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. <u>Enzyme immunoassay (EIA/ELISA)</u>
    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 <u>antibodies</u> 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" <u>antibody</u> detection; see example below
      a) <u>Antigen</u> (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

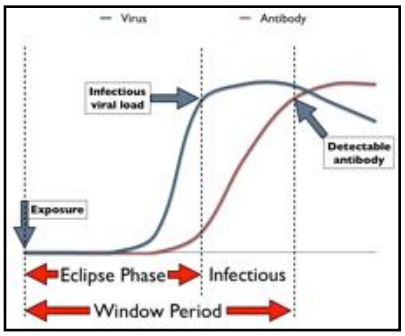
E. Recombinant Immunoblot Assay (RIBA)
1. a.k.a. "Strip Immunoblot Assay" (SIA)
2. Used for confirmation of anti-HCV EIA results
3. Similar in concept to western blot above
4. General procedure
a) Synthetic and recombinant HCV antigens are immobilized on a test strip by the manufacturer
b) Donor sample 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

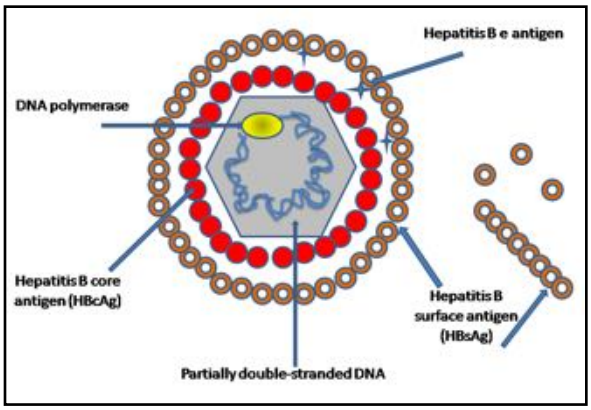
6. Deferral
a) If confirmed; one year after treatment completed
b) If not confirmed, left to discretion of medical director

C. Hepatitis B Virus (HBV)

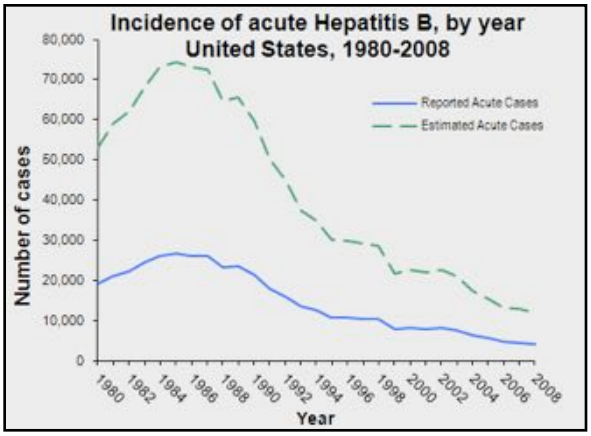
1. History
a) 1943 report of multiple soldiers with post-transfusion hepatitis
b) HBsAg first discovered in 1965; called "Australia antigen"
c) Testing for HBsAg implemented around 1970
(1) Initial radioimmunoassay replaced quickly by EIA; ChLIA used too
(2) 2006: Test with increased sensitivity introduced
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) <u>HBsAg</u>
(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) <u>Anti-HBc (total)</u>
(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) <u>NAT HBV</u>
(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

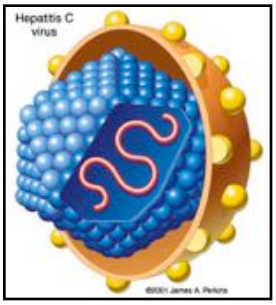
a) Transmitted through transfusion as "non-A, non-B" hepatitis (NANBH)

(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



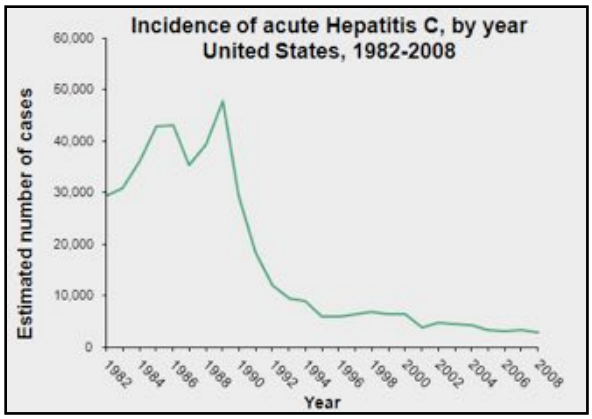
2. Organism

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 <u>normal</u> 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

