

# Transfusion-transmitted Diseases Part 1

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- I. Outline:
  - A. General philosophy
  - B. Basic screening test descriptions
  - C. Terminology (phases/periods)
  - D. Specific organisms
- II. General Philosophy
  - A. Build a wall of safety between donor and recipient
    1. FDA's Five Layers of Safety
      - a) Donor screening by history and physical exam
      - b) Donor deferral lists to prevent donations from previously deferred donors
      - c) Donor blood testing (see below for specifics)
      - d) Quarantine of units until testing and any issues with the above complete
      - e) Problem and deficiency investigation to ensure quality
    2. Direct treatment of blood products
      - a) Pathogen reduction/inactivation
      - b) Examples:
        - (1) Heat treatment of factor concentrates
        - (2) Solvent-detergent treatment to inactivate enveloped viruses
        - (3) Methylene blue treatment of plasma
        - (4) Specific technologies targeted to nucleic acid of organisms
          - (a) Current focus of study in the US (already approved in Europe)
          - (b) Substance binds to nucleic acid, then UV light exposure makes the nucleic acid incapable of being translated
          - (c) Will probably lessen irradiation, leukoreduction, culture needs
  - B. Zero-risk
    1. Reaching a "zero-risk" blood supply is not feasible with current (or foreseeable future) technology
    2. The battle to prevent TTDs is always a struggle between science, economics, and donor availability, and some level of trade-off is inevitable
- III. Basic Screening Test Descriptions
  - A. Enzyme immunoassay (EIA/ELISA)
    1. Most commonly used method for infectious disease donor screening
    2. Can be done in multiple ways, searching either for antibodies or antigens
      - a) Used for antibodies most often (anti-HIV-1,2, anti-HTLV-I/II, anti-HCV, anti-HBc, anti-*T. cruzi*); also used for antigens(HBsAg)
      - b) 2010 approval of a combination antibody and antigen detection EIA for HIV (Abbott); not yet approved for blood donor use
    3. Method: "Indirect" antibody detection; see example below
      - a) Antigen (purified natural or recombinant) bound by test manufacturer to a microwell (capture reagent)
      - b) Sample added, incubated, then washed to remove unbound antibody
      - c) Anti-human antibody conjugated to an enzyme like horseradish peroxidase added; will bind to antibody attached to test antigen above
      - d) Chromogen solution added; color change occurs in proportion to amount of detected antibody

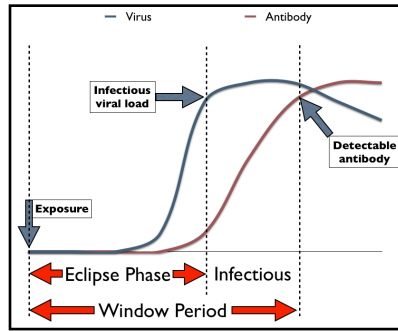
4. Method: Antigen detection (“sandwich technique”)
    - a) Antibody against a target antigen bound to microwell as capture reagent
    - b) Donor sample added, incubated, washed; target antigen should be bound
    - c) Second antibody (conjugated to an enzyme) against a different part of the antigen is added; will bind to antigen/capture antibody complex
    - d) Detection via chromogen solution
  5. In general, all forms of EIA are highly sensitive and specific
- B. Chemiluminescent immunoassay (ChLIA)
1. Chemiluminescence: Generation of light from a chemical reaction occurring close to ambient temperature
  2. Similar in principle to EIA, but visible light is generated and detected rather than color
    - a) As with EIA, can be used to detect either antigens or antibodies
    - b) ChLIA methods in current blood donor screening primarily use microparticles coated with antigen or antibody rather than microwells
  3. Acridinium is most commonly used for detection; can be bound to proteins such as antibodies and gives off light in the presence of hydrogen peroxide
  4. Used in Abbott Prism testing for anti-HIV-1,2, anti-HCV, anti-HBc, anti-HTLV-I/II, and HBsAg
- C. Western blot (WB)
1. Used as confirmatory test after reactive EIA/ChLIA tests for anti-HIV-1,2
  2. Procedure is actually similar to EIA using antigen as capture reagent, but reaction occurs on a membrane rather than in a microwell
  3. General procedure:
    - a) HIV proteins separated by high-resolution electrophoresis and “blotted” onto a membrane
      - (1) This separation of proteins is what adds specificity to WB over EIA
      - (2) Minimizes cross-reactivity
    - b) Donor sample added and incubated
    - c) Labeled anti-human antibody binds to any membrane/antigen bound donor antibody present
    - d) Visible “bands” examined through a variety of detection methods (colorimetry, chemiluminescence, fluorescence, radioactivity) and compared to controls
  4. Main difficulty: “Indeterminate” tests
    - a) Not positive, not negative, and usually NOT related to HIV!
- D. Immunofluorescence Assay (IFA)
- a) Used as confirmatory test for anti-HIV-1 (considered essentially equivalent to WB for confirmation, but fewer indeterminate results)
  - b) General procedure:
    - (1) HIV antigens expressed on surface of T-cells bound to wells on a glass slide
    - (2) Donor sample added to wells, incubated with cells, then washed to remove unbound antibody
    - (3) Anti-human antibody conjugated to fluorescent marker (FITC) added
    - (4) UV light activates the fluorescence for detection under a UV microscope
    - (5) Pattern determines positive, indeterminate, and negative results

