

Donor Infectious Disease Testing (methods, interpretation, re-entry)

C. Michael Knudson, MD, PhD

Dept of Pathology

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Infectious Disease Testing Required Tests

- ◆ **Hep B**
 - Hep B Surface Antigen**
 - Hep B Core Antibody**
- ◆ **HIV**
 - HIV 1/2 antibody**
 - HIV p19 antigen**
- ◆ **HTLV 1/2 antibody**
- ◆ **Hep C antibody**
- ◆ **Syphilis**
 - Serological test.**

Optional Testing

- ◆ **NAT-GAT amplification testing**
 - Hep C**
 - HIV**

Hepatitis B

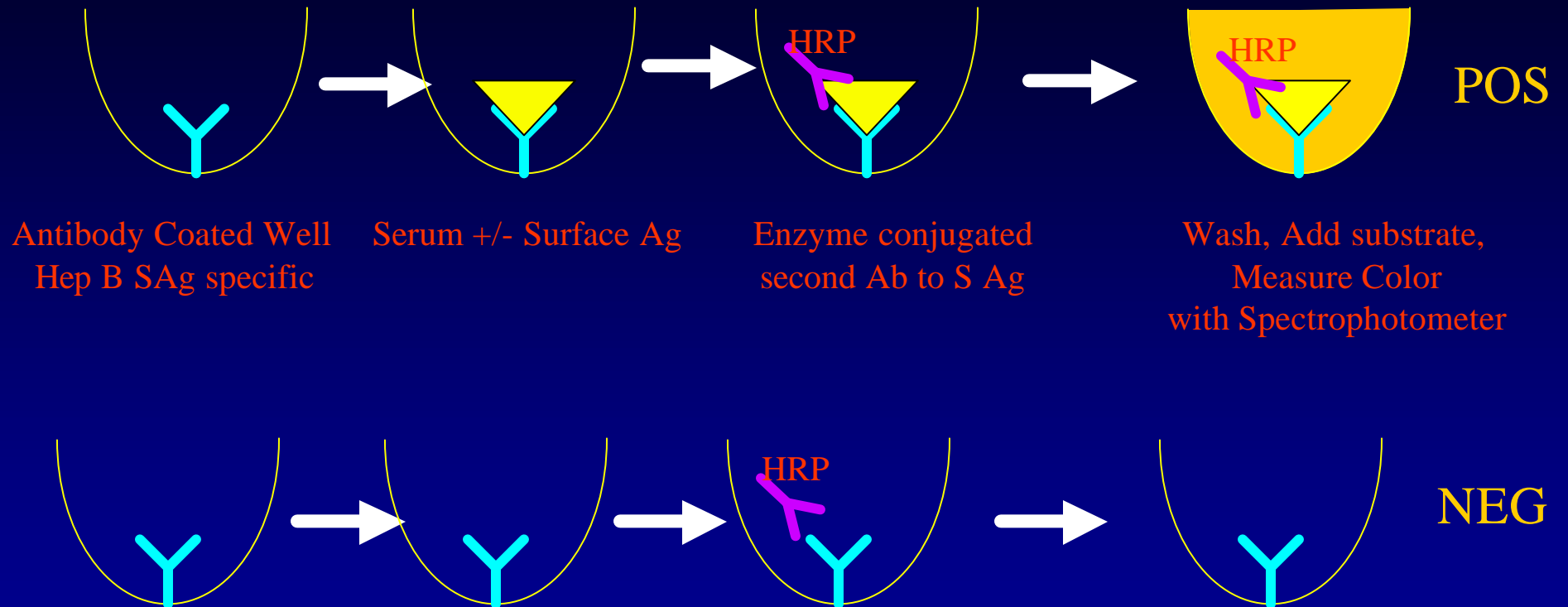
- ◆ Serious infection that is clearly caused by blood transfusion and transmitted by other parental routes and sexually.
- ◆ 50% infections symptomatic/0.2% lethal.
- ◆ 6-10% result in chronic infections.
- ◆ Vaccine available and has decreased the incidence of this disease.

