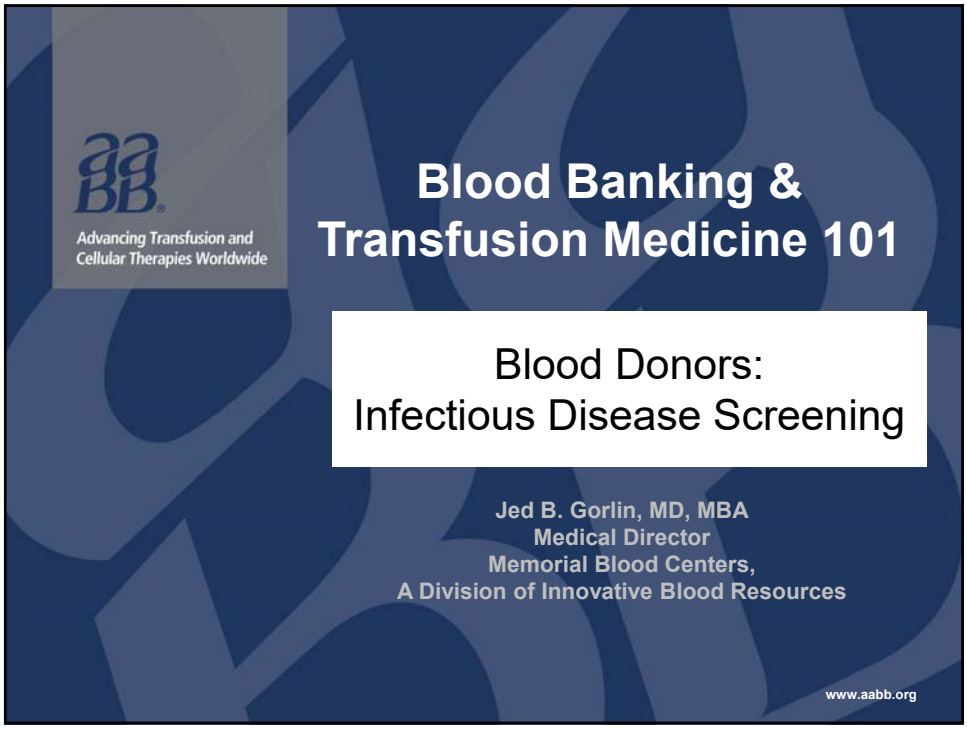


1

Faculty Disclosure

- I am VP and Medical Director of Innovative Blood Resources that performs blood donor (and other donor) screening for many other blood, tissue and milk banks
- I am on medical Advisory board for Quotient/MosaiQ (that is not discussed in this presentation!)

2

Learning Objectives

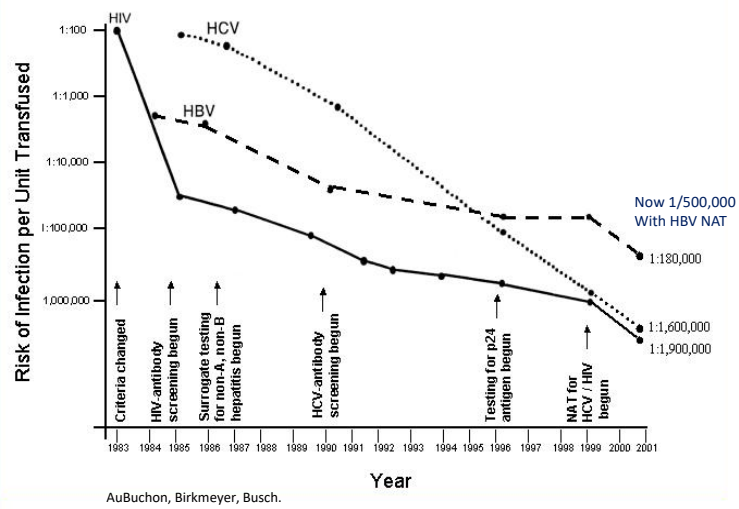
After participating in this program you should be able to….

- Review US-FDA requirements for donor screening (blood)
- Explain how testing is performed
- Explore concepts of sensitivity, specificity and positive predictive value
- Discuss challenges for donor management
- Discuss blood donor re-entry in US

www.aabb.org 3

3

New Test Implementation and Declining Risk of Viral Infections from Transfusion



AuBuchon, Birkmeyer, Busch.
Ann Intern Med 1997;127:904-9.

4

Blood Donor Screening

- Historical Perspective – 1940's
  - ABO/Rh (blood type), Red Cell antibody Screen
  - Syphilis Serology (Reagin methods)
- 1965-1985:
  - 1965: Australian Antigen (Hep B Surface Antigen)
  - 1969-1972: Immunodiffusion Test
  - 1972: Fed Regs Require HBsAg Testing
  - **Status Quo from 1972-1985**
  - **Then HIV was found in blood!**

5

Historical Perspective: Since 1985

- 1985: Anti-HIV-1
- 1986: ALT, Anti-HBc (Hep-B Core Ab)
- 1987: HIV-1 (conf) Western Blot licensed
- 1989: Anti-HTLV-I
- 1990: Anti-HCV1.0 (Hepatitis C Virus)
- 1992: Anti-HCV 2.0, Anti-HIV-1/HIV-2
- 1993: HCV-(conf) RIBA 2.0 blot licensed

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MAJ1

Historical Perspective: Since 1985

- 1994: FDA discusses molecular testing
- 1996: HIV-1 p24 Antigen, Anti-HCV 3.0
- 1997: HTLV-I/II
- 1999: HCV-RIBA 3.0 licensed
- 1999: HCV-NAT, HIV-NAT clinical trials licensed in 2003, 2004
- 2002: Hep. B-NAT US clinical trials
- 2003: West Nile Virus by NAT
- 2006: Chagas test licensed-
  - (Most US centers only test first time donors)
- 2015 Babesia EIA under IND
- 2016 Zika NAT research ('17 licensed)
- 2018 Babs NAT (regional approach) under IND, licensed 2019

7

Current Blood Donor Tests 2019

*CMV testing is available, but not required

**FDA requires testing on first time donors only

Test	Methodology
ABO/Rh	Agglutination
Red Cell Ab screen	Agglutination
Syphilis	Agglutination
CMV*	Agglutination
HBsAg	EIA
Anti-HBc	EIA
Chagas**	EIA
Anti-HIV-1/2 + group O	EIA
Anti-HTLV-I/II	EIA
Anti-HCV	EIA
HBV,HIV,HCV-NAT	Pooled PCR
WNV-NAT	IND or Pooled PCR
Babesia microti	IND or Pooled PCR
Zika Virus	IND or Pooled PCR

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Slide 7

MAJ1 Mark A. Janzen, 11/13/2019

ABO/Rh Screening

- Commonly used screening devices are Beckman PK 7300 and Immucor Neo/Iris (NEO upgrade)
- Both offer syphilis testing and CMV
- We also perform Rh (C,c,E,e,K) typing in addition to ABO and Rh(D) on selected 1st time donors
  - Full RBC genotyping on some repeat donors
- Antibody screening – we use the Immucor NEO)

9

Blood Center EIA Testing Requirements, per FDA

- Sample tests neg = **Non-Reactive (NR)**
- Sample tests pos = **Initially Reactive (IR)**
- FDA requires all IR samples be retested in duplicate (NR samples are not retested).
- If final results are positive 2/3 or 3/3 times, the sample is **"repeatedly reactive" (RR).**
- **This provides opportunity to r/o false +!**

10

Screening and Confirmatory Tests

- Donor ***screening tests*** are designed to detect infections in as many donors as possible.
  - Because these tests are ***so sensitive***, some donors may have a false positive result, meaning a positive test result despite the fact that the donor was never exposed to the particular infection.
- Confirmatory tests help determine whether a donor is truly infected
  - Repeat reactive screening tests are followed up with more specific tests called ***confirmatory tests***.
- Performing confirmatory tests when a positive or reactive screening test result is obtained is used for donor counseling or investigating discordant test results.
- If you perform a confirmatory test, negative or nonreactive results on a confirmatory test would not override a positive or reactive screening test but may allow a donor to be later re-entered.

