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IMPROVING THE CLINICAL SPECIFICITY OF ALANINE AMINO TRANSFERASE (ALT) TEST RESULTS WITHIN THE NORMAL BLOOD DONOR POPULATION OF QUEENSLAND. D. DeLeacy*, C. Hyland, M. Hourn, D. Battistutta. Queensland Red Cross Blood Transfusion Service, Queensland; Queensland Institute of Medical Research.

ALT testing is used by Blood Banks to screen for potentially infective liver disease not caused by currently identified agents. In an attempt to overcome some benign factors that influence this very sensitive but non specific test, a statistical analysis of ALT results from 31,006 routine blood donors was carried out. The effects of gender, age, Quetelet's body mass index ($BMI=KG/M^2$) and season were examined. On the basis of all available observations, reference ranges (95%) for ALT levels in the different subsets were calculated as the mean ±1.96 times the standard deviation of the logarithmically transformed data. At the univariate level, Student's t-test was used to compare two means and where more than two groups were involved, one way analysis of variance was used. Multiple linear regression analyses then considered the independent contributions of any of the variables which were found by univariate analyses to significantly affect ALT levels. At the univariate level, ALT values were significantly effected by all four paramaters ($p<0.0001$ in all cases). However multivariate analyses identified only gender and season as important independent predictors of an ALT level. Finally an interactive effect between gender and BMI was noted to be highly significant ($p<0.001$). The Queensland Red Cross Blood Transfusion Service now employs ALT reference ranges, tailored to each donor's BMI index (or gender if the former information is unavailable). This has allowed for the more precise exclusion of donors with elevated ALT values which are clinically significant and has reduced the incorrect deferral of healthy donors.

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when the donor is to be the recipient, the rules that govern donor acceptance should be remembered.

KEITH L. DUNCAN, MD, PHD
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Hepatitis C virus antibody seroconversion in a volunteer blood donor

To the Editor:

We report findings in a volunteer blood donor who seroconverted from negative for antibody to hepatitis C virus (HCV) to positive and who presented with elevated alanine aminotransferase (ALT) levels while in the window phase. The blood unit collected during the window phase was subsequently shown to be viremic as judged by polymerase chain reaction (PCR) but was negative in existing enzyme-linked immunosorbent assay (ELISA) for anti-HCV.

A man, aged 30 years, had donated 10 units of blood between the commencement of HCV screening in February 1990 and April 1992. All were negative for antibodies to HCV, human immunodeficiency virus (HIV), and syphilis and for hepatitis B surface antigen (HBsAg). All showed plasma ALT levels below 40 IU per L. In April 1992, the donor presented with an ALT level of 301 IU per L but was negative for anti-HCV in second-generation ELISA (Ortho Diagnostic Systems, Inc., Raritan NJ). The donated unit was not used for transfusion. The unit donated next, on July 21, 1992, was anti-HCV ELISA positive, although the second-generation anti-HCV recombinant immunoblot assay (RIBA-2, Chiron, Emeryville, CA) showed an indeterminate pattern with reactivity observed against the c22c HCV peptide only. The RIBA profile progressed to a positive pattern by March 30, 1993 (Table 1).

PCR assays were performed on plasma samples retrieved from storage at –30°C. RNA was extracted by a guanidinium hydrochloride method[1]; HCV cDNA synthesis and PCR were performed using anti-sense primer, GGTGCACGGTCTACGAGACCT, and a sense primer, GGCGACACTCCACCATAGAT, which amplified a 324-base pair product from the highly conserved 5′-noncoding region of the HCV genome.[2] Specificity of the PCR product was established using Southern blotting and probing with an internal oligonucleotide, CAATTCCGGTGTACTCACCGGTTCCGC (anti-sense position 159 to 1332), labeled with digoxigenin-dUTP.