- c) Fairly simple to perform and gives results in 90 minutes or so
  - d) Drawbacks:
    - (1) Some subjectivity in interpretation
    - (2) Increased false positives in SLE patients
- E. Recombinant Immunoblot Assay (RIBA)
- 1. a.k.a. “Strip Immunoblot Assay” (SIA)
  - 2. Used for confirmation of anti-HCV EIA results
  - 3. Similar in concept to western blot above
  - 4. General procedure
    - a) Synthetic and recombinant HCV antigens are immobilized on a test strip by the manufacturer
    - b) Donor sample incubated with strip, antibodies (if present), bind to specific HCV antigens
    - c) After washing, peroxidase-labeled anti-human antibody added, which binds to the strip-bound HCV antigen/antibody complexes
    - d) Detection accomplished by addition of peroxide-chromogen solution
    - e) Visible bands examined and compared to controls
- F. Nucleic Acid Testing (NAT)
- 1. In general, a test that directly identifies the genetic material of an organism
  - 2. Polymerase chain reaction (PCR); what most think of when you say “NAT”
    - a) A procedure that greatly amplifies a *selected portion* of genetic material into large quantities of that segment for easier detection
    - b) Sequence:
      - (1) Denaturing/melting: Sample is heated to cause DNA to denature into strands of single stranded DNA
      - (2) Annealing: Specific single stranded DNA (SS-DNA) “primers” are used to bind adjacent to the particular sequence of interest
      - (3) Extension/elongation: DNA polymerase synthesizes complementary single stranded DNA after binding to the primer-SS target DNA complex
    - c) The cycle is repeated, and the number of copies of the target sequence (“amplicon”) are amplified exponentially (chain reaction)
    - d) Variant: Reverse transcriptase PCR (RT-PCR):
      - (1) Used for retrovirus detection (e.g., HIV, HTLV); required because these viruses contain RNA rather than DNA
      - (2) RT creates a SS-DNA copy (complementary DNA or “cDNA”) that can then be amplified using basic sequence outlined above
  - 3. Transcription-mediated Amplification (TMA)
    - a) Variant of NAT approved for use for HIV-1, HCV, HBV, and WNV NAT (Novartis Diagnostics testing platform; developed by Gen-Probe)
      - (1) Does not require heating/cooling cycles like PCR
      - (2) Produces RNA amplicon in greater quantities faster than PCR (10 billion copies in an hour or so)
    - b) Specific primer used to isolate sequence of interest
    - c) Reverse transcriptase is used to make a DNA copy of the sequence (cDNA)
    - d) RNA polymerase then amplifies abundant RNA copies

## IV. Definitions in TTD transmission and testing

## A. Phases/periods (see bbguy.blogspot.com blog entry March 2011):

1. Window period
  - a) Time from *infection* to *laboratory detection* of organism
  - b) May be longer or shorter than incubation period
2. Eclipse phase
  - a) Time from entry into the cell until the appearance of new virus within the cell
  - b) No detectable evidence of infection in this phase by definition



3. Incubation period
  - a) Time from *exposure* to appearance of *clinical symptoms*
  - b) May be longer or shorter than window period
4. Latent phase
  - a) Multiple definitions (confusing)
  - b) To a virologist: Time from eclipse phase end until infectious virus is present
  - c) To most blood bankers: Time from infection until symptoms appear (often years as is seen with HIV)

## B. Reactive patterns on screening EIA/ChLIA

1. “Initially reactive”
  - a) First screening test is reactive (value exceeds cutoff)
  - b) Leads to performance of repeat test in duplicate
2. “Repeat reactive”
  - a) If reactive on either repeat test, donor is called “repeat reactive”
  - b) Unit discarded, donor deferred as appropriate (see organisms and assays below)
3. “Negative” or “non-reactive”
  - a) Either of the following:
    - (1) Initial screening test is non-reactive (value below cutoff)
    - (2) Initial test is reactive, but NONE of the repeat tests are reactive
4. “Reactive” vs. “Positive” terminology; in general...
  - a) Screening test results are reactive or non-reactive (NAT reported both ways)
  - b) Confirmatory results and disease status are positive or negative

## V. Specific Organisms

## A. General requirements for an agent to be a TTD:

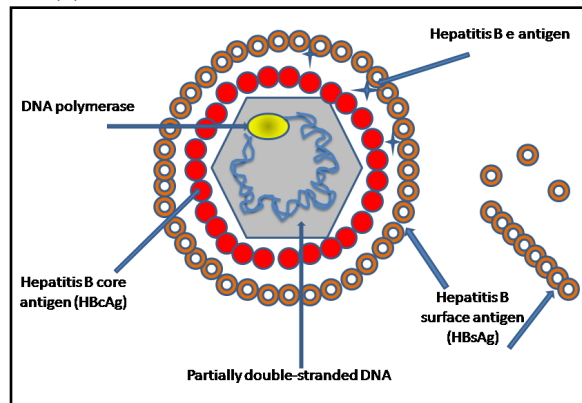
1. Asymptomatic phase
2. Agent survival in blood
3. IV transmission route
4. Susceptible recipients
5. Detectable disease in recipients

**B. Syphilis**

1. History
  - a) Possibly brought to Europe via Columbus return from the New World in 1493 (controversial), probably named from a poem “Syphilus” (1532)
  - b) Blood transmission discovery 1940s; first testing of blood for TTD transmission
2. Organism
  - a) *Treponema pallidum*
  - b) Helical coiled gram negative bacterium (spirochete); cannot be cultured
3. Disease when transmitted sexually:
  - a) Primary: Chancre at infection site, self-heals in 3-6 weeks
  - b) Secondary: Spirochetemia with fever, rash, fatigue, joint pain, mucus patches, lymphadenopathy
  - c) Tertiary: Spread to CNS, heart, bones, liver, skin
4. Risk of transmission:
  - a) Very low; syphilis is rare in population (0.03% annual new infection rate)
  - b) Also, spirochete does not survive refrigerated storage for >96 hours
  - c) Only two cases documented since 1950 (most recent in 1966)
5. Testing (required by 21CFR 640.5 and AABB Standard 5.8.4):
  - a) Infection induces two types of antibodies
    - (1) Non-treponemal antibodies are usually not specific and usually turn negative with treatment
    - (2) Treponemal antibodies appear earlier, are more specific for syphilis, and usually stay positive for life (85%)
  - b) Traditionally, donor testing used non-treponemal tests as screening, then FTA-ABS (treponemal test) as confirmatory, but now, treponemal tests like microhemagglutination are more commonly used initially
  - c) Non-treponemal tests; detect antibody vs. lipid antigens (cardiolipin)
    - (1) General
      - (a) False positives more common than true positives
        - i) Pregnancy
        - ii) Immunoglobulin production abnormalities (rheumatoid arthritis, ulcerative colitis, cirrhosis)
        - iii) Other infections (HIV [possible surrogate test], EBV, TB, rickettsia, endocarditis)
      - (b) Lacks sensitivity as well (antibody lags behind spirochetemia)
    - (2) Tests
      - (a) Rapid plasma reagin (**RPR**)
        - i) Anti-cardiolipin demonstrated by agglutination of coated carbon particles by patient serum
        - ii) “Cardiolipin” = “reagin”
        - iii) Simplified version of VDRL
      - (b) Venereal disease research lab (**VDRL**)
        - i) Mixture of serum and cardiolipin antigens in well on glass slide
        - ii) Positive = flocculation (loose aggregates or flakes formed)