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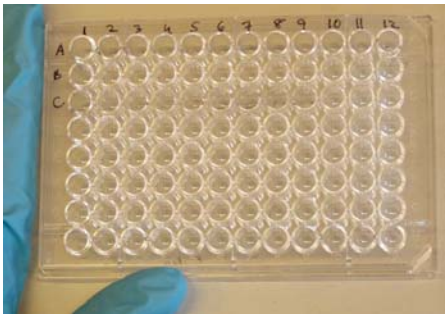
EIA Test Methodology



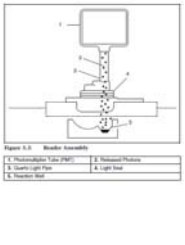
•The Microtiter Plate
Flat plate with multiple "wells" used as small test tubes (e.g. 96 wells)

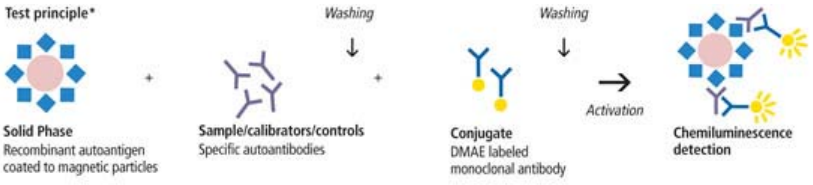
- Standard tool in clinical laboratories
- Commonly used for EIA
- Automation developed to:
  - dispense liquid samples to and from these plates,
  - "plate movers" which transport them between instruments.
- Plate Readers: detect specific biological or chemical reactions in the individual wells.

12

Chemiluminescent Immunoassay (ChLIA) Method

- ChLIA methods result in a release of photons (light)
- Higher sensitivity and specificity than EIA method





*if not otherwise stated on instructions for use

13

INDIRECT EIA

List of Assays:

- Antibody to HTLV-I/II
- Antibody to HCV
- Antibody to HBC core
- Antibody to HIV-1/-2

Antigen Sources:

- Viral lysate
- Recombinant
- Synthetic Peptide

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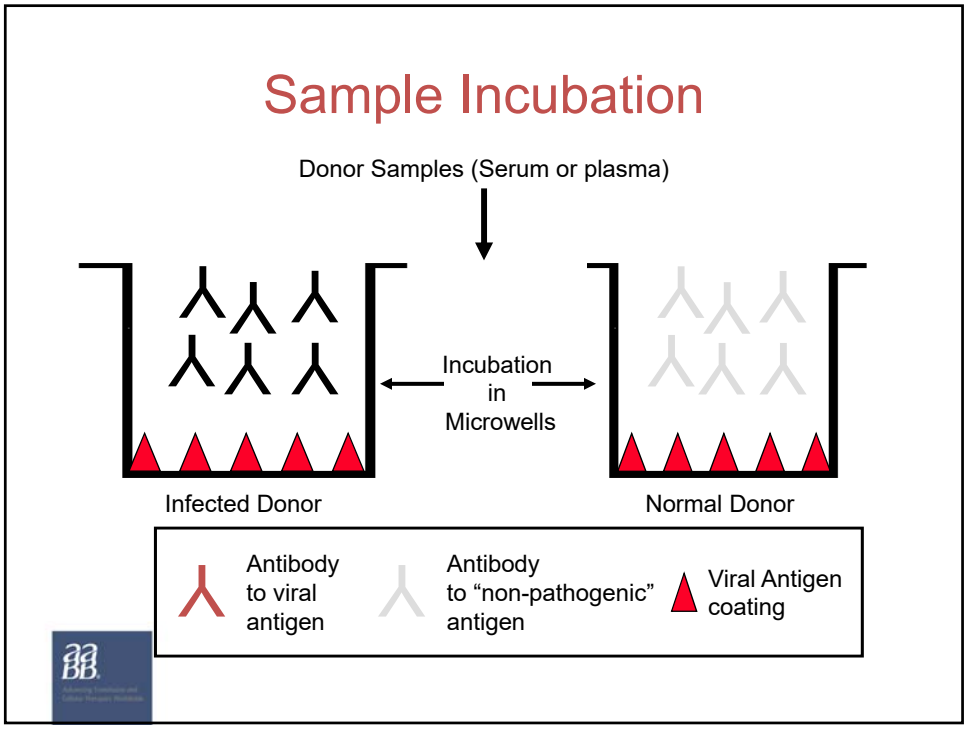

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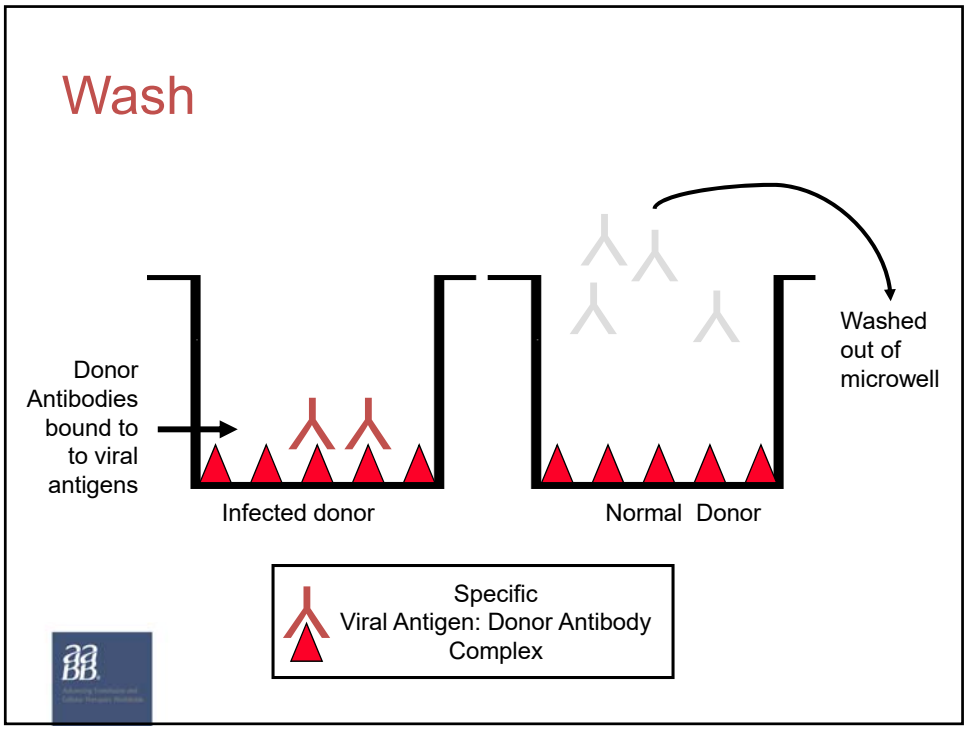