Hepatitis B Testing Surface Antigen

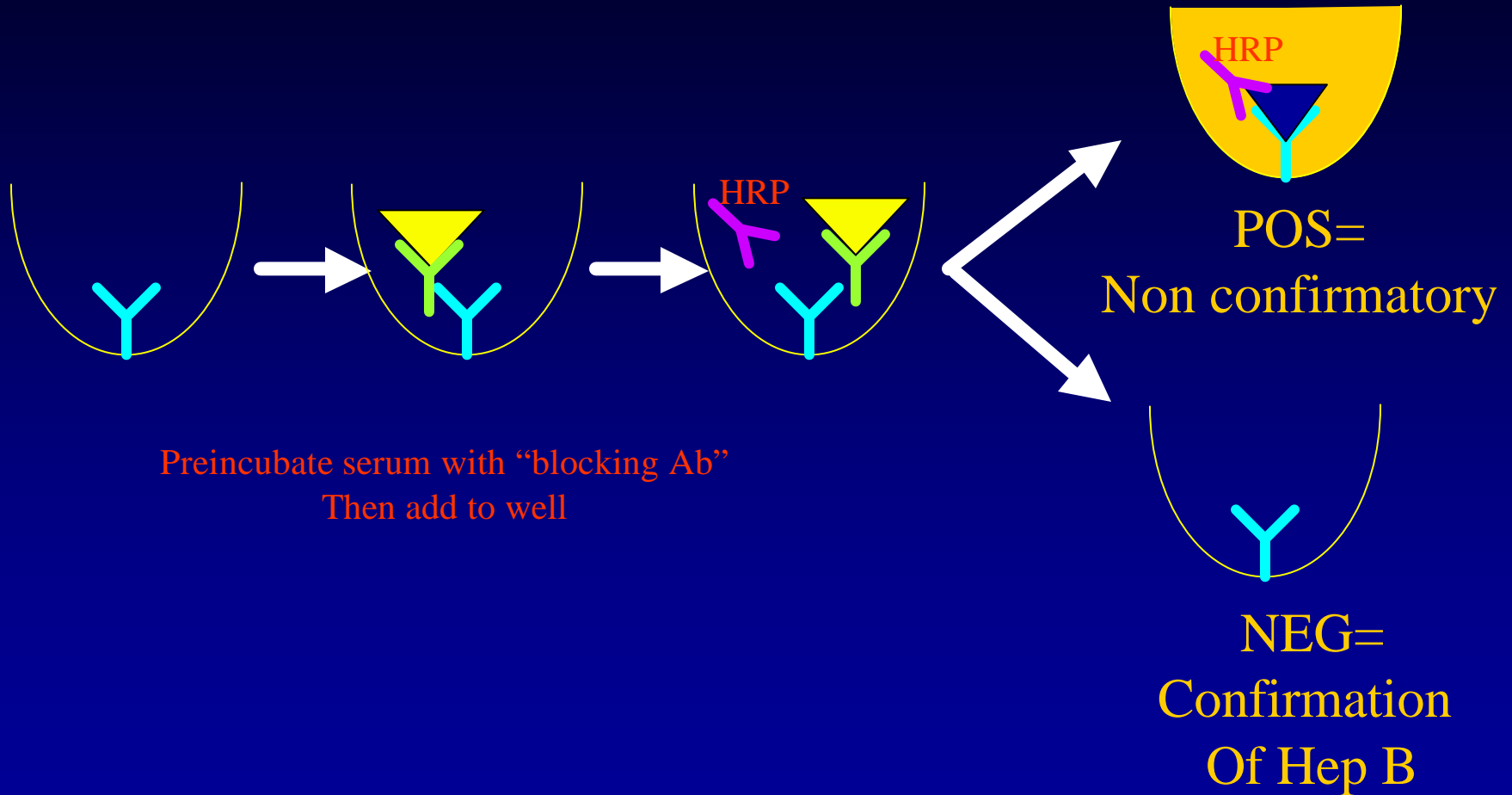
- ◆ We use an Abbot Immunoassay which is a sandwich EIA using two different antibodies that recognize Hep B Surface Antigen.
- ◆ Positives are repeated in Duplicate and if one is positive a neutralization step is performed.
- ◆ If HepB sAg can be neutralized, this indicates a true positive and indicates the donor has an active infection and the donors are strongly encouraged to see a physician. Deferral is permanent.
- ◆ If HepB sAg is not neutralized the original test is presumed to be falsely positive and the donor is temporarily deferred for at least 8 weeks.
- ◆ Reentry possible if test becomes negative at or after this time.

