
4. Serological Diagnosis of Infectious Diseases

I. Spirochete Diseases

A. Introduction

1. Morphology

- a. Spirochetes are long, slender, helically coiled bacteria containing axial filaments, or periplasmic flagella, that wind around the bacterial cell wall and are inclosed by an outer sheath.
- b. Gram-negative, micro-aerophilic bacteria.
- c. Corkscrew motility.

2. Spirochete diseases have many similarities.

- a. Localized skin infection disseminates to other organs.
- b. Latent stage, no signs/symptoms apparent.
- c. Cardiac and neurological involvement in untreated cases.

B. Serological testing important in diagnosis

1. Isolation of organism very difficult
2. Clinical symptoms not always apparent.

II. Syphilis

A. Introduction

1. The most commonly acquired spirochete disease in the U.S.
2. Syphilis is a complex sexually transmitted disease that has a highly variable clinical course.
3. Over 50,000 cases reported in 1990 in the U.S.

B. Characteristic of the organism

1. Causative agent is *Treponema pallidum*, member of the family Spirochaetaceae.
2. No natural reservoir in the environment, requires living host.

3. Morphology
 - a. Spiral shaped and motile due to periplasmic flagella.
 - b. Variable length.

4. Three other pathogens in the group *Treponema* which are morphologically and antigenically similar to *T. Pallidum*, differences are in characteristics of lesions, amount of systemic involvement and course of the disease.
 - a. ***T. pertenue*** found in tropics, causes disease **Yaws**.
 - 1) Non-venereal transmission, transmitted by direct contact.
 - 2) Disease of bone and skin, rarely viscera
 - 3) Persistent lesions, wart-like, occur primarily in children, causes and ulcerative necrosis, scar formation, disfiguring.
 - 4) untreated disease not as severe as syphilis, but lesions are more persistent.
 - 5) treat with penicillin
 - 6) serologic syphilis test will be reactive.

 - b. ***T. endemicum*** causes **non-venereal syphilis**
 - 1) treat with penicillin.
 - 2) serologic syphilis test will be reactive.

 - c. ***T. carotum*** found in central and South America, causes **pinta**.
 - 1) non-venereal, direct contact, disease of skin.
 - 2) lesion is initially a scaly patch, becomes red-blue, later become depigmented and atrophy.
 - 3) treat with penicillin
 - 4) serologic syphilis test will be reactive.

 - d. ***T. cuniculi***, not pathogenic for humans, causes **rabbit syphilis**.
 - 1) rabbit VD
 - 2) organism morphologically identical to human syphilis spirochete.
 - 3) rabbits produce antibodies that immobilize the spirochete.

- C. Mode of Transmission
 1. Organism is very fragile, destroyed rapidly by heat, cold and drying.
 2. Sexual transmission most common, occurs when abraded skin or mucous membranes come in contact with open lesion.
 3. Can be transmitted to fetus.
 4. Rare transmission from needle stick and blood transfusion.

D. Stages of the Disease

1. **Primary Stage**

- a. Organism enters directly through skin or through mucosal tissue.
- b. Carried by blood throughout the body.
- c. Organisms remaining at the site begin to multiply.
- d. After variable incubation period of 10 days to several months, a primary lesion, **chancre**, forms at the entrance site.
- e. Chancre begins as a small, usually singular nodule; as it enlarges, the overlying epithelial tissues begins to necrose, resulting in a relatively painless ulcer.
- f. Unlike other bacterial infections, there is **no** formation of pus unless a secondary bacterial infection sets in.
- g. Chancre is most frequently seen on the external genitalia, but in women the lesions may form in the vagina or on the cervix. In men it may be inside the urethra, resulting in a serous discharge.
- h. *The lesion heals spontaneously after 1-5 weeks.*
- i. Swab of chancre smeared on slide, examined under dark-field microscope, spirochetes will be present.
- j. Thirty percent become serologically positive one week after appearance of chancre, *90% positive* after three weeks.

2. **Secondary Stage**

- a. Occurs 6-8 weeks after initial chancre, *becomes systemic, patient highly infectious.*
- b. Characterized by localized or diffuse mucocutaneous lesions, often with generalized lymphadenopathy.
- c. Primary chancre may still be present.
- d. Secondary lesions subside in about 2-6 weeks.
- e. Serology tests *nearly 100%* positive.

3. **Latent Stage**
 - a. Stage of infection in which organisms persist in the body of the infected person without causing symptoms or signs.
 - b. This stage may be further subdivided.
 - 1) Early latent, initial infection occurred within previous 12 months.
 - 2) Late latent, initial infection occurred greater than 12 months.
 - 3) Latent of unknown duration, date of initial infection cannot be established as having occurred in the previous year.
 - c. This stage may last for years.
 - d. One-third of untreated latent stage individuals develop signs of tertiary syphilis.
 - e. After four years it is rarely communicable sexually but can be passed from mother to fetus.
4. **Tertiary Stage** divided into three manifestations.
 - a. Occurs anywhere from months to years after secondary stage, typically between 10 and 30 years.
 - b. **Gummatous syphilis**
 - 1) Gummas are localized areas of granulomatous inflammation found on bones, skin and subcutaneous tissue.
 - 2) Contain lymphocytes, plasma cells and perivascular inflammation.
 - c. **Cardiovascular syphilis**
 - 1) Inflammatory lesions of the cardiovascular system.
 - 2) Usually involve the ascending aorta, symptoms due to destruction of elastic tissue.
 - 3) May result in aortic aneurysm, thickening of valves, or angina pectoris.
 - d. **Neurosyphilis**
 - 1) Most often associated with tertiary stage but can occur any time after primary stage.
 - 2) Acute meningitis.
 - 3) **Tabes dorsalis**, degeneration of lower spinal cord, general paresis and chronic progressive dementia.
4. Congenital Syphilis
 - a. Transmitted from mother to fetus.

- b. Fetus affected during second or third trimester.
- c. Forty percent result in syphilitic stillbirth-fetal death that occurs after a 20 week gestation and the mother had untreated or inadequately treated syphilis at delivery.
- d. Live-born infants show no signs during first few weeks.
 - 1) Sixty to 90 % develop clear or hemorrhagic rhinitis.
 - 2) skin eruptions (rash) especially around mouth, palms of hands and soles of feet.
 - 3) Other signs: general lymphadenopathy, hepatosplenomegaly, jaundice, anemia, painful limbs, and bone abnormalities.

E. Diagnosis of Syphilis

1. Evaluation based on three factors:
 - a. Clinical findings.
 - b. Demonstration of spirochetes in clinical specimen.
 - c. Present of antibodies in blood or cerebrospinal fluid.
 - 1) more than one test should be performed.
 - 2) no serological test can distinguish between other treponemal infections.
2. Laboratory testing
 - a. Direct examination of clinical specimen by *dark-field microscopy* or fluorescent antibody testing of sample.
 - b. *Non-specific or non-treponemal* serological test to detect *reagin*, utilized as screening test only, not diagnostic.
 - 1) Reagin is an antibody formed against cardiolipin.
 - 2) Found in sera of patients with syphilis as well as other diseases.
 - 3) This type of reagin not to be confused with same word originally used to describe IgE.
 - 4) Non treponemal tests become positive 1 to 4 weeks after appearance of primary chancre, in secondary stage may have false negative due to prozone, in tertiary 25% are negative, after successful treatment will become nonreactive after 1 to 2 years.
 - c. *Specific Treponemal antibody tests* are used as a confirmatory test for a positive reagin test.

