

I. D. SIGNIFICANCE OF HIV INDETERMINATE WESTERN BLOT RESULTS

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HIV Plasma RNA for Confirmation of HIV p24 Ag / anti-HIV Reactivity in Seroconverting (SC) Donors

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Background

Current supplemental assays for HIV-1 p24 Ag and antibody yield rare false-negative and false-positive and frequent indeterminate results.

This results in confusing notification messages to reactive donors, with required follow-up testing to resolve their infection status.

Objectives

1. Project rates of HIV infection among donors with various p24 Ag/Ab-reactivity patterns.
2. Evaluate sensitivity of HIV RNA and p24 Ag assays for confirming infection in early SC.

Methods

- 51 SC plasma donor panels (439 samples)
- Analyzed by
 - HIV RNA PCR (Roche Monitor)
 - p24 Ag EIA (Abbott, Coulter)
 - anti-HIV-1/2 EIA (Ab EIA: Abbott)
 - Western blot (WB: Cambridge Biotech)
- Window periods (WP) estimated by Markov modeling
- Probability of true HIV infection among reactive donors projected based on HIV incidence and donor reactivity rates
- RNA and p24 Ag sensitivities and levels evaluated for each SC stage

Definition of SC Stages

	RNA	p24 Ag	anti-HIV EIA	WB
RNA(+) only	+	-	-	-
RNA / p24(+), Ab(-)	+	+	-	-
Ab EIA(+), WB(-)	(+)	(+)	+	-
Ab EIA(+), WB(Ind)	(+)	(+ / -)	+	Ind
Ab EIA(+), WB(+)(-p31)	(+)	(+ / -)	+	+ (no p31)
Ab EIA(+), WB(+)	(+ / -)	(+ / -)	+	+ (w/ p31)

Duration of Progressive Stages of Seroconversion

Stage	WP (days) (95% CI)
RNA(+) only	3.1 (1.7-5.5)
RNA / p24(+) / Ab(-)	5.0 (3.4-7.3)
Ab EIA(+), WB(-)	3.1 (2.1-4.7)
Ab EIA(+), WB(ind)	5.0 (3.4-7.4)
Ab EIA(+), WB(+)(-p31)	35 (23 - 47)

Projected Number of HIV SC Donors Presenting Annually in US Volunteer Donor Setting During Each Stage of Early SC

Stage	Duration Days (Years)	Incidence Rate * / 1 million P-Y	# / 12 million Donations
RNA(+) only	3.1 (.0082)	4.0	3.9
RNA / p24(+), Ab(-)	5.0 (.014)	4.0	6.6
Ab EIA(+), WB(-)	3.1 (.0082)	4.0	3.9
Ab EIA(+), WB(Ind)	5.0 (.014)	4.0	6.6
Ab EIA(+), WB(+)(-p31)	35 (.096)	4.0	46

* from Retrovirus Epidemiology Donor Study (REDS); Schreiber et al, NEJM 1996

**Probability of HIV Infection in Blood
Who Test Reactive by p24 Ag or
(based on 5 REDS centers, 1991)**

Reactive Pattern	Reactivity Rate In Donors		
p24 Ag RR	0.03 %		
Ab EIA(+)	0.088 %	10	
Ab EIA(+),WB(-)	0.060 %	7,200	
Ab EIA(+),WB(ind)	0.019 %	2,280	
Ab EIA(+),WB(+)(-p31)*	0.00081 %	97	46
Ab EIA(+),WB(+)	0.0083 %	996	996 (100)

* no p31 band, possible false-positive WB pattern

**Sensitivity of HIV Plasma RNA and p24 Ag for
Confirmation of HIV-1 Reactivity
in Seroconverting (SC) Blood Donors**

Stage	Number of Samples	Number (%) RNA-pos	Number (%) p24 Ag-pos
RNA(+) only	19	19 (100)	0
RNA / p24(+), Ab(-)	51	51 (100)	51 (100)
Ab EIA(+), WB(-)	23	23 (100)	23 (100)
Ab EIA(+), WB(ind)	41	41 (100)	31 (76)
Ab EIA(+), WB(+)(-p31)	143	143 (100)	55 (38)

Conclusions

- 1. RNA PCR accurately detects HIV infection in Ag / Ab - reactive samples from all stages of early SC.**
- 2. p24 Ag detects infection during early Ab SC, but sensitivity is reduced as WB evolves toward positivity.**
- 3. A licensed RNA PCR assay would be useful for establishing infectious status of donors with reactivity patterns suggestive of early HIV seroconversion.**