EIA-Enzyme Immunoassay

The basics



Hep SAg Neutralization Test



Hepatitis B Core Ab testing

- ◆ The test detects people in the window period after they become sAg negative and before they become sAg antibody positive.
- ◆ This test was implemented in the 80's as a surrogate test for HIV and then for Hep C and HTLV.
- ◆ Hepatitis sAg testing prevents transmission of Hepatitis B and better tests exist for HIV/HepC and HTLV.
- ◆ The positive predicted value is low due to the poor specificity of this test.
- ◆ One 1993 study (Transfusion V33 Pg 212) found that 0/158 Core Ab Pos -sAg negative blood donors was PCR positive for DNA.
- ◆ Similar study in Greece also found that 282 Core positive samples were DNA negative and the Recipients were all negative between 6-9 months.
Transfusion V41, Pg 652, 2001
- ◆ Therefore the utility of the Hep B Core Ab testing has been questioned and debate continues about dropping this test.

Hepatitis B Core Ab testing

- ◆ This is a competitive EIA used to detect antibodies to the hepatitis B Core Antigen.
- ◆ Positives are repeated in duplicate and if both are negative the sample is considered negative and donor and product are ok.
- ◆ If 1 of 2 repeats are positive the product is discarded and the donor is temporally deferred. After at least one week the testing can be repeated along with HBsAG antibody testing.
- ◆ If Core antibody is again Positive they are permanently deferred.
- ◆ If repeat Core antibody Neg and HBsAG antibody positive due to immunization they can reenter.

HIV Testing

- ◆ Serious infection that is clearly caused by blood transfusion and transmitted by other parental routes and sexually.
- ◆ Major efforts to decrease transmission include:
 - ▶ Screening out High risk individuals~1983
 - ▶ HIV 1/2 Antibody testing-1985. Residual Risk 1:2-500,000
 - ▶ p24 Antigen Testing 1996. Residual Risk 1:0.5-1 million
 - ▶ NAT testing 1999 (optional/IND). Residual Risk??
- ◆ With each additional level of testing the “returns” on these interventions have diminished.

Donor Questions:

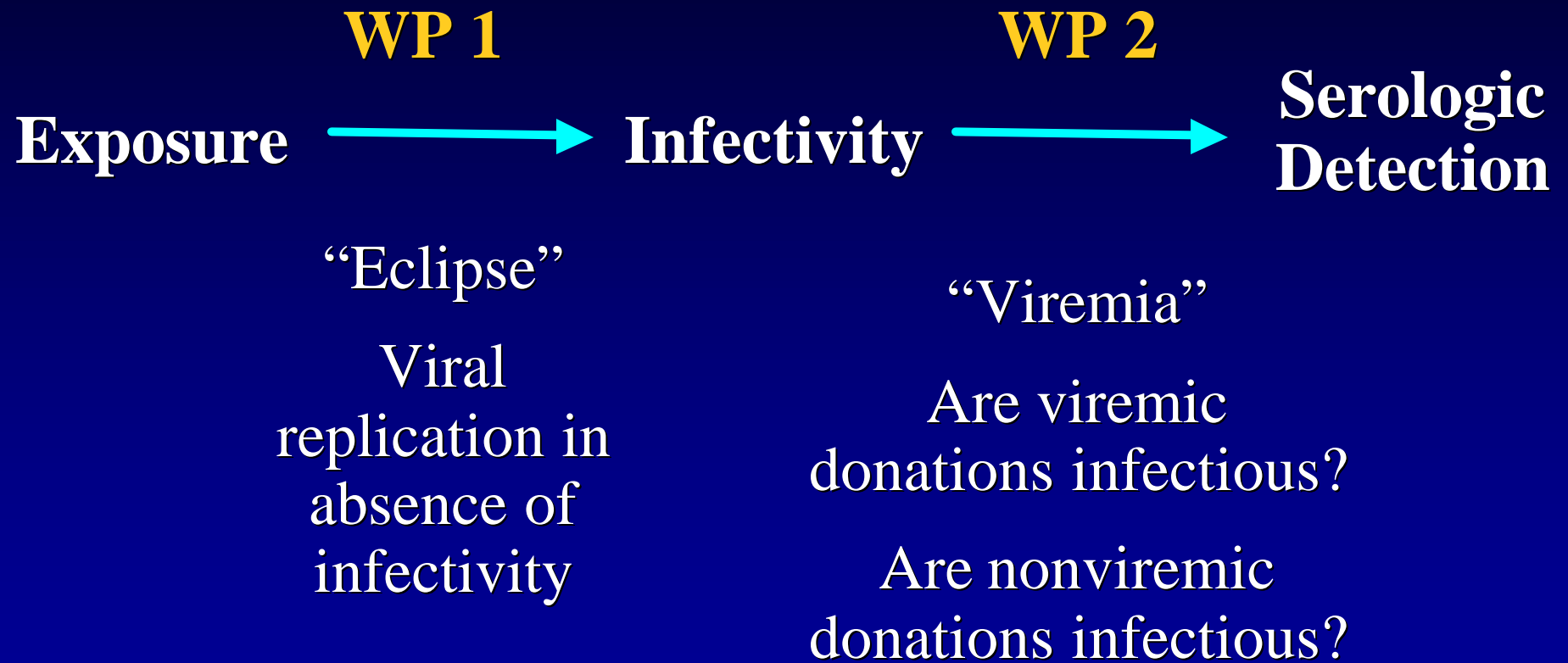
Donors are asked the following questions relative to HIV

