Objective:

- Methods to detect viruses
- Detection of specific viral infections (skin and mucosa, respiratory, CNS and gastrointestinal infections)
- Detection of chronic viral infections including CMV, EBV, Hepatitis, and HIV-1
- Detection of emerging viral diseases
Rational for Diagnostic Virology

• Until recently, DV was outside of clinical laboratory practice (lack of tissue culture methods, commercial reagents and medication)
• Emergence of HIV-1 and AIDS; and success in stem cells and solid organ transplantation increased pool of patients with viral infections
• Progress in antiviral treatment and availability of antiviral therapeutics
• Development of advanced technologies (PCR, RT-PCR and ELISA, Multiplex analysis)
• Increased risk of opportunistic viral infections in HIV-1 epidemics
• Management of HIV-1 and hepatitis C infection required molecular diagnostics
• Rapid test often determines the management of the case
History

• 1898, Loeffler and Frosch found a filterable agent, they called virus, that caused foot and mouth disease of cattle

• 1892, Guarnieri found intranuclear and intracytoplasmic inclusion in smallpox lesions

• 1929, complement fixation test by Bedson and Bland to detect antibodies to smallpox, vaccinia and varicella zoster virus

• 1948, Weller and Enders reported growth of viruses in tissue culture

• 1956, Liu and colleagues introduced fluorescent staining for the detection of influenza virus

• 1970s, development of monoclonal antibodies

• 1980s, development of ELISA and PCR assays for diagnostics
Under attack!

(From Medical Microbiology, 4th ed., Murray, Rosenthal, Kobayashi & Pfaller, Mosby Inc., 2002, Fig. 65-1.)
Specimen for the Viral Diagnosis

• Acute viral infection – from the site of the disease (I.e. CSF for viral meningitis; swab for mucosal infection)

• Consider the time required for transportation, adverse effects of freezing or prolonged storage for the perseverance of the viral specimen

• Blood: whole blood, leukocytes or plasma (EDTA – purple-top tubes; heparin – green top tubes or acid-citrate-dextrose – yellow top tubes) or serum (no anti-coagulant, red-top tubes)

• Swab, Dacron or rayon swabs, can be places in media and cooled for transportation

• Fluids, transported in sterile containers

• Tissues, transport with some media or PBMS

• Stool, transport in a sterile container
Diagnostics Most Often Used:

• Screening of donors’ blood: presence of HIV-1, HBV, HCV, etc.

• Prenatal diagnostics of CMV and HIV-1

• Viral opportunistic infections in immunocompromised patients

• Chronic infections, HIV-1 and HCV

• EBV in teenagers

• Immunization status in immigrants (measles, mumps and rubella)
DIAGNOSTIC METHODS

• Viral detection and isolation in cell culture
• Antigen detection
• Detection of viral DNA and RNA
• Serology
• Electron microscopy
• Cytology
• Histology
Use of Cell Culture to Detect Viruses

- Maintain continuous cell lines: primary green monkey kidney cells (Vero), human fibroblasts (NIH 3T3), primary rabbit kidney cells, human epithelial cells (HEP-2), engineered cell lines (Heal-MAGI, CEM-GFP, …) etc.

- Inoculate a specimen (blood, sputum, urine, feces etc) and incubate up to 14 days

- Observe cytopathic effect (CPE), hemadsorption, interference, electron microscopy

- Or isolate virus and perform antigen or nucleic acid detection
Use of Cell Culture to Detect Viruses – cont’d

Enteroviruses
Respiratory viruses
Herpes Simplex Virus (HSV)

Cytomegalovirus (CMV)
Varicella-zoster virus (VZV)
Rhinoviruses

Respiratory Scintitval Virus (RSV)

green monkey kidney cells (Vero)

human fibroblasts (NIH 3T3)

human epithelial cells (HEP-2)
Detection of Viral Replication

- **Cytopathic Effect (CPE):** microscopically visible changes in cultured cells; which cells are affected; timing and rate of progression; nature of the morphological changes

- **Hemadsorption:** production by some viruses (I.e. paramyxoviruses: influenza, parainfluenza and mumps) of antigens that can bind erythrocytes of certain species (guinea pig, rat or monkey)