F. *Non treponemal* Serological Tests - Reagin test1. *Venereal Disease Research Laboratory - VDRL*

- a. Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin.
- b. Utilizes an antigen which consists of *cardiolipin, cholesterol and lecithin*.
 - 1) Antigen very technique dependent.
 - 2) Must be made up fresh daily.
- c. **Serum must be heated to 56 C for 30 minutes** to remove anti-complementary activity which may cause false positive, if serum is not tested **within 4 hours** must be **reheated for 10 minutes**.
- d. Calibrated syringe utilized to dispense antigen **must deliver 60 drops/mL +/- 2 drops**.
- e. Performing the test:
 - 1) 0.05 mL of serum added to circle on ceramic slide and spread.
 - 2) Add one calibrated drop of antigen to each circle.
 - 3) Rotate at **180 rpms** for 4 minutes.
 - 4) **Read microscopically** at 100x and grade reaction if positive.
 - 5) Perform titer on positive samples, report out titer.
- f. *VDRL used primarily to screen cerebral spinal fluid.*
- g. Quality control:
 - 1) Run three levels of control: Non-reactive, weakly reactive and reactive.
 - 2) Glass syringe with 18g delivery needle must be checked daily to ensure delivery of **60 drops/mL**.
 - 3) Rotator rpms must be checked to ensure 180 rpms.
 - 4) Room temperature must be 23-29 C.

2. *Rapid Plasma Reagin test - RPR*

- a. General screening test, can be adapted to automation.
- b. **CANNOT be performed on CSF.**
- c. The VDRL cardiolipin antigen is **modified with choline chloride** to make it more stable and is attached to charcoal particles to allow macroscopic reading, the antigen comes prepared and is very stable.
- d. **Serum or plasma** may be used for testing, serum is **not** heated.

- e. Test Procedure:
 - 1) Serum or plasma added to circle on card and spread.
 - 2) One drop of antigen from a needle **capable of delivering 60 drops/mL** is added.
 - 3) Rotate at **100 rpms/minute for 8 minutes**.
 - 4) Results are read **macroscopically**.
 - f. Daily quality control:
 - 1) 20 gauge needle checked for delivery of 60 drops/mL
 - 2) Rotator checked for 100 rpms/minute
 - 3) Room temperature must be 23-29 C.
 - 4) Three levels of control must be run and give appropriate results.
 - g. RPR appears to be more sensitive than the VDRL.
5. Other tests which use modified VDRL antigen.
- a. USR-unheated serum reagin test
 - 1) modified VDRL antigen, uses choline chloride/EDTA
 - 2) microscopic flocculation test.
 - b. RST-reagin screen test
 - 1) Modified VDRL antigen with Sudan Black
 - 2) Sudan black makes flocculation reaction macroscopically visible.
- G. Specific Treponemal Tests
1. ***Treponema Pallidum Immobilization*** test - TPI
 - a. Live *T. pallidum* become immobilized by antibody in serum of infected persons.
 - b. Cumbersome and expensive, no longer used in US.
 2. ***Treponema pallidum hemagglutination*** (TPHA)
 - a. Adapted to microtechniques (MHA-TP)
 - b. Tanned sheep RBCs are coated with *T. pallidum* antigen from Nichol's strain.
 - c. Agglutination of the RBCs is a positive result.
 3. ***Fluorescent treponemal antibody absorption test*** (FTA-ABS)
 - a. One of the most used confirmatory test.
 - b. Diluted, heat inactivated serum added to Reiter's strain of *T. pallidum* to remove cross reactivity due to other *Treponemes*.

- c. Slides are coated with Nichol's strain of *T. pallidum* and add absorbed patient serum.
- d. Slides are washed, and incubated with antibody bound to a fluorescent tag.
- e. After washing again the slides are examined for fluorescence.
- f. Requires experienced personnel to read.
- g. Highly sensitive and specific, but time consuming to perform.

4. **ELISA**

- a. Tubes coated with *T. pallidum* antigen.
- b. Antibody in serum attaches to antigen.
- c. Following washing, add an anti-antibody tagged with enzyme alkaline phosphatase.
- d. Detectable color change occurs.

H. Problem Areas

- 1. **Biologic False Positives (BFP)**, reactive screening tests when patient does not have syphilis.
 - a. Collagen diseases such as arthritis, LE, etc., sometimes result in increased amount of reagin.
 - b. Certain infections: infectious mononucleosis, malaria leprosy.
 - c. Other treponemal infections.
- 2. False negatives
 - a. Very early in disease or latent, inactive stage
 - b. Immunosuppressed patients
 - c. Consumption of alcohol prior to testing (temporary)
- 3. Congenital syphilis
 - a. Non treponemal tests on cord blood or baby serum detect IgG antibody, may be of maternal origin.
 - b. Detection of IgM lacks sensitivity.
 - c. Wester Blot has demonstrated high sensitivity and specificity.
 - d. Recommended that all mothers be tested.

4. Cerebrospinal Fluid tests
 - a. Used to determine if *Treponemes* have invaded CNS.
 - b. VDRL utilized to confirm neurosyphilis.
 - c. Lacks sensitivity.

I. Treatment

1. *Drug of choice penicillin, if allergic, doxycycline.*
2. Correlation of treatment with test results.
 - a. Treatment at the primary stage, serology tests become non-reactive after 6 months.
 - b. Treatment at secondary stage, *tests usually non-reactive after 12-18 months.*
 - c. If treatment is not initiated until 10 or more years, the reagin tests probably positive for life.

III. Lyme's Disease

A. Characteristic of the Organism

1. Disease first recognized in 1977 in Lyme, Connecticut
2. Causative agent ***Borrelia burgdorferi***
 - a. Loosely coiled spirochete 5-25 micrometers.
 - b. Can be cultured, but it is very difficult.
 - c. Organism has been isolated from blood, CSF, skin lesions and joint fluid.
 - d. Disease rarely, if ever, fatal
 - e. Can be transmitted perinatally, causing intrauterine death.
3. Reservoir in nature is the white footed mouse and white tailed deer.
 - a. Vector of transmission is the ***Ixodes tick.***
 - b. Humans acquire the disease from a bite from the nymphal or adult tick.
 - c. Must remain attached a minimum of 24-48 hours for transmission to occur.

4. Incidence
 - a. Most prevalent tick-borne disease in US.
 - b. All states have reported the disease, 92% of the cases are in 8 NE states - NY, NJ, PA, CT, MA, RI, WI, and MN.
- B. Stage of the Disease
 1. **Localized rash**
 - a. Sixty to 80% of adult and 50% of children develop characteristic red, ringed rash known as **erythema chronicum migrans** appears 2 days to 2 weeks after the bite.
 - b. Starts as small red papule, expands to form a red ring with a central area of partial clearing, gradually fades after 3 to 4 weeks.
 - c. Fifty percent develop secondary skin lesions.
 - d. Develop nonspecific flu like symptoms.
 2. **Dissemination to multiple organ systems**
 - a. Dissemination occurs by way of the bloodstream.
 - b. May occur weeks to months after infection.
 - c. Migratory pain may occur in the joints, tendons and bones.
 - d. Neurologic
 - 1) Bell's palsy most prevalent neurologic sign.
 - 2) If CNS involved patient may develop mild confusion or difficulty with memory.
 - 3) Aseptic meningitis characterized by headache, photophobia and elevated CSF protein.
 - e. Cardiac include carditis and arrhythmia.
 3. **Chronic disseminated**
 - a. Characterized by chronic arthritis.
 - b. Chronic axonal polyneuropathy causes cognitive disorders, sleep disturbance, fatigue and personality changes.

- c. Affects the large joints, especially the knees.