- ▶ Are you feeling well today.
- ▶ Had sex with another male since 1977. (Females sex with male who had sex with male).
- ▶ Travel to Africa since 1977.
- ▶ Swollen lymph glands for > 1 month.
- ▶ Ever used needles to take illegal drugs
- ▶ Giving blood for purpose of being tested
- ▶ In the past 12 months:
 - Have you snorted cocaine in the past 12 months
 - Tattoo's/needle sticks/skin piercing
 - Given money or drugs for sex
 - Had sex with someone who has used a needle for drugs
 - Sex with hemophilia pt who has taken Factor concentrates
 - Treated or tested for Syphilis or gonorrhea
 - Received blood transfusions
 - Been in jail or prison

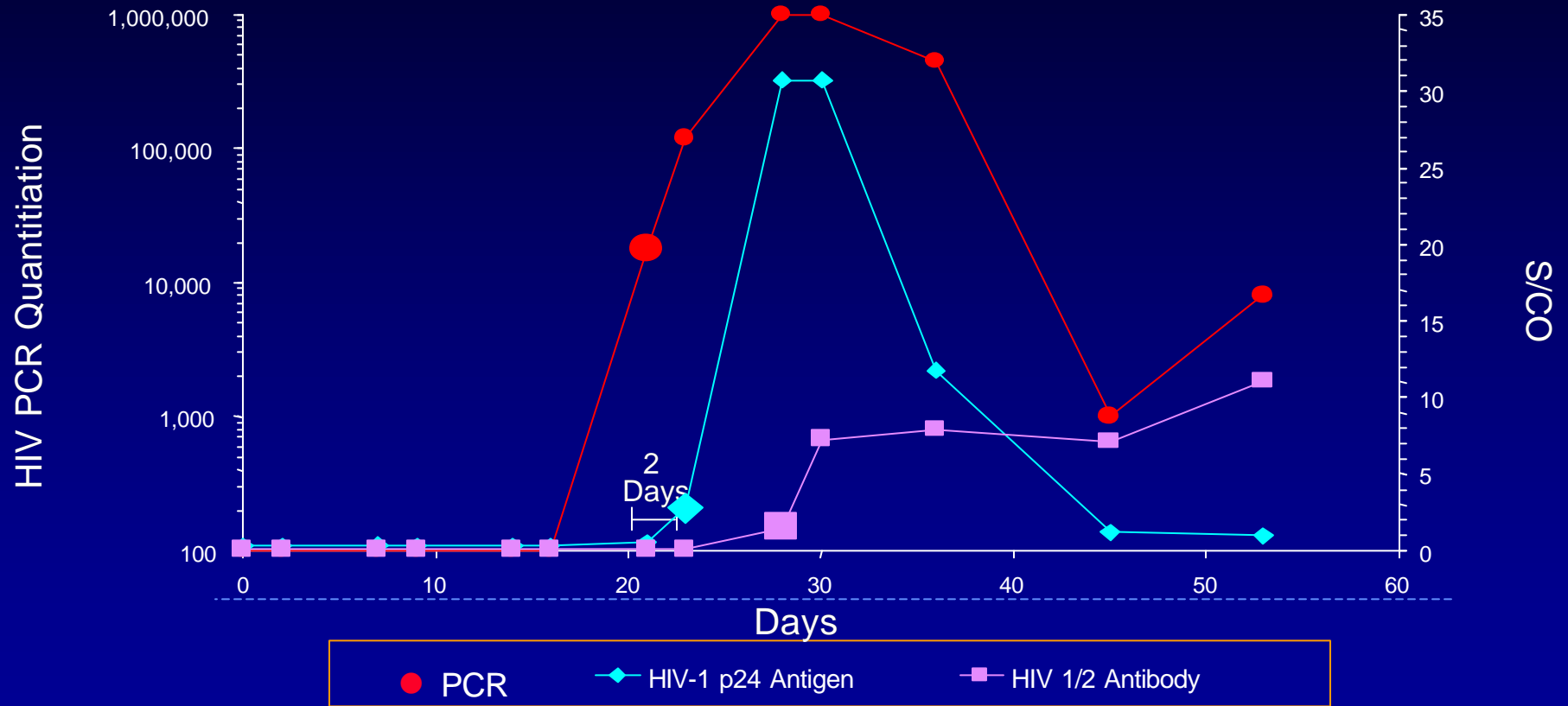
HIV 1/II Immunoassay

- ◆ All donors have been screened for antibodies to HIV since 1985.
- ◆ This reduced the risk of HIV transmission to ~1:100,000. subsequent improvements in the test have lowered this to an estimated 1:500,000.
- ◆ The residual risk it thought to be due to the window period between infection and antibody production. This averages ~22 days for the current HIV I/II EIA's.
