

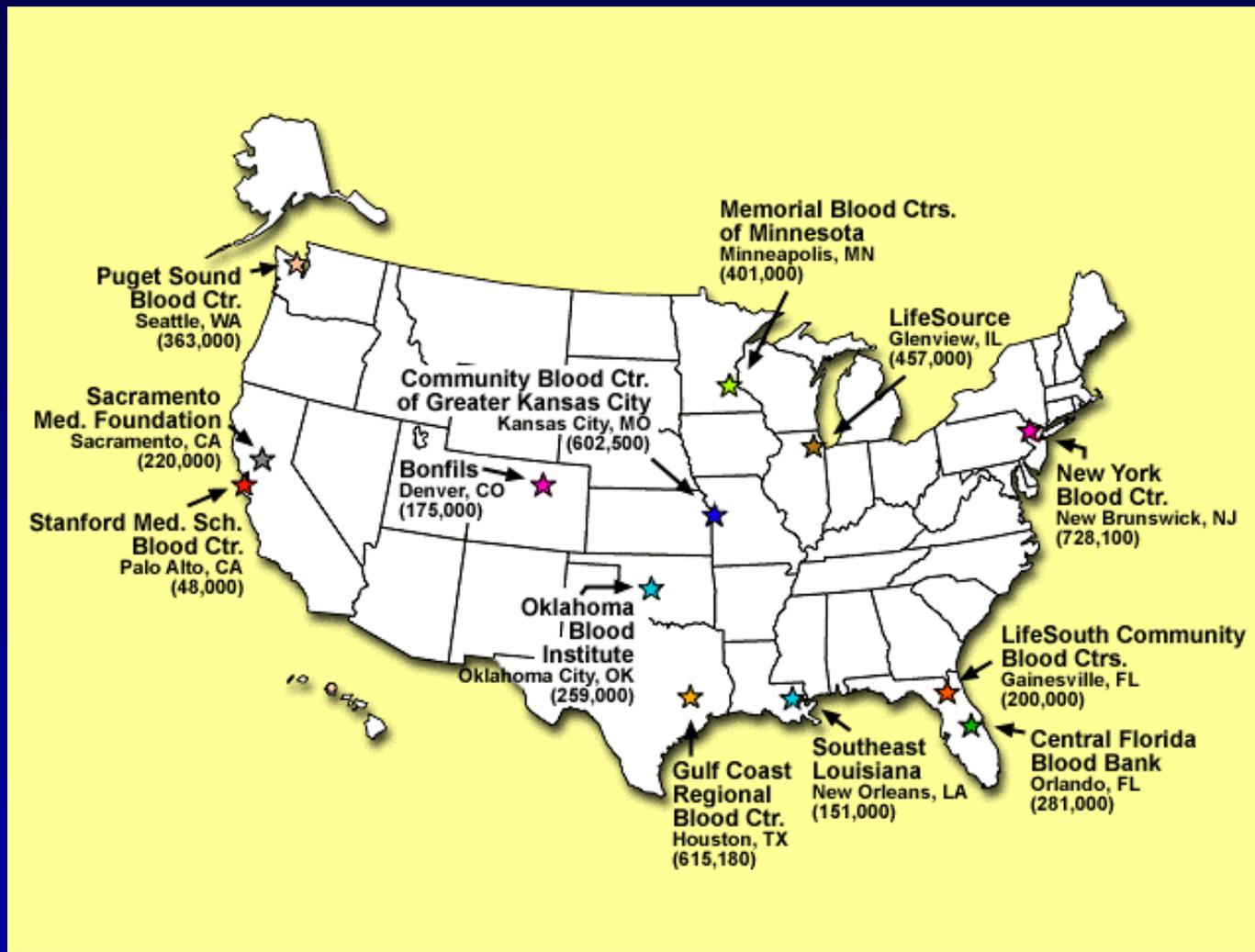
Roche COBAS AmpliScreen<sup>TM</sup>

HCV Test v2.0

HIV Test v1.5

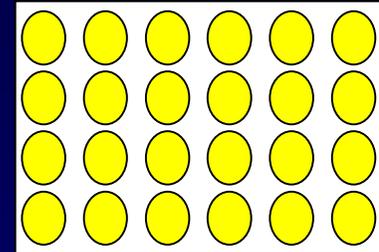
Clinical Trials of Minipool Testing in 13  
U.S. Blood Centers