C. Immune Response

1. IgM antibodies develop against flagellar antigens.
2. Antibody production peaks between 3 and 4th weeks of infection.
3. IgG lags, not detectable until 4 to 6 weeks after erythema migrans.

D. Laboratory Diagnosis

1. Diagnosed clinically, confirmed serologically.
2. Antibodies to antigens of *B. burgdorferi* can be detected by IFA, ELISA and Western Blot.
3. Serological tests are often falsely negative during early weeks.
4. Western Blot is most sensitive.
5. IFA and ELISA are more commonly performed due to ease of procedure, but are subject to false positives due to other spirochete diseases and some autoimmune diseases.

E. Treatment

1. Penicillins, cephalosporins, tetracyclines, and macrolides..
2. For early Lyme disease doxycycline drug of choice followed by amoxicillin.

IV. Streptococcal Serology

A. Introduction

1. Streptococci are gram-positive, beta hemolytic, spherical, ovoid or lancet-shaped organisms which are catalase negative and seen in pairs or chains.
2. Divided into groups or serotypes based on cell wall components.
 - a. Two major proteins in outermost cell wall known as M and T.
 - b. Interior proteins divided into 20 defined groups A-H and K-T, known as Lancefield grouping.
 - c. *Streptococcus pyogenes* belongs to Lancefield group A
 - d. It is believed the M protein is the chief virulent factor of this group.

3. Numerous exoantigens are produced and excreted as the cell metabolizes:
 - a. Streptolysin O
 - b. Deoxyribonuclease B DNase
 - c. Hyaluronidase
 - d. Nicotinamide Adenine dinucleotidase (NADase)
 - e. Streptokinase
 4. Organism found only in man.
 5. This organism is the principle cause of oropharyngitis which can lead to complications.
 6. Culture and rapid screening tests detect early infection.
 7. Sequelae include **Rheumatic Fever** and **Acute Glomerulonephritis** which can only be diagnosed through testing for specific antibodies.
- B. Characteristics of Group A Streptococcal Infection
1. Two major sites of infection: *upper respiratory tract and skin*.
 2. Upper respiratory tract
 - a. Fever, chills, severe sore throat, headache, tonsillar exudate, and petechiae on soft palate.
 - b. Twenty percent of school children are carriers and are asymptomatic.
 3. Skin infections
 - a. Pyoderma or impetigo
 - b. Impetigo-lesions on extremities become pustular and crusted, more common in children.
 4. Suppurative complications
 - a. Otitis media, erysipelas, cellulitis, puerperal sepsis and abscess formation (necrotizing fasciitis).
 - b. Scarlet fever characterized by rash with subsequent peeling.
 - c. Septic arthritis, acute bacterial endocarditis and meningitis can result from pharyngitis.
 - d. Evidence that *Streptococcus pyogenes* is responsible for a toxic shock-like syndrome.

5. Non-suppurative complications
 - a. Inflammatory response elsewhere in the body.
 - b. Acute rheumatic fever or post-streptococcal glomerulonephritis.
 6. Rheumatic Fever
 - a. Only certain serotypes of *S. pyogenes* involved.
 - b. *Develops as sequel in 2-3% untreated upper respiratory infections, not well understood.*
 - c. Symptoms occur about 20 days after sore throat.
 - d. Group A Streptococcus share antigenic determinants with host tissue, especially heart and even joints.
 - e. Inflammation of the mitral valve most serious.
 - f. Thirty to 60% of patients may suffer permanent disability.
 7. Post-streptococcal Glomerulonephritis
 - a. Follows Streptococcal infection of **skin or pharynx**.
 - b. Occurs about 10 days following initial infection.
 - c. Characterized by damage to glomeruli of the kidneys.
 - d. Renal function impaired due to reduction in glomerular filtration rate, results in edema and hypertension.
 - e. Renal failure not typical.
 - f. Damage caused by antigen-antibody complexes depositing in kidneys, activates complement resulting in inflammatory response.
 - g. Complement is activated resulting in hypocomplementemia.
- C. Laboratory Testing
1. Most reliable test is culture and identification of the organism from infected site.

2. Rapid Streptococcal screening tests from throat exudates have *high specificity* but *low sensitivity*, 60 - 95%.
 - a. Antigen from a swab is extracted and tested by ELISA or latex agglutination method.
 - b. If negative, perform C&S.
3. Detection of Streptococcal antibodies most useful in Streptococcal sequelae.
 - a. Group A Streptococci elaborate more than 20 exotoxins that may invoke an antibody response.
 - b. The antibodies found most useful are:
 - 1) anti-Streptolysin O (ASO)
 - 2) anti-DNase B
 - 3) anti-NADase
 - 4) anti-Hyaluronidase
 - c. Serological evidence of disease is based on elevated or rising titer of Streptococcal antibodies.
 - d. Four fold (2 tube dilution) rise in titer is considered clinically significant.
5. Anti-Streptolysin O Titer (ASO Titer)
 - a. Two of the toxins produced are **Streptolysin S, which is oxygen stable, non-antigenic and Streptolysin O (SLO), which is oxygen labile and antigenic.**
 - b. SLO is a hemolysin which is toxic to many tissues, including heart and kidneys.
 - c. Evokes an antibody response (anti-SLO) which neutralizes the hemolytic action of SLO.
 - d. Specific for ASO, it does not test for antibodies to any other Streptococcal exotoxins.
 - e. ASO procedure (Must Know Principle of Each Step)
 - 1) Patient serum is diluted with buffer.
 - 2) A measured amount (0.5 mL) of SLO is added, this reagent must be used within 15 minutes.
 - 3) Tubes are incubated at 37 C, if ASO is present in the patient serum it will bind to the SLO, neutralizing its' hemolytic action.
 - 4) After incubation 0.5 mL of fresh RBCs are added and an additional incubation is performed.

- 5) If the SLO was neutralized no hemolysis of the RBCs will occur, once the ASO is sufficiently diluted out, the *SLO is no longer neutralized and hemolysis of the RBCs will occur.*
 - 6) *Reported out in Todd units.*
- f. Normal values will vary, <125 Todd units for adults, 5-125 Todd units for children, recent Strep infections 250 Todd units for adults, 333 Todd units for children.
- g. Test Controls and Standard-Must know contents and purpose of each of the following:
- 1) **Tube 13** is RBC control, contains only buffer and RBCs, no hemolysis is expected in this tube.
 - 2) **Tube 14** is SLO control, contains SLO and RBCs, complete hemolysis is expected, proves SLO is active.
 - 3) A **standard** solution is diluted out and must give results of 166 Todd units, plus or minus one tube, ensures proper performance of all reagents.
- h. A single titer is of little significance unless extremely elevated, titers performed over a period of time will give the most information.
4. Anti-DNase B Testing
- a. May appear earlier than ASO.
 - b. Increased sensitivity for detection of glomerulonephritis preceded by streptococcal skin infections.
 - c. Macro- and micro-titer, ELISA and neutralization techniques are available.
 - d. Neutralization technique has advantage of stability of reagents.
5. Streptozyme Testing
- a. Hemagglutination procedure to detect antibodies to numerous Streptococcal antigens.
 - b. Sheep RBCs are coated with: Streptolysin, streptokinase, Hyaluronidase, DNASE, and NADase.
 - c. Patient serum diluted 1:100, mixed with sheep RBCs and observed for agglutination.

- d. Good screening test as it can detect any of a number of antibodies produced in a Streptococcal infection.
- e. Rapid and simple to perform, more false positive and negative results occur.