- ◆ Physicians in France have gone to jail because they did not implement this test as rapidly as possible. This has created some fear that we need to do everything possible at any cost to assure the safety of blood transfusion.
- ◆ Assay itself is an EIA that detects antibodies to both HIV I or II.

Definition of Window Periods (WP)



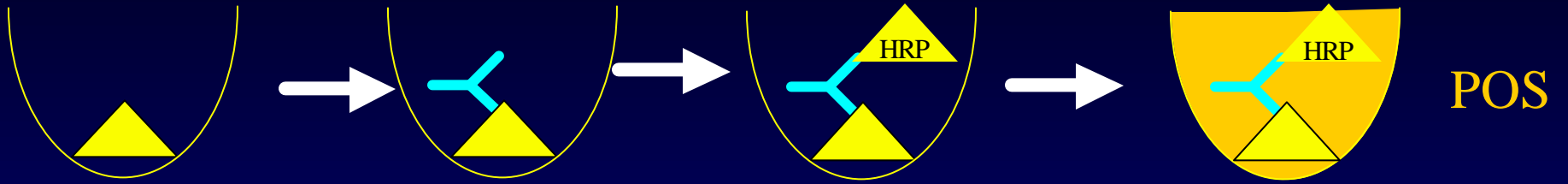
Example of HIV Window Period



HIV 1/II Immunoassay

- ◆ We use an Abbot Immunoassay which is a sandwich EIA that detects antibodies to HIV 1 (env and gag) or HIV II (env) proteins.
- ◆ Positives are repeated in duplicate and if one repeat is positive the test is considered positive and the sample discarded (2 of 3).
- ◆ The sample is then sent for confirmatory testing by Western Blot. If positive or indeterminate the donor is permanently deferred.
- ◆ If negative the donor is deferred for 6 months. After this they can reenter if the both the HIV I/II testing and the Western Blot are negative.