The first positive PCR result was seen at the time of the April 1992 donation, coinciding with the ALT elevation, and persisted following anti-HCV seroconversion. Immunoblot assays (RIBA and Matrix [Abbott Laboratories, Chicago, IL]) showed reactivity against a single HCV peptide when both the anti-HCV ELISA and HCV PCR tests were positive. RIBA-indeterminate results, which represent individuals infected with HCV in the early phase of infection, have been reported elsewhere.[3]

ELISAs are now available that include an additional HCV peptide from the NS5 region of the HCV genome. An ELISA that included the NS5-derived peptide (Murex Diagnostics, Ltd., Dartford, UK) gave a negative result on retrospective testing of the April 1992 sample. Fortunately, on that occasion, routine ALT screening identified the infectious unit. However, the ALT test is nonspecific, and furthermore, ALT levels in HCV-infected individuals may fluctuate or remain normal.[4] Other workers[5] previously expressed concern about the sensitivity of assays for anti-HCV detection in blood donors. It remains to be seen whether PCR can be used as a routine screening test to eliminate HCV-viremic components, assuming that current problems of cost, contamination, and mass screening can be surmounted.

C.A. HYLAND, MS
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Table 1. *ALT levels and HCV profile of anti-HCV-seroconverting blood donor*

	Donation date					
	08/27/91	02/04/92	04/28/92	07/21/92	10/27/92	03/03/93
ALT (IU/L)	27	27	301	164	144	151
ELISA*						
Ortho	0.1	0.1	0.2	4.4	4.6	4.5
Abbott	NT†	NT	0.4	4.4	5.7	5.8
Murex	NT	NT	0.2	3.7	3.9	NT
Immunoblot						
RIBA-2	NT	NT	NT	Ind‡	NT	Pos§
Matrix	Neg‖	NT	Neg	Ind¶	Ind¶	Ind¶
PCR	Neg	Neg	Pos	Pos	Pos	Pos

* Absorbance-to-cutoff value.
† Not tested.
‡ Indeterminate: reactive (4+) against c22c peptide.
§ Reactive (4+) against c22c, (2+) against c33, and ± against c100-3.
‖ Negative.
¶ Reactive to c22c (sample-to-cutoff value absorbance ratio = 22.0).

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Vox Sanguinis (2003) 85, 1–8

ORIGINAL PAPER

Follow-up of six blood donors highlights the complementary role and limitations of hepatitis C virus antibody and nucleic acid amplification tests

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NOTE ALT RESULTS

Vox Sanguinis

Background and Objectives The purpose of this study was to analyse the follow-up results for six blood donors who screened positive for hepatitis C virus (HCV) by nucleic acid amplification technology (NAT) but were non-reactive in the primary antibody immunoassay (HCV NAT yield).

Materials and Methods Volunteer blood donations were screened, in parallel, for antibodies to hepatitis C virus (anti-HCV) and for human immunodeficiency virus (HIV)/HCV RNA using the Abbott PRISM HCV Chemiluminescent immunoassay (ChLIA) and the Chiron Procleix HIV-1/HCV RNA assays, respectively. NAT yield donor samples were further tested using supplemental assays, including an alternate HCV antibody enzyme immunoassay (EIA) (Abbott Murex anti-HCV Version 4), an immunoblot (Ortho RIBA-3 or Genelabs Diagnostics HCV Blot 3·0) and two alternative HCV NAT assays [Roche HCV Amplicor and an assembled HCV polymerase chain reaction (PCR)]. Five of the six donors were available for follow-up testing.

Results The six NAT yield donations were identified as constituents of 24-member minipools among 2 212 695 donations screened over the 28-month study period. All samples were positive when tested, undiluted, using the Roche Amplicor and assembled reverse transcription–polymerase chain reaction (RT–PCR) alternate NAT assays. One of the donors, subsequent to seroconversion, showed RNA levels that fluctuated above and below the limit of detection of the NAT screening assay. Three of the six were reactive on the secondary EIA and showed reactivity to the core c22(p) antigen by immunoblot at the index donation. Two others subsequently became reactive in the ChLIA prior to the EIA, showing reactivity against c100 and/or c33c antigens by immunoblot. The remaining donor became reactive in the ChLIA and EIA at the same time, showing RIBA reactivity against all of the following three peptides: c100; c33c; and c22(p).