V. Serology of Viral Infections

A. Hepatitis

1. Introduction

- a. *General term meaning inflammation of the liver*, usually accompanied with fever, nausea, vomiting and jaundice..
- b. Can be caused by radiation, chemicals, disease processes such as autoimmune disease, viruses and cancer.
- c. Focus here will be on 5 distinct hepatitis viruses, A, B, C, D and E which cause chronic and acute hepatitis.
- d. All of these are RNA viruses with the exception of **hepatitis B which is a DNA virus**.
- e. Initial infection may be clinically silent.
- f. Chronic carrier state may develop and may result in liver failure due to cirrhosis, hepatocellular carcinoma, or fulminant hepatitis.
- g. Fulminant hepatitis is the term used when the number of hepatocytes destroyed is so great that too few remain to maintain basic liver function, results in liver failure.

2. Hepatitis A virus (HAV) - formerly known as “short incubation” or “infectious hepatitis”

- a. ***Transmitted by fecal-oral route***, usually associated with unsanitary conditions or contaminated food or water.
- b. Occurs worldwide, less common in industrialized nations.
- c. Most hepatitis epidemics are due to HAV and may show seasonal patterns.
- d. Progress of infection:
 - 1) Incubation of 2-7 weeks, may be asymptomatic or may include jaundice.
 - 2) clinical illness develops abruptly and include: fever, anorexia, vomiting, fatigue and malaise.
 - 3) Increase in serum transaminases.
 - 4) Right upper quadrant pain, dark urine and pale stool.

- 5) Recovery 2-4 weeks, no carrier state.
 - 6) Mortality 0-1%
- e. Antibody and antigen markers.
- 1) *First and most clinically useful is IgM antibody to HAV.*
 - 2) IgM indicates acute infection, appears 4-5 weeks after exposure.
 - 3) IgM disappears in 3-6 months, replaced by IgG anti-HAV.
 - 4) IgG peaks during convalescence and may remain detectable for life.
- f. HAV vaccine is available and highly effective.
3. Hepatitis B (HBV)
- a. Old term “serum hepatitis”, incubation time of 4-26 weeks.
- b. ***Route of infection is usual parenteral, direct inoculation***
- 1) small breaks in skin/mucosa come in contact with infected blood or serum.
 - 2) blood transfusions
 - 3) Needles or sharps exposure
 - a) IV drug abusers
 - b) tattoos
 - c) dental equipment
 - d) accidental needle stick
 - 4) homosexual or heterosexual contact
 - 5) maternal to fetus or newborn
 - 6) sharing razors, toothbrushes, etc.
- c. Incidence of infection is 140-320,000 cases per year resulting in 5-6,000 deaths per year.
- 1) Duration of acute infection ranges from 4-8 weeks with symptoms similar to HAV.
 - 2) Ten percent progress to chronic.
 - 3) One-third of chronic at risk of developing chronic active hepatitis, liver cirrhosis, and/or hepatocellular carcinoma.
- d. Laboratory diagnosis involve the detection of three marker systems.
- 1) **Hepatitis B surface antigen (HBsAg) is the first to appear**, appears 2 to 4 weeks during late incubation, marker of choice for recent infection.
 - 2) **Anti-Hepatitis B surface antigen (anti-HBs) is the last antibody to appear**, may persist for life.
 - 3) Between disappearance of HbsAg and appearance of anti-HBs is known as the **core window**.
 - 4) **IgM antibody to hepatitis B core antigen (anti-HBc)** may be the *only detectable marker during the core window*, differentiates recent infection from chronic carrier state.

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- 5) Third marker is hepatitis Be antigen (HBeAg), appearance of HbeAg and anti-HBe, closely coincide with HbsAg.
4. Hepatitis D Virus
 - a. ***Requires infection with Hepatitis B.***
 - b. Route of transmission the same as HBV
 - c. Can occur as coinfection or superinfection
 - d. Serological markers
 - 1) HDAg found early, disappears rapidly, not very useful
 - 2) IgM anti-D and total anti-HD (IgM and IgG) detected during acute phase.
 - 3) Presence of IgM anti-D and HbsAg together with IgM anti-HBc indicates coinfection.
 - 4) Absence of IgM anti-HBc indicates superinfection.
 - 5) Persistence of IgG anti-HD indicates chronic infection.
 6. Hepatitis C Virus
 - a. Clinically and epidemiologically similar to HBV.
 - b. Sixty to 70% of HCV patients will develop chronic hepatitis, 10-20 % cirrhosis and 15% hepatocellular carcinoma.
 - c. HCV and HBV may be present as coinfections.
 - d. Serological markers
 - 1) Serological profile not fully developed.
 - 2) Present of HCV antibodies only indicates present or past infection.
 - 3) Can have false negative in some patients.
 7. Hepatitis E Virus
 - a. Similar to HAV in transmission (*fecal-oral route*) and clinical course.
 - b. Found primarily in developing countries, Africa and Asia, and related to poor sanitation and contaminated water.
 - c. Results in acute hepatitis, no risk of chronic hepatitis.

- d. Pregnant women with HEV may develop fulminant liver failure and death.
 - e. No distinctive markers, diagnosis based on symptoms for exposed individuals in endemic countries.
8. Hepatitis G Virus
- a. Independently discovered 1995-1996 by 2 separate research groups.
 - b. RNA virus
 - c. Transmissible by bloodborne route.
 - d. found in patients with acute or chronic liver diseases.
 - e. Exact clinical significance needs to be further defined.
 - f. ELISA and Western blot methods have been developed.
- B. Herpes virus Group
1. Introduction
- a. Includes:
 - 1) Epstein-Barr virus
 - 2) Cytomegalovirus
 - 3) Herpes simplex virus type I and II
 - 4) Varicella-zoster virus
 - b. DNA viruses that remain within nucleus while completing life cycle.
 - c. Most infections are subclinical and result in latent stage.
2. **Epstein-Barr Virus (EBV)**
- a. Spread through oral transmission of infective saliva and is the cause of *Infectious Mononucleosis*.
 - b. Other diseases caused by this virus include:
 - 1) African or Burkitt's lymphoma
 - 2) nasopharyngeal carcinoma
 - 3) B-cell lymphoma
 - c. AIDS patients may develop oral hairy leukoplakia or lymphocytic interstitial pneumonia.

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- d. Virus may become reactivated and is the suggested cause of chronic fatigue syndrome.
 - e. Characteristics of infection:
 - 1) Acute self-limiting infection of the RE system
 - 2) Enlarged lymph nodes in the neck.
 - 3) Sore throat, fever, rash
 - 4) Malaise, lethargy, extreme tiredness
 - 5) Liver and spleen involvement and enlargement
 - 6) Hematological picture-atypical, reactive, or Downey cell lymphocytes.
 - f. Serological testing may involve screening tests to detect heterophile antibodies.
 - 1) **Heterophile antigens** are a group of similar antigens found in unrelated animals, IE, man, sheep, horse, dog cat, mouse.
 - 2) **Heterophile antibodies** produced against heterophile antigens of one species will cross react with others.
 - 3) **Forssman antigen** is an example of a heterophile antigen and is found on the RBCs of many species (guinea pig, dog, cat, mouse, sheep, fowl, horse)
 - 4) **Forssman antibodies** formed against Forssman antigens will agglutinate sheep RBCs.
 - g. **Davidson Differential** classic test to distinguish between antibodies produced to Forssman antigen, serum sickness and IM.
 - 1) Patient serum added to one tube containing Guinea Pig (GP) kidney and one tube containing beef RBCs and allowed to incubate.
 - 2) The GP kidney will absorb out IM antibody, the beef RBCs will absorb out Forssman antibody, serum sickness antibodies will be absorbed.
 - 3) Absorbed serum is removed and tested with sheep RBCs, no agglutination is a positive reaction, indicating presence of the antibody.
 - 4) Agglutination indicates that the antibody was not absorbed out so is still available to agglutinate the sheep RBCs in the test system