- **Interference:** infection with a virus prevents an infection by a test virus.
Cytopathic Effect (CPE)

Figure 17.1 Cytopathic effect caused by viruses growing in cell culture. Herpes simplex virus growing in primary rabbit kidney cells (A); uninfected primary rabbit kidney cells (D). Cytomegalovirus growing in human embryonic lung fibroblast cells (B); uninfected human embryonic lung fibroblast cells (E). Respiratory syncytial virus growing in HEp-2 cells (C); uninfected HEp-2 cells (F).
Cytopathic Effect (CPE) of Primary Severe Acute Respiratory Syndrome–Associated Coronavirus (SARS)

A, uninfected Vero cells form a continuous monolayer of spindle-shaped cells. B, a strong CPE was observed after 24 hours of incubation of Vero cells with the patient sputum sample (primary isolate).

Additinal Cell Cultures to Detect Viruses

- **Shell Viral Culture**: specimen is centrifuged on the top of the cell monolayer and viral growth is detected by antigen detection.

- **Mixed Cell Culture**: simultaneous growth of several cell lines for the detection of several viruses.

- **Genetically Engineered cell lines**: usually a stably transfected cell line containing a reporter gene under the control of viral promoter element that can be activated by viral proteins.
Figure 17.2 Shell vial assay for cytomegalovirus. (From Shuster EA, et al. *Mayo Clin Proc* 1985;60:577–585, with permission.)
Shell Viral Culture – Cont’d

• Inoculate the specimen, centrifuge the shell vial and then incubate for 24 hours
• Observe CPE or detect virus by immunological methods

Genetically Engineered cell lines

**Figure 17.3** ELVIS herpes simplex virus (HSV), a genetically engineered cell line for the detection of HSV clinical samples. ELVIS cells are baby hamster kidney (BHK) cells that contain a β-galactosidase gene under the control of the HSV promoter UL39. If HSV is present in the specimen, viral particles enter the cells and the HSV proteins ICP0 and VP16 activate the UL39 promoter, leading to synthesis of β-galactosidase. The presence of β-galactosidase is detected by adding X-gal, a substrate for the enzyme. The activity of β-galactosidase on X-gal results in blue staining of ELVIS cells, visible microscopically.

**HeLa-MAGI Cells to Detect HIV-1**

- Culture an indicator cell line to determine the presence of a particular virus.
- Inoculate the specimen, incubate for 24 hours or longer
- Detect the expression of a reporter gene (β-galactosidase or Luciferase)

**A. Control**

**B. Adeno-Tat**

**C. HIV-1**

**A**, Monolayer of uninfected HeLa-MAGI cells. **B**, HeLa-MAGI cells infected with adenovirus expressing HIV-1 transcriptional activator (Tat). **C**, HeLa-MAGI cells infected with T-tropic HIV-1. All cells were fixed and exposed to β-galactosidase substrate, X-Gal, which shows as blue color when processed by β-galactosidase. Magnification 100x.

Ammosova et al., J. Biol. Chem. 2003
Use of Viral Culture to Detect Viruses

Plus
• Well established methods
• Reliable viral detection

Minus
• Maintaining cell lines is expensive and requires special equipment and trained personnel
• Relatively slow detection unless combined with other methods
Electron Microscopy

• Staining with electron-contrast material (phosphotungstic acid or uranyl acetate)

• Evaluation of stool specimens from patients with gastroenteritis (rotaviruses, astroviruses, adenoviruses, noroviruses)

• Direct detection of viral particles when viral culture conditions or reagents are not available (early detection of SARS)

• Direct examination of specimens for herpesvirus, poxvirus or Ebola

• Examination of fixed tissues obtained from biopsy or autopsy if histology is performed as well

• Examination of infected tissue culture
Light Microscopy

• **Cytology:** visual evidence of viral infection (HSV, CMV, adenoviruses, polyomaviruses and measles virus)

• **Tzanck smear:** rapid detection of HSV or VZV viruses by fixing and staining of specimens with Write, Giemza and other stains. Detect the presence of multinucleated giant cells