Conclusions This study demonstrated that at least five of six HCV NAT yield donors were in the pre- or early antibody seroconversion phase of infection. The observation that one yield donor demonstrated HCV RNA that fluctuated above and below the limit of detection of the primary NAT-screening assay supports the maintenance of both NAT and antibody screening for HCV. Follow-up testing of suspected yield donors revealed that the primary and alternate anti-HCV immunoassays had different performance characteristics, depending on the specificity of the donor's early anti-HCV response.

Key words: blood donor, hepatitis C, NAT yield.

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Introduction

Donor screening by nucleic acid amplification technology (NAT) for hepatitis C virus (HCV) RNA has now been implemented in a number of countries worldwide [1]. NAT augments the existing HCV antibody immunoassays by reducing the window period (time between viral infection and marker detection) from approximately 66 days, for third-generation antibody assays, to approximately 23 days [2]. In the Australian donor population, this equates to a reduction in the estimated risk 'per unit' of post-transfusion hepatitis C from approximately 1 in 317 000 to 1 in 911 000 [3]. NAT, as a direct viral test, also has other potential advantages, including the identification of infected donors without a detectable antibody response (immunosilent infections) [4], and identification of individuals who produce antibodies that may not be detected by the antigen configurations used in some currently available immunoassays [5].

Although HCV NAT has demonstrated very high specificity in our donor population [6], the possibility that a NAT-positive but antibody-negative immunoassay result represents a biological false-reactive result, cannot be excluded. It is important therefore to have strategies to quickly resolve such results so that appropriate donor counselling and follow-up procedures can be given in a timely manner. Whilst HCV NAT-positive but antibody-negative (HCV NAT yield) donors have been previously reported [1,4,7–11], there have been few reports of follow-up studies or the strategies used to confirm infection, in such donors [4,12–16]. Over the first 28-month period of NAT screening in Australia, six HCV NAT yield donations were detected. The purpose of the current study was to present the results of follow-up testing of five of these donors, examine the usefulness of concomitant HCV NAT and antibody screening, and describe an effective strategy for confirming the HCV infection status of these donors on their index donation.

Materials and methods

Population studied

Between June 2000 and September 2002 (duration of the study period), the Australian Red Cross Blood Service (ARCBS) collected 2 212 695 donations from homologous volunteer blood donors.

HCV antibody screening

The primary anti-HCV immunoassay used during the study period by all ARCBS testing sites was the PRISM HCV chemiluminescent immunoassay (ChLIA) (Abbott GmbH & CO.KG, Weisbaden-Delkenheim, Germany), which was performed in parallel with NAT screening for HCV RNA. Samples that were reactive in the NAT assay, but non-reactive in the PRISM ChLIA, were also tested using the Abbott Murex anti-HCV version 4 enzyme immunoassay (EIA) (Murex EIA; Murex Biotech., Kyalami, South Africa). The Murex EIA was selected as the secondary immunoassay as it has a similar sensitivity to the primary immunoassay but differs in assay format and antigenic source. Selection of a secondary immunoassay with a similar sensitivity to the primary immunoassay minimizes the possibility of false-negative results, whilst assay differences potentially minimize overlap of false reactivity between the immunoassays [17]. To confirm the specificity of the anti-HCV reactivity, samples were further tested in an immunoblot using either the Ortho recombinant immunoblot assay (RIBA) 3·0 (Ortho Clinical Diagnostics, Raritan, NJ) or Genelabs Diagnostics HCV blot 3·0 (Genelabs Diagnostics, Singapore).

NAT for HCV and HIV RNA

Details of the NAT procedure used by the ARCBS, including the algorithms for resolving NAT-reactive pools and discriminating between HCV and HIV reactivity, have been described previously [6]. Briefly, all ARCBS sites used the Procleix HIV-1/HCV multiplex assay (Chiron Blood Testing Emeryville, CA). Approximately 80% of samples were screened at three testing sites (Sydney, Melbourne and Brisbane) where most donations are tested in pools of 24; the remaining 20% of samples were screened at two sites (Perth and Adelaide) exclusively by using individual-donation testing.