# COBAS AmpliScreen Clinical Study Sites

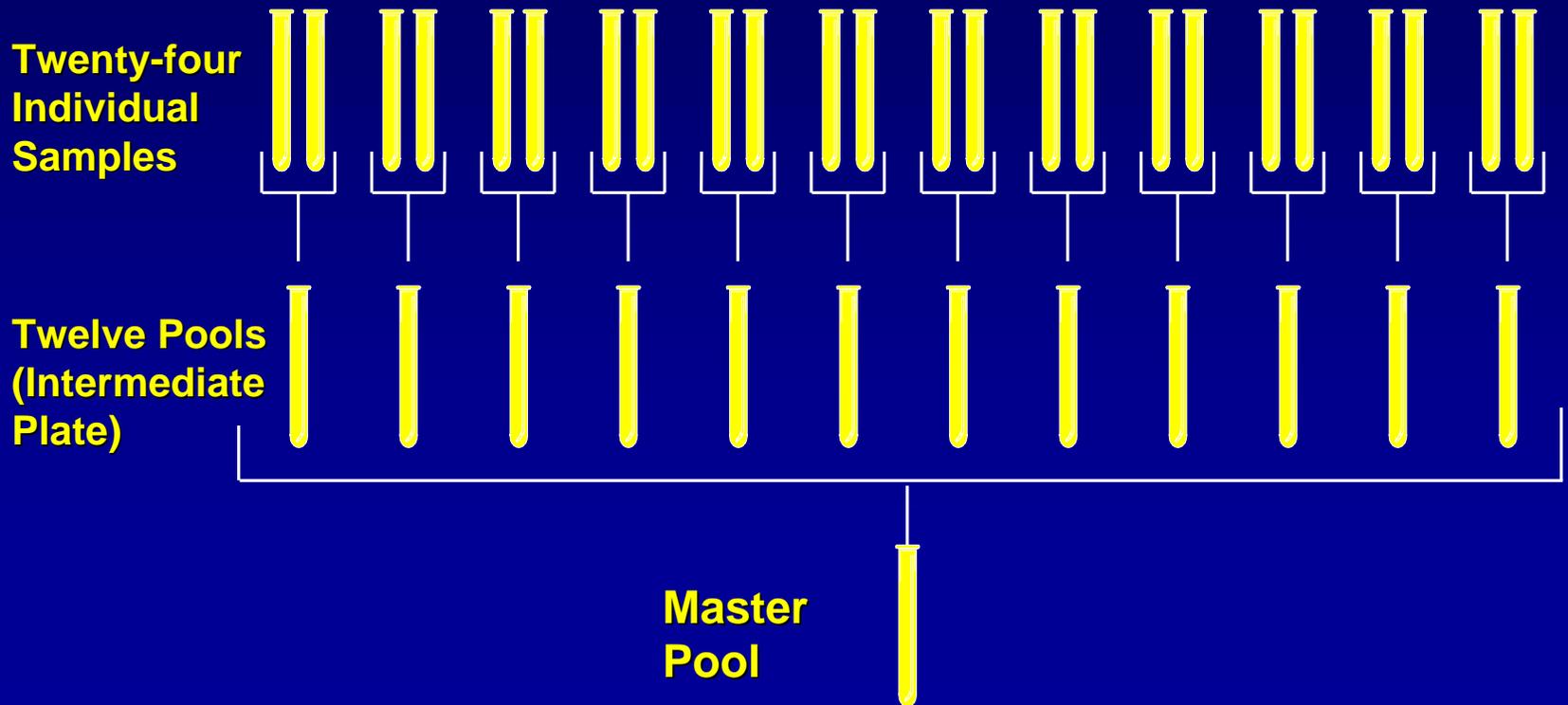


# Roche Pooling Scheme

Twenty-four individual samples  
create pools and archive plate

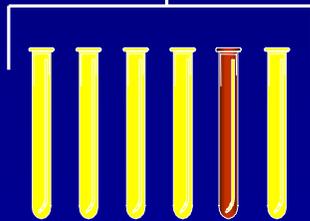
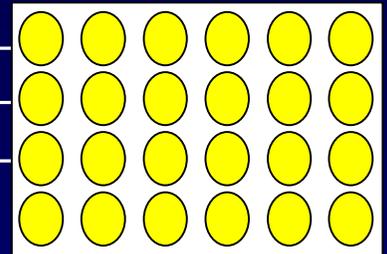


Archive Plate



# Roche Resolution Testing

**NAT +  
Master  
Pool  
(HIV or  
HCV)  
Secondary  
Pools  
Tested for  
HIV or HCV**



**Sample  
identified  
as NAT  
positive**

# 6 Month Dataset

## Roche COBAS Ampliscreen™ HCV Test v2.0

- 1.7 Million donations screened
- 1.67 Million (98%) are Primary Pool (MP) negative
- 30,000 (1.8%) are Primary Pool (MP) positive
- 7,100 (0.42%) are Secondary Pool (subpool) positive
- 1,297 (0.08%) are Tertiary Pool (Individual Donation) positive

Note: Data presented are preliminary results from the HCV study and represent the samples from all 13 clinical sites from 6 consecutive months.

## Individual donor HCV NAT positive, N = 1297 EIA and RIBA results on index donation

EIA	RIBA	Total	% of NAT Positive	% of donors screened
Repeat Reactive	Positive	1122	86.5%	0.066
Repeat Reactive	Indeterminate	10	0.8%	0.0006
Repeat Reactive	Negative	6	0.5%	0.0004
Repeat Reactive	Unknown	26	2.0%	0.0015
Negative	N/A	130	10.0%	0.0076
Unknown	Unknown	3	0.2%	0.0002
		1297	100%	0.076