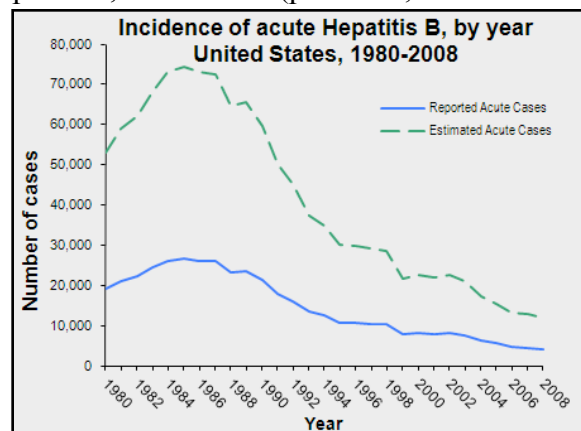
- d) Treponemal tests; detect anti-*T. pallidum*
    - (1) General
      - (a) Most true positive treponemal tests persist for life (85%)
      - (b) Often used as confirmation for a reactive non-treponemal test
      - (c) Reactive non-treponemal test + non-reactive treponemal test = unit can be used but must be labeled as syphilis +
      - (d) Today, TPHA commonly used, with TP-PA confirmation
    - (2) Specific tests:
      - (a) Fluorescent treponemal antibody absorption (**FTA-ABS**)
        - i) *T. pallidum* fixed to slide, flooded with patient serum
        - ii) Indicator fluorescent-labeled anti-human antibody added
        - iii) See positives under fluorescent light
      - (b) *T. pallidum* hemagglutination (**TPHA**)/microhemagglutination assay (**MHA**)
        - i) Sheep or bird RBCs coated with *T. pallidum* antigens
        - ii) Antibodies in donor plasma agglutinate sheep RBCs
        - iii) A variant (using chicken RBCs) is widely used on Olympus PK7200 machine (automated blood group test equipment)
          - (1) Approximately equal sensitivity to FTA-ABS
      - (c) *T. pallidum* particle agglutination/aggregation (**TP-PA**)
        - i) Like TPHA except antigen bound to colored gelatin particles
        - ii) Fewer false positives than other treponemal tests
        - iii) CDC believes TP-PA to be most suitable confirmatory test (*MMWR*, 2/11/11)
      - (d) *T. pallidum* EIA
        - i) Standard indirect (antibody detection) EIA, using recombinant *T. pallidum* antigens coated to a microwell
        - ii) Similar sensitivity to FTA
6. Deferral
  - a) If confirmed; one year after treatment completed
  - b) If not confirmed, left to discretion of medical director
- C. **Hepatitis B Virus (HBV)**
  - 1. History
    - a) 1943 report of multiple soldiers with post-transfusion hepatitis
    - b) HBsAg first discovered in 1965; called “Australia antigen”
    - c) Testing for HBsAg implemented around 1970
      - (1) Initial radioimmunoassay replaced quickly by EIA; ChLIA used too
      - (2) 2006: Test with increased sensitivity introduced
    - d) Anti-HBc introduced in 1986
      - (1) Originally a surrogate for non-A, non-B hepatitis
      - (2) Later a marker for window period infections (see below)
  - 2. Organism
    - a) Enveloped DNA virus, *Hepadnaviridae* family
    - b) Unusual DNA virus that replicates in hepatocyte through an RNA intermediate using an inaccurate, error-prone reverse transcriptase

- c) Parts of the virus (see figure below):
- (1) Outer envelope: Hepatitis B Surface Antigen (HBsAg)
    - (a) Active infection leads to marked overproduction of circulating HBsAg
    - (b) This acts as “natural amplification” and makes HBsAg ideal for early detection of infection
  - (2) Virus capsid = Hepatitis B Core Antigen (HBc)
    - (a) Not found circulating free in serum/plasma
  - (3) HBe antigen is found circulating during active infection
    - (a) Indicator of more severe disease



### 3. Disease

- a) US incidence rate has declined from 11.5 per 100,000 in the US in 1985 to 1.3 per 100,000 in 2008 (per CDC; see chart below)



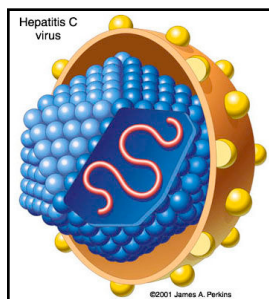
- b) Symptoms:
- (1) Incubation period = 8-12 weeks
  - (2) Majority are asymptomatic (65%); those with symptoms usually only have jaundice
  - (3) Fulminant infection in 0.5-1%
- c) Chronic hepatitis B is uncommon but more likely in younger patients
- (1) 90% chronic for perinatal infection
  - (2) 20-50% chronic for those infected at ages 1-5
  - (3) <5% chronic for those infected as adults
- d) Modes of transmission (note that all are parenteral):
- (1) Horizontal:
    - (a) IV drug use

