
AABB 53rd Annual Meeting

November 7, 2000

**Lack of Evidence to Support the Presence of
NAT Inhibitors in Donor Specimens**

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CHIRON Blood Testing

Background (1)

Currently, the routine NAT blood screening for HIV-1 and HCV is performed (under INDs) on plasma pools of 16 or 24 donations

Samples in a reactive pool must be further tested individually to resolve the pool reactivity

Samples in a non-reactive pool and samples from a reactive pool that tested individually and found non-reactive are considered NAT-negative

Background (2)

When all samples in a reactive pool are found to be non-reactive, the original pool reactivity is defined as having an “unresolved” reactive result

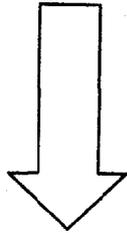
One concern is that an unresolved reactive pool may involve a viremic sample that contains NAT inhibitors

As a result, this sample can only be detected in a plasma pool where the inhibitors are diluted out more rapidly than viral nucleic acids

Dilution of NAT Inhibitors in Pool Testing

Individual Sample

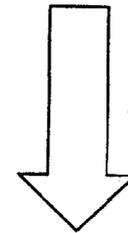
160X Enzymes
160X Inhibitors



No Amplification

Pool of 16

160X Enzymes
10X Inhibitors



Amplification

wouldnt be an issue
due to IC

CHIRON Blood Testing

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Experiment #1

6 Chiron Procleix HIV-1/HCV Assay unresolved reactive pools

6 X 24 = 144 non-reactive samples

All samples were tested once at 1:24 dilution in negative base matrix

The original pools were re-tested in duplicate

New pools made from the same samples were re-tested once

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Experiment #1

Pool No.	Repeat Testing (S/CO)	New Pool (S/CO)	Constituent Samples	
			Undiluted* (# reactive / # tested)	1:24 Dilution (# reactive / # tested)
1	0.19/9.38 ✓	0.17	0/24	0/24
2	✓ 8.22/0.20	0.13	0/24	0/24
3	✓ 4.71/0.18	0.16	0/24	0/24
4	0.18/4.71 ✓	0.22	0/24	0/24
5	0.18/QNS	QNS	0/24	0/24
6	✓ 7.14/0.26	0.17	0/24	< 2/24**

* Re-test by another lab

** S/CO = 4.05 and 7.01. Both NR in discriminatory assays

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Experiment #1

5 newly formed pools from the same original constituent samples were tested negative in Chiron Procleix HIV-1/HCV Assay

The original samples for the remaining one pool were not all available to form a new pool

All 144 samples were found to be non-reactive when re-tested undiluted with Chiron Procleix HIV-1/HCV Assay by another laboratory

All but 2 samples were tested non-reactive at 1:24 dilution by another laboratory with Chiron Procleix HIV-1/HCV Assay

These two samples were non-reactive in Chiron Procleix HIV-1 and HCV Discriminatory Assays

These two samples and their 1:24 dilution and the original pools were tested by PCR and found to be negative

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Experiment #2

6 HCV RIBA-positive samples, each involved in a pool that was tested repeatedly reactive (3X) with the Chiron Procleix HIV-1/HCV Assay

All 6 samples were tested undiluted and found to be non-reactive with the Chiron Procleix HIV-1/HCV Assay

These 6 samples were diluted 1:24 in negative base matrix and tested with the Chiron Procleix HIV-1/HCV Assay by another laboratory

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Experiment #2

Sample No.	HCV Serology	HCV TMA		HCV TMA Re-testing*	
		Pool	IDS	Undiluted* (S/CO)	1:24 Dilution (S/CO)
1	Positive	RR	NR	0.11	0.21
2	Positive	RR	NR	0.18	0.31
3	Positive	RR	NR	0.33	0.14
4	Positive	RR	NR	0.28	0.32
5	Positive	RR	NR	4.46	QNS
6	Positive	RR	NR	5.77	0.16

* By another lab

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Experiment #2

When re-tested undiluted, 2 (33%) of these 6 samples were reactive in Chiron Procleix HIV-1/HCV Assay

When these 6 samples were tested at 1:24 dilution with the Chiron Procleix HIV-1/HCV Assay, 5 were non-reactive, one was QNS

Small percent of anti-HCV positive samples contain very low level of viral load

All of these 6 repeatedly reactive pools contained another HCV viremic sero-positive sample

The original reactivity of these 6 pools were not from the sero-positive, NAT-negative samples since they were tested non-reactive at 1:24 dilution

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Experiment #3

From 4/99 to 5/00, 10 (0.07%) unresolved reactive pools were identified at BCSEW after testing over 360,000 donations using the Chiron Procleix HIV-1/HCV Assay

Two new pools were made and re-tested using the same samples from each of these 10 unresolved reactive pools

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Experiment #3

Pool No.	Original Pool (S/CO)	New Pool #1 (S/CO)	New Pool #2 (S/CO)
1	1.42	0.25	0.43
2	1.36	0.45	0.24
3	2.57	0.33	0.29
4	2.21	0.54	0.33
5	1.87	0.24	0.39
6	3.53	0.28	0.42
7	1.04	0.46	0.39
8	1.68	0.34	0.36
9	1.48	0.48	0.40
10	1.46	0.21	0.45

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Experiment #3

All 20 newly formed pools (2 for each of the 10 unresolved reactive pool) were found non-reactive in the Chiron Procleix HIV-1/HCV Assay

The original pool reactivity could not be reproduced

Conclusions

Our data do not support the presence of inhibitors in donor samples when tested with the Chiron Procleix HIV-1/HCV Assay

The testing process of the Chiron Procleix HIV-1/HCV Assay involves a specific target capture step and a washing step prior to amplification

The unresolved reactivity in pool testing may be caused by either technical errors and/or intra-assay cross contamination

When all samples in a reactive pool are tested negative with the Chiron Procleix HIV-1/HCV Assay, these samples can be considered as NAT-negative and no further testing is needed