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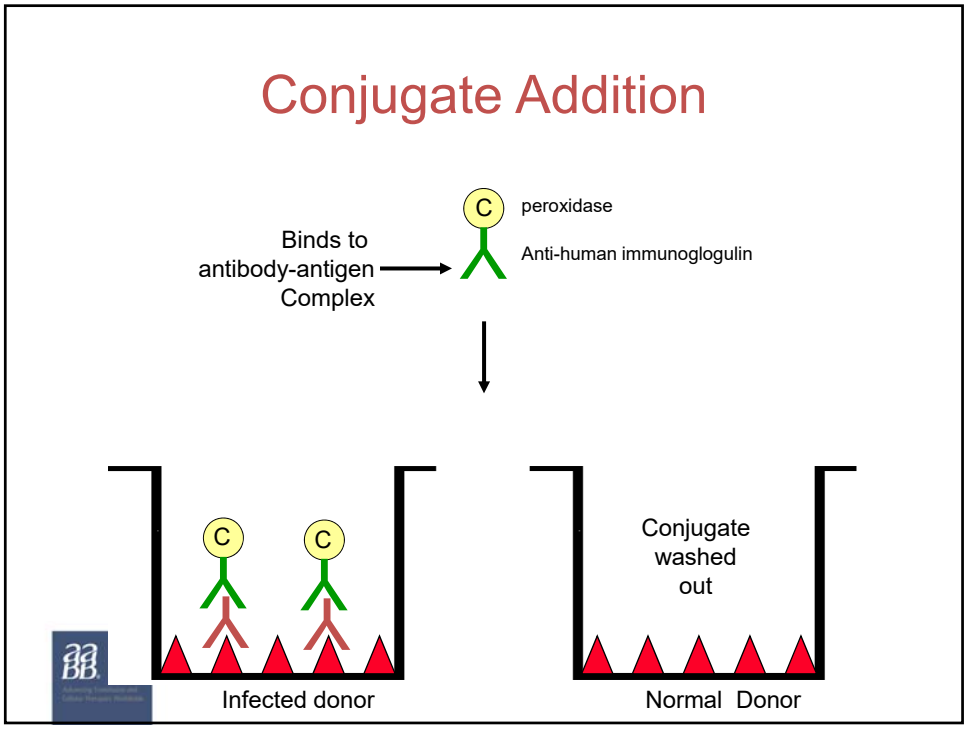

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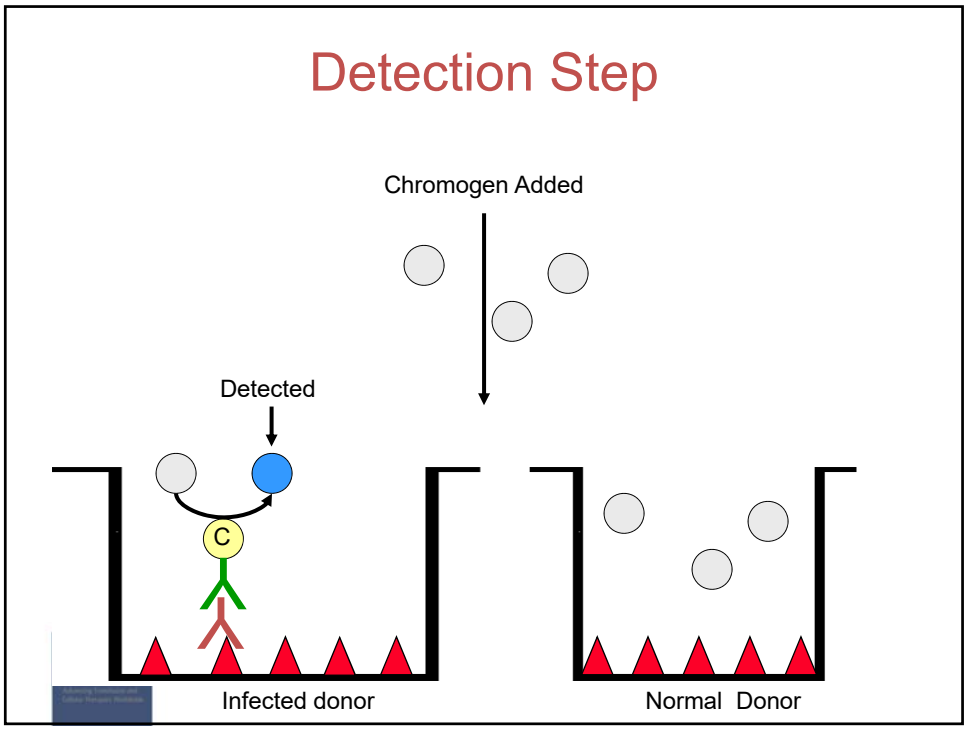

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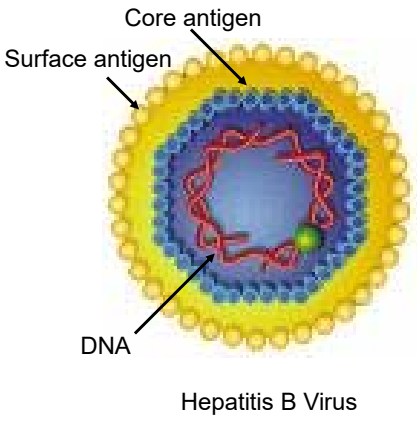
Sandwich EIA

- Detection of the Hepatitis B Surface Antigen



Hepatitis B Virus

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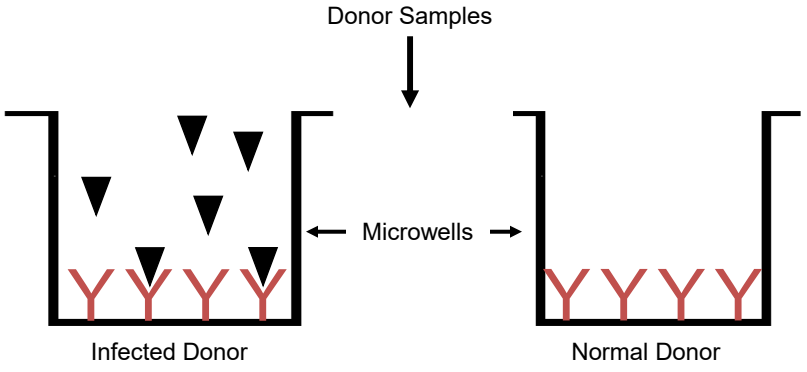
Sample Addition



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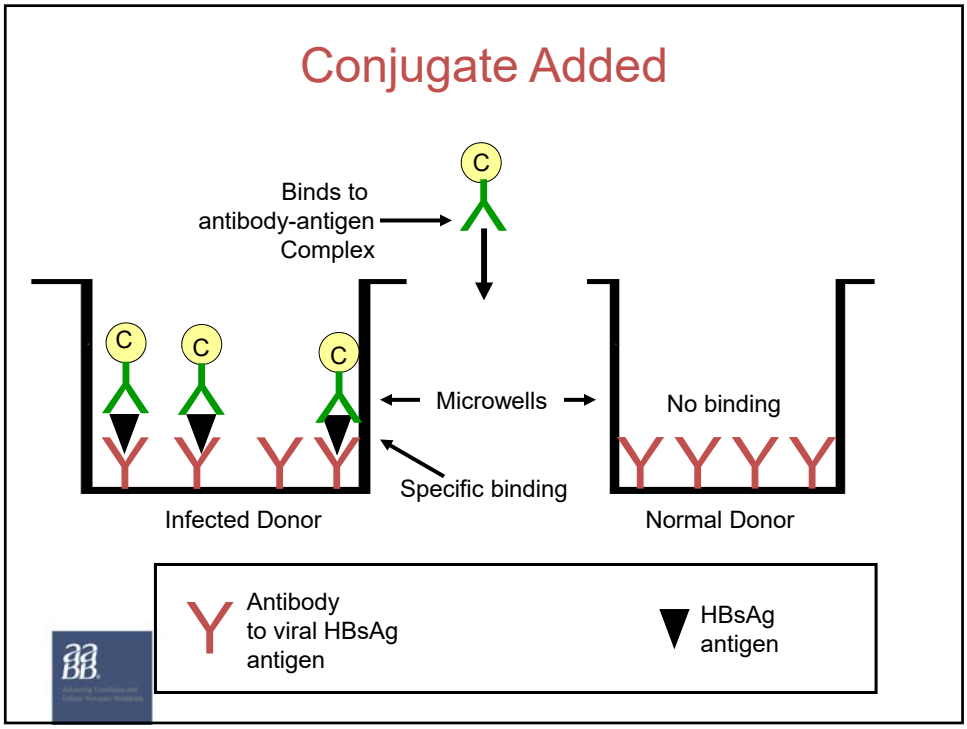