- (b) Sexual exposure
- (c) Blood/body fluid exposure
- (d) Tattoos/piercings without single needles
- (e) Familial exposure
- (2) Vertical
  - (a) Mother-child during birth possible
- e) Prevalence:
  - (1) US: 5.6% with either HBsAb or anti-HBc, but 0.1% with HBsAg alone
  - (2) Much higher elsewhere
- f) New treatment regimens lead to improvement in about 1/3
- 4. Risk
  - a) Historic: 1:205,000 for repeat donors (1:144,000 for all donors)
  - b) Current: 1:355,000 to 1:357,000 donations (TRANSFUSION 2009;49:1609-20)
- 5. Testing
  - a) HBsAg
    - (1) EIA or ChLIA using monoclonal anti-HBsAg to capture antigen
    - (2) First testable marker to become reactive in acute HBV infection
      - (a) Window period: 4-8 weeks (30-60 days); may be just before symptoms
    - (3) In self-limited infections, usually disappears after about 4-6 months
    - (4) Confirmatory test: *HBsAg neutralization*
      - (a) Repeat reactive samples incubated with reagent anti-HBs, then run in parallel with regular serum test
      - (b) If test result strength is then decreased by 50% or more, the test is *neutralized*, confirming the repeat reactive result
  - b) Anti-HBc (total)
    - (1) Introduced as surrogate marker for non-A, non-B hepatitis
    - (2) EIA or ChLIA using recombinant HBc antigen for capture
    - (3) Becomes reactive about the time symptoms start (10-12 weeks)
    - (4) Positive initially due to IgM antibody; persists indefinitely as IgG
    - (5) Test plagued by non-specificity
      - (a) Less than 1% of anti-HBc reactive donors have detectable HBV DNA by NAT
    - (6) Originally thought to be only marker positive after HBsAg disappears but before anti-HBs seen as evidence of immunity; given increased HBsAg test sensitivity, probably not useful for that purpose anymore
  - c) NAT HBV
    - (1) Not currently required in U.S. but commonly done
    - (2) As with HIV and HCV, done in minipools rather than individually
      - (a) In minipools, sensitivity is similar to that of HBsAg (remember HBsAg natural “amplification” above)
      - (b) Improved sensitivity in individual testing, but expensive
    - (3) Probably not as much benefit as for HIV or HCV
      - (a) Does not substantially change window period
      - (b) Levels of HBV may decrease enough to be missed by NAT; so it is hard to envision a NAT-alone strategy

- i) 3-6% false negative NAT in HBsAg/anti-HBc reactive donors
  - (c) January 2011 study in NEJM (Stramer S et al. "Nucleic Acid Testing to Detect HBV Infection in Blood Donors," *NEJM* 2011; 364:236-247); higher than expected NAT-only detection rate
  - (d) Mandate from FDA believed to be on near horizon
- 6. Deferral
  - a) History of Hepatitis B infection = permanent deferral
  - b) History of any viral hepatitis after age 11 = permanent deferral
  - c) Testing results:
    - (1) Anti-HBc+, HBsAg-
      - (a) No deferral for first time
      - (b) Permanent deferral for second time
      - (c) Re-entry possible as of FDA Guidance from May 2010
        - i) Requires at least 8 week wait after second positive
        - ii) All of the following are required and must be negative: Anti-HBc, HBsAg, and NAT HBV
    - (2) Anti-HBc+, HBsAg+
      - (a) Permanent deferral, regardless of neutralization result
    - (3) Anti-HBc-, HBsAg+ (not neutralized)
      - (a) Indefinite deferral, may attempt re-entry after 8 weeks
      - (b) This re-entry may be associated with a donation
      - (c) Technically, attempts can continue indefinitely
    - (4) Anti-HBc-, HBsAg+ (neutralized)
      - (a) Permanent deferral
    - (5) NAT HBV reactive
      - (a) Permanent deferral

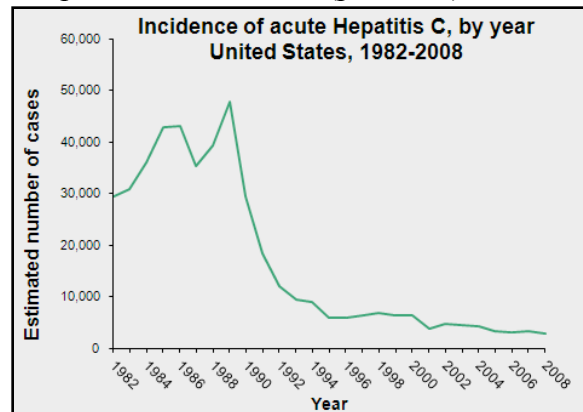
#### D. Hepatitis C Virus (HCV)

- 1. History
  - a) Transmitted through transfusion as "non-A, non-B" hepatitis (NANBH)
    - (1) Most post-transfusion hepatitis from WWII probably HCV rather than HBV
    - (2) 10% of blood recipients had evidence of NANBH in 1970s
  - b) HCV identified in 1989
  - c) Major "lookback" effort in late 1990's to identify recipients of blood from HCV-positive donors was not incredibly productive



- 2. Organism
  - a) Enveloped, single stranded RNA virus (see illustration above)
  - b) Has a core antigen like HBV, but not as useful for testing

- c) *Hepacivirus* species within Flaviviridae family
  - d) Six major genotypes (1-6), with considerable genetic variability
    - (1) 1a and 1b are most frequent subtypes in US (~75%)
3. Disease
- a) Transmission: Parenteral
    - (1) 60% of HCV transmission in US currently is from IV drug use (shared needles)
    - (2) US incidence rate has declined from 2.4 per 100,000 in the US in 1992 to 0.3 per 100,000 in 2008 (per CDC)