NAT yield samples were also tested, undiluted, in two different NAT assays [the HCV Amplicor version 2·0, Roche Molecular Systems, Pleasanton, CA; and an assembled HCV reverse transcription–polymerase chain reaction (RT–PCR), National Serology Reference Laboratory, Melbourne, Australia]. Confirmation of HCV infection in NAT yield samples required reactivity in at least two independent NAT assays or confirmed seroconversion, defined by reactivity in at least one antibody screening assay with an indeterminate/positive immunoblot.

HCV genotyping and viral load

Genotyping was performed using the Versant HCV (LiPA) genotype assay (Bayer Corporation, Ghent, Belgium). HCV RNA levels were determined using the Amplicor HCV Monitor v2·0 assay (Roche Molecular Diagnostics, Pleasanton, CA).

Alanine aminotransferase (ALT) testing

ALT activity was determined by using standard methods.

Table 1 Additional testing results for six NAT yield donations (when first identified on primary screening as NAT HCV RNA reactive and chemiluminescent immunoassay (ChLIA) non-reactive[a])

	Abbott PRISM Anti-HCV ChLIA		Murex HCV EIA		Immunoblot Anti-HCV reactivity[d]		HCV RNA Roche Amplicor 2·0	HCV RNA Assembled RT–PCR	HCV RNA Roche Monitor
Donor	S : CO[b]	Result[c]	S : CO	Result[c]	HCV antigen	Interpretation	Result	Result	IU/ml
1	0·07	NR	0·15	NR	None	Negative	R	Not tested	220 000
2	0·09	NR	0·2	NR	None	Negative	R	R	1 530 000
3	0·75	NR	≥ 4·14	R	c22(p)	Indeterminate[e]	R	R	Not tested
4	0·25	NR	≥ 4·14	R	c22(p)	Indeterminate[e]	R	R	850 000
5	0·34	NR	3·9	R	c22(p)	Indeterminate[e]	R	R	5 100 000
6	0·06	NR	0·22	NR	None	Negative	R	R	Not tested

[a]All samples were NAT reactive when initially screened in a pool of 24 donations using the Chiron Procleix HIV-1/HCV assay.
[b]Ratio of the sample to cut-off value (S : CO), defined according to the manufacturer's instructions.
[c]Result: NR, non-reactive where the S : CO is < 1·0; R, reactive where the S : CO is ≥ 1·0.
[d]All samples tested using the Ortho recombinant immunblot assay (RIBA) 3·0, except for sample 2 which was tested using the Genelabs Diagnostics HCV 3·0 blot.
[e]Indeterminate, defined as antibodies to HCV (anti-HCV) reactivity observed against one HCV antigen only with a reactivity of ≥ 1+ when compared with the manufacturer's controls.

Results

Donor samples reactive for HCV NAT, but ChLIA non-reactive (NAT-only yield)

Six donations were identified as HCV NAT reactive in the Procleix assay and non-reactive in the primary screening immunoassay (ChLIA), among 2 212 695 donations screened over the 28-month study period. All six were identified when tested in a 24-member minipool in the Procleix assay and all were positive when tested undiluted in the Roche Amplicor and assembled RT–PCR alternate NAT assays. Three of the six donations were reactive in the secondary immunoassay (EIA) and showed reactivity to the c22p (core) antigen in the RIBA (Table 1).

Follow-up testing of NAT-only yield donors

Results on serial follow-up samples for five of the six donors are shown in Tables 2 and 3 and in Fig. 1. Donor 1 (Table 2, Fig. 1) demonstrated a developing antibody response along with initially high, but a subsequently declining and eventually undetectable (in all but the assembled PCR assay), viral load. HCV antibody was first detected in the ChLIA between days 16 and 23, by which time the RIBA had become positive (c100 and c33), but the EIA remained non-reactive until days 35–52.

By day 35, RNA was undetectable in the two commercial NAT assays (but still detectable by the assembled PCR assay). It was again detectable on day 52 in all NAT assays, although only by single (not pooled) donor testing in the Procleix assay, and then not detectable in the following two blood samples in any of the NAT assays.

