Dramatic Reduction in Indeterminate Results from HIV Antibody Screening of Blood Donors by Conversion from Western Blot to an Immunofluorescence Assay

SK Vanderpool,, NE Kiely, SJ Cyrus; HT Kamel, PA Tomasulo, M Busch

Blood Systems, Tempe and Scottsdale, AZ and San Francisco CA.

Background – Donor Screening

- ◆ In the US, ~16 million blood donations and 15 million source plasma donations screened annually.
- ◆ Information on abnormal infectious disease test results on blood donations should be accurate and informative.
- This is particularly important for HIV, given the health implications for the donor and consequences for hospitals who must notify recipients of prior donations from donors with positive or indeterminate HIV test results (an FDA mandated process called "lookback").

Indeterminate and "unreadable" results on HIV-1 Western blot (WB) are especially troublesome to convey since donors and hospital customers are left with a confusing and non-specific message.

Background – HIV Confirmation

- HIV-1 algorithms utilize immunoassays for confirmation of EIA/ChIA repeat reactive blood donors
 - Most commonly used test: whole HIV lysate Western Blot (WB)
 - HIV-1 Immunofluorescence (also uses viral lysate)
 - Not widely used in blood centers due to difficulty in interpretation
 - HIV-2 confirmation also required for HIV-1 WB ind/neg samples
 - Licensed HIV-2 EIA => Research based HIV-2 WB (CA)
 - HIV Multi-Spot rapid test
 - 10 or fewer HIV-2 confirmed pos blood donors in the US reported since 1992
 - No confirmation for HIV-1 type O
 - HIV-1 RNA screening (NAT) implemented in 1999

Background – Problems with WBs

◆ Issues with HIV confirmation using WBs

- Sensitivity to early SC good with current CDC/FDA criteria, but a WP between 3rd (and 4th!) generation EIAs/ChIAs and WB SC
- Poor specificity
 - Unreadable, uninterpretable or invalid results generated
 - Misclassification of donors
 - False positive and false negative results
 - High rates of indeterminate results (neither pos or neg) in healthy individuals
 - Donor deferral, anxiety, and use of complicated donor reentry algorithms, when available
 - Mixed message, "You are healthy and not HIV infected, but you cannot donate blood"

Background - Problems with WBs

- Issues with HIV confirmatory assays, cont'd
 - High costs
 - Inconsistent availability
 - No new products for "antibody confirmation" on the market for HIV-1 or HIV-2
 - Screening tests ongoing improvements since 1985
 - Costs associated with approval of new products extremely high
 - Can alternate algorithms be validated?
 - HIV RNA (NAT), use of high/low screening S/CO ratios, dual EIA algorithm, IFA

Human Immunodeficiency Virus Type 1 (HIV-1) Western Blot Kit

March 16, 2000 BPAC

- Any bands present but pattern does not meet criteria for POSITIVE = INDETERMINATE
- Non-viral bands have been observed with certain specimens. These bands are not usually accompanied by any of the major viral bands of diagnostic significance (p24, gp41/120/160). The non-viral bands appear to be cell related with the most common in the molecular weight range of 70K, 51-55K (possible HLA-DR) and 43K (possible HLA-ABC).

Cambridge Biotech HIV-1 Western Blot



Correlation of routine HIV NAT screening with supplemental HIV serological data 5,972 HIV-EIA RR of 7.6 million donations at BSL, 04/99 - 07/03

Western Blot Results

NA I Results	Pos	Ind	Neg	Total
Pos (dHIV R)	267 (93%)	2** (0.1%)	0	269
Neg (mTMA on MPs)	18 [*]	2216	3468	5702
Total	286	2218	3468	

9/18 autologous donations, 6/9 from 3 donors. 3 false positive WB, f/u EIA R, WB(-), NAT(-).
1 w/ 1+ p24 band-only; 1 w/ +/- p24, p55, gp120 & 2+ gp160 bands.