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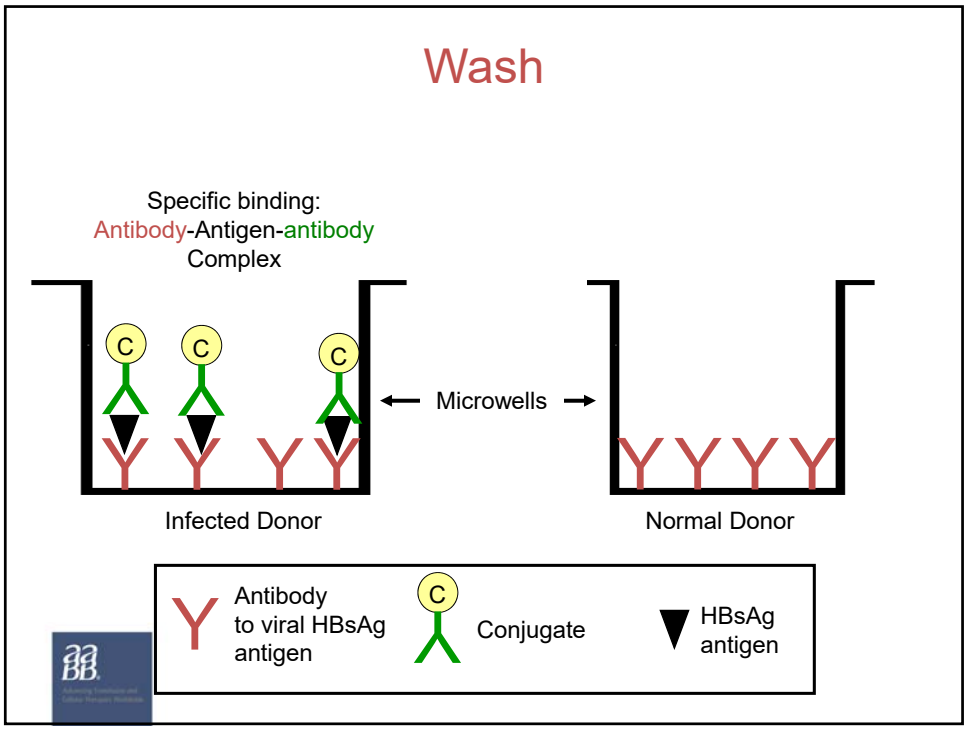

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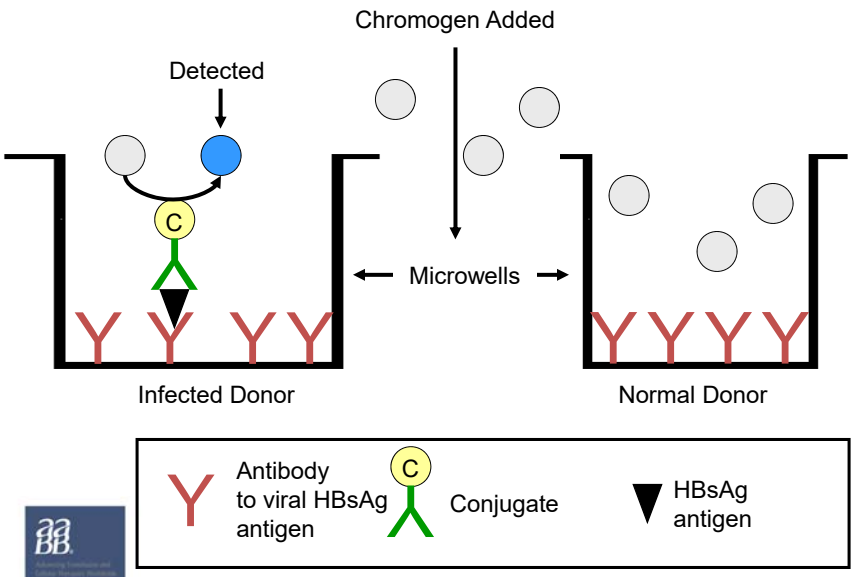
Detection Step



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Syphilis Testing

- Non-Treponemal Tests (RPR, VDRL) have been largely replaced by Treponemal Tests (Ab to T. palidum)
- Treponemal methodologies include: EIA, MHA-TP (Beckman Coulter)
- Confirmatory testing often referred to outside laboratories

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Nucleic Acid Testing (NAT) Lab

Using Molecular Methods

Most NAT testing in the US is performed as a pooled testing, either in pools of 16 or 6
This works well when both the assay is very sensitive and when there is a low background prevalence of the infection tested for in the tested population
Where used in high prevalence areas, individual donor testing is better with respect to sensitivity and not having to retest.

Cobas Hamilton STAR Pipettor

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Pooled Testing & Resolution Of Positive – Roche & Grifols

- Roche: Uses a pool of 6 in US. If the pool tests positive, the 6 individual samples are tested.
- Grifols: If pool of 16 is +, then each individual sample is tested
- Hence, for both platforms the secondary testing allows ruling out false positives

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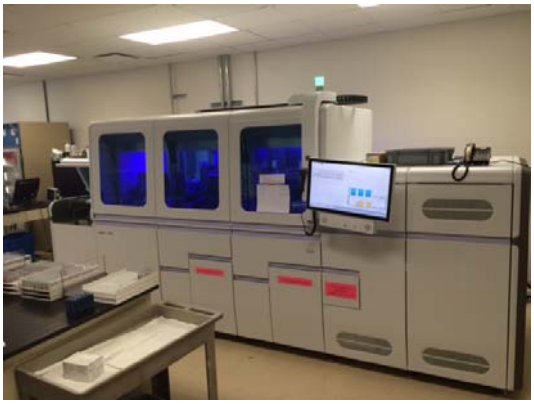
Roche NAT Testing System

Current NAT platform performs nucleic acid extraction and PCR multiplex assays. Used to screen for HIV, Hepatitis B,C, WNV, Zika and regionally for Babesia. Generally screening performed in pools of 6 for blood donors, individually for tissue/cell therapy donors.

Roche 8800



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Grifols Blood Donor Screening Assay: Babesia species

- BARCELONA, Spain, U.S. Food and Drug Administration (FDA) approved the Procleix Babesia assay, a qualitative assay for the detection of the ribosomal RNA from 4 *Babesia* species (*B. microti, B. duncani, B. divergens, B. venatorum*) in individual samples or up to 16 pooled lysed specimens from human donors.

"The assay runs on the Procleix Panther system — a fully automated platform utilizing Nucleic Acid Testing (NAT) for blood screening." Same platform is used for multiplex testing for HIV, Hep B,C and WNV and Zika.
https://www.biospace.com/article/releases/grifols-receives-fda-approval-for-procleix-babesia-assay-for-donor-screening-on-procleix-panther-system/

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Confirmatory Testing

- **Samples testing RR, are generally confirmed with a licensed test:**
  - Neutralization Assay (HBsAg)
  - HIV-1 Western Blot and Licensed EIA (anti-HIV, HIV-2)
  - Alternative Licensed Screen Assay (Abbott PRISM HCV)
  - New guidance on HCV confirmatory requires individual NAT if EIA + and NAT pooled neg
  - 2nd mfr. Licensed EIA (CAPTIA syphilis)
  - Immunoblot (MP Diagnostics HTLV blot 2.4)
  - ESA (Abbott Chagas ESA)
  - Note: No licensed confirmatory assays for anti-HBc, NAT testing!