Frequency of human immunodeficiency virus (HIV) infection
among contemporary anti-HIV-1 and anti-HIV-1/2
supplemental test-indeterminate blood donors

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C.-P. PAU, G.B. SCHREIBER, AND THE RETROVIRUS EPIDEMIOLOGY DONOR STUDY

False-Positive HIV-1 Test Results in a Low-Risk Screening Setting of Voluntary Blood Donation

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Public Health

**WHAT DO WESTERN BLOT INDETERMINATE
PATTERNS FOR HUMAN
IMMUNODEFICIENCY VIRUS MEAN IN
EIA-NEGATIVE BLOOD DONORS?**

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False-positive human immunodeficiency virus type 1 Western blot tests in noninfected blood donors

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Received for publication April 18, 1995; revision received July 28, 1995, and accepted August 29, 1995.

TRANSFUSION 1996;36:45-52.

Donor reactions to notification of abnormal test results

S. Kleinman, A. Williams, M. Busch, Y. Wu for the Retrovirus Epidemiology Donor Study

Previous studies

- HIV positive donors in 1980s
- REDS publication on psychological distress in donors informed of positive HTLV results
 - Guiltinan et al Trans 1998;38:1056-62
- No systematic studies for full range of infectious disease test results

Background

- Estimated annual number of donor notifications is 100,000 – 200,000 depending on notification policies for anti-HBc
- Blood safety policy decisions often fail to consider the adverse impacts of donor notification

Background

- Notification messages for different test results vary in content, in clarity, and by blood center
- FDA has recently proposed a rule regarding the general content of and specific methods of donor notification

Methods

- Anonymous mail survey of donors notified of abnormal test results from Jan to May 1997
 - Notification by routine blood center procedures, usually letter
 - Survey conducted 6 to 12 months after notification

Categories of Test Notifications

- | | |
|-------------------------|----------------------------|
| • Confirmatory Positive | HIV, HCV, HTLV Ab
HBsAg |
| • Indeterminate | HIV, HCV, HTLV Ab |
| • Confirmatory Negative | HIV, HCV, HTLV Ab
HBsAg |
| • Anti- HBc | |
| • Elevated ALT | |

Selected survey questions

- Was the information provided by the blood center staff:
 - Easy to understand
 - A little hard to understand
 - Very hard to understand

Selected survey questions

- Were you confused when you were first told about your test results by blood center staff ?
- Are you confused now ?
- Possible answers were:
 - No, not at all; Yes, a little; Yes, a lot

Selected survey questions

- Were you emotionally upset when you were first told about your test results?
- Are you emotionally upset now ?
- Possible answers were:
 - No, not at all; Yes, a little; Yes, a lot

Eligible and responding donors

<u>Group</u>	<u>Eligible</u>	<u>Responding</u>
Conf Pos	369	109 (30%)
Indeterm	461	169 (37%)
Conf Neg	256	118 (46%)
Anti-HBc	1997	759 (38%)
High ALT	652	259 (40%)

Understanding of message

<u>Group</u>	<u>Not easy to understand</u>
Conf Pos	40%
Indeterm	66%
Conf Neg	62%
Anti-HBc	39%
High ALT	51%

p = 0.001

Donor confusion at and post notification

<u>Group</u>	<u>At</u>	<u>Post</u>
Conf Pos	82%	40%
Indeterm	90%	62%
Conf Neg	90%	62%
Anti-HBc	79%	45%
High ALT	80%	40%

p = 0.001 p = 0.001

Emotional upset at and post notification

<u>Group</u>	<u>At</u>	<u>Post</u>
Conf Pos	86%	51%
Indeterm	91%	51%
Conf Neg	84%	51%
Anti-HBc	68%	30%
High ALT	77%	30%

p = 0.001 p = 0.001

Result summary

- Confusion and emotional upset were present at the time of notification in 68 - 91% of donors
- Although both reactions diminished at 6 to 12 months, they were still reported by 30-62 % of donors, depending on donor group

Result summary

- Confusion was most prevalent in Indeterm and Conf Neg donors at time of (90%) and post notification (62%)
- Emotional upset was most prevalent in Indeterm, Conf Neg, and Conf Pos donors at time of (84 - 91%) and post notification (51%)

Conclusions

- Confusion and emotional upset are a feature of the notification process, even for results without medical significance (i.e. anti-HBc)
- These reactions persist for 6 to 12 months post notification

Conclusions

- These reactions are more frequent when the meaning of the test result is less clear
- Policy makers should be aware that donor notification adversely impacts many donors

Possible ways to reduce the number of donors with adverse reactions

- Increase specificity of screening assays
- Improve confirmatory assays so as to:
 - be able to conclude that confirmatory negatives are not infected
 - minimize indeterminate results

Value of routine Nucleic Acid Test (NAT) results for resolution of infectious status of HIV and HCV seroreactive donors.