Characteristics of HIV-1 WB Pos/TMA Nonreactive Samples

Sample	Pool	Neat	HIV-1/HIV-2 S/CO	WB	HIV PCR
1		NR	1.43	41, 120, 160	Neg
2	NR		1.03	41, 160	Neg
3		NR	1.62	41, 160	Neg
4		NR	1.11	41, 55, 160	Neg
5		NR	2.50	24, 41, 51, 61, 160	Neg
6		NR	20.18	all bands	Neg
7		NR	1.22	24, 41, 160	Neg
8	NR		1.11	17, 41, 120, 160	Neg
9	NR		1.84	41, 160	Neg
10		NR	1.40	24, 41, 160	Neg
11		NR	1.70	17, 24, 41, 51, 160	Neg
12	NR		20.00	all bands	Pos (200 copies/mL)
13		NR	17.84	all bands	Neg

All samples p24 Ag negative; lack of HIV infection in those weakly EIA Rx with follow up

Characteristics of HIV-1 WB Pos/TMA Nonreactive Samples

Sample	Pool	Neat I	HIV-1/HIV-2 S/CO	WB	HIV PCR
14		NR	8.73	17, 24, 160	Neg
15	NR		10.56	24, 55, 120, 160	Neg
16	NR		19.30	all bands	Neg
17	NR		19.47	all bands	Pos (200 copies/mL)
18	NR		1.04	41, 66, 120, 160	Neg
19	NR		4.72	41, 160	Neg
20	NR		1.50	24, 41, 160	Neg
21	NR		1.53	24, 41, 160	Neg
22	NR		1.60	24, 160	Neg
23	NR		17.60	all bands	Neg
24		NR	19.30	all bands	Neg

All samples p24 Ag negative; lack of HIV infection in those weakly EIA Rx with follow up

False Positive WBs vs Elite Controllers 374 ARC donations that tested WB+/NAT-, '99-09

Allogeneic Donors	Confirmed infected (EC)	106
	False Positive	120
	Unable to Determine	8
	Total	234

Autologous Donors	Confirmed infected (EC)	130
	False Positive	2
	Unable to Determine	0
	Total	132

Methods

- Blood Systems testing laboratories replaced the HIV-1 WB, used for over 20 years, with the HIV-1 immunofluorescence assay (IFA) on 02/19/07.
- We compared HIV-1 confirmatory results performed using WB over 34 months prior to IFA implementation (4/19/04-2/18/07) with HIV-1 confirmatory results performed with IFA between 2/19/07-12/18/09.

	WB	IFA
	(4/04 - 2/07)	(2/07 - 12/09)
Anti-HIV RR	2,143	905
Positive	72 (4%)	82 (9%)
Indeterminate	807 (38%)	25 (3%)
Unreadable	351 (16%)	0 (0%)
Negative	901 (42%)	321 (85%)

	WB	IFA
	(4/04 - 2/07)	(2/07 - 12/09)
# Donations	2,333,447	2,560,862
Anti-HIV RR	2,143 (0.092%)	905 (0.035%)
Positive	72 (0.0031%)	82 (0.0032%)
Indeterminate	807 (0.035%)	25 (0.0009%)
Unreadable	351 (0.015%)	0 (0%)
Negative	901 (0.038%)	321 (0.012%)

	WB	IFA
	(4/04 - 2/07)	(2/07 - 12/09)
# Donations	2,333,447	2,560,862
Anti-HIV RR	2,143 (0.092%)	905 (0.035%)
Positive	72 (0.0031%)	82 (0.0032%)
Indeterminate	807 (0.035%)	25 (0.0009%)
Unreadable	351 (0.015%)	0 (0%)
Negative	901 (0.038%)	321 (0.012%)

	WB	IFA
	(4/04 - 2/07)	(2/07 - 12/09)
# Donations	2,333,447	2,560,862
Anti-HIV RR	2,143 (0.092%)	905 (0.035%)
Positive	72 (0.0031%)	82 (0.0032%)
Indeterminate	807 (0.035%)	25 (0.0009%)
Unreadable	351 (0.015%)	0 (0%)
Negative	901 (0.038%)	321 (0.012%)

HIV-1 Confirmatory Results - ARC



*06/24/2008 Converted to HIV IFA

Impact of conversion to IFA

- 13-fold reduction in indeterminate rates and elimination of unreadable results.
- Donor notifications of indeterminate or unreadable results decreased from 343/year with WB to 12/year with IFA.
- Lookback notifications to hospitals triggered by indeterminate or unreadable results decreased from 588/year with WB to <40/year with IFA.</p>



- WB indeterminate and unreadables results rarely represent true infection with HIV-1 in EIA reactive donors, yet convey message of uncertainty when reported to donors and hospitals.
- Conversion to HIV-1 IFA led to dramatic reduction in donor notifications and lookbacks resulting from indeterminate or unreadable confirmatory results.
 - In addition, eligibility for re-entry simplified, since donors with negative confirmatory test are eligible for donor re-entry while donors with indeterminate results are not eligible.