- (3) Risk factors for HCV aside from IVDA:
    - (a) History of clotting factor concentrate infusion
    - (b) Health care exposures (needle sticks, scalpel injuries)
    - (c) Household exposures
    - (d) Multiple sex partners
    - (e) Low socioeconomic level
    - (f) Blood transfusions before 1990
  - b) Course of infection:
    - (1) 80% of infections are chronic
    - (2) Of chronic cases, 30% have stable/favorable hepatitis, 30% severe, progressive hepatitis, 40% variable progression
    - (3) Long-term effects
      - (a) Chronic symptomatic hepatitis: Up to 10 years after infection
      - (b) Cirrhosis: Up to 20 years after infection
      - (c) Hepatocellular carcinoma: Up to 30 years after infection (rare)
  - c) New treatment options lead to response in 42-82% of patients
4. Risk
- a) In US, 1 in 1.149 million transfusions (TRANSFUSION 2010;50:1495-1504)
  - b) This risk is somewhat increased in recent years, likely due to increased incidence in the US
5. Testing
- a) Alanine transferase (ALT)
    - (1) Used prior to anti-HCV assay; no longer required
    - (2) Nonspecific, but likely did prevent infections
    - (3) One third of HCV-infected have normal ALT

- b) **Anti-HCV EIA/ChLIA**
    - (1) Introduced in 1990 (version 1.0)
    - (2) Standard EIA/ChLIA tests using various recombinant HCV antigens
    - (3) Most current version is 3.0, which detects antibodies against multiple HCV proteins (core, NS3, NS4, NS5)
    - (4) Window period = 70-80 days
  - c) **Recombinant immunoblot assay (RIBA 3.0)**
    - (1) Confirmatory test for reactive anti-HCV EIA/ChLIA
    - (2) See description earlier
    - (3) “3.0” implies that antibodies against the same antigens as above are detected
  - d) **NAT HCV**
    - (1) First test licensed in 2001
    - (2) Detects HCV RNA 20-40 days after infection
    - (3) 2004 report in *NEJM* (Stramer, et al): 1 HCV NAT positive donation per 240,000 anti-HCV-negative donations
    - (4) Methods:
      - (a) Reverse transcriptase PCR (RT-PCR); Roche platform
      - (b) Transcription-mediated amplification (TMA); Novartis platform
    - (5) Samples tested in 16-24 donor minipools first (usually with NAT HIV +/- NAT HBV), then individually if minipool is reactive
6. Deferral
- a) Anti-HCV reactive, HCV NAT reactive
    - (1) Permanent deferral
  - b) Anti-HCV reactive, NAT non-reactive
    - (1) RIBA negative or indeterminate (“non-positive”)
      - (a) Donor is indefinitely deferred
      - (b) Eligible for re-entry testing after 6 months
        - i) Re-entry tests (done on a *sample*, not a donation):
          - (1) Individual donor NAT (ID-NAT); not minipool NAT (MP-NAT)
          - (2) Licensed anti-HCV EIA/ChLIA
        - ii) If re-entry testing is identical, FDA allows continued attempts at six month intervals
        - iii) Many centers permanently defer after one failed re-entry attempt
    - (2) RIBA positive
      - (a) Permanent deferral
      - (b) No option for re-entry
  - c) Anti-HCV nonreactive, NAT reactive
    - (1) Treated nearly the same as the EIA+/NAT- donor with non-positive RIBA
      - (a) Considered by FDA a “likely false positive”
    - (2) Indefinite deferral
    - (3) Eligible to re-enter in six months
      - (a) Distinction: These donors MAY donate a unit of blood rather than just have a sample drawn for re-entry testing

- (b) Re-entry testing must include ID-NAT and anti-HCV EIA as above
  - i) If identical results (or if anti-HCV is reactive as well), defer donor permanently
  - ii) If anti-HCV is reactive but NAT is now negative, FDA allows continued re-entry attempts at six month intervals

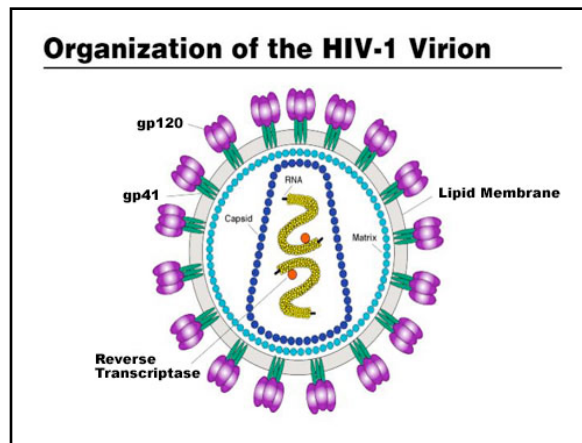
## E. Human Immunodeficiency Virus (HIV)

### 1. History

- a) Possible origin in Congo (area of greatest genetic diversity of the virus)
- b) In US, seen first in San Francisco in late 1970's-early 80's
- c) Discovered by Gallo and Montagnier, established cause of AIDS in 1984
  - (1) Montagnier called HIV "lymphadenopathy virus" (LAV)
  - (2) Gallo designated it "HTLV III"
- d) Testing history
  - (1) Anti-HIV: 1985
    - (a) 56 day window period
  - (2) Anti-HIV 1,2: 1992
    - (a) 20-22 day window period currently
  - (3) p24 antigen test: 1996
    - (a) 16 day window period
  - (4) NAT HIV-1: 1999-2000
    - (a) 10-11 day window period

### 2. Organism

- a) Two main species
  - (1) HIV-1 (see illustration below)
    - (a) Divided into three main groups
      - i) Group M (main)
        - (1) Obviously, the most common form of HIV worldwide
        - (2) Group M has at least 11 subclasses (clades A-K)
          - (a) In US/developed countries, clade B by far most common
          - (b) Worldwide, clade C most common (50% of infections)
      - ii) Group O (other)
        - (1) Found in West Africa (Cameroon especially)
        - (2) Rare in other areas
        - (3) Detected by most modern HIV screening
      - iii) Group N (new, or "non-M, non O")
        - (1) Similar distribution to group O
  - (2) HIV-2
    - (a) Identified in 1985 (one year after HIV-1)
    - (b) Found most frequently in West Africa and Europe
    - (c) Very similar immunologic features as HIV-1
    - (d) Causes similar disease as HIV-1, but clinical manifestations and disease progression are slower, and transmission (while via the same pathways) appears less likely