• **Papanicolau staining:** staining of the cells from uterine cervix. Papillomavirus (HPV) produces characteristic changes (condensed nucleus) in keratinocytes

• **Urine cytology:** staining of the cells present in urinary sediments (CMV or polyomaviruses, JC and BK infection)
Cytology

Figure 17.4 Cytologic findings suggestive of viral infection. A: Cervical smear showing multinucleated cells and the Cowdry type A intranuclear inclusions of herpes simplex infection. B: Papanicolaou smear showing binucleate squamous epithelial cells with distinct perinuclear halos. These characteristics, described as karyocytosis, are the cellular features associated with human papillomavirus infection. C: Urinary epithelial cell containing an enlarged nucleus with smudgy chromatin and a small pale glassy intranuclear inclusion indicative of polyomavirus infection. D: Cell from bronchoalveolar lavage with a large intranuclear inclusion with a perinuclear clear space (owl’s eye cell) indicative of cytomegalovirus infection. (Photographs provided by Dr. Leslie Boucher, Department of Pathology, Washington University, St. Louis, Missouri.)
 ANTIGEN DETECTION

• Fluorescent antibody (FA) staining

• Immunoperoxidase staining

• Enzyme Immunoassay (EIA)/ Enzyme-Linked Immunoassay (ELISA)
Fluorescent antibody (FA) staining of cell cultures infected with viruses:

- Tissue culture cells are grown on coverslips
- Infected with viral specimen and incubated
- Cover slips are washed and the cells are fixed with acetone or some other suitable fixative
- The cover slips are then stained with fluorescein-labeled antibody solution

Cover slips are washed, mounted and observed under the fluorescent microscope
ANTIGEN DETECTION- cont’d

Immunoperoxidase staining of cell cultures infected with viruses:

- Tissue culture cells are grown on cover slips
- Infected with viral specimen and incubated
- Cover slips are washed and the cells are fixed with formaldehyde or another fixative
- Cover slips are incubated with 0.1% H$_2$O$_2$ to quench endogenous peroxidase activity
- Cover slips are incubated with primary antibody
- Cover slips are incubated with peroxidase-conjugated secondary antibody, and then with a peroxidase substrate (DAB) that became insoluble in the presence of peroxidase.
- Cover slips are counter stained with hematoxylin, dehydrated and mounted
ANTIGEN DETECTION- cont’d

Enzyme Immunoassay (EIA)/ Enzyme-Linked Immunoassay (ELISA)

- Independently developed in 1971 by Peter Perlmann and Eva Engwall (Stockholm University) and Anton Schuurs and Bauke van Weemen (The Netherlands)

- Coat the microtiter plate with purified antibody to the antigen
- Add sample to be tested for antigen to plate and allow antigen to bind antibody
- Add enzyme-labeled specific antibody to a different epitope of the antigen to make a "sandwich"
- Add chromogenic substrate for enzyme that will be converted to a colored product
ANTIGEN DETECTION- cont’d

Antibodies linked to ▲, to which enzymes are attached.

Liquid containing various substances

Liquid turned from clear to yellow by enzymes

Clear → Yellow

Washing

Vessel in which antibodies linked to ▲ were linked.

Vessel only antibody-substances and antibody-enzymes remain.

Concentration of ▲ indicated by intensity of yellow

*Yellow deepens according to concentration of ▲.
ANTIGEN DETECTION- cont’d

<table>
<thead>
<tr>
<th>Suitable Targets</th>
<th>Poor Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>• RSV</td>
<td>• Enteroviruses</td>
</tr>
<tr>
<td>• Influenza</td>
<td>• Rhinoviruses</td>
</tr>
<tr>
<td>• Parainfluenza</td>
<td></td>
</tr>
<tr>
<td>• adenovirus (respiratory specimens)</td>
<td></td>
</tr>
<tr>
<td>• HSV and VZV (cutaneous specimens)</td>
<td></td>
</tr>
<tr>
<td>• rotavirus (stool specimens)</td>
<td></td>
</tr>
<tr>
<td>• CMV and hepatitis B (blood)</td>
<td></td>
</tr>
</tbody>
</table>
ANTIGEN DETECTION

