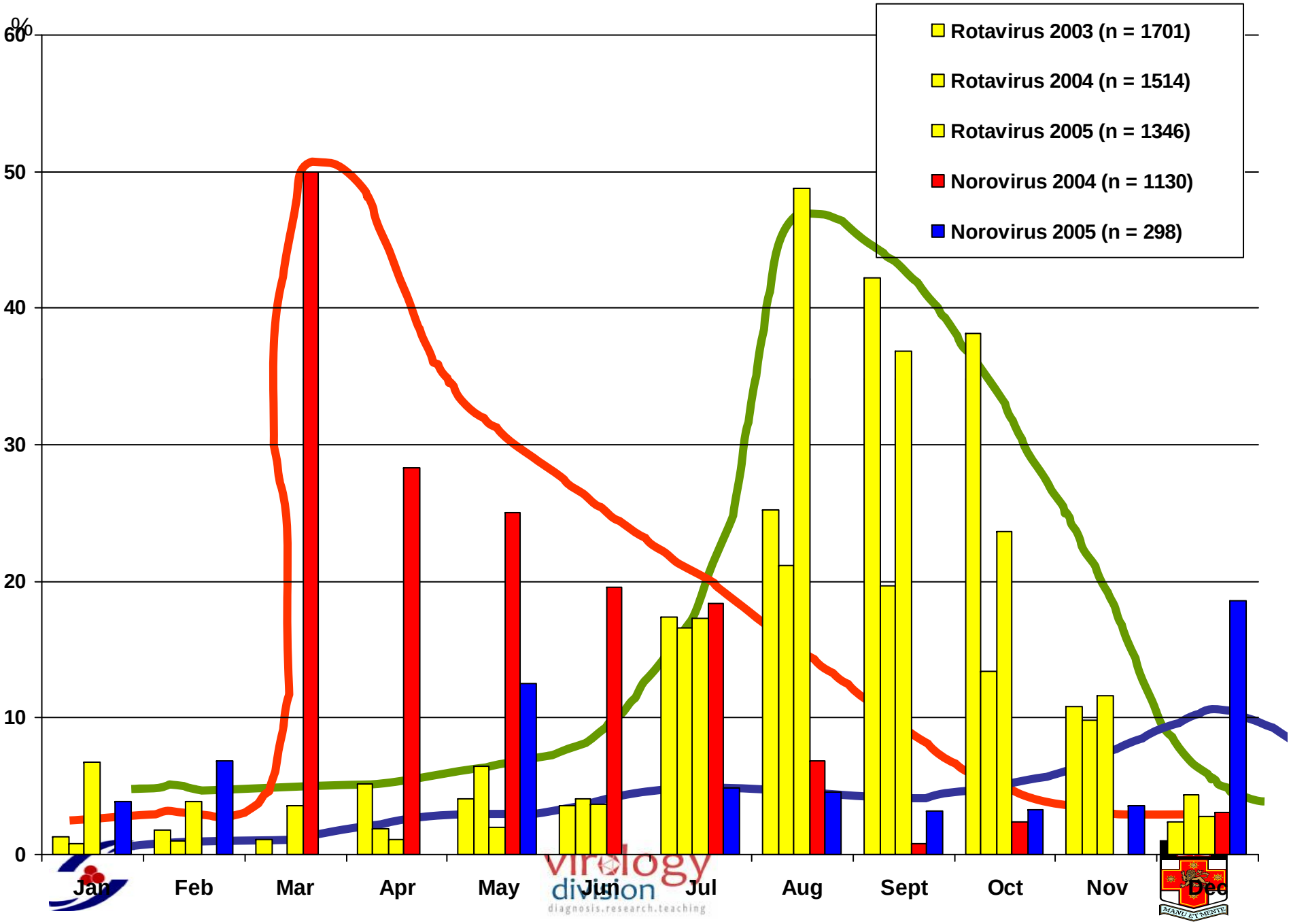
%
60
50
40
30
20
10
0
Rotavirus 2003 (n = 1701)
Rotavirus 2004 (n = 1514)
Rotavirus 2005 (n = 1346)
Norovirus 2004 (n = 1130)
Norovirus 2005 (n = 298)
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
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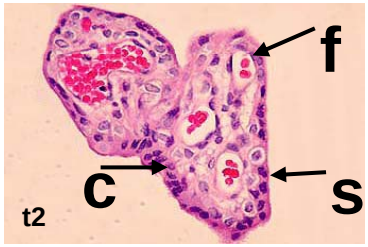
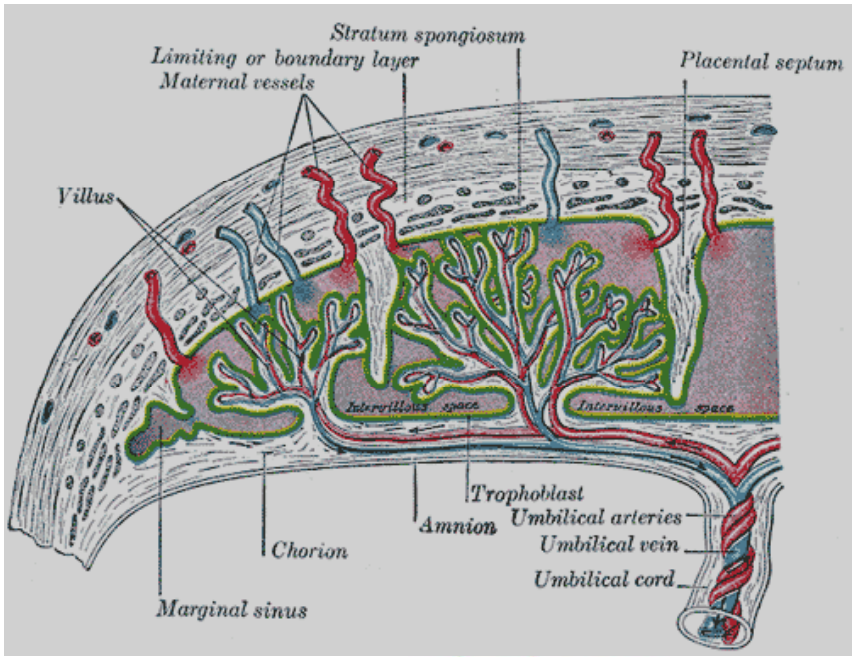
MANU ET MENTE