Donors 2 and 6 showed a similar serological pattern to donor 1, with anti-HCV detection by ChLIA occurring either before or around the same time as detection in the EIA, although donor 6 was lost to follow-up after returning only twice (Table 2). However, unlike donor 1, RNA was detectable in all follow-up bleeds from these two donors by the Procleix assay (viral load testing and assembled PCR were not performed on follow-up samples).

In contrast to donors 1, 2 and 6, two of the remaining NAT-only yield donors [4,5] showed anti-HCV reactivity by EIA and RIBA prior to the ChLIA. In fact, EIA and RIBA (c22p only) reactivities were detected at the index donations of both donors. Although the RNA levels of these two donors remained detectable throughout follow-up, there was a significant decline in viral load.

Summary of the seroconversion profiles

Table 4 summarizes the time to antibody seroconversion, as determined by the different assays, for five [1,2,4–6] of the six NAT yield donors, confirming that all were in the preantibody seroconversion phase at the time of their index donation.

Donor 3 was lost to follow-up, but the index donation, although negative in the ChLIA, was reactive by EIA and indeterminate on immunoblot, with reactivity to the c22(p) antigen only. This result profile was similar to that for donors 4 and 5, suggesting that donor 3 was also in the early seroconversion phase. The index donation of these three donors was non-reactive in the ChLIA, but reactive by EIA. In all three donors this early EIA reactivity was associated with reactivity to the c22(p) antigen on immunoblot.

Table 2 Follow-up testing for NAT yield donors 1, 2 and 6 (non-reactive in the supplemental Murex enzyme immunoassay (EIA) at the index donation)

Index and follow-up bleeds (days)	PRISM Anti-HCV ChLIA S : CO[a]	Murex Anti-HCV EIA S : CO[a]	Immunoblot Anti-HCV reactivity to HCV peptide[b]					Procleix S : CO[a]	Assembled PCR Result	HCV viral load IU/ml	Plasma ALT IU/ml[c]
			c100	c33c	c22(p)	NS5	Interpretation				
Donor 1											
0	0·07 NR	0·15 NR	Neg	Neg	Neg	Neg	Negative	*18·34 R*	Not tested	220 000	*97*
8	0·05 NR	0·16 NR	Neg	Neg	Neg	Neg	Negative	18·74 R	R	1500	*214*
16	0·47 NR	0·19 NR	1+	+/–	Neg	Neg	Indeterminate[c]	*21·16 R*	R	3800	*201*
23	*2·6 R*	0·52 NR	4+	3+	Neg	Neg	Positive	*19·58 R*	Not tested	8000	*136*
35	*2·5 R*	0·67 NR	4+	3+	Neg	Neg	Positive	0·92 NR	R	Not tested	29
52	*3·1R*	*1·55 R*	4+	3+	Neg	Neg	Positive	*18·01 R*	R	< 600	*68*
80	*4·0 R*	*≥ 4·27 R*	4+	3+	Neg	Neg	Positive	0·40 NR	NR	Not tested	31
150	*4·4 R*	*≥ 4·15 R*	4+	3+	Neg	Neg	Positive	0·19 NR	NR	Not tested	15
Donor 2[d]											
0	0·09 NR	0·2 NR	Neg	Neg	Neg	Neg	Negative	*25·4 R*	R	Not tested	Not tested
7	0·09 NR	0·2 NR	Neg	Neg	Neg	Neg	Negative	*22·5 R*	Not tested	1 530 000	40
14	0·1 NR	0·3 NR	Neg	Neg	Neg	Neg	Negative	*22·5 R*	Not tested	Not tested	*102*
21	0·07 NR	0·3 NR	Neg	Neg	Neg	Neg	Negative	*22·5 R*	Not tested	Not tested	*263*
28	0·32 NR	0·76 NR	Neg	+/–	1+	+/–	Indeterminate	*19·7 R*	Not tested	Not tested	*248*
35	*2·64 R*	*4·3 R*	+/–	+/–	2+	+/–	Indeterminate	*21·3 R*	Not tested	Not tested	*261*
42	*3·67 R*	*≥ 4·3 R*	2+	+/–	2+	1+	Positive	*22·5 R*	Not tested	Not tested	*497*
91	*6·62 R*	*≥ 4·7 R*	2+	+/–	4+	+/–	Positive	*20·4 R*	Not tested	Not tested	Not tested
Donor 6											
0	0·06 NR	0·22 NR	Neg	Neg	Neg	Neg	Neg	*22·92 R*	R	Not tested	31
6	0·08 NR	0·18 NR	Neg	Neg	Neg	Neg	Neg	*23·04 R*	R	Not tested	*97*
13	*1·7 R*	0·71 NR	Neg	1+	Neg	Neg	Indeterminate	*21·10 R*	Not tested	Not tested	*267*