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Confirmatory Tests

Screening Test	Confirmatory Test
Hepatitis B Surface Antigen (HBsAg)	HBsAg Neutralization assay
Antibody to Hepatitis B core	None
Antibody to HIV	HIV Western Blot +
HIV NAT	None
Antibody to HCV	Other manufacturer's EIA
Antibody to HTLV I/II (manufacturer's kit #1)	"Antibody to HTLV I/II (manufacturer's kit #2)"
West Nile Virus NAT	None
Antibody to Cytomegalovirus (Anti-CMV)	None
Syphilis (RPR, MHA-TP)	FTA-ABS

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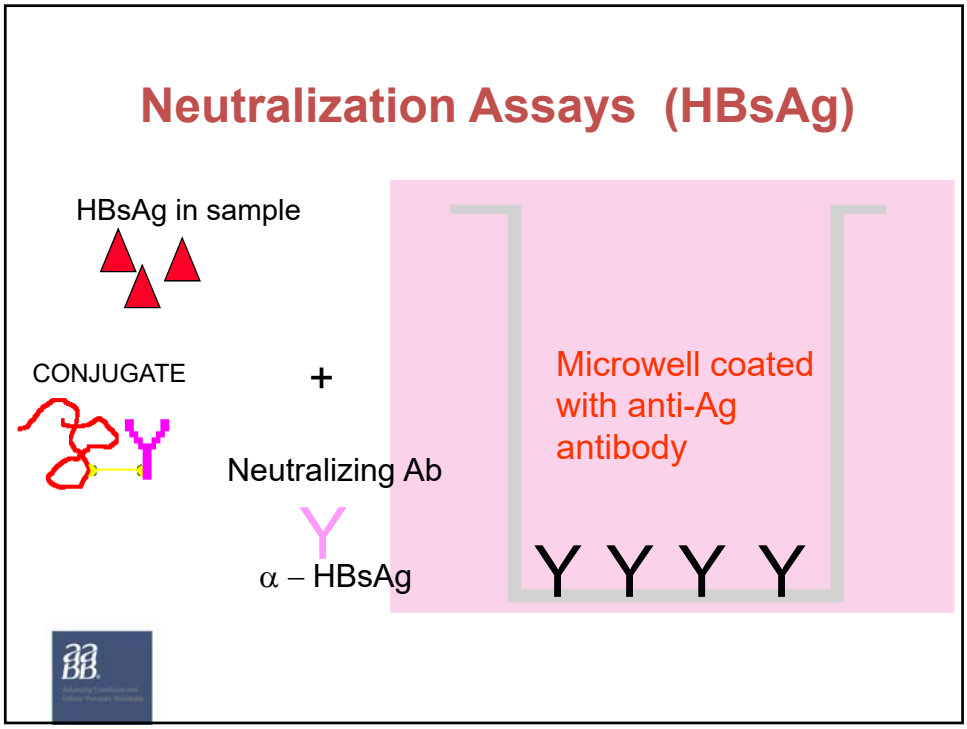

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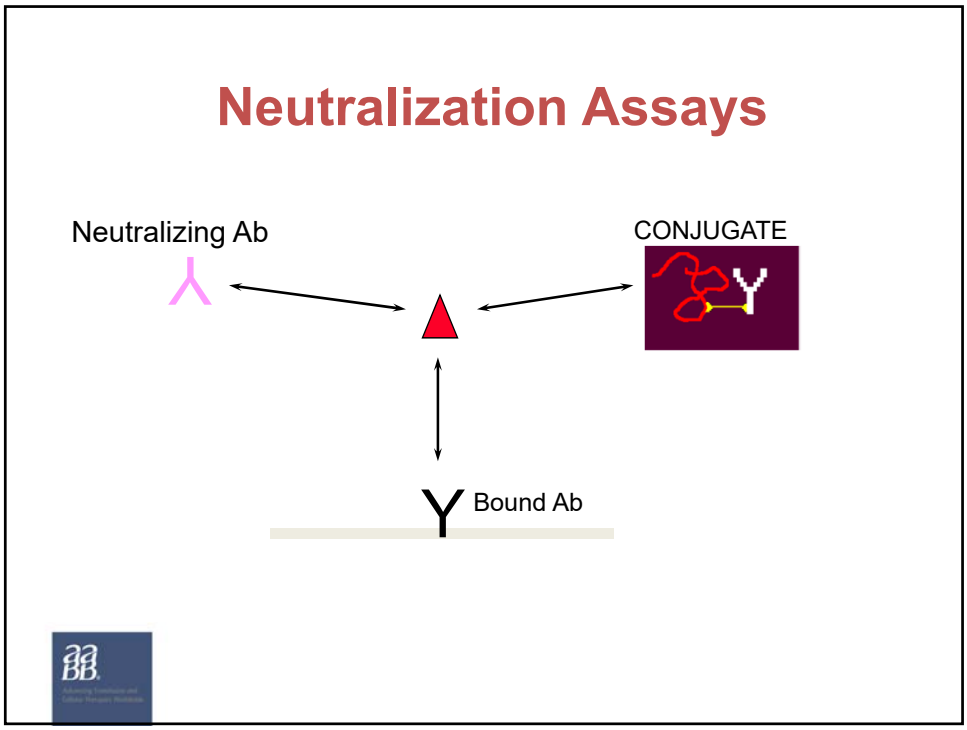

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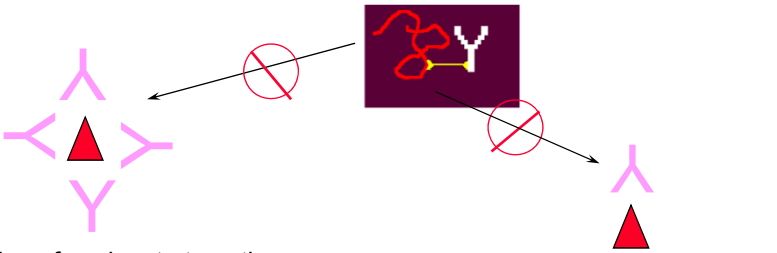
Neutralization Assays



Binding of conjugate to antigen is reduced due to interference by neutralizing antibody.

Y Bound Ab

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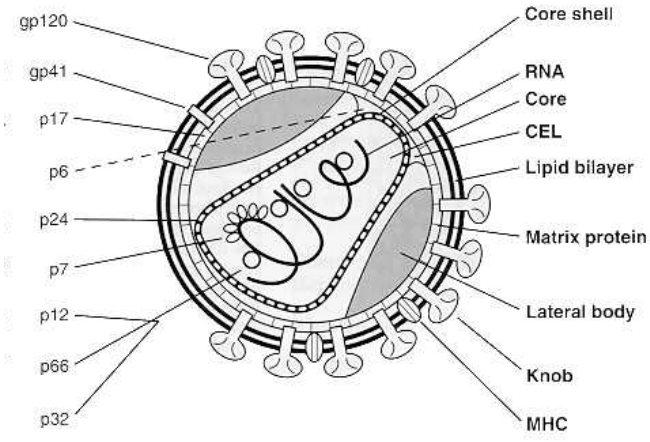
WESTERN BLOT



