

Effectiveness of the HCV blood screening strategy through eighteen years of surveillance of HCV infection in blood donors in France

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Background - The question of maintaining blood screening based on both Hepatitis C virus (HCV) infection antibodies (Ab) and Nucleic Acid Testing (NAT) has been raised in several countries. The French blood donor surveillance database was used to address this issue.

Materials and methods - In France, HCV-NAT was implemented in mini pools (MP) in 2001 and in individual testing (ID) in 2010. HCV-positive donations are further investigated including detection of RNA with an alternative polymerase chain reaction assay: AmpliCor HCV v2.0 (Roche; LOD95 50 IU/mL) from 2001 to 2006 and CobasTaqMan (CTM) HCV 2.0 assay (Roche; LOD95 9.3 IU/mL) since 2007.

Results - From 2001 to 2018, 3,058/48.8 million donations were confirmed HCV positive: 64.4% were Ab+/NAT+, 35.1% Ab+/NAT- and 0.5% Ab-/NAT+. From 2001 to 2018, the NAT yield decreased from 0.65 per million donations to 0, and NAT+ donations dropped from 77% to 46% of the total of HCV donations. 2,491/3,058 were further tested for HCV-RNA: 1,032 (816 NAT+, 216 NAT-) with AmpliCor and 1,459 (897 NAT+, 562 NAT-) with CTM. Four (3 MP and 1 ID-NAT, 0.5%) of the 778 NAT negative donations had low viral loads.

Discussion - The decline in HCV-NAT yield cases raises the question of the relevance of NAT. Conversely, the increase in Ab+/NAT- donors, suggesting a growing number of resolved infections, argue for Ab discontinuation. In our experience, at least 0.5% of Ab+/NAT- donations had low RNA level when retested. Although the risk of viral transmission by such donations is probably low, the uncertainty associated with their infectivity goes against the removal of Ab in blood screening in our country.

Keywords: HCV, blood donations, NAT, viral load.

INTRODUCTION

Hepatitis C virus (HCV) infection is highly prevalent worldwide with more than 70 million infected people, for a global prevalence of 1.0%¹. Chronic hepatitis, cirrhosis and hepatocellular carcinoma are severe complications of HCV infection and are responsible for approximately 350,000 deaths per year worldwide². HCV is mainly transmitted by

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blood and people particularly at risk of infection are injection drug users (IDU) and subjects with transfusion history before the implementation of the screening for anti-HCV antibodies (Ab) in blood donations in the early '90s². Alongside Ab screening, nucleic acid testing (NAT) has been implemented in the blood-bank setting of many high-resource countries to further prevent HCV transmission by transfusion with the main objective of compensating for the failure of serological assays during the window period³. In low-income areas, where NAT is not affordable, Ag/Ab combination assays have proved their efficacy in reducing the residual risk⁴ by closing the window period from 37 to 45% when compared to Ab assays^{5,6}.

It is known that 20-40% of people who acquire HCV infection spontaneously resolve their infection keeping anti-HCV Ab in their plasma as a marker of past infection^{7,8} while some of them exhibit seroreversion⁹. Moreover, anti-HCV treatment is able to cure more than 95% of chronic HCV infection^{10,11} leading the World Health Organization (WHO) to recommend treatment with pan-genotypic direct-acting antivirals to all individuals >12 years of age diagnosed with HCV infection, irrespective of disease stage; this aims to reduce the number of deaths by two-thirds and to increase treatment rates to up to 80% by 2030¹². Thus, in the future, more and more HCV infected subjects will be expected to be non-viremic due to natural or anti-viral drug induced recovery.

This raises the question of maintaining antibody in HCV blood screening once highly sensitive NAT has been introduced, particularly in high prevalence countries where maintaining an adequate blood supply could be challenging. Moreover, as reported by Bruhn *et al.* the incremental safety provided by serological testing is minimal once ID-NAT has been introduced¹³. We have previously shown that 1.1% of anti-HCV Ab positive blood donations collected in France were RNA negative when tested in mini-pool NAT but retrospectively positive with individual (ID) NAT¹⁴. Based on another approach consisting of multiple replicate ID-NAT tests, El Kiaby *et al.* reported that, in Egypt, 1.1% of initially ID-NAT negative/Ab positive blood donations were later found to be RNA positive¹⁵.