- b) Transmission routes
  - (1) Sexual exposure
    - (a) Both homosexual and high-risk heterosexual
      - i) Males having sex with other males = most frequent risk by factor of two
  - (2) Parenteral exposure
    - (a) Sharing IV drug needles
    - (b) Blood transfusion
  - (3) Vertical transmission
    - (a) Childbirth
    - (b) Breastfeeding
3. Disease
  - a) Early infection
    - (1) Incubation period: 2-4 weeks
    - (2) Majority have a flu-like illness at some point
    - (3) “Window period” from infection to detection 10-11 days (see below) corresponding with period of cell-free viremia
  - b) Asymptomatic phase (latent)
    - (1) Extended period with no clinical symptoms
    - (2) Virus is replicating and CD4 lymphocyte count may gradually decline
    - (3) May have reservoir in monocytes
  - c) Acquired Immune Deficiency Syndrome (AIDS)
    - (1) Gradual CD4 decline becomes rapid
    - (2) Opportunistic infections occur
    - (3) Damage to other organs
    - (4) Death in approximately ten years untreated
      - (a) Those who get HIV from transfusion often progress faster, but that is likely due to underlying disease
    - (5) Current antiretroviral therapy (high activity anti-retroviral therapy; HAART) prolongs survival significantly, but does NOT eradicate virus
4. Risk
  - a) San Francisco circa ~1980, risk roughly 1 per 100 units
  - b) Current: 1:1,467,000 units (TRANSFUSION 2010;50:1495-1504)
  - c) Almost zero for HIV-2

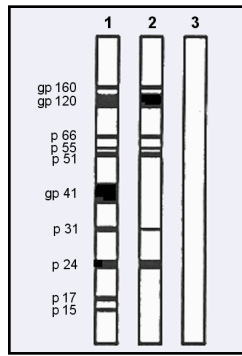
## 5. Testing

a) Anti-HIV-1,2

- (1) Required by FDA and AABB
- (2) EIA/ChLIA using “sandwich” technique
- (3) Seronegative window phase approximately 20-22 days
- (4) False positives occur: 1-5 per 100,000 donors
- (5) Confirmatory tests

## (a) Western blot (WB)

- i) Technique described earlier; see diagram below
- ii) Interpretative criteria have varied, but currently:
  - (1) Positive: At least TWO of the following: p24, gp41, gp120/160 (#1 & 2 below)
  - (2) Indeterminate: Not all of the above
  - (3) Negative: NONE of the above (#3 below)



## (b) Immunofluorescence assay (IFA)

- i) Discussed earlier
- ii) Similar sensitivity to WB; fewer indeterminates than WB

## (c) Anti-HIV-2 EIA

- i) Specific test for anti-HIV-2 required if anti-HIV-1,2 is RR
- ii) This test is rarely positive, as HIV-2 is rarely seen
- iii) No confirmatory test available

b) HIV-1 Nucleic Acid Testing (HIV-1 NAT)

- (1) Same basic methods as outlined for HCV NAT above
- (2) Detects HIV RNA 10-11 days after infection
- (3) Fewer seronegative, NAT positive donors than seen with HCV NAT
  - (a) Same NEJM study referenced above (Stramer et al, 2004) showed 1 HIV NAT positive donation per 3.1 million anti-HIV-negative donations

## c) p24 antigen testing

- (1) Useful in its time
- (2) No longer mandated, due to NAT sensitivity

## 6. Donor deferral

## a) Based on donor history

- (1) Males having sexual contact with other males (abbreviated “MSM”) have been banned from donating blood since 1983
  - (a) Current ban includes anyone with such contact *even once* since 1977

- (b) MSM is a very controversial topic, with numerous advocacy groups and many blood bank groups recommending allowing MSM donors to donate after 1 year; no change as of now
- (2) Other “high risk” activities/occurrences that lead to permanent deferral:
  - (a) IV drug use at any time
  - (b) Treatment with clotting factor concentrates
  - (c) Accepting money or drugs for sex even once since 1977
- (3) Less “high risk” activities leading to a 12 month deferral:
  - (a) Sexual contact with anyone in the above categories
  - (b) Accidental blood exposure (needle sticks, scalpel injuries, etc)
  - (c) Receiving a transfusion of someone else’s blood
  - (d) Victim of sexual assault/rape
  - (e) Incarceration/lockup for more than 72 consecutive hours
- b) Based on donor testing
  - (1) **Anti-HIV-1,2 repeat reactive (RR), NAT HIV negative**
    - (a) Not confirmed with western blot or IFA (neg or indeterminate)
      - i) Though their donated blood is destroyed, these donors are NOT considered to have HIV, and most likely are false positive
      - ii) FDA requires at least an eight week wait until the donor is re-tested for eligibility
        - (1) Donor may be tested before 8 weeks, but those results can’t be used to re-qualify the donor
      - iii) If all tests are negative after 8 weeks, donor may be re-entered at the discretion of the medical director
        - (1) Re-entry testing must include ID-NAT and licensed anti-HIV
      - iv) Most centers will permanently defer a donor that tests in the same way rather than re-test them ad nauseum
    - (b) Confirmed with western blot or IFA
      - i) Donor is considered to HAVE HIV, and is permanently deferred from donating blood
      - ii) Lookback: Prevent transfusion of blood from previous donations and notify recipients to 1 year from last negative donation
  - (2) **HIV NAT positive, with non-reactive anti-HIV EIA**
    - (a) Donor is indefinitely deferred and notified
    - (b) May attempt to re-enter after eight weeks
      - i) Testing includes ID-NAT and anti-HIV EIA as above
      - ii) May NOT donate a unit at time of testing (unlike HCV re-entry with NAT only)
    - (c) If same result, defer permanently
      - i) Also defer permanently if EIA is reactive as well as ID-NAT, regardless of WB/IFA results
      - ii) If EIA reactive ONLY, FDA allows continued attempts to re-enter at eight week intervals
  - (3) **Anti-HIV repeat reactive, HIV NAT positive**
    - (a) Permanent deferral