Plus
• Quick and reliable viral detection
• ELISA is well established, inexpensive and easy to perform
• Can be used for multiply sample analysis (100 samples or more at a time)

Minus
• Difficult or impossible to detect chronic viral infections
• Not always antibodies are available
• Sometimes antibodies produce false-positives
NUCLEIC ACID DETECTION

DNA detection – by PCR (since 1985)
RNA detection – RT-PCR

- RNA is copied to cDNA by reverse transcriptase
- cDNA is mixed with heat-stable polymerase, specific primers and deoxynucleotides
- PCR is performed
- After 30 to 40 cycles of amplification, the reaction products are analyzed by agarose gel electrophoresis
NUCLEIC ACID DETECTION- cont’d

Quantitative RT-PCR

• During each step of PCR, a fluorescent dye is incorporated into DNA and quantified

• Normalization is performed using housekeeping genes as references against the expression level of a gene under investigation
**NUCLEIC ACID DETECTION- cont’d**

<table>
<thead>
<tr>
<th>Viruses detected by PCR:</th>
<th>Viral RNA detected by RT-PCR:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Epstein-Barr (EBV)</td>
<td>• HIV-1</td>
</tr>
<tr>
<td>• HSV</td>
<td>• Hepatitis C (HCV) RNA</td>
</tr>
<tr>
<td>• Varicella-zoster (VZV)</td>
<td>• CMV pp65 RNA</td>
</tr>
<tr>
<td>• Colorado tick fever</td>
<td>• Human papillomavirus (HPV)</td>
</tr>
<tr>
<td>• Cytomegalovirus (CMV)</td>
<td>• Rabies</td>
</tr>
<tr>
<td>• Herpes simplex (HSV)</td>
<td>• Enterovirus</td>
</tr>
<tr>
<td>• JC (John Cunningham)</td>
<td>• Hantavirus</td>
</tr>
<tr>
<td>• BK virus</td>
<td>• Dengue virus</td>
</tr>
<tr>
<td>• Parvovirus</td>
<td></td>
</tr>
<tr>
<td>• Human herpes virus-6</td>
<td></td>
</tr>
<tr>
<td>(HHV-6)</td>
<td></td>
</tr>
<tr>
<td>• Lassa, Marburg and</td>
<td></td>
</tr>
<tr>
<td>Ebola</td>
<td></td>
</tr>
<tr>
<td>• Yellow fever</td>
<td></td>
</tr>
</tbody>
</table>
Target Amplification Assay

Figure 17.6 Transcription-mediated assay, also called 3SR. The 3SR reaction depends on a continuous cycle of reverse transcription and transcription reactions to replicate an RNA target by means of complementary DNA (cDNA) intermediates. Oligonucleotides A and B prime DNA synthesis and encode the promoter sequence for the T7 RNA polymerase (black boxes). Steps 1–6 depict the synthesis of a double-stranded cDNA, which is a transcription template for T7 RNA polymerase. Complete cDNA synthesis is dependent on the digestion of the RNA in the intermediate RNA–DNA hybrid (step 4) by ribonuclease (RNase) H. Transcription-competent cDNA yield antisense RNA copies of the original target (step 7, right). These transcripts are converted to cDNA containing double-stranded promoters on both ends in an inverted repeat orientation (steps 7–12). These cDNA can yield either sense or antisense RNA, which can reenter the cycle. Thin lines, RNA; thick lines, DNA. RT, reverse transcription. (From ref. 76 with permission.)
Figure 17.7 Branched chain DNA assay as used for ultrasensitive detection of human immunodeficiency virus (HIV). After liberation of viral nucleic acid from the clinical specimen by a lysis buffer, viral nucleic acid is hybridized with two sets of bifunctional oligonucleotide probes, each of which contain sequences complementary to the target. One set of probes also contains a generic sequence complementary to a capture probe that is bound to the surface of a microtiter tray, and serves to bind the target to the solid surface. The other set of probes contains a sequence complementary to preamplifier molecules. Additional specificity is achieved because two of the target-specific probes must be juxtaposed in the correct orientation to stabilize the binding of the preamplifier molecule. Each preamplifier molecule binds numerous amplifier molecules, each of which binds many subsequently added alkaline phosphatase-labeled probes. A chemiluminescent substrate is added and generated light is read by a luminometer. The cascade effect results in amplification of the signal generated from initial binding of probes to the target and allows for detection and quantitation of nucleic acid present in the specimen. (Courtesy of Bayer Diagnostics, Emeryville, California.)
Figure 17.8 Hybrid capture assay. In step 1, target DNA is released and denatured. In step 2, RNA probe hybridizes with target DNA. In step 3, RNA–DNA hybrids are captured by an antibody to RNA–DNA hybrids that is bound to the sides of the reaction vessel. In step 4, an enzyme-conjugated antibody that recognizes RNA–DNA hybrids binds to the hybrids. In step 5, a chemiluminescent substrate is added, and light is emitted if hybrids have been formed. (Courtesy of Digene Corporation, Beltsville, Maryland.)
NUCLEIC ACID DETECTION