The virological and epidemiological surveillance of HCV infection in the French blood donor population introduced in 1990 is performed in partnership with the National Reference Centre (NRC) for transfusion infectious risks,

the National Blood Service (*Etablissement Français du Sang* - EFS), the Army Blood Service (*Centre de Transfusion des Armées* - CTSA), and the National Public Health Agency (*Santé publique France* - SpF). We use information gathered from the database from this extensive surveillance focusing on donations collected after the introduction of HCV-NAT to investigate the effectiveness of the current HCV blood screening strategy in France and to suggest what should be taken into account when considering a potential evolution.

MATERIALS AND METHODS

HCV screening in blood donations

In France, blood donations are tested for anti-HCV Ab with Prism HCV assay (Abbott, Rungis, France) in mainland France, ARCHITECT HCV assay (Abbott) in overseas territories, and Ortho HCV 3.0 ELISA TEST (Ortho Clinical Diagnostics, Raritan, New Jersey, NJ, USA) at the CTSA. Confirmation assay (InnoLIA HCV score, Innogenetics Gent, Belgium) is systematically applied in repeatedly Ab reactive samples to exclude false-positive antibody reactivity. Donations are considered as HCV Ab positive when reactive with both assays. HCV-NAT was introduced in July 2001 in mini pools (MP): 60% of blood donations were screened in 24-sample MP using Nuclisens Extractor/Ampliscreen Cobas ([Roche, Branchburg, NJ, USA] LOD 95%: 13 IU/mL per donation), and 40% of blood donations were screened in 8-sample MP using Procleix HIV-1/HCV assay ([Chiron Blood Testing, Emeryville, CA, USA] LOD 95%: 15 IU/mL per donation)¹⁶, except overseas and at the CTSA where it was performed individually. Individual NAT with Procleix Ultrio HIV-1/HCV/HBV assay ([Grifols, Barcelona, Spain] LOD95%: 7.5UI/mL)¹⁷ was progressively implemented in 2010 and all blood donations have been individually tested since 2014.

Investigations on HCV-positive blood donations

The NRC for transfusion infectious risks has a large volume of plasma for each positive donation to complete viral investigations. In HCV-positive blood donations, detection of RNA was performed using Amplicor HCV (Roche, LOD 50%: 50 IU/mL) from 2001 to 2006, and the plasma viral load (VL) was determined with Cobas TaqMan (CTM) HCV test 2.0 assay (Roche; LOD95%: 9.3IU/mL LOQ95%: 25 IU/mL) from 2007.

Hepatitis C virus epidemiological surveillance

Blood donor surveillance is conducted on a national level, based on questionnaires gathering data on the whole blood donor population (total number of donations, donor status [first-time/repeat], gender, age), and on demographic characteristics and risk factors identified during the post-donation interview of each donor found positive for viral infections. These demo-epidemiological data are matched with virological results obtained at the NRC to provide a global analysis.

The HCV positivity rate is defined as the ratio between the number of positive donations over the number of donations tested. It was calculated for first-time, repeat and all blood donors, respectively.

RESULTS

In 2018, the HCV positivity rates were 1.70, 0.04 and 0.26, per 10,000 donations in first time, repeat, and all blood donors, respectively. These figures were 5 times lower than in 2001 (Table I).

From July 2001 to December 2018, among 48.8 million donations tested, 3,058 were confirmed HCV positive: 1,970 (64.4%) were Ab and NAT positive, 1,073 (35.1%) were Ab positive but NAT negative, and 15 (0.5%) were NAT-only positive. Interestingly, the proportion of NAT positive donations declined over time from 77% in the period 2001-2003 to 46% in 2016-2018 (Figure 1). NAT screening alone prevented 1-3 donations per year from 2001 to 2010 while only one was further identified NAT positive only in 2015, corresponding to a drop in NAT yield cases from 0.65 per million donations tested in the period 2001-2003 to 0 in 2016-2018 (Figure 1).