## F. Human T-cell Lymphotropic Virus (HTLV)

1. Organism/History
  - a) Two retrovirus species, with approximately 60% phenotypic similarity:
    - (1) HTLV-I
      - (a) Discovered in 1978 (first known human retrovirus)
    - (2) HTLV-II
      - (a) Discovered in 1982 in a patient with hairy cell leukemia (coincidence, as HTLV-II does not cause HCL)
  - b) Both are *cell-associated* viruses, with neither showing significant periods of plasma viremia
    - (1) Both primarily infect lymphocytes
    - (2) As a result, it is likely that leukocyte reduction significantly decreases transmission of these viruses
  - c) More prevalent in female donors than male (72% positives are female)
2. Disease
  - a) HTLV-I
    - (1) Endemic in Japan, Caribbean countries, South America, West and Central Africa; with infections also seen in southeastern US
    - (2) Associated with two main diseases:
      - (a) **Adult T-cell leukemia/lymphoma (ATL or ATLL)**
        - i) Usually fast-growing, ultimately aggressive malignancy of T-lymphocytes
          - (1) 50% mortality rate within 6 months of diagnosis; 20% 5 year survival with traditional chemotherapy
          - (2) Antiviral therapy (zidovudine and alpha interferon) in addition to chemo tested; possible increased survival reported in 2010
        - ii) Can involve only the blood (leukemia) or only the lymph nodes (lymphoma) or a combination of the two
        - iii) Two distinguishing clinical factors:
          - (1) Extensive solid organ involvement with malignant cells (especially spleen and liver)
          - (2) Hypercalcemia is extremely common
        - iv) Estimates range from 95-99.75% of those infected with HTLV-I will NEVER develop ATL
          - (1) Of those that do, the incubation period may be 40 years!
          - (2) Two reported cases of ATL following transfusion transmission of HTLV-I
      - (b) **Tropical spastic paraparesis (TSP)/HTLV-associated myelopathy (HAM);** together abbreviated “TSP/HAM”
        - i) Progressive degeneration of portions of the spinal cord (lateral and posterior columns)
        - ii) Bilateral leg muscle spasms (“spasticity”) and weakness, with eventual decreased or absent sensation in the legs
        - iii) Not generally fatal, but is progressive
        - iv) Antiretroviral therapy shows minimal success

- v) 96-99% of those infected will NEVER develop TSP/HAM
- (3) Causes disease by infecting CD4 lymphocytes (like HIV), but can also infect CD8 lymphs without causing clinical disease
- (4) Transmission routes (none through casual contact)
  - (a) Transfusion
    - i) Inefficient, however; only 1 in 3 positive units actually transmits
  - (b) IV drug use
  - (c) Mother-child transmission (vertical); probably breast-feeding rather than transvaginal
  - (d) Rare cases of possible sexual transmission reported
- (5) HUGE numbers of infected people worldwide (estimates as high as 10-20 million), with VERY few expected to ever get disease
- b) HTLV-II
  - (1) Endemic in IV drug users in the US, and among Native Americans
  - (2) Disease association is much less clear than with HTLV-I
    - (a) Probably NOT associated with ATL, like HTLV-I
    - (b) MAY be associated with a few cases of TSP/HAM
- 3. Risk
  - a) Estimated as 1:641,000 (1996)
  - b) Risk is almost certainly lower (estimated at 1 in 3,000,000) now due to near-universal leukoreduction and improved testing sensitivity
    - (1) Residual risk due to approximately 50 day window period
- 4. Testing
  - a) Begun in 1988
  - b) Currently approved testing uses ChLIA technology (Abbott PRISM platform) and inactivated viral lysate as capture reagent
    - (1) Because of FDA requirements, test detects antibodies to either HTLV-I or -II
  - c) NAT in development; unlikely to be implemented
  - d) No confirmatory test is licensed by the FDA
    - (1) Western blot, radioimmunoprecipitation (RIPA) can be used for informational purposes
    - (2) This makes counseling reactive donors difficult!
- 5. Donor deferral
  - a) One-time anti-HTLV-I/II repeat reactive
    - (1) No deferral from future donations required
      - (a) In-date previously donated products must be destroyed
    - (2) Lookback to test recipients of previous products from this donor not required
  - b) Second-time anti-HTLV I/II repeat reactive OR repeat reactive using a different test on the FIRST reactive donation
    - (1) Permanent deferral

## G. Cytomegalovirus (CMV)

- 1. History
  - a) Long-known disease; more recent discovery of significance
  - b) Increased prevalence in lower socioeconomic groups

2. Organism
  - a) DNA virus
  - b) Human herpesvirus family (HHV-5)
3. Disease
  - a) Mild in most healthy patients, may be severe in at-risk groups
  - b) At-risk:
    - (1) Patients:
      - (a) Transplant recipients
      - (b) Fetuses receiving intrauterine transfusion
      - (c) Low-birthweight neonates
      - (d) Patients with severe immunodeficiencies
    - (2) Disease
      - (a) Hepatitis, pneumonitis, retinitis
      - (b) If disease occurs in CMV-negative pregnant female, she may pass it on to fetus, with severe disease resulting, including:
        - i) Blindness and/or deafness
        - ii) Mental retardation
4. Risk
  - a) Prevalence averages about 50%; greater in lower socioeconomic areas
  - b) For CMV-negative, at risk recipients, risk minimization has two main strategies:
    - (1) Blood from CMV-seronegative donors
      - (a) Negative for antibody detection listed below
      - (b) Still a small risk of infection (1-4%)
    - (2) Leukoreduced blood products
      - (a) CMV resides in WBCs; removal likely makes products noninfectious
      - (b) Still a small risk of infection (1-4%)
    - (3) Both categories likely see failures due to acutely infected donors, who have not formed antibodies and have a short plasma viremia
5. Testing (not required)
  - a) Antibody detection:
    - (1) EIA
      - (a) Standard antibody detection EIA methods can be used
      - (b) Highly sensitive and specific
    - (2) Hemagglutination
      - (a) Performed on blood analyzers (analogous to syphilis testing)
  - b) Direct organism detection:
    - (1) PCR
      - (a) Not in wide use and definitely not required
      - (b) Probably not a huge benefit over current serologic methods
6. Donor deferral
  - a) None mandated