Plus
• Very high sensitivity of PCR (can detect as low as several molecules)
• Relatively quick and reliable viral detection
• Can be used for multiple sample analysis (100 samples at a time)

Minus
• Require special equipment and trained personnel
• Relatively expensive procedures (especially quantitative RT-PCR)
SEROLOGY

• Detection of viral-specific IgM antibodies (EBV, viral capsid antigen), CMV, hepatitis A, hepatitis B (IgM to core antigen), parvovirus B19, measles, rubella, mumps, arboviruses (St. Louis encephalitis virus)

• Detection of chronic infections: HIV or HCV

• Defining specific antiviral immunity (VCV, CMV, EBV, HSV, measles and rubella, parvovirus B16, hepatitis A and hepatitis B
Detection of Viral-specific Antibodies

• wells coated with purified antigens

• adding of sera
• incubation

• adding of conjugate
• incubation

• adding of chromogen
• incubation
• reaction termination
• result reading
SEROLOGY

Plus
• Quick and reliable viral detection
• The only available method for certain viruses
• Can be used for multiply sample analysis (100 or more samples at a time)

Minus
• Prone to false positives
Other Detection Methods

• Electron microscopy
  • Highly laborious, requires special equipment and training
  • Very sensitive, accurate detection

• Cytology
  • The cytopathic changes of HSV, VZV and human polyomaviruses in smears
  • Morphology of virus-infected cells is established
  • Cheap and reproducible but requires experience

• Histology
  • Immunohistochemistry and in situ hybridization
  • Requires experience
# Specific Viral Infections

## Infections of the Skin and Mucosa

**Symptoms:** vesicular or ulcerative lesions  
**Specimen:** scrapes from the base of a lesion  
**Cause:** usually HSV or VZV

<table>
<thead>
<tr>
<th>Virus</th>
<th>Detection</th>
<th>Detection time, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV</td>
<td>Culture, FA</td>
<td>1-3</td>
</tr>
<tr>
<td>VZV</td>
<td>FA, culture</td>
<td>4-10</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Culture</td>
<td></td>
</tr>
<tr>
<td>HPV</td>
<td>Hybrid capture assay, PCR, histology, cytology</td>
<td></td>
</tr>
<tr>
<td>Poxvirus</td>
<td>Histology, electron microscopy</td>
<td></td>
</tr>
</tbody>
</table>
**Respiratory Infections**

Specimen: nasopharyngeal aspirates, washings, swabs, bronchial washings or bronchoalveola fluids

<table>
<thead>
<tr>
<th>Virus</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>Antigen detection, culture</td>
</tr>
<tr>
<td>Influenza (A&amp;B)</td>
<td>Antigen detection, culture</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Antigen detection, culture</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Antigen detection, culture</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>Culture</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>None</td>
</tr>
</tbody>
</table>

Sensitivity of antigen detection ~80%
Infections of CNS

- Acute meningitis (inflammation of the protective membranes covering the brain and spinal cord) in immunologically normal hosts

- Acute encephalitis in immunologically normal hosts (inflammation of the brain)