Note: Data presented are preliminary results from the Roche Ampliscreen™ HCV study and represent the samples from all 13 clinical sites from 6 consecutive months.

# 6 Month Dataset

## 130 NAT pos/EIA neg donations

### Additional Testing

<u>Alternate Source</u>	<u>Follow-up*</u>	<u>Number</u>	<u>%</u>
Negative	----	33	25
----	Negative	32	25
Negative	Negative	9	7
Unknown	Unknown	46	35
Positive	---	3	2
----	Positive	7	5

\*Follow-up defined as positive if any HCV NAT or EIA3 is reactive

# Details of suspected contamination events

## Community Blood Center of Greater Kansas City

- ~980,000 donations screened
- 8 “true positive” NAT pos/EIA neg (NAT pos on 2nd specimen)
  - ~ 1/123,000
- 48 NAT pos/EIA neg suspected contamination (NAT negative on 2nd specimen)
  - ~ 1/20,000

## Proximity of 48 specimens to EIA pos/NAT pos specimen

- 44 with EIA pos/NAT pos specimen on archive plate
  - 5 archive plate dropped
  - 16 adjacent, 4 diagonal
  - Remainder scattered through archive plate
- 4 with no EIA pos/NAT pos specimen on archive plate
  - 1 neg on tube
  - 1 neg on tube/unit
  - 2 neg on tube/unit/follow-up

# Value of Additional Testing on Index Donation

## Community Blood Center of Greater Kansas City

- 36 tubes tested
  - 30 negative
    - 22 tested on additional specimen(s), all neg
  - 6 positive
    - Additional specimens all neg
- 25 units tested neg
  - 9/9 neg on follow-up