HIV Virus Structure

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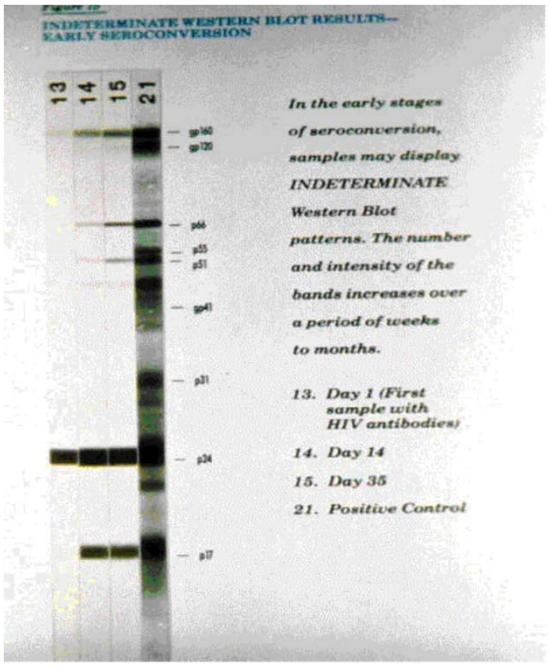

35

Geenius™ HIV 1/2 Supplemental Assay

Intended Use/Indications for Use:

- The Geenius™ HIV 1/2 Supplemental Assay is a single-use immunochromatographic assay for the confirmation and differentiation of individual antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) in serum, or plasma samples from blood donors.
- The Geenius™ HIV 1/2 Supplemental Assay is intended for use as an additional, more specific test for human serum and plasma samples with repeatedly reactive results by an FDA licensed blood donor screening test for antibodies to HIV-1/HIV-2.

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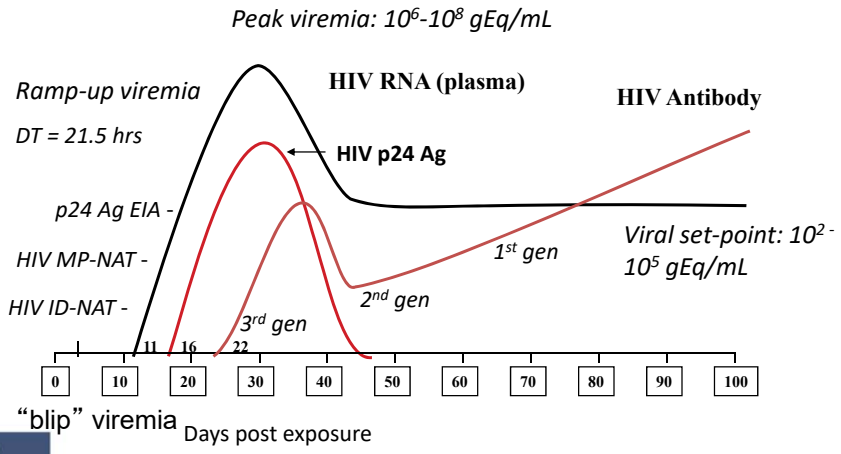
HIV Viremia During Early Infection



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Hepatitis B Virus (HBV)

- 25% chance of Post-Transfusion Hepatitis existed prior to 1965
- Majority due to Hepatitis B Virus
- Few remaining cases due to serologic variants and early infections
- Many US citizens now vaccinated

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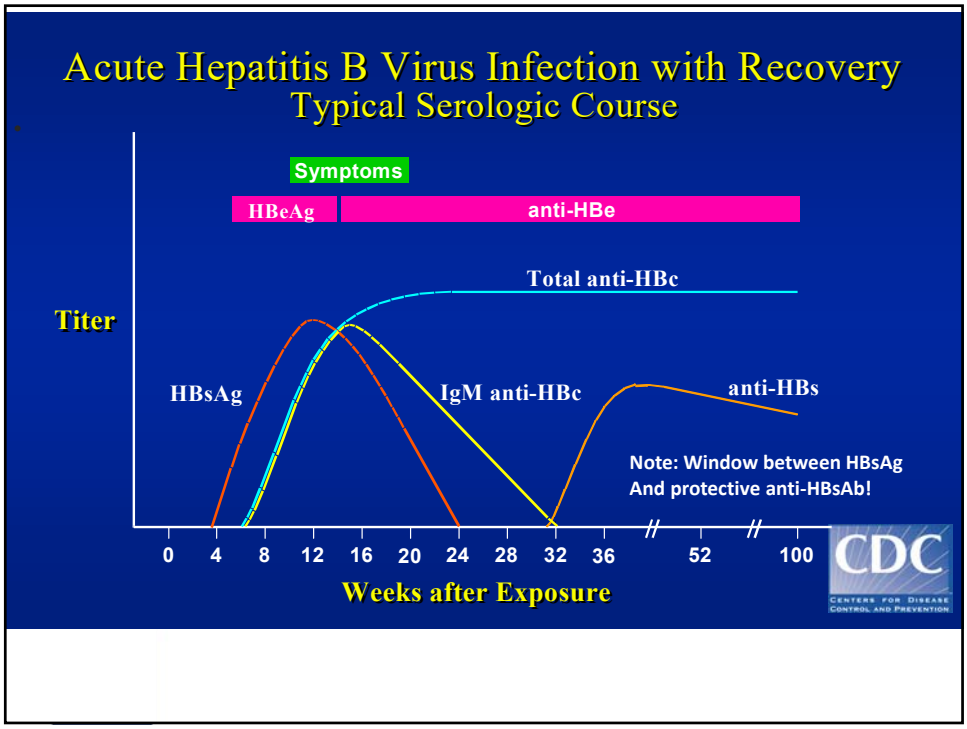

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So why does FDA still require the Hep B core test?: Hepatitis B Virus (HBV) DNA COBAS AmpliScreen HBV Test –

- **STUDY DESIGN AND METHODS:**
- A multicenter study COBAS AmpliScreen HBV test (Roche Mol. Systems) on minipools (MP) of 24 samples
- **RESULTS:**
  - Window-period rate =1 in 352,451 Assay specificity was high (99.9964%)
  - HBV DNA was detected in 84 percent of HBsAg-+, anti-HBc+ donations by MP NAT and in 94 percent using individual donation (ID) NAT
  - HBV DNA was detected in 0.41 % (about 1/250) of Hep B Sag negative but Hep Bc Ab+ donors using ID NAT

Transfusion. 2005 Aug;45(8):1247-57.

40

Hepatitis C Virus (HCV)

- 5-10% risk of post-transfusion hepatitis (PTH) prior to 1990, due primarily to HCV
- HCV was detected and made into a diagnostic test entirely by molecular techniques
- ~ 1.6% of US population exposed and 1.2% carry hepatitis C virus (CDC fact sheet)

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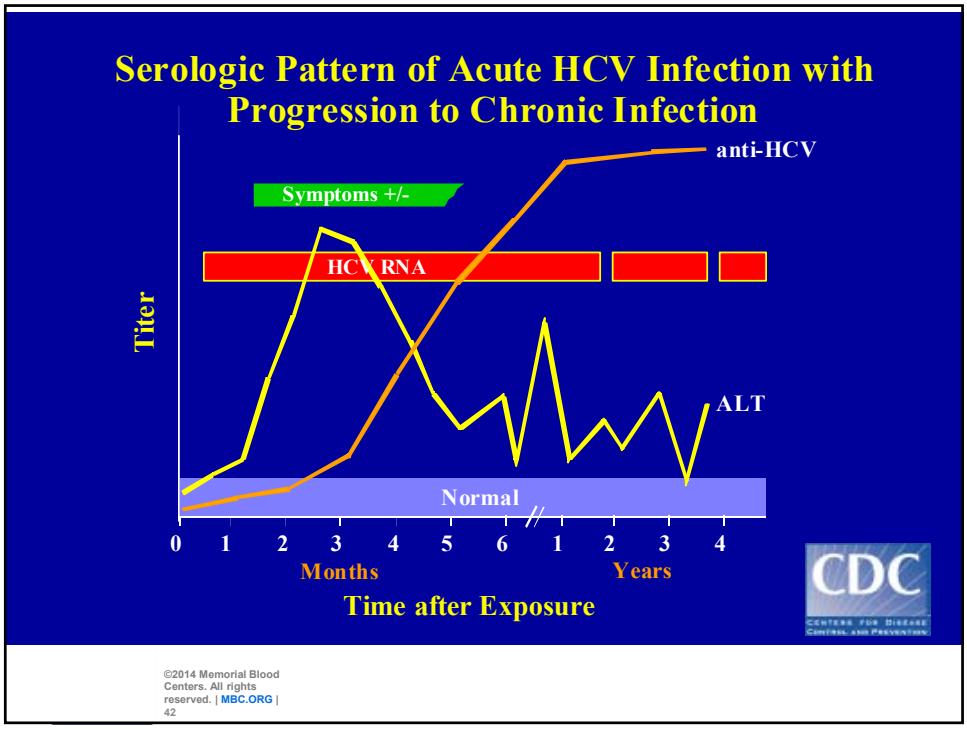