[a]The sample to cut-off ratio (S : CO) was determined according to the manufacturer's instructions. Result: NR, non-reactive where the S : CO was < 1·0; R, reactive where the S : CO was ≥ 1·0.
[b]All samples were tested using the Ortho recombinant immunoblot assay (RIBA) 3·0, except for sample 2 which was tested on the Genelabs Diagnostics HCV blot.
[c]Indeterminate: defined as anti-HCV reactivity observed against one HCV antigen only with a reactivity of ≥ 1+ when compared with the manufacturer's controls.
[d]Several additional follow-up samples from this donor were not included in the table because they duplicated a profile already apparent in an earlier sample.
[e]Values higher than the reference range are shown in italics.
ALT, alanine aminotransferase; anti-HCV, antibodies to hepatitis C virus; ChLIA, chemiluminescent immunoassay; HCV, hepatitis C virus; PCR, polymerase chain reaction.

Table 3 Follow-up testing for NAT yield donors 4 and 5 [antibody reactive in the supplemental Murex enzyme immunoassay (EIA) at the index donation]

Index and follow-up bleeds (days)	PRISM Anti-HCV ChLIA S : CO[a]	Murex Anti-HCV EIA S : CO[a]	Immunoblot Anti-HCV reactivity to HCV peptide[b]				Procleix S : CO[a]	Assembled PCR Result	HCV viral load IU/ml	Plasma ALT IU/ml[d]
			c100	c33c	c22(p)	NS5	Interpretation			
Donor 4										
0	0·25 NR	≥ *4·14 R*	Neg	Neg	3+	Neg	Indeterminate[c]	*23·23 R*	R	850 000	*303*
16	*3·1 R*	≥ *4·14 R*	Neg	3+	3+	Neg	Positive	*17·97 R*	R	17 500	*1054*
23	*3·9 R*	≥ *4·13 R*	Neg	4+	4+	Neg	Positive	*20·72 R*	Not tested	< 600	*166*
Donor 5										
0	0·339 NR	*3·9 R*	Neg	Neg	3+	Neg	Indeterminate	*20·78 R*	R	5 100 000	*80*
7	0·963 NR	*4·2 R*	Neg	+/-	3+	Neg	Indeterminate	*21·05 R*	R	360 000	*127*
15	*3·415 R*	*3·9 R*	1+	2+	4+	Neg	Positive	*22·66 R*	R	730 000	*139*
21	*4·614 R*	*4·132 R*	2+	3+	4+	+/-	Positive	*24·32 R*	Not tested	Not tested	*407*
35	*4·245 R*	*3·716 R*	2+	2+	4+	Neg	Positive	*21·71 R*	Not tested	2/1 000	*205*
56	*5·283 R*	*3·86 R*	3+	4+	4+	Neg	Positive	*21·96 R*	Not tested	219 000	*523*

[a]The sample to cut-off ratio (S : CO) was determined according to the manufacturer's instructions. Result: NR, non-reactive where the S : CO was < 1·0; R, reactive where the S : CO was ≥ 1·0.

[b]All samples were tested using the Ortho recombinant immunoblot assay (RIBA) 3·0.

[c]Indeterminate: defined as anti-HCV reactivity observed against one HCV antigen only with a reactivity of ≥ 1+ when compared with the manufacturer's controls.

[d]Values higher than the reference range are shown in italics.

ALT, alanine aminotransferase; anti-HCV, antibodies to hepatitis C virus; ChLIA, chemiluminescent immunoassay; HCV, hepatitis C virus; PCR, polymerase chain reaction.