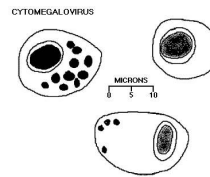
Type of Heterophile Antibody	Guinea Pig Kidney		Beef RBCs	
	Absorbed	Aggt of Sheep RBCs	Absorbed	Aggt of Sheep RBCs
Infectious Mononucleosis	No	Agglutination	Yes	No Agglutination
Forssman Antibody	Yes	No Agglutination	No	Agglutination
Serum Sickness	Yes	No Agglutination	Yes	No Agglutination

- h. Mononucleosis slide tests
- 1) It was discovered that horse RBCs possess antigens which react with the antibody associated with IM.
 - 2) Patient serum mixed with horse RBCs, agglutination is positive.
 - 3) *Not diagnostic, must look at total clinical picture.*
- i. EBV specific antibodies may be measured.
- 1) Must know pattern of appearance of EBV antigens.
 - 2) Most valuable is IgM antibody to viral capsid antigen (VCA).
 - 3) Can also detect anti-early antigen (EA) and anti EB nuclear antigen (EBNA).
 - 4) ELISA and immunofluorescence techniques most commonly used.

3. Cytomegalovirus

- a. Transmitted through saliva, sexual contact, perinatally or blood transfusions or organ transplantation.
- b. Clinical course
- 1) Symptoms resemble IM.
 - 2) In babies may cause life threatening illness resulting in CNS involvement, hearing loss, and mental retardation.
 - 3) Patients with deficient immune systems CMV infection may cause ocular disease, pneumonia, neurologic disorders, febrile illness and hepatitis.
 - 4) CMV in AIDS patients may cause ocular disease or pneumonia.
 - 5) In transplantation patients life-threatening infection may occur.

- c. Immunologic response
 - 1) IgM antibodies produced against early and intermediate-early (IE) CMV antigens, last for 3 to 4 months.
 - 2) IgG appear shortly after and peak at 2 to 3 months.
- d. Laboratory Diagnosis
 - 1) Range from culture and cytologic techniques to DNA probes, PCR and serologic techniques.
 - 2) Detection of antibodies indicator of recent or active infection.
 - 3) Viral cultures lack sensitivity and are time consuming, expensive.
 - 4) Microscopic examination of biopsy specimens, urine sediment or peripheral blood may reveal the typical cytomegalic cell with “owls eye” inclusion.



The morphologic appearances of cytomegalovirus are diagrammed here. The cell is so large that in histologic sections, the plane of section may not reveal all or part of the nucleus in every cytomegalic cell, and the cytoplasmic inclusions, though characteristic for CMV, are not always present.

- 5) These morphologic changes have been reported in a number of cells.
- 6) Detection of CMV antigen in cells more appropriately detected by immunofluorescent techniques using monoclonal antibodies.
- 7) The enzyme-linked immunosorbent assay (or ELISA) is the most commonly available serologic test for measuring antibody to CMV.
- 8) The result can be used to determine if acute infection, prior infection, or passively acquired maternal antibody in an infant is present.
- 9) Other tests include various fluorescence assays, indirect hemagglutination, and latex agglutination.
- 10) Screening tests using coated latex particles compare favorably to more complex tests for antibody detection.

11) False positives can occur due to RA and Epstein-Barr antibodies.

4. Herpes Simplex Virus (HSV)

a. Introduction

- 1) Most of us are exposed to the herpes virus during childhood, about 90% of adults are positive for prior viral exposure-primary infection.
- 2) The herpes virus however, is unique in that it possess *viral latency*.
- 3) HSV has the ability, once inside the host, to go into hibernation, usually in specific nerve tissue only to become active at a later date.
- 4) **Two types: HSV-1 and HSV-2**

b. *HSV-1 results in lesions above the waist.*

- 1) HSV-1 is usually transmitted from person to person by saliva or direct contact.
- 2) **Cold sores** around the mouth are most often caused by the herpes simplex virus - type 1 (HSV-1 or gingivostomatitis), may have several episodes of cold sores during a lifetime
 - a) When the HSV-1 reactivates again, it causes classic cold sores around the mouth.
 - b) Symptoms may begin with tingling and numbness in the mouth area, followed by a blister that eventually breaks and forms a crust.
 - c) Cold sores or fever blisters caused by reactivation of HSV-1 usually follow some type of stress, either to the mouth area alone or to the whole body, including
 - (1) a cold or other infection,
 - (2) a stressful period of life,
 - (3) unusually long exposure to sunlight;
 - (4) hormone changes in pregnancy;
 - (5) during menstrual periods,
 - (6) or after a tooth extraction.
- 3) Conjunctivitis, keratitis and herpetic whitlow may occur.

c. *HSV-2 results in lesions below the waist-herpes genitalis.*

- 1) HSV-2 is usually transmitted person to person by intimate sexual contact or perinatally.

- 2) Symptoms typically begin with pain, tenderness, or an itch in the genital area and occur with fever, headache, lymphadenopathy and malaise.
 - a) Blisters soon appear on the penis in males and on the area around the vagina in females.
 - b) In females, blisters may spread to the vagina and cervix, and in both sexes they may appear on the thighs and buttocks.
 - c) Blisters soon erupt to form painful sores that last 1 to 3 weeks.
 - d) The HSV-2 virus of genital herpes can lie dormant in nearby nerves and be reactivated later in life
 - 3) HSV-2 can cause potentially fatal infections in infants if the mother is shedding virus at the time of delivery.
 - a) If a woman has active genital herpes at delivery, a cesarean delivery is usually performed.
 - b) In infants results in localized infections of skin, eyes and mucosa or may develop systemic infection with 70% mortality.
 - c) Disseminated neonatal herpes is the most common and most lethal form of neonatal herpes
 - 4) Clinical findings lead to diagnosis, laboratory tests important for monitoring.
- d. Laboratory Testing
- 1) Recovery of the virus in cell culture is considered the "gold standard" for detection of this virus from sources other than cerebrospinal fluid, culture helpful in differentiating types of HSV.
 - 2) Direct examination using immunofluorescence or immunoperoxidase staining of cells from lesion.
 - 3) DNA probes, ELISA, latex agglutination, RIA and indirect immunofluorescence.
 - 4) Serology is not very useful because there is a high prevalence of antibody in the normal population.
5. Varicella-Zoster Virus
- a. Two different manifestations of the same virus.
 - 1) **Varicella** is the primary infection, *causes chicken pox*
 - 2) **Herpes Zoster causes shingles** is due to reactivation of the latent virus.