## H. West Nile Virus (WNV)

1. History
  - a) Before 1999, disease was primarily seen in Africa and Asia
  - b) In US, rapid east-west spread after first outbreak in Queens, NY in 1999

- c) Transfusion transmission proven in 2002
  - (1) Other transmission (organ transplantation, breast milk, transplacental) also proven around the same time
- d) NAT developed on an urgent basis; approved under FDA investigational new drug (IND) application in 2003, formally approved in 2005
- 2. Organism
  - a) RNA virus, flavivirus family
  - b) Arthropod vector (mosquito)
  - c) Virus causes epidemic disease in birds; humans are incidental hosts
- 3. Disease
  - a) Majority with WNV are asymptomatic (estimated as high as 80%)
  - b) Nearly 20% have mild infection (“West Nile Fever”) that is flu-like, mild
  - c) 1 in 150 infected get meningitis, a few of those get encephalitis
  - d) Fatal in 5% of those with serious disease
  - e) Infectious period is generally from days 3-10 after mosquito bite
    - (1) Viral nucleic acid detectable by NAT within 2-3 days of infection (window)
    - (2) By the time antibodies develop, the virus may already be gone!
    - (3) Short incubation and short window period (with NAT)
- 4. Risk
  - a) Essentially zero since current testing strategy implemented in 2004
- 5. Testing
  - a) NAT technology used (because of short window period above)
    - (1) Both TMA and standard PCR methods are licensed
  - b) Routinely, testing is done in minipools (MP-NAT)
  - c) Under specific “trigger” conditions, testing moves to individual (ID-NAT)
    - (1) Centers are required to define these conditions
    - (2) Can be triggered by health department notifications of disease (both in birds and humans), donors testing positively, mosquito studies, or other evidence of increased activity
    - (3) The definition includes how long to test using ID-NAT, and under what conditions the center will revert to MP-NAT
  - d) No supplemental test licensed by FDA
    - (1) Anti-WNV EIAs available, but may not add substantially
- 6. Donor deferral
  - a) Donors with known WNV disease are deferred until 120 days after symptoms resolve
  - b) Donors with positive WNV NAT are deferred 120 days from test date if asymptomatic, or 120 days from symptom development if they get sick

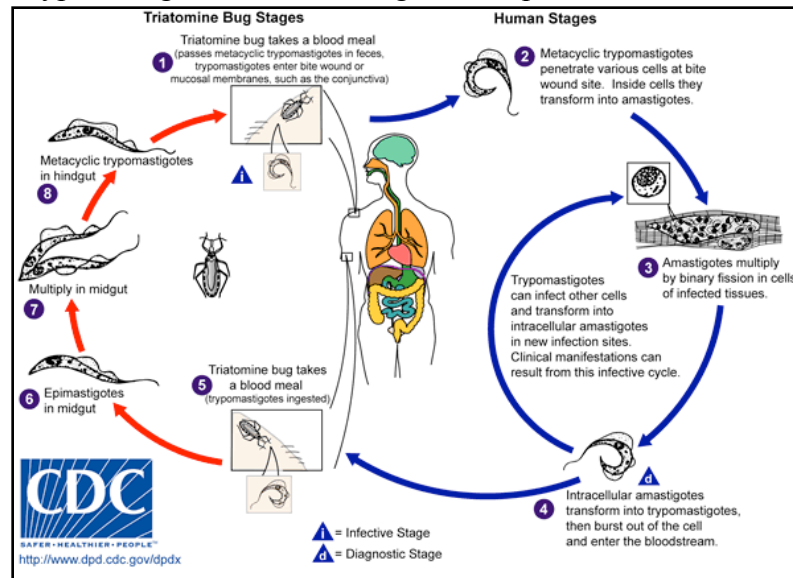
## I. Chagas’ Disease

- 1. History
  - a) Discovered by Carlos Chagas in 1909 in Brazil
  - b) Endemic in Mexico, Central and South America
    - (1) 8 to 11 million infected in these areas
  - c) Growing potential problem in southern US (due to migration of vector described below) and areas of high immigration from above areas

## 2. Organism

a) *Trypanosoma cruzi*

- (1) Parasite with life cycle inside insect vector and humans
- (2) Vector: Triatomine bug (called “kissing bugs” because they feed on people’s faces at night; no kidding!)
- (3) Trypomastigotes infect via bug feces deposited near bite wound



## 3. Disease

- a) Transmission (see diagram above)
- b) Acute phase mild and may be asymptomatic
  - (1) Mild flu-like symptoms described
- c) Chronic phase is lifelong
  - (1) Often asymptomatic (70% or so)
  - (2) When symptomatic, usually involves two areas:
    - (a) Cardiac system
      - i) Cardiomyopathy
      - ii) Arrhythmia
      - iii) Heart failure
    - (b) Gastrointestinal system
      - i) Megacolon
      - ii) Megaesophagus
- d) Symptoms and disease are more dramatic in immunocompromised individuals

## 4. Risk

- a) Appears extremely low in US
- b) Only nine currently known transmissions (two since beginning of testing outlined below)

## 5. Testing

- a) Most blood centers began testing in 2007
- b) Antibody detection EIA using lysate of *T. cruzi* as capture reagent
  - (1) FDA guidance in December 2010 mandated use by December 2011
- c) No current confirmatory test licensed by FDA

- (1) Some facilities use RIPA for unofficial confirmation and counseling
6. Donor deferral
  - a) One-time anti-T. cruzi repeat reactive leads to permanent deferral
  - b) Lack of confirmatory test makes re-entry impossible for now

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