April 99-July 00  
~ 5.5 million donations screened

- 23 donors HCV NAT positive/EIA negative with a second specimen NAT positive
- ~1/240,000

# COBAS AmpliScreen HCV Test, v2.0

## Yield Data Comparison for EIA 2.0 vs. EIA 3.0

Index Sample Testing Criteria	PCR Positive EIA 2.0 Negative *	PCR Positive EIA 3.0 Negative**	PCR Positive EIA 2.0 or EIA 3.0 Negative
No. Window Cases***	17	6	23
Total Samples Tested	2,269,004	3,242,498	5,511,502
Yield	1:133,000	1:540,000	1:239,630

\* Data from sites using EIA 2.0 as test of record

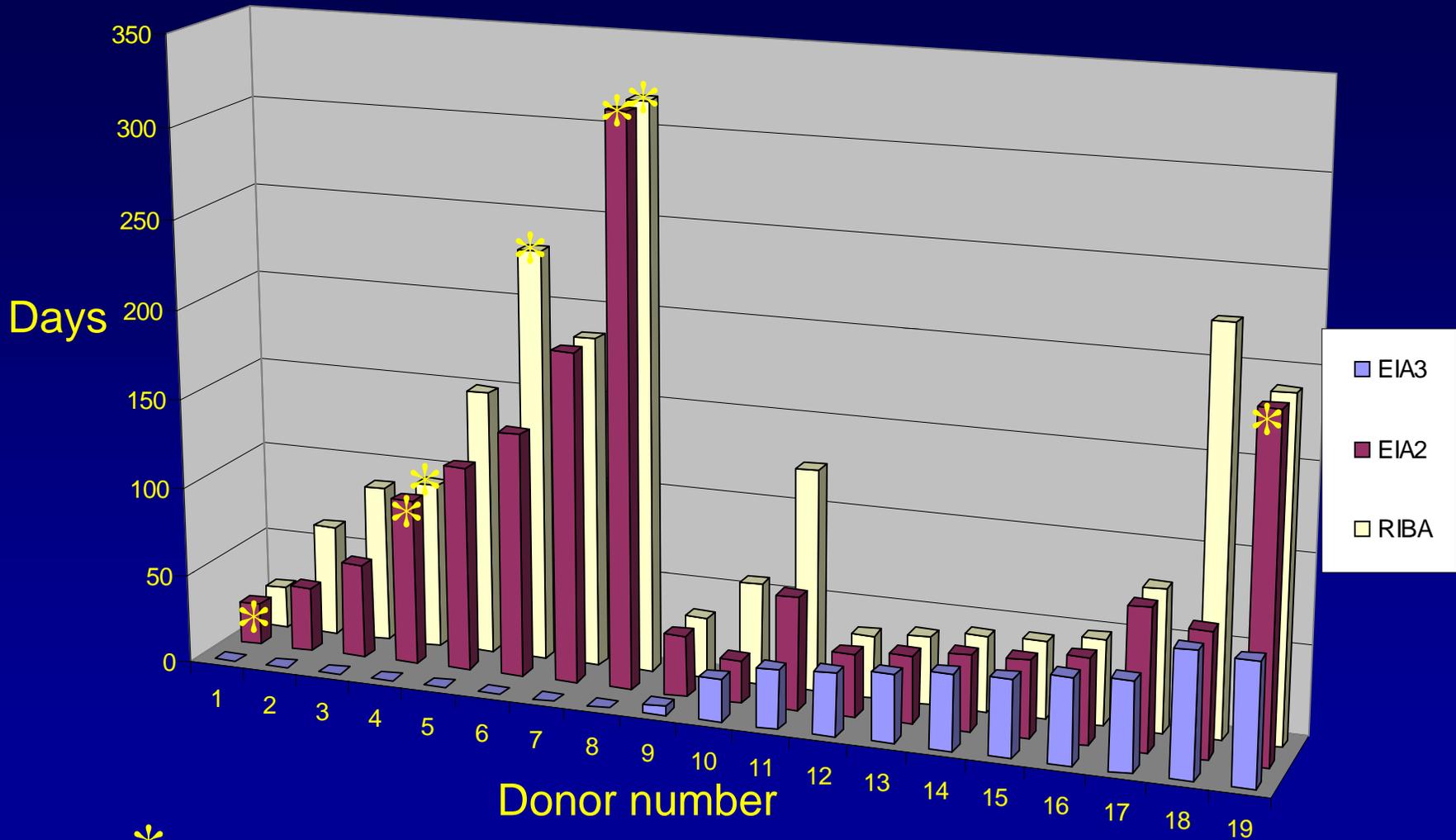
\*\* Data from sites using EIA 3.0 as test of record