- b. **Chicken pox**, self-limiting infection causes fever and vesicular exanthem.
- 1) The typical rash of chickenpox is made up of groups of small, itchy blisters surrounded by inflamed skin.
 - 2) The rash usually begins as one or two lesions, quickly spreading throughout the body including the trunk, scalp, face, arms, and legs.
 - 3) The total number of blisters varies greatly from person to person.
 - 4) Over four days, each blister tends to dry out and form a scab, which then falls off between 9 to 13 days later.
 - 5) Secondary complications due to infection most common.
 - a) May also result in pneumonia, encephalitis and hepatitis.
 - b) Very serious for immunocompromised children.
 - 6) Vaccine now available.
- c. **Shingles** causes painful eruptions of vesicular lesions forming.
- 1) After a chickenpox infection, the virus lives in a dormant state in nerve cells along the spine.
 - a) Later in life, when it is reactivated, usually from a weakened immune system, aging, or other risk factor.
 - b) The virus travels down the tract of the particular nerve where it was "hibernating", first causing the pain and other sensations followed by the rash.
 - c) The pattern or path that the symptoms follow is called a dermatome, which essentially means the area of the skin that the nerve supplies.
 - 2) The typical rash of shingles begins as redness (erythema) followed by the appearance of blisters that cover one concentrated area of the body on either the face, trunk, shoulders and neck, or legs.
 - 3) These eruptions follow the path of an infected nerve, usually only a single nerve is involved, confining the rash to one side and one section of the body (called a dermatome).
 - 4) The trunk is the area affected in 50% to 60% of cases. The next most common site is one side of the face, which may even involve the tongue, the eye, or the ear.

- 5) Skin may be extremely sensitive to touch, causing great pain, pain reduces once rash appears.
- d. Laboratory testing important to distinguish VZV from other infections, selection of antiviral drugs, or determining immune status of individuals.
 - 1) PCR is now the routine testing method for varicella zoster virus (VZV).
 - 2) Direct fluorescent antibody staining and viral culture techniques may be used for the detection of VZV in most specimen types.
 - 3) IgG and IgM antibody tests by ELISA may be used.

C. Rubella Virus

1. RNA virus with 3 major structural proteins, E1, E2, and C.
2. Incubation 2-3 weeks
3. Highly contagious, spread through respiratory tract cause when infected person coughs or sneezes and is also spread by direct contact with the nasal or throat secretions of an infected person.
4. Causes **German measles** infection which is self limiting with symptoms ranging from subclinical to erythematous rash, low grade fever, headache, arthralgia and conjunctivitis.
5. *Rubella vaccine has resulted in 99% decline in infections.*
6. Congenital rubella most serious.
 - a. Congenital Rubella Syndrome (CRS) is caused by infection of the fetus in utero during the first trimester of pregnancy.
 - b. If a pregnant woman gets rubella during the first 3 months of pregnancy may result in miscarriage or stillbirth, live-born baby at risk of having serious birth defects or dying.
 - c. 20% of the children born after such an infection suffer the severe congenital abnormalities associated with CRS which include
 - 1) neurosensory deafness,
 - 2) blindness,
 - 3) congenital heart disease,
 - 4) microcephaly with mental retardation,
 - 5) growth retardation,
 - 6) and hepatosplenomegaly.
 - d. 10-20% of these children die within the first year of life.
 - e. Rubella vaccine contraindicated during pregnancy.

7. Antibody response
 - a. IgG and IgM antibodies may form at same time in acquired infections.
 - b. IgM antibodies persist for 4 to 5 weeks, IgG for life.
8. Laboratory Testing
 - a. Performed primarily for diagnosis of acquired infections and to determine immune status of pregnant patients.
 - b. Some tests detect IgG antibodies, other IgM.
 - c. Methods include: hemagglutination inhibition, passive hemagglutination, neutralization, hemolysis in gel, complement fixation, fluorescence immunoassay, RIA, ELISA and latex agglutination.
 - d. Method depends on volume of testing, turn around time, complexity, expense and whether a qualitative or quantitative test is needed.

D. Rubeola

1. Introduction
 - a. *Measles, also called rubeola*, is a single stranded RNA virus best known for its typical skin rash, although it is primarily a respiratory infection.
 - b. Incubation time is about 10 days, varying from 8 to 13 days from exposure to onset of fever; about 14 days until rash appears.
 - c. Hospital cases require airborne precautions.
 - d. Symptoms include irritability, runny nose, eyes that are red and sensitive to light, hacking cough, and high fever
 - e. Fever peaks with the appearance of the rash, which typically begins on the forehead, then spreads downward over the face, neck, and body.
 - f. Rash appears on face first and consists of large flat red to brown blotches that often flow into one another to completely cover the skin, especially on the face and shoulders, then moves down to legs and feet.
 - g. Rash fades in the same order that it appeared, forehead first and feet last.
 - h. The total time for the rash, from beginning to end, head to toe, is usually about 6 days.
2. Complications
 - a. Croup, bronchitis, bronchiolitis, pneumonia, conjunctivitis, myocarditis, hepatitis, and encephalitis.

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- b. Can also make the body more susceptible to ear infections or pneumonias caused by bacteria.
 - c. The disease can be severe, with bronchopneumonia or brain inflammation leading to death in approximately 2 of every 1,000 cases.
 - d. Symptoms and complications of measles are usually most severe in adults.
3. Measles vaccine
- a. Measles vaccine contains live, attenuated measles virus.
 - b. Should not be given to pregnant women, or to persons with active tuberculosis, leukemia, lymphoma, or depressed immune systems.
 - c. Persons with severe allergies to eggs, or to the antibiotic neomycin, may risk life-threatening reactions to measles vaccine.
 - d. Measles vaccine occasionally causes side effects in persons with no underlying health problems, in about 10% of cases there is a fever between 5 and 12 days after vaccination, and in about 5% of cases there is a rash.
4. Laboratory Testing
- a. Serology testing provides best means of confirming a measles diagnosis.
 - b. Methods to detect rubeola antibodies include: hemagglutination inhibition, endpoint neutralization, complement fixation, IFA and ELISA.
 - c. In addition to signs and symptoms diagnosis confirmed by presence of Rubeola specific IgM antibodies antibodies or four-fold rise in IgG antibody titer in paired samples taken after rash to 10 to 30 days later.
 - d. IgM test highly depended on time of sample collection with 3-11 days after rash being optimal.
 - e. IgM false positive due to RA.
- E. **Mumps**
1. Introduction
- a. Single stranded RNA virus.
 - b. Mumps is transmitted by direct contact with saliva and discharges from the nose and throat of infected individuals, incubation 16-18 days.
 - c. The virus can infect many parts of the body, especially the parotid salivary glands.

- d. The parotid salivary glands, which produce saliva for the mouth, are found toward the back of each cheek, in the area between the ear and jaw, in cases of mumps, these glands typically swell and become painful.
- e. The glands usually become increasingly swollen and painful over a period of 1 to 3 days, pain gets worse when the child swallows, talks, chews, or drinks acidic juices.
- f. Both the left and right parotid glands may be affected, with one side swelling a few days before the other, or only one side may swell.
- g. In rare cases, mumps will attack other groups of salivary glands instead of the parotids, if this happens, swelling may be noticed under the tongue, under the jaw, or all the way down to the front of the chest.

2. Complications

- a. Mumps can lead to inflammation and swelling of the brain and other organs.
- b. Symptoms of encephalitis appear in the first week after the parotid glands begin to swell and may include:
 - 1) high fever,
 - 2) stiff neck,
 - 3) headache,
 - 4) nausea and vomiting,
 - 5) drowsiness,
 - 6) convulsions,
 - 7) and other signs of brain involvement.
- 8) Mumps in adolescent and adult males may also result in the development of orchitis, an inflammation of the testicles.
 - 1) Usually one testicle becomes swollen and painful about 7 to 10 days after the parotids swell.
 - 2) Accompanied by a high fever, shaking chills, headache, nausea, vomiting, and abdominal pain that can sometimes be mistaken for appendicitis if the right testicle is affected.
 - 3) After 3 to 7 days, testicular pain and swelling subside, usually at about the same time that the fever passes.
 - 4) Even with involvement of both testicles, sterility is only a rare complication of orchitis.
- d. Mumps may affect the pancreas or, in females, the ovaries, causing pain and tenderness in parts of the abdomen.