- Opportunistic infections in immunocompromised individuals
### Acute meningitis in immunologically normal hosts

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterovirus</td>
<td>PCR analysis of a conserved 5-non-translated region of enetrovirs detects 60 out of 67 enteroviruses or culture of SCF</td>
</tr>
<tr>
<td>HSV-2</td>
<td>PCR analysis for HSV-2, but not culture, potentially RT-PCR</td>
</tr>
<tr>
<td>VZV</td>
<td>Complication of Varicella (Chicken Pox) or Zoster (herpes virus), detected by PCR</td>
</tr>
</tbody>
</table>

Cause: usually enteroviruses and HSV-2 (and possibly VZV)
### Sporadic encephalitis in immunologically normal hosts

**Cause:** usually HSV-1, 70% M&M in untreated cases

<table>
<thead>
<tr>
<th>Virus</th>
<th>Detection:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>PCR analysis of SCF fluid, earlier – brain biopsies and synthesis of HSV antibodies</td>
</tr>
<tr>
<td>Arboviruses</td>
<td>including California (LaCross), St.Louis, eastern equine, western equine, Venezuelan equine, and West Nile, detected by serology of serum and SCF</td>
</tr>
<tr>
<td>Coltivirus (Colorado tick fever)</td>
<td>infects erythrocytes, detected by culture, FA and PCR from blood clot</td>
</tr>
</tbody>
</table>
## Encephalitis of unknown etiology

<table>
<thead>
<tr>
<th>Virus</th>
<th>Detection:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabies</td>
<td>Early in illness, detection of antigen or nucleic acid in saliva, SCF or skin. After 8 days, also in serum</td>
</tr>
</tbody>
</table>
### CNS infection in immunocompromised individuals

**Cause:** CMV, EBV, VZV and JC

<table>
<thead>
<tr>
<th>Virus</th>
<th>Syndrome</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>encephalitis, radiculomyelitis</td>
<td>PCR</td>
</tr>
<tr>
<td>EBV</td>
<td>Primary CNS Lymphoma in AIDS</td>
<td>PCR</td>
</tr>
<tr>
<td>JC</td>
<td>progressive multifocal leukoencephalopathy</td>
<td>PCR</td>
</tr>
<tr>
<td>VZV</td>
<td>encephalitis, myelitis</td>
<td>N/A</td>
</tr>
<tr>
<td>HSV</td>
<td>meningoencephalitis</td>
<td>PCR</td>
</tr>
</tbody>
</table>
## Gastrointestinal Tract Infections

**Cause:** rotaviruses, enteric adenoviruses, caliciviruses and astroviruses. Originally discovered by electron microscopy of feces

<table>
<thead>
<tr>
<th>Virus</th>
<th>Detection:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>EIA/ELISA of stool specimen (children)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>EIA/ELISA (serotype 40 and 41), mild disease</td>
</tr>
<tr>
<td>Calicivirus</td>
<td>causes outbreaks, RT-PCR</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>EIA/ELISA, RT-PCR, no commercial assays</td>
</tr>
</tbody>
</table>
## Cytomegalovirus Infection

Traditional methods—viral culture and serology

<table>
<thead>
<tr>
<th>Diagnostic need</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune status</td>
<td>serology (IgG)</td>
</tr>
<tr>
<td>Congenital infection</td>
<td>culture of urine</td>
</tr>
<tr>
<td>Mononucleosis, Normal host</td>
<td>serology (also for EBV)</td>
</tr>
<tr>
<td>Systemic infection, Immunosuppressed host</td>
<td>pp65 antigenemia assay (indirect fluorescence), PCR</td>
</tr>
<tr>
<td>Retinitis</td>
<td>PCR of vitreous fluid</td>
</tr>
<tr>
<td>CNS (AIDS)</td>
<td>PCR of SCF</td>
</tr>
</tbody>
</table>
Epstein-Barr Virus

- Causes Burkitt lymphoma, a cancer of B lymphocytes, in equatorial Africa (not typical for US)
- Causes infectious mononucleosis (clinical findings – atypical lymphocytes, heterophile antibodies, often false positive for CMV)
- Primary CNS lymphoma in AIDS patients
- Posttransplantational lymphoproliferative syndrome in transplant recipients
Specific Viral Infections - cont'd