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Human T-Lymphotropic Viruses I & II (HTLV-I/II)

- HTLV-I associated with:
  - ATL-Adult T-cell Leukemia, TSP-Tropical Spastic Paraparesis, HAM-HTLV Associated Myelopathy
- Endemic in Japan, Caribbean, IV drug users
- No licensed confirmatory test to distinguish HTLV-I from HTLV-II – the blot listed below does this
- Confirmation by positive test with MP Diagnositics HTLV blot 2.4

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Zika Screening via NAT

- Zika emerged as a potentially transfusion transmitted disease in major outbreaks during summers of 2015,2016
- Recognition that it was causing disproportionate adverse effects on newborns put significant pressures on FDA to implement transfusion safety measures.
- Implementation of Intercept
- Testing started under IND using individual donor NAT
- Replaced by pooled testing
- Consideration to drop test in light of
- dramatic drop in incidence

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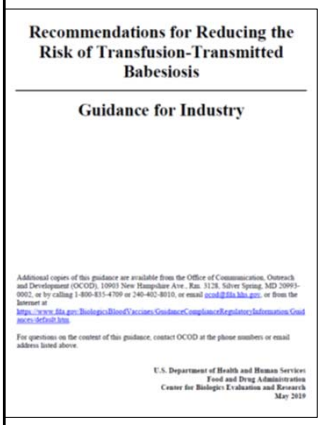
FDA Babesia Guidance (2019)


Recommendations for Reducing the Risk of Transfusion-Transmitted Babesiosis

Guidance for Industry

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
May 2019

Key Recommendations:

- Babesiosis is a relevant transfusion-transmitted infection under 21 CFR 630.3(h)(2)
- All donations must be tested for Babesia year-round or the blood products be pathogen reduced.
  - Note this includes plasma and apheresis platelets!
- Connecticut, **Delaware**, Maine, Maryland, Massachusetts, **Minnesota**, New Hampshire, New Jersey, **New York**, Pennsylvania, **Rhode Island**, Vermont, Virginia, **Wisconsin**, Washington D.C.

https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/blood-guidances

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Babesia Guidance Details

- All donations including WB, RBC, Plasma and Apheresis Platelets
- Donors with + *Babesia* NAT results are deferred for 2 years
- Donors notified and counseled on significance of the results.
- Donors previously deferred for a history of Babesiosis may be eligible for reentry.
- Products from donations testing + Babesia not to be used for transfusion.
- Implemented as soon as feasible but no later than 12 months after the guidance issuance date, i.e. “drop dead date” = May 9, 2020.
- Grifols and Roche NAT Babesia donor screening assays licensed

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Other Testing we do on blood donors

- TRALI mitigation: Anti HLA antibody screen: female donors with one or more prior pregnancies donating single donor platelets or plasma
- Mitigation of hemolysis from incompatible plasma: Using “low” titer anti-A (or A,B)
  - Iso A @ different cutoffs, eg whole blood, group O plts
- Hemoglobin S
  - Interferes with leukoreduction
  - Is contraindicated if being transfused into sickle cell patients or settings with low FIO2 (ECMO, NICU)

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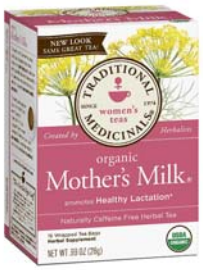
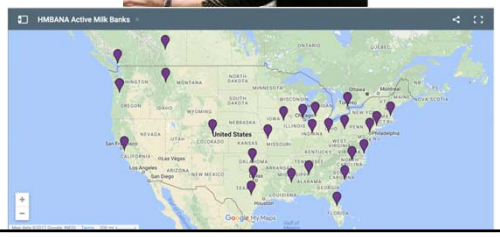
Milk Bank testing!

We perform infectious disease testing on the donors of mother’s milk.
Infants in NICUs benefit by the antibodies and other protective components of human mother’s milk. Iowa and Minnesota, among other states have non-profit programs to share mother’s milk.





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Bacterial Screening of Platelets

- FDA requirement to screen platelets for ~1/2000 risk of bacterial contamination
- Currently screen after 24 hours of incubation by culture
- Point of care screening
- Final guidance 2019 requires additional testing or treatment if transfused day 4 or after

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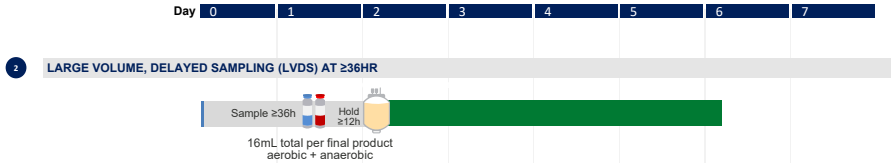
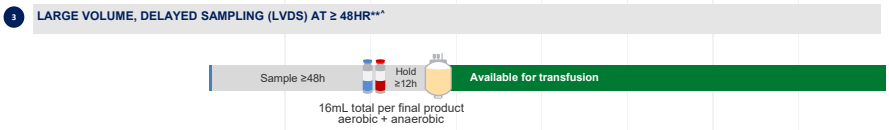
Large Volume Delayed Sampling One Step Strategies



- All Apheresis and pre-storage pools
- Can extend to 7 days with additional bacterial sampling or rapid testing
  - Rapid testing not available for platelets in PAS



- For apheresis platelets that are approved for 7-day storage
  - Not yet available, waiting for safety claim

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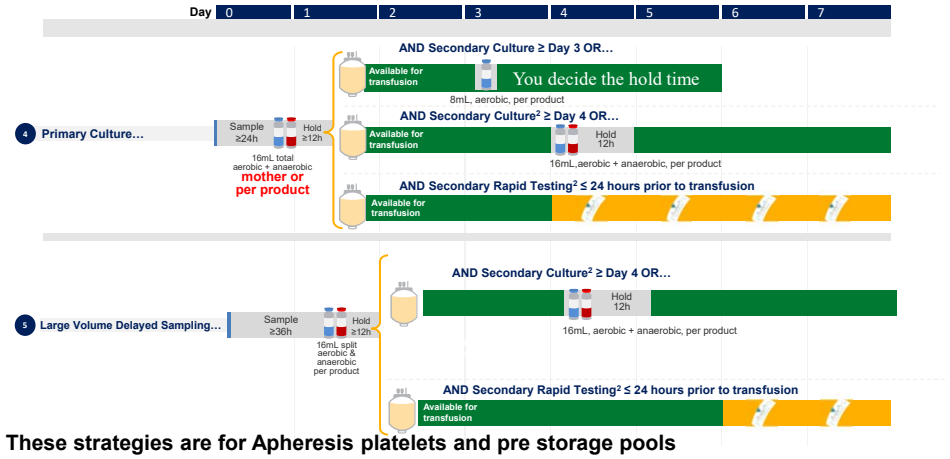
Two Step Strategies