- e. Mumps infection in pregnant women may result in increased risk for fetal death when it occurs in first trimester but is not associated with congenital abnormalities.

3. Laboratory Testing

- a. Methods to detect mump antibodies include: complement fixation, hemagglutination inhibition, hemolysis-in-gel, neutralization assays, IFA and ELISA.
- b. Current or recent infections indicated by presence of specific IgM antibody in single sample which can be detected within 5 days of illness.
- c. Fourfold rise in specific IgG antibody in 2 samples collected during acute and convalescent phases.
- d. Fluorescent antibody staining for mumps antigens developed but not widely used.
- e. Cross-reactivity between antibodies to mumps and parainfluenza viruses has been reported in tests for IgG, not a problem since symptoms differ.

V. Human Immunodeficiency Virus (HIV)

A. Introduction

1. Etiologic agent of Acquired Immunodeficiency Syndrome (AIDS).
2. Discovered independently by Luc Montagnier of France and Robert Gallo of the US in 1983-84.
3. Former names of the virus include:
 - a. Human T cell lymphotropic virus (HTLV-III)
 - b. Lymphadenopathy associated virus (LAV)
 - c. AIDS associated retrovirus (ARV)
4. HIV-2 discovered in 1986, antigenically distinct virus endemic in West Africa.
5. One million people infected in US, 30 million worldwide are infected.
6. Leading cause of death of men aged 25-44 and 4th leading cause of death of women in this age group in the US.

B. Characteristics of the virus

1. Icosahedral (20 sided), enveloped virus of the lentivirus subfamily of *retroviruses*.
2. Retroviruses transcribe RNA to DNA.

3. Two viral strands of RNA found in core surrounded by protein outer coat.
 - a. Outer envelope contains a lipid matrix within which specific viral glycoproteins are imbedded.
 - b. These knob-like structures responsible for binding to target cell.
- C. Structural Genes
 1. Three main structural genes:
 - a. **Group Specific Antigen (Gag)** is *p55* from which three **core** structural proteins (*p15*, *p17* and *p24*) are formed.
 - 1) Located in nucleocapsid of virus.
 - 2) Icosahedral capsid surrounds the internal nucleic acids made up of *p24* and *p15*.
 - 3) *p17* lies between protein core and envelope and is embedded in the internal portion of the envelope.
 - 4) Two additional *p55* products, *p7* and *p9*, are nucleic acid binding proteins closely associated with the RNA.
 - b. **Envelope (Env)** gene codes for envelope proteins *gp160*, *gp120* and *gp41*.
 - 1) *gp160* cleaved to form *gp120* and *gp41*.
 - 2) *gp120* forms the 72 knobs which protrude from outer envelope.
 - 3) *gp41* is a transmembrane glycoprotein antigen that spans the inner and outer membranes and attaches to *gp120*.
 - 4) *gp120* and *gp41* both involved with fusion and attachment of HIV to CD4 antigen on host cells.
 - c. **Polymerase (Pol)** codes for *p66* and *p51* subunits of reverse transcriptase and *p31* an endonuclease.
 - 1) Located in the core, close to nucleic acids.
 - 2) Responsible for conversion of viral RNA into DNA, integration of DNA into host cell DNA and cleavage of protein precursors.
- D. Viral Replication
 1. First step, HIV attaches to susceptible host cell.
 - a. Site of attachment is the CD4 antigen found on a variety of cells
 - 1) helper T cells
 - 2) macrophages
 - 3) monocytes
 - 4) B cells
 - 5) microglial brain cells
 - 6) intestinal cells

- b. Macrophages predominant cell infected in primary infection, may serve as reservoir for virus.
 - c. T cells infected later on.
2. The gp120 protein on virus binds specifically to CD4 receptor on host cell with high affinity.
3. Gp41 causes fusion of the virus to the cell membrane.
 - a. After fusion virus particle enters cell.
 - b. Viral genome exposed by uncoating particle.
4. Reverse transcriptase produces viral DNA from RNA.
 - a. Becomes a provirus which integrates into host DNA.
 - b. Period of latency occurs.
5. After a period of latency lasting up to 10 years viral replication is triggered and occurs at high rate.
6. CD4 cell may be destroyed in the process, body attempts to replace lost CD4 cells, but over the course of many years body is unable to keep the count at a safe level.
7. Destruction of large numbers of CD4 cause symptoms of HIV to appear with increased susceptibility to opportunistic infections, disease and malignancy.

E. HIV Transmission/Symptoms

1. Methods of transmission:
 - a. Sexual transmission, presence of STD increases likelihood of transmission.
 - b. Exposure to infected blood or blood products.
 - c. Use of contaminated clotting factors by hemophiliacs.
 - d. Sharing contaminated needles (IV drug users).
 - e. Transplantation of infected tissues or organs.
 - f. Mother to fetus, perinatal transmission variable, dependent on viral load and mother's CD 4 count.
2. ***Primary HIV syndrome***
 - a. Mononucleosis-like, cold or flu-like symptoms may occur 6 to 12 weeks after infection.
 - 1) lymphadenopathy
 - 2) fever
 - 3) rash
 - 4) headache
 - 5) fatigue

- 6) diarrhea
 - 7) sore throat
 - 8) neurologic manifestations.
 - 9) no symptoms may be present
- b. Symptoms are relatively nonspecific, may not be severe and misdiagnosis may occur.
 - c. HIV antibody test often negative but becomes positive within 3 to 6 months.
 - d. Primary HIV can be diagnosed using viral load titer assay or other tests.
 - e. Primary HIV syndrome resolves itself and HIV infected person remains asymptomatic for a prolonged period of time, often years.
3. ***Clinical latency period***, HIV continues to reproduce, CD4 count gradually declines from its normal value of 500-1200.
- a. Once *CD4 count drops below 500*, HIV infected person at risk for **opportunistic infections**.
 - b. The following diseases are ***predictive*** of the progression to AIDS:
 - 1) persistent herpes-zoster infection (shingles)
 - 2) oral candidiasis (thrush)
 - 3) oral hairy leukoplakia
 - 4) Kaposi's sarcoma (KS)
5. AIDS
- a. *CD4 count drops below 200* person is considered to have advanced HIV disease
 - b. If preventative medications not started the HIV infected person is now at risk for:
 - 1) Pneumocystis carinii pneumonia (PCP)
 - 2) cryptococcal meningitis
 - 3) toxoplasmosis
 - c. *If CD4 count drops below 50*:
 - 1) Mycobacterium avium
 - 2) Cytomegalovirus infections
 - 3) lymphoma
 - 4) dementia
 - 5) Most deaths occur with CD4 counts below 50.
6. Infants with HIV may exhibit the following:
- a. Failure to thrive
 - b. Persistent oral candidiasis
 - c. Hepatosplenomegaly

- d. Lymphadenopathy
- e. Recurrent diarrhea
- f. Recurrent bacterial infections
- g. Abnormal neurologic findings.

F. Immunologic Manifestations

1. Early stage slight depression of CD4 count, few symptoms, temporary.
2. Window of up to 6 weeks before antibody is detected, by 6 months 95% positive.
3. During window p24 antigen present, acute viremia and antigenemia.
4. Antibodies produced to all major antigens.
 - a. First antibodies detected produced against gag proteins p24 and p55.
 - b. Followed by antibody to p51, p120 and gp41
 - c. As disease progresses antibody levels decrease.
5. Immune abnormalities associated with increased viral replication.
 - a. Decrease in CD4 cells due to virus budding from cells, fusion of uninfected cells with virally infected cells and apoptosis.
 - b. B cells have decreased response to antigens possibly due to blockage of T cell/B cell interaction by binding of viral proteins to CD4 site.
 - c. CD8 cells initially increase and may remain elevated.
 - d. As HIV infection progresses, CD4 T cells drop resulting in immunosuppression and susceptibility of patient to opportunistic infections.
 - e. Death comes due to immuno-incompetence.