**Epstein-Barr Virus - diagnostics**

- Cultures are too slow for diagnostics
- Can be characterized by clinical findings – atypical lymphocytes, heterophile antibodies
- EBV-serological detection mostly in children < 4yo, IgG and IgM antibodies to viral capsid antigen (VCA), and antibodies to early antigen (EA) and nuclear antigen (EBNA)
- HIV-1 infection – EBV PCR analysis
- Lymphoproliferative disease, quantitative PCR in peripheral blood leukocytes
# Viral Hepatitis

<table>
<thead>
<tr>
<th>Virus</th>
<th>Detection:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A</td>
<td>IgM (acute infection), total antibody (past, current infection or immunization)</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>surface antigen (acute or chronic infection), total core antibody (current or past infection), core antibody (positive in current infection), e antigen (current infection or correlation with infectivity), antibody to e antigen (less current infection with lower infectivity)</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>ELISA (current or past infection); RNA (current infection); genotype (affects response to interferon)</td>
</tr>
<tr>
<td>Hepatitis D</td>
<td>antibody (current or recent infection)</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>IgM (current infection), IgG (past or current infection-immunization status)</td>
</tr>
</tbody>
</table>
# HIV and Other Retroviruses

<table>
<thead>
<tr>
<th>Use</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine diagnosis</td>
<td>ELISA/Western blot</td>
</tr>
<tr>
<td>Acute infection</td>
<td>RNA load analysis</td>
</tr>
<tr>
<td>Congenital infection</td>
<td>PCR (HIV DNA), plasma HIV RNA (Amplicor RT-PCR, capture assay, branched-chain DNA)</td>
</tr>
<tr>
<td>Response to therapy</td>
<td>plasma HIV RNA</td>
</tr>
<tr>
<td>Resistance to ART</td>
<td>genotyping or phenotypic susceptibility assay</td>
</tr>
<tr>
<td>Screening of donors blood</td>
<td>ELISA, p24 antigen assay, plasma HIV RNA</td>
</tr>
</tbody>
</table>
## Miscellaneous Viral Infections

<table>
<thead>
<tr>
<th>Virus</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles, acute infection</td>
<td>measles IgM antibody, culture</td>
</tr>
<tr>
<td>Rubella</td>
<td></td>
</tr>
<tr>
<td>acute infection</td>
<td>Rubella IgM antibody</td>
</tr>
<tr>
<td>congenital inf. in utero</td>
<td>culture, RT-PCR of amniotic fluid</td>
</tr>
<tr>
<td>Mumps, acute infection</td>
<td>Mumps IgM, culture</td>
</tr>
<tr>
<td>Parvovirus</td>
<td></td>
</tr>
<tr>
<td>Acute infection</td>
<td>parvovirus B19 IgM</td>
</tr>
<tr>
<td>Chronic infection</td>
<td>PCR of serum</td>
</tr>
<tr>
<td>Aplastic crisis</td>
<td>PCR of serum</td>
</tr>
<tr>
<td>congenital inf. in utero</td>
<td>PCR of amniotic fluid</td>
</tr>
<tr>
<td>congenital inf. Postpartum</td>
<td>Parvovirus IgM antibody</td>
</tr>
<tr>
<td>BK: hemorrhagic cystis, nephropathy</td>
<td>PCR analysis of urine (hem. Cys.) or plasma (nephr.)</td>
</tr>
</tbody>
</table>
Antiviral Susceptibility Testing

- **HIV**, sensitivity for ART (discussed above)
- **HSV**, tests for sensitivity to acyclovir, foscarnet and cidofovir
  - Quickly grow in culture, culture-based method: plaque reduction assay
  - Synthesis of HSV DNA
  - Growth of genetically engineered cell line
- **CMV**, test for resistance to gancyclovir. Slow growth in culture makes culture impractical
  - Detection of mutation in UL79 gene
  - DNA sequencing to detect mutation in DNA polymerase
Questions:

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