These strategies are for Apheresis platelets and pre storage pools

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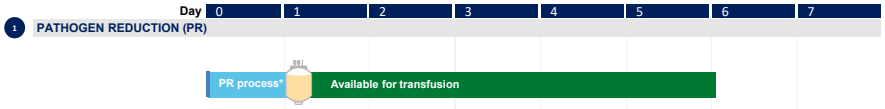
Pathogen Reduction



- Available for Trima apheresis platelets in plasma
- Available for Amicus apheresis platelets in platelet additive solution (PAS)
- Licensed for singles and doubles only
  - May split some doubles and triples to meet acceptable guard bands
- 7-day product is not available at this time

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Donor Deferral Algorithms

- Generic FDA Algorithm:
  - Test once: If +, repeat in duplicate:
    - If one or both repeats are positive = repeat reactive, (RR) then discard unit, temporarily defer donor
  - Perform confirmatory testing, if positive, permanently defer donor
  - If negative, donor eligible for re-entry at some later date

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Donor Deferral: HIV

- If RR, discard unit, contact and temporarily defer donor
- Perform Western Blot or other confirmatory test
  - Challenges include:
    - Limited test kit choices
    - Most results indeterminate, with non-specific bands
    - Note: Western blot indeterminate are eligible for re-entry

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HIV Deferral

- If WB Positive Counsel
- If WB indeterminate defer indefinitely (15% of individuals tested at random will test HIV WB indeterminate!)
- If WB negative, eligible for re-entry after 6 months: BUT many US blood centers do not re-enter deferred blood donors since there is regulatory risk if all steps not carefully documented

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HCV Deferral

- If RR, alternate screening assay and ID NAT performed (If NAT + permanently defer)
- If neither alternate screen or NAT are positive, may re-enter donor after 6 months. Many centers have donor return, provide a sample and if sample is negative, then re-enter.

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Our center's re-entry history

- Historically many centers were reluctant to perform re-entry as FDA was very critical of centers performing this task.
- In 2010 FDA eased pathway for re-entry
- In 2011 our center sent hundreds of letters to potentially re-enterable past donors.

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Donor re-entry US FDA requirements

- If a donor has a repeat reactive test, the unit is discarded and the donor deferred, typically indefinitely
- Re-entry is typically allowed after 6 months if all prior confirmatory testing was negative
- Prior to re-entry additional testing, both the original screening test and a licensed confirmatory test must be negative.

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Specific re-entry algorithms

- HIV: FDA allows re-entry as early as 8 weeks after initial RR, our center waits 6 months
  - Requires testing with HIV EIA, ID NAT
  - Re-entry only available for HIV-1, not HIV-2 RR
- HCV: Re-entry after 6 months
  - Requires testing with HCV EIA and ID NAT
- HBV: Re-entry after 6 months
  - Requires testing with Hep B Surface antigen, Hepatitis B core and Hepatitis B ID NAT

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HIV re-entry at our center: 2016

- Re-entry may be performed after 8 weeks, but we wait 6 months to give more chance for clearance of any antibody causing a false positive EIA test.
- Re-entry require –EIA HIV1/2+O and NAT-ID
  - 15 donors tested positive
  - 16 returned for testing (one from previous yr)
  - 2 (13%) donors remained ineligible
  - 14 (88%) donors re-entered

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HCV Re-entry by our center: 2016

- Re-entry may be performed as soon as 6 months after prior presumed false positive test. Testing includes Anti-HCV EIA +individual donor NAT –
  - 46 tested positive for anti-HCV
  - 13 (28%) returned for retesting
  - 4 (9%) remained positive on screening test
  - 9 (19%) successfully re-entered

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Sensitivity vs. Specificity

- FDA and US Blood Industry want most “Sensitive” tests available
- Sensitivity usually comes at the cost of Specificity
- Majority of marker positive donors are false positive reactions
- True positive reactions are typically first-time or autologous donors

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Airport Security, Screening and Confirmatory Testing

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Sensitivity vs. Specificity

- **Sensitivity:** ability of the test to correctly identify those who have a specific disease
- **Specificity:** ability of the test to correctly identify those who do not have the disease
- **Positive predictive value:** the likelihood that a positive test result represents a true positive

The balancing act

sensitivity specificity

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Sensitivity, Specificity and Positive Predictive Value

- Sensitivity = A/A+C
- Specificity = D/ B+D
- Positive Predictive Value = A/A+B
- Note PPV is highly affected by disease prevalence.

	PERSON HAS DISEASE	PERSON DOES NOT HAVE DISEASE
Test Result +	A True Positives	B False Positives
Test Result -	C False Negative	D True Negative

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Positive Disease Marker Rates: 10/2018-09/19

(Screening Results-~140,000 blood donors)

	Init. Reactive Total	% Initially Reactive	Rpt. Reac. Total	% Rpt. Reactive
HBsAg	45	0.03	37	0.03
HBcAb	601	0.43	462	0.33
HIV-1/2	33	0.02	24	0.02
HCV-Ab	295	0.21	172	0.12
HTLV-I/II	13	0.01	9	0.01
Syphilis			78	0.06
Total Donations			797	0.57

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Confirmed + Result Rates

	Total	% of Total
HBsAg-Neut. Pos	11	0.008
HIV-WB/NAT-Pos	0	0.000
HCV-PRISM-POS	17	0.012
HTLV-Abbott Pos	0	0.000
HBc high S/CO	224	0.159

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RR Disease Marker Rates

- Memorial Blood Center has some of the lowest positive marker rates in the US, with some variance due to late fall seasonal peak possibly due to cross reactivity following influenza vaccine in HBc, HCV assays.

Test	MBC	National Average (ARC)
HBc	0.33*	0.59%
HBsAg	0.03	0.06
HIV-1/2	0.02	0.15
HCV	0.12*	0.12
T. Cruzi	0	0.004

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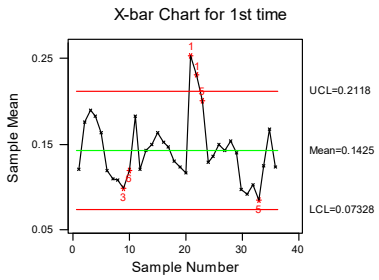
First Time Donation Rates

Seasonal Effects & 9/11

- More first time donors in Fall and Spring: blood drives at HS & colleges.
- Lowest in summer we depend on fixed site blood donations.
- 9/11 = big spike from ~14 to 25%!
- *Data = 2000-2002*



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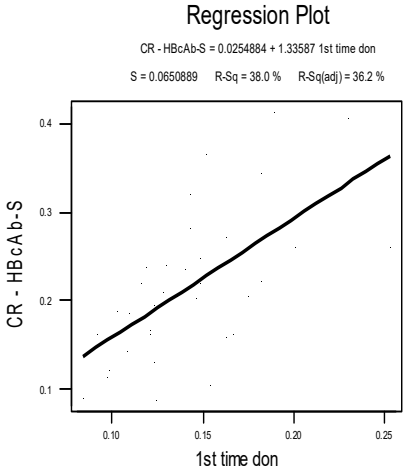
Do first time donation rates matter?

- First time donors reflect the prevalence of the disease rates of the population
- Hence, more first time donors means more deferrals for hepatitis B core antibody.



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Infectious Disease Testing

Summary

- Repeat donors are lowest risk because donors who previously tested positive are not allowed to continue to donate- this requires a computer tracking system and shared data base
- Having confirmatory testing allows donor counseling for false positives
- Redundant methodologies (NAT) have most value in setting of higher background prevalence of tested analyte

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Questions?

Contact

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