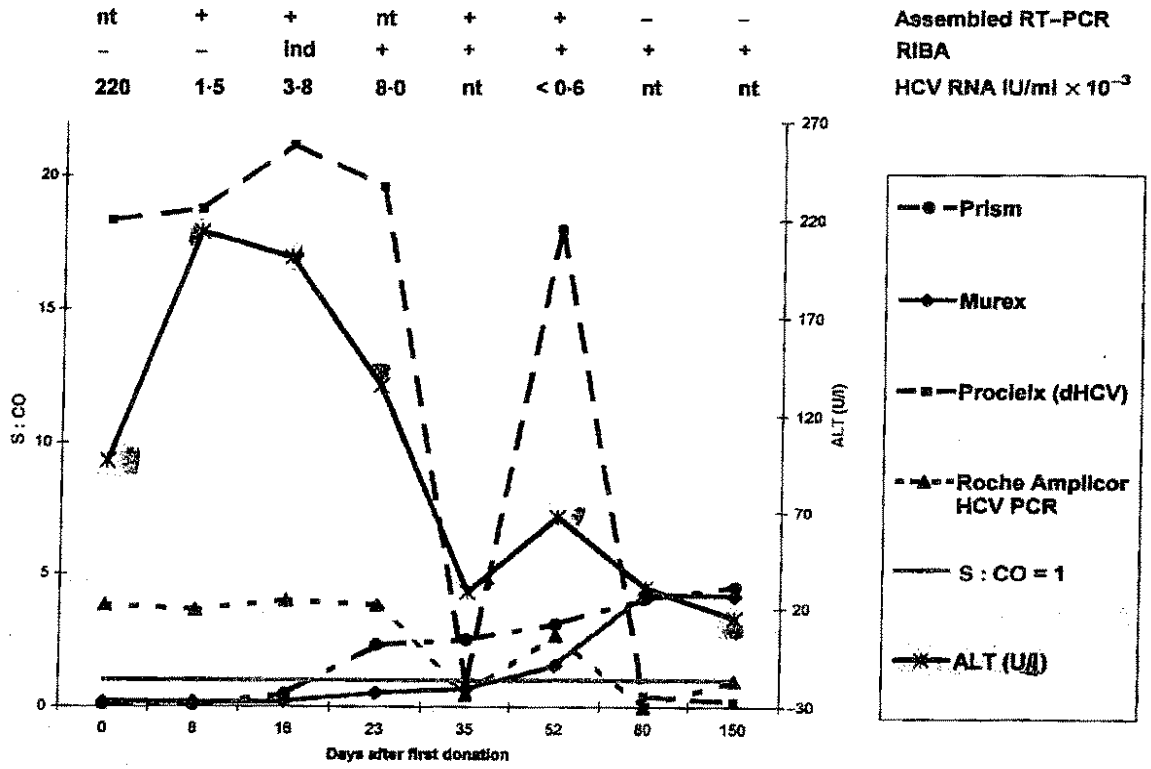

Fig. 1 Hepatitis C virus (HCV) serology profile for donor 1 following the index donation, which was non-reactive for antibodies to HCV (anti-HCV) and reactive for HCV RNA. The figure shows the sample to cut-off (S : CO) ratios, where values ≥ 1 indicate that the sample was reactive for the respective test. Corresponding plasma alanine aminotrasferase (ALT) levels are also indicated. Note the declining RNA levels from the index donation (day 0) to non-detectable levels (non-reactive) on day 35 by the commercial Procleix and Roche nucleic acid amplification technology (NAT) assays. At this time-point, anti-HCV were detected only in the chemiluminescent immunoassay (ChLIA). The Murex enzyme immunoassay (EIA) detected anti-HCV on day 52, when HCV RNA also reappeared before decreasing again.

Table 4 Comparison, by assay, of time to first reactivity (in days) from index donation for all serological assays

Donor	ChLIA Abbott PRISM	EIA Murex	Ortho RIBA[a] c100	c33c	c22(p)	NS5	HCV genotype
3	Not available	1					3a
4	1–16	1		16	1		3a
5	7–15	1	15	7	1	21	3a
6	6–13	> 13		13			1
1	15–22	34–53	15	15[b]			2b
			Genelabs HCV blot[a] NS3	Core	NS5	NS4	
2	28–35	28–35	35	28	28[c]	28	3a

[a]All samples tested using the Ortho recombinant immunoblot assay (RIBA) 3·0, except for sample 2 which was tested using the Genelabs Diagnostics hepatitis C virus (HCV) blot.