HIV EIA

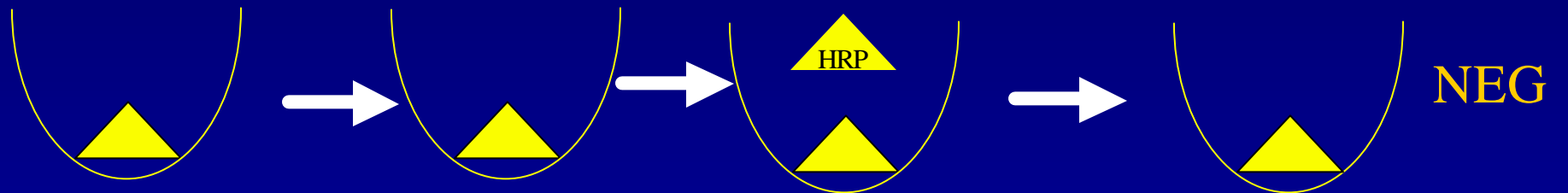


Antigen Coated Well
or Beads

Serum +/- Antibodies

Enzyme conjugated
antigens

Wash, Add substrate,
Measure Color
with Spectrophotometer

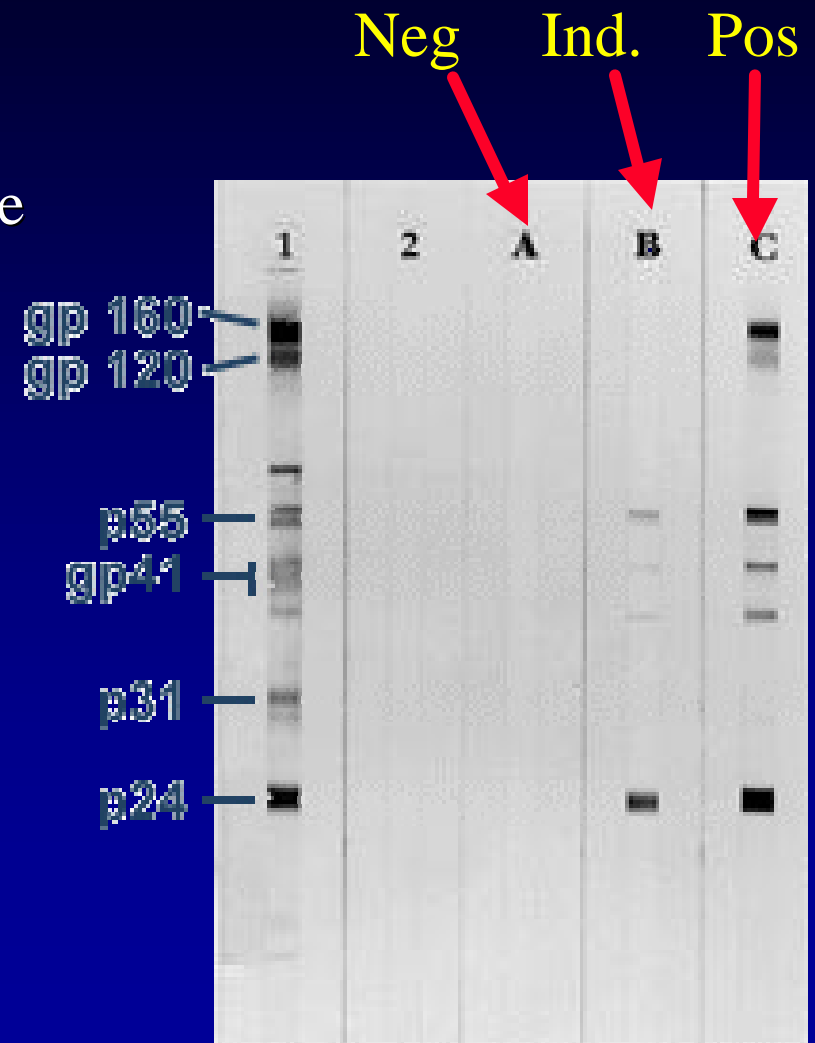


NEG

Western Blot for HIV

Confirmatory testing

- ◆ Protocol: HIV antigens are separated by size on an acrylamide gel.
- ◆ The proteins are transferred to membrane and then incubated with pt serum.
- ◆ Positive reactions are detected with enzyme conjugated secondary antibodies.
- ◆ Specific guidelines are followed for interpretations of the bands with pts defined as Neg, Indeterminate or Pos depending on the number of bands detected.