G. Treatment

1. Zidovudine (azidothymidine or AZT) is the most commonly prescribed anti-HIV treatment.
 - a. In class of anti-HIV drugs known as nucleoside analogs.
 - b. A number of other nucleoside analog drugs with similar action are (2 are required):
 - 1) ddI (Videx)
 - 2) ddC (Hivid)
 - 3) d4T (Zerit)
 - 4) 3TC (EpiVir)

- c. A newer class of anti-HIV drugs are protease inhibitors and include (1 required):
 - 1) saquinavir (Invirase)
 - 2) ritonavir (Norvir)
 - 3) indinavir (Crixivan)
 2. Recently completed studies indicate that combinations of these anti-HIV drugs are much more effective than AZT alone in preventing disease progression and death.
 3. Combination anti-HIV treatment has become the standard of care for HIV patients (drug cocktail).
 4. It has not been determined which of the anti-HIV drug combinations are most effective.
 5. The stronger the anti-HIV effects of a combination therapy, the less likely it is that HIV will become resistant to the effects of the drugs.
 6. When HIV becomes resistant to a drug, that drug no longer works against the virus.
 7. Combinations usually include a protease inhibitor, 3TC, and one other nucleoside analog anti-HIV such as AZT or d4T
- H. Laboratory Diagnosis of HIV Infection
1. Methods utilized to detect:
 - a. Antibody
 - b. Antigen
 - c. Viral nucleic acid
 - d. Virus in culture
 2. ELISA Testing
 - a. First serological test developed to detect HIV infection.
 - 1) Easy to perform.
 - 2) Easily adapted to batch testing.
 - 3) Highly sensitive and specific.
 - b. ELISA tests useful for:
 - 1) Screening blood products.
 - 2) Diagnosing and monitoring patients.
 - 3) Determining prevalence of infection.
 - 4) Research investigations.
 - c. Antibodies detected in ELISA include those directed against: p24, gp120, gp160 and gp41, detected first in infection and appear in most individuals

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- d. Different types of ELISA techniques used:
 - 1) indirect
 - 2) competitive
 - 3) sandwich
 - e. ELISAs are for screening only, false positives do occur and may be due to AI disease, alcoholism, syphilis, and immunoproliferative diseases.
4. Other Screening Tests
- a. Agglutination tests using latex particles, gelatin particles or microbeads are coated with HIV antigen and will agglutinate in the presence of antibody.
 - b. Dot-Blot Testing utilizes paper or nitrocellulose impregnated with antigen, patient serum is filtered through, and anti-antibody is added with enzyme label, color change is positive.
5. Western Blot Testing
- a. Most popular confirmatory test.
 - 1) Utilizes a lysate prepared from HIV virus.
 - 2) The lysate is electrophoresed to separate out the HIV proteins (antigens).
 - 3) The paper is cut into strips and reacted with test sera.
 - 4) After incubation and washing anti-antibody tagged with radioisotope or enzyme is added.
 - 5) Specific bands form where antibody has reacted with different antigens.
 - 6) Most critical reagent of test is purest quality HIV antigen.
 - 7) The following antigens must be present: p17, p24, p31, gp41, p51, p55, p66, gp120 and gp160.
 - b. Antibodies to p24 and p55 appear earliest but decrease or become undetectable.
 - c. Antibodies to gp31, gp41, gp 120, and gp160 appear later but are present throughout all stages of the disease.
 - d. Interpretation of results.
 - 1) No bands, negative.
 - 2) In order to be interpreted as positive a minimum of 3 bands directed against the following antigens must be present: p24, p31, gp41 or gp120/160.
 - 3) CDC criteria require 2 bands of the following: p24, gp41 or gp120/160.

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- e. Indeterminate results are those samples that produce bands but not enough to be positive, may be due to the following:
 - 1) prior blood transfusions, even with non-HIV-1 infected blood
 - 2) prior or current infection with syphilis
 - 3) prior or current infection with malaria
 - 4) autoimmune diseases (e.g., diabetes, Grave's disease, etc)
 - 5) infection with other human retroviruses
 - 6) second or subsequent pregnancies in women.
 - 7) run an alternate HIV confirmatory assay.
 - f. Quality control of Wester Blot is critical and requires testing with strongly positive, weakly positive and negative controls.
8. Indirect immunofluorescence assay can be used to detect both virus and antibody to it.
- a. Antibody detected by testing patient serum against antigen applied to a slide, incubated, washed and a fluorescent antibody added.
 - b. Virus is detected by fixing patient cells to slide, incubating with antibody.
9. Detection of p24 HIV antigen
- a. P24 antigen only present for short time, disappears when antibody to p24 appears.
 - b. Anti-HIV-1 bound to membrane, incubated with patient serum, second anti-HIV-1 antibody attached to enzyme label is added (sandwich technique), color change occurs.
 - c. Optical density measured, standard curve prepared to quantitate results.
 - d. Positive confirmed by neutralizing reaction, preincubate patient sample with anti-HIV, retest, if p24 present immune complexes form preventing binding to HIV antibody on membrane when added.
 - e. Test not recommended for routine screening as appearance and rate of rise are unpredictable.
 - f. Sensitivity lower than ELISA.
 - g. Most useful for the following:
 - 1) early infection suspected in seronegative patient
 - 2) newborns
 - 3) CSF
 - 4) monitoring disease progress

5. Polymerase Chain Reaction (PCR)
 - a. Looks for HIV DNA in the WBCs of a person.
 - b. PCR amplifies tiny quantities of the HIV DNA present, each cycle of PCR results in doubling of the DNA sequences present.
 - c. The DNA is detected by using radioactive or biotinylated probes.
 - d. Once DNA is amplified it is placed on nitrocellulose paper and allowed to react with a radiolabeled probe, a single stranded DNA fragment unique to HIV, which will hybridize with the patient's HIV DNA if present.
 - e. Radioactivity is determined.
6. Virus isolation can be used to definitively diagnose HIV.
 - a. Best sample is peripheral blood, but can use CSF, saliva, cervical secretions, semen, tears or material from organ biopsy.
 - b. Cell growth in culture is stimulated, amplifies number of cells releasing virus.
 - c. Cultures incubated one month, infection confirmed by detecting reverse transcriptase or p24 antigen in supernatant.
7. Viral Load Tests
 - a. Viral load or viral burden is the quantity of HIV-RNA that is in the blood.
 - b. RNA is the genetic material of HIV that contains information to make more virus.
 - c. Viral load tests measure the amount of HIV-RNA in one milliliter of blood.
 - 1) Take 2 measurements 2-3 weeks apart to determine baseline.
 - 2) Repeat every 3-6 months in conjunction with CD4 counts to monitor viral load and T-cell count.
 - 3) Repeat 4-6 weeks after starting or changing antiretroviral therapy to determine effect on viral load.
8. Testing of Neonates
 - a. Difficult due to presence of maternal IgG antibodies.
 - b. Use tests to detect IgM or IgA antibodies, IgM lacks sensitivity, IgA more promising.
 - c. Measurement of p24 antigen.
 - d. PCR testing may be helpful but still not detecting antigen soon enough: 38 days to 6 months to be positive.