Congenital CMV Infection

- Hepatosplenomegaly
- Jaundice
- Microcephaly
- Prematurity
- Chorioretinitis
- Petechiae
- Mental retardation
- Hearing loss

Normal (uninfected) placental tissue





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Blood Supply testing

- Mainly viral
- Ignores persistent non-threatening viruses
- Constantly changing
- Role of emerging viruses

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HEPATITIS VIRUS	ACUTE HEPATITIS	CHRONIC HEPATITIS	FULMINANT HEPATITIS	CIRRHOSIS	HCC
A	+	0	+	-	-
B	+	5-10%	+	+	+
C	+	75%	+	+	+
D	+	<5% 50%	+	+	+
E	+	0	+	-	-
G	+	20%	-	-	-
TTV	+	60%	-	-	-

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Some Emerging Issues

- New respiratory virus – SARS
  - hMPV
  - Associations with chronic conditions
- Transfusion – HCV
  - Non A non B non C
  - New viruses (GBV, TTV, Sen V)
- Old viruses re-emerging
  - Smallpox
- Zoonoses
  - SARS
  - Rabies
  - Arenaviruses
  - Hantaviruses

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What are the names of the seven dwarves, and what was the woman.

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What are the names of the seven dwarves, and what was the woman.

Dopey, Grumpy, Doc, Happy, Bashful, Sneezy, Sleepy

An only child, maternal death, somnolence, hallucinations
Queen you are full fair, ‘tis true, but Snow White is fairer than you
The fairest of them all