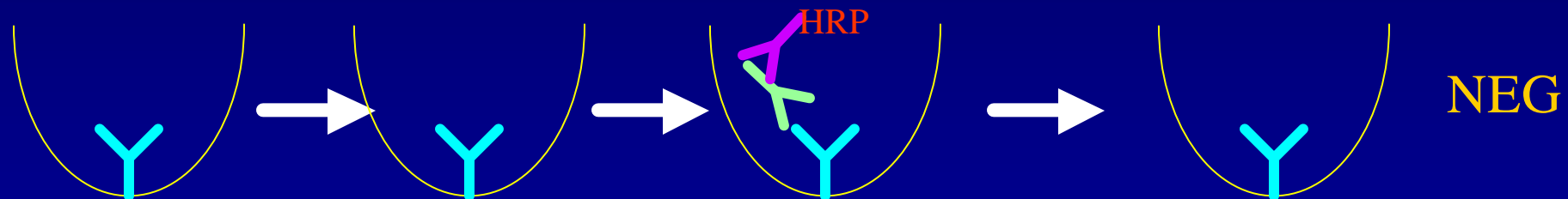
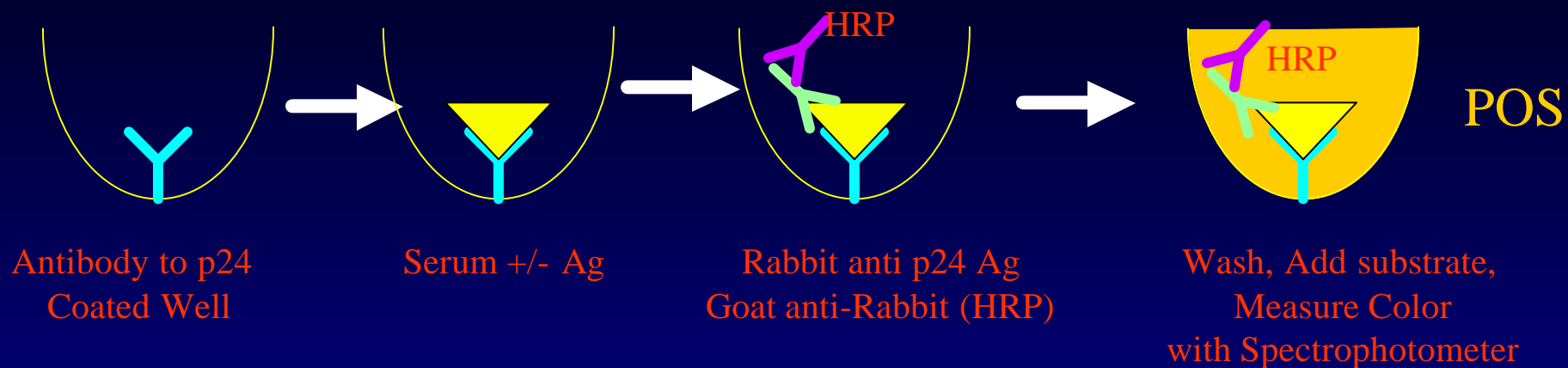


HIV 1 p24 Antigen

- ◆ All donors have been screened for antibodies to HIV-1 p24 Antigen since 1996.
- ◆ Although controversial this is estimated to have reduced the risk of HIV transmission to ~1:1,000,000.
- ◆ The test has lowered the Window period to ~ 15-16 days from 22 days for antibody testing.

p24 Immunoassay

From Abbot



p24 Antigen Testing

- ◆ Positives are repeated in duplicate and if both second tests are negative the test is considered negative and the donor and unit are acceptable.
- ◆ If one repeat is positive the test is considered positive and the product(s) discarded and the sample sent for confirmatory neutralization testing.
- ◆ If confirmatory testing is positive the donor is permanently deferred.
- ◆ If confirmatory testing is indeterminate the donor is deferred for 8 weeks. After this they can reenter if all testing is negative.

Hepatitis C

- ◆ Serious infection (8-10,000 deaths/yr in US) that is clearly caused by blood transfusion and transmitted by other parental routes and sexually (Rarely).
- ◆ Most common chronic blood born infection in US with ~150,000 new cases/year.
- ◆ Cause of Non-A Non-B Hepatitis discovered in 1989. Testing started in 1990.
- ◆ Serious infection and most common cause of liver transplants due to the chronic nature (~75%) of the disease.
- ◆ The availability of treatments (although not uniformly effective) has resulted in a “look back” to identify infected patients.

Hepatitis C Antibody Testing

- ◆ We use an Abbot Immunoassay which is an EIA that detects antibodies to Hep C.
- ◆ Positives are repeated in duplicate and if both second tests are negative the test is considered negative and the donor and unit are acceptable.
- ◆ If just one is positive the sample is considered positive and the product is discarded and the sample sent for confirmatory testing (RIBA=Recombinant Immunoblot Assay).
- ◆ If confirmatory test is positive (or indeterminate) the donor is permanently deferred.
- ◆ If confirmatory test is negative the donor is eligible for reentry after 6 months if both screening and confirmatory testing are negative at that time.

Syphilis Testing

- ◆ Complication of Syphilis are well know.
- ◆ Is rarely transmitted by blood transfusion. 2-3 reported cases in 27 years. Most commonly transmitted sexually.
- ◆ Serological testing may not prevent disease as seroconversion occurs after spirochetemia.
- ◆ Nonetheless testing is required by AABB and has been performed for ~50 years.
- ◆ Purpose of test twofold: Potentially decrease Syphilis and as surrogate marker for other diseases.
- ◆ Like Hep B core is a pore surrogate marker and dropping this test has been considered.