\*\*\* Window case defined as 1) PCR positive on index sample and seroconversion on subsequent sample; or 2) PCR positive on index sample and PCR positive on index plasma unit; or 3) PCR positive for index sample and PCR positive on subsequent sample. Data from 4/8/99 – 7/31/00

# 19 Donors in Follow-Up: Days to Positive Test

<u>Donor</u>	<u>EIA3</u>	<u>EIA2</u>	<u>RIBA</u>
1	0	>23	23
2	0	36	62
3	0	53	88
4	0	>93	>93
5	0	115	149
6	0	137	>231
7	0	185	185
8	0	>317	>317
9	5	34	34
10	24	24	57
11	33	63	124
12	35	35	35
13	38	38	38
14	42	42	42
15	43	43	43
16	48	48	48
17	50	79	79
18	70	70	224
19	68	>190	190

# Days to Positive Test



\* Still not positive for this marker at last follow-up

## EIA3 vs. EIA2 vs. RIBA, observations

- About 30% of donors showed a significant time lapse (>90 days) between EIA3 positivity and EIA2 positivity
- During the EIA3 positive/EIA2 negative interval, most specimens are RIBA3 indeterminate (c33c)
  - One donor was EIA3 positive/EIA2 negative/RIBA negative on days 17 and 54; RIBA positive on day 115
- It is not clear whether all infected donors will ultimately become EIA2 positive and RIBA positive

## 19 HCV EIA neg/NAT positive\* donors enrolled in follow-up

- All 19 donors were EIA3 reactive by the second follow-up visit
- 5/19 donors had one or more (individual) NAT negative samples during follow-up
  - 3 had a positive NAT on further follow-up,
  - 2 had two consecutive negative NAT with no further follow-up

\*NAT positive on two specimens

*Question 1: Is it useful to consider reentry for donors with individual donation NAT Positive / Anti-HCV EIA RR / RIBA 3.0 Indeterminate or Negative results?*

Answer: Probably not

- Donors who are NAT pos/EIA RR/RIBA indeterminate are most likely in the process of seroconverting.
- Donors who are NAT pos/EIA RR/RIBA neg may also be seroconverting.
  - A false positive on both NAT and EIA would be a rare event; re-entry algorithm not worth pursuing?

# Update June 2001

## Preliminary Data

### HCV

- 8.1 million donations screened
- 32 “window cases” (1/253,000)
  - 24 in follow-up
  - All NAT pos or EIA3 pos or both in every f/u sample
- Est.~ 300 suspected false pos (1/27,000)
  - 97 donors with 2 or more f/u samples, no evidence infection
  - 21 donors with neg unit and f/u, all no evidence of infection

### HIV

- 5.4 million donations screened
- 1 “window case”
- 1 suspected false positive

*Question 2: .Should reentry be considered for donors with (pooled) NAT Negative / Anti-HCV EIA RR / RIBA 3.0 Indeterminate results ?*

Answer: Probably not, because:

- These donors could be in the process of seroconverting
  - Pooled NAT is less sensitive than individual unit NAT
  - Some donors are intermittently NAT neg (individual sample) during seroconversion
  - Agree that individual NAT testing (optional) would be useful for donor counseling and defer if positive
- Even if not infected, these donors are likely to remain reactive on screening test
  - Worth reconsidering when next generation screening test is licensed

*RE: Proposed “option” of following up with an HCV NAT test at any time up to 6 months after the index donation*

- Agree that testing of a second specimen is very useful for donor counseling
- Suggest that plasma from index donation may be used for this purpose (if validated)
- Additional testing of tubes from original donation (including supplemental NAT) should not be cause for deferral because of likelihood of contamination
- Agree that NAT positive result on a second “pristine” specimen should be cause for deferral

### *Question 3: What should be the minimum time period for waiting prior to follow-up testing ?*

- All 19 “window case” donors were positive for either EIA 3 or individual NAT or both at every follow-up visit.
- All donors were EIA3 positive by day 70.
  - 8 donors were EIA3 positive on index specimen
  - Remaining 11 donors were EIA 3 positive on first or second follow-up (median 42 days, range 5-70)
- 8 weeks for follow-up should be sufficient if both individual NAT and EIA are done and EIA 3 is used
  - If you wish to allow time for EIA3 to become positive, six months should be more than sufficient
- Agree that RIBA should not be required for reentry as long as EIA 3 is negative; (RIBA less sensitive than EIA3)

*Question 4: Should the blood establishment have the option of continuing to follow-up a donor with individual sample NAT Negative / persistent Anti-HCV EIA RR results for possible reentry ?*

Answer: absolutely

- These donors may be non-reactive on the next generation screening test (e.g., EIA 2.0 RR / EIA3 NR) or on another manufacturer's licensed screening test

## Other comments

- Suggest use of terminology such as “Licensed serological screening assay” rather than “EIA”

# Comparison of HIV NAT to HIV-1 Supplemental Test Results

## HIV Supplemental Test Results\*

HIV NAT	Pos.	Ind.	Neg.
Pos.	23 (4.3%)**	4 (0.75%)	0 (0.0%)
Neg.	7 (1.3%)	116 (21.8%)	383 (71.9%)

\*Includes HIV-1 W. Blot and HIV-1 IFA.

\*\*Values in ( ) indicate % relative to anti-HIV 1/2 EIA repeat reactive samples  
n=1,077,035 donations

533 donors reactive by anti-HIV 1/2 EIA.

anti-HIV 1/2 EIA reactive rate = 0.049%

% NAT POS./Supplemental Test POS. = 77%

*Question 1: Is it useful to consider reentry for donors with NAT Positive/Anti-HIV-1/2 EIA RR/ HIV-1 Western Blot Indeterminate or Negative results?*

Answer: Probably not

- In Roche system, false positive NAT for HIV is extremely rare event.
- Probability of false positive EIA and false positive NAT is unlikely

*Question 2: Should reentry be attempted for a donor with (pooled NAT negative)/ Anti-HIV-1/2 EIA RR/ HIV-1 Western Blot Indeterminate, Viral Bands Present Results?*

Answer: Yes

- Clear from literature that most HIV-1WB indeterminate donors are uninfected

*Question 3: What should be the minimum time period for waiting prior to follow-up testing?*

- Follow-up testing prior to 8 weeks OR testing of second specimen from time of donation may be useful for counseling
- For re-entry, 8 weeks should be sufficient
- For Group 3 donors, (i.e., reactive only on EIA and not NAT), suggest that donor could be re-entered if EIA reactivity disappears, without performing individual NAT
- Agree that WB should not be required if repeat EIA is NR
- Agree that positive individual NAT on a “pristine” specimen should be cause for permanent deferral

*Question 4: Should the blood establishment have the option of continuing to follow-up a donor with NAT Neg/persistent Anti-HIV-1/2 EIA RR results for possible reentry?*

Answer: Absolutely

- Donor may be non-reactive on another licensed serological screening assay

# Other comments

- Include IFA in re-entry strategy