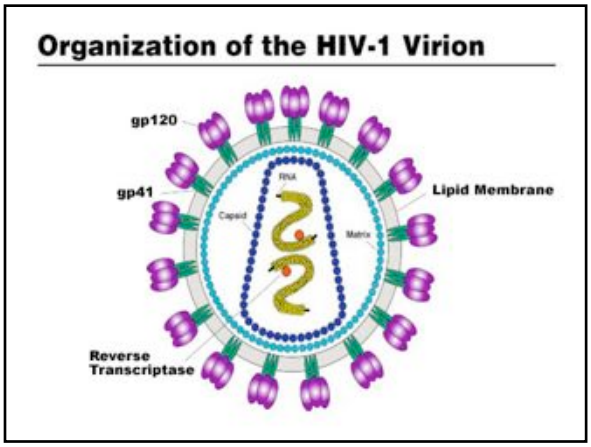
6. Deferral
a) Anti-HCV reactive, HCV NAT reactive
(1) Permanent deferral
b) Anti-HCV reactive, NAT non-reactive
(1) RIBA negative or indeterminate ("non-positive")
(a) Donor is indefinitely deferred
(b) Eligible for re-entry testing after 6 months
i) Re-entry tests (done on a *sample*, not a donation):
(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

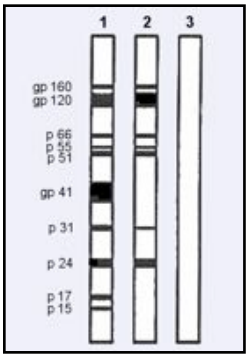
2. Organism
a) Two main species
(1) HIV-1 (see illustration below)
(a) Divided into three main groups
i) Group M (main)
(1) Obviously, the most common form of HIV wordwide
(2) Group M has at least 11 subclasses (clades A-K)
(a) In US/developed countries, clade B by far most common
(b) Worldwide, clade 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) <u>Blood from CMV-seronegative donors</u>
         (a) Negative for antibody detection listed below
         (b) Still a small risk of infection (1-4%)
      (2) <u>Leukoreduced blood products</u>
         (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

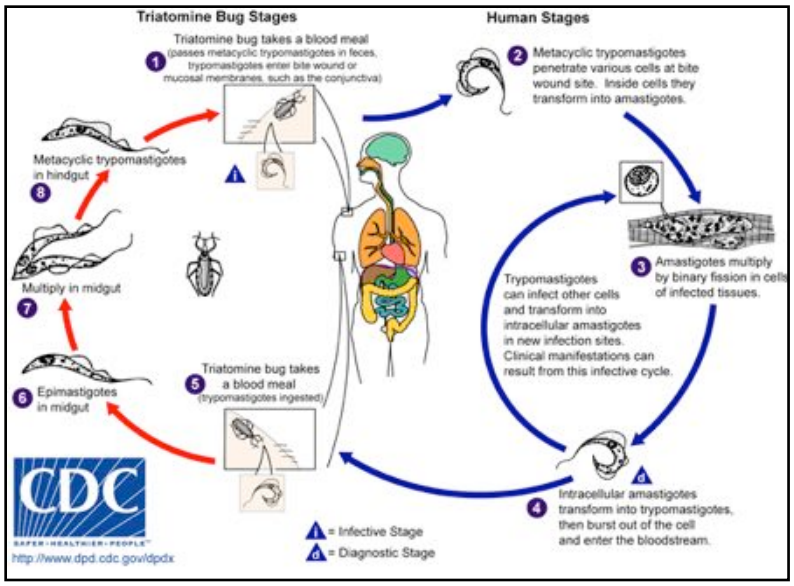
5. Testing
a) NAT technology used (because of short window period above)
(1) Both TMA and standard PCR methods are licensed
b) Routinely, testing is done in minipools (MP-NAT)
c) Under specific "trigger" conditions, testing moves to individual (ID-NAT)
(1) Centers are required to define these conditions
(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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