Syphilis Testing

- ◆ UIHC- the RPR (Rapid Plasma Reagin) test is performed. This detects anti-cardiolipin antibodies or Reagin.
- ◆ Positives are repeated in duplicate and if both second tests are negative the test is considered negative and the donor and unit are acceptable.
- ◆ If just one is positive the sample is considered positive and the sample is sent for RPR titer and FTA testing. Donor is temporarily deferred and blood components quarantined.
- ◆ If FTA non reactive the product is used and the donor can reenter.
- ◆ If FTA is positive the product is discarded and the donor is deferred for at least 12 months. May reenter if appropriate treatment and RPR is negative.

HIV and HCV NAT Testing

- ◆ Currently the ARC and others are using NAT for HIV and Hep C.
- ◆ This may reduce the risk of HIV transmission below the currently estimated 1:1,000,000. These studies have identified patients who were NAT positive, p24 Ag Neg and HIV I/II Ab Neg. Follow up has confirmed these patients were in the window period.
- ◆ If animal studies are correct the residual risk may be near zero as monkeys were not infectious prior to the detection of HIV nucleic acid.
- ◆ I have discussed this testing previously so will not go into more details.
- ◆ Likely to be required at some point. May replace the p24 antigen testing as these are thought to be redundant.
- ◆ Currently at UIHC we are performing NAT testing through the Univ. of Minnesota. If positive, donors are permanently deferred regardless of serological testing. Products are not released until NAT results except with physician approval.

HTLV I/II

- ◆ Rare infection that can be caused by blood transfusion and transmitted by other parental routes and sexually.
- ◆ Generally the infection is mild or asymptomatic but in a minority of patients is linked to more severe sequela.
- ◆ HTLV-I causes adult T-cell leukemia/lymphoma (ATL) (~.1%/year), neurological disorders (HAM-HTLV associated myelopathy and TSP=Tropical spastic paraparesis), uveitis and Arthropathy (total penetrance is <5%).
- ◆ Association of HTLV-II and neurological disorders has been suggested but the evidence is not as clear.

HTLV 1/II Immunoassay

- ◆ We use an Abbot Immunoassay which is a sandwich EIA that detects antibodies to HTLV I/II.
- ◆ Positives are repeated in duplicate and if one repeat is positive the test is considered positive and the blood donation is discarded (2 of 3).
- ◆ The sample is then sent for repeat testing using another FDA approved screening test. If positive the donor is permanently deferred. (These are the tough donors to council.)
- ◆ ARC data suggest 65% of donors will be negative on 2nd test.
- ◆ If negative, the unit is still discarded and the donor is not deferred. However if there is ever a repeat positive sample the donor is then indefinitely deferred at that time.

Donor Counseling

With the new reentry criteria for HTLV, we have stopped performing the confirmatory testing for HTLV (RIBA/western). How to counsel the donors who are indefinitely deferred?

Donor Counseling

You are using an ELISA for HTLV that is 99% specific and 99% Sensitive. You screen blood donors from Iowa who have given blood (ie Risk factors eliminated) and have an estimated prevalence of HTLV of 1:1000. The initial screening HTLV Elisa is positive. What are the chances that this donor has been infected with HTLV??

99%

~50%

~9%

~1% ?????

False Negative Testing

Remember 2 x 2 table

		Disease			
		YES	NO		
Test	yes	A	B	$=A/(A+B)$	Positive Predictive Value
	NO	C	D	$=D/(C+D)$	Negative Predictive Value
		$=A/(A+C)$	$=D/(D+B)$		
		Sensitivity	Specificity		
Prevalance = $(A+C)/(A+B+C+D)$					

False Negative Testing

Remember 2 x 2 table

		Disease	
		YES	NO
Test	YES	99	999
	NO	1	98901
		99%	99%

9.02% Positive Predictive Value

99.999% Negative Predictive Value

Sensitivity Specificity

Prevalance 0.10%

Prevalance = $(A+C)/(A+B+C+D)$

samples= 100000

Donor Counseling

- ◆ It is difficult to counsel these donors as their true status is not clear.
- ◆ Nonetheless they need to know they should no longer donate blood.
- ◆ They should be strongly encouraged to consult a specialist or their primary care physician for additional evaluation and testing.