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Background

- NAT introduced to detect seronegative HCV and HIV window-phase infections
- NAT data is useful for resolving true infections status of seroreactive donors, particularly donors with indeterminate and negative supplemental results

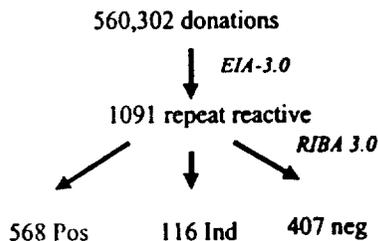
Objective

- Evaluate performance of routine NAT screening, in context of minipool and single donation screening strategies, for resolving true infectious status of donors who test seroreactive for HIV or HCV antibody

Methods

- Study period: 4/12/99 - 10/12/99
- Serological screening/confirmation
 - HIV: Abbott HIV-1/2 EIA, CBT Western blot
 - HCV: Ortho 3.0 EIA, Chiron RIBA-3
- NAT
 - Genprobe/Chiron HIV-1/HCV TMA system
 - 24 donation pools (all repeat, most FT donors)
 - reactive pools resolved to SD, followed by HIV and HCV discriminatory testing

Results of HCV serological screening
Blood Systems Laboratory, 4/99-10/99



Correlation of HCV NAT screening with supplemental HCV serological results (1,091 HCV RR Donations, BSL, 4/99-10/99)

NAT Result	RIBA Result		
	Pos	Ind	Neg
Pos	463 (82%)	8* (7%)	0
Neg	105	108	407
Total	568	116	407

* 2 SOD rx w/ 4 HCV bands; 3 c22 (4+); 2 c33 (2+); 1 NS5 (3+)

Relative sensitivity of HCV NAT on RIBA positive donations screened in minipools vs as single donations

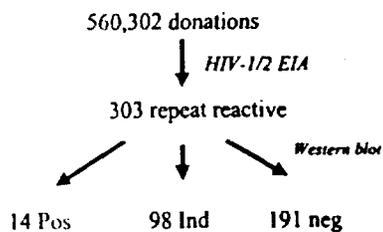
NAT Screening	# RIBA+ units screened	# (%) NAT+
Minipool	468	385 (82%)
Single donation	100	82 (82%)

Results of SD-NAT retesting of HCV-RIBA-3 positive specimens that tested negative by MP- vs SD-NAT

Original NAT Screening	# RIBA+NAT- specimens tested	# (%) pos on SD-NAT
Minipool	60	15 (25%) *
Single Donation	15	2 (13%)

* - Mean SD-NAT s/co = 5.0 (range 1.05-9.2)

Results of HIV serological screening
Blood Systems Laboratory, 4/99-10/99



Correlation of routine HIV NAT with supplemental HIV serological data (303 HIV-EIA RR donations, BSL, 4/99-12/99)

NAT Result	Western Blot Result		
	Pos	Ind	Neg
Pos	15 (94%)	2* (2%)	0
Neg	1	96	189
Total	16	98	189

* Both cases had non-viral bands only on WB and tested negative on HIV discriminatory TMA

Summary - HCV

- Sensitivity of MP-NAT similar to that of SD-NAT for HCV RNA detection among RIBA-pos donor specimens (82%)
- 25% of RIBA-pos units that tested RNA neg on MP-NAT screening, tested pos on SD-NAT (low-level viremia).
- 7% of RIBA-ind donations tested HCV RNA pos by routine NAT
- 0/407 RIBA-neg samples tested NAT pos

Summary - HIV

- 94% of HIV WB-pos donations tested HIV RNA pos by routine NAT
- Although 2 of 98 HIV WB-ind donations tested multiplex NAT reactive, these were neg by HIV discriminatory NAT (probable false positives)
- 0/189 HIV WB-neg donations tested NAT pos

Conclusion

- Routine NAT screening data is useful for confirming the infectious status of HCV and HIV EIA-reactive donors, and should be incorporated into donor notification and counseling programs