[b]On day 15, reactivity against c100 was '1+' and against c33c was +/–, therefore interpretation was 'indeterminate'.

[c]On day 28, reactivity was defined as indeterminate because the reactivity against core antigen was 1+, whilst against C33c and NS5 it was only +/–.

ChLIA, chemiluminescent immunoassay; EIA, enzyme immunoassay.

Discussion

This is one of few studies to describe the comprehensive follow-up of HCV NAT-only yield donors and reveals a number of important features about the pre- and early seroconversion phases of HCV infection. Furthermore, it highlights the efficacy of a testing strategy based on alternate HCV RNA and antibody assays in rapidly assessing the HCV-infection status of suspected NAT yield donors.

At least five of the six HCV NAT-only yield donors were either in the preantibody or early seroconversion phases of HCV infection when donating. None of the five donors had immunosilent or immunovariant viral infections, a finding consistent with other reports indicating that the majority of NAT-only yield donors are in the preseroconversion phase [4,5]. A sixth donor had also mounted a limited HCV antibody response at the index donation but was not available for further follow-up tests to monitor the subsequent antibody response.

Although all six NAT-only yield donors had high viral titres at their index donation, donor 1 showed a subsequent decrease in viral load around the time of antibody seroconversion, predictable if the immune response is effectively reducing the viral load. This observation raises the possibility that a donor in the early seroconversion phase of HCV infection could present with RNA levels undetectable by pooled NAT testing and without detectable antibody. Therefore, pooled NAT testing, in combination with antibody screening, may not completely eliminate the risk of HCV transmission from early seroconverting donors. Subsequent to seroconversion, the RNA levels of this donor further declined, fluctuating above and below detectable levels, although antibody remained detectable. This phenomenon has been reported recently [18] in pooled NAT-negative, HCV antibody-positive donors and has important implications for the maintenance of both HCV RNA and antibody screening. During the short time course that donor 1 was studied, there were points when only NAT and subsequently only serological testing detected markers for ongoing viral infection.

It is interesting to note that in the four NAT-yield donors where the EIA detected antibody earlier than or in the same bleed as the ChLIA, the initial antibody response was directed against the c22(p) antigen, as indicated by the immunoblot results. In contrast, for the two donors where the ChLIA detected antibody earlier than the EIA, the first antibodies detected by immunoblot were directed against the c33c or c100 antigens. These results suggest that the specificity of the antibody response against the HCV antigens in the early seroconversion phase of HCV infection can vary between infected donors and this may, in turn, result in sensitivity differences between antibody-screening immunoassays in early seroconverting donors.

Finally, the study demonstrates the utility of a confirmatory strategy combining carefully selected primary and secondary NAT and antibody assays. The secondary NAT assays accurately confirmed HCV infection (based on subsequent seroconversion or antibody detection by alternate immunoassay in the index sample) in all six donors before anti-HCV was detected by either, or both, immunoassays (ChLIA or EIA). The secondary alternate immunoassay assay detected antibody in three of the six donors, at the index donation, an unexpected but valuable finding that increased confidence in the specificity of the NAT results.

In conclusion, our study confirms previous observations that the majority of HCV NAT yield donors are in the preseroconversion phase of infection. Furthermore, follow-up of one yield donor demonstrated fluctuating HCV RNA levels after seroconversion, undetectable at times even with all but the most sensitive individual-donation NAT. This observation reinforces the case for maintaining both NAT- and antibody-screening assays for HCV, as either alone may fail to detect such potentially infectious donations. Finally, the ARCBS testing strategy for follow-up of suspected yield donors revealed that the use of both a primary and an alternate anti-HCV immunoassay was useful in rapidly confirming seroconversion and therefore the specificity of the NAT result in suspected yield samples, leading to timely resolution of the donor's HCV-infection status.

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