Of the 3,058 HCV positive donations collected during the 18-year study period, 2,491 (81.5%) could be further tested for the presence of HCV-RNA with a polymerase chain reaction (PCR) assay: 1,032 (816 MP-NAT positive and 216 MP-NAT negative) with Amplicor and 1,459 (436 MP-NAT and 461 ID-NAT positive, and 200 MP-NAT and 362 ID- NAT negative) with Cobas Taq Man. The results were in agreement with NAT screening

Table I - Rate of HCV positive donations (per 10,000) in first time, repeat, and all blood donors in France from 2001 to 2018

Year	First time blood donors		Repeat donors		All donors	
	N. Positive donations	Positive rate per 10,000 donations	N. Positive donations	Positive rate per 10,000 donations	N. Positive donations	Positive rate per 10,000 donations
2001	322	8.00	43	0.21	365 ¹	1.51
2002	262	7.20	27	0.13	289	1.17
2003	290	7.60	16	0.08	306	1.24
2004	227	6.02	28	0.13	255	1.02
2005	210	5.61	14	0.07	224	0.89
2006	188	4.95	17	0.08	205	0.80
2007	178	4.06	14	0.06	192	0.71
2008	182	3.43	19	0.08	201	0.71
2009	181	3.23	13	0.05	194	0.65
2010	103	2.19	15	0.06	118	0.39
2011	124	2.41	16	0.06	140	0.44
2012	129	2.64	11	0.04	140	0.46
2013	109	2.42	8	0.03	117	0.42
2014	120	2.67	7	0.03	127	0.45
2015	101	2.48	9	0.04	110	0.37
2016	74	2.03	12	0.05	86	0.29
2017	84	2.20	15	0.06	99	0.33
2018	66	1.70	9	0.04	75	0.26

¹185/365 were not tested with Nucleic Acid Testing (NAT) (implemented on the 1st July 2001). HCV: hepatitis C virus.

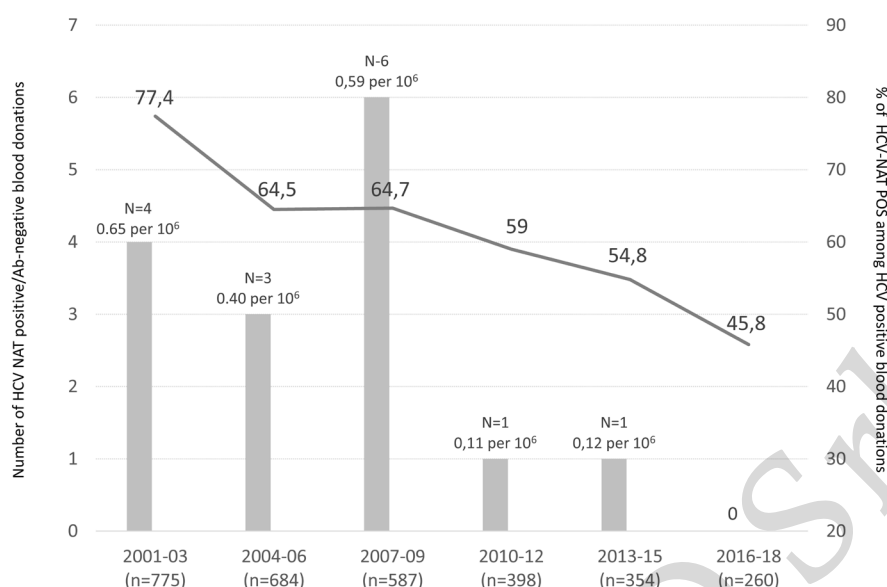


Figure 1 - Results of HCV-NAT in France between 2001 and 2018

Percentage of HCV-NAT positive (black line) among all HCV positive donations. Number of HCV-NAT yield donations (grey bars), over three-year periods of time.

with an overall concordance of 99.8% and 99.5% for NAT-positive and NAT-negative donations, respectively (Table II). However, 7 samples were discordant: 3 were NAT screening positive (2 with ID-NAT and 1 with 24 MP-NAT) and negative with the Amplicor assay, and 4 were NAT screening negative (3 with 24-MP-NAT and 1 with ID-NAT) and positive with CTM (Table III). One of these latter samples was collected from a 52-year-old first-time donor who disclosed at the post-donation interview a chronic HCV infection treated one year before the donation.

Three of the 4 NAT negative/CTM positive discordant samples have been retrospectively tested individually in triplicate with Procleix assay and resulted negative.

Among the 2,491 donations investigated, 901 were tested for plasma VLs ranging from 1.38 to 7.67 log IU/mL (median: 5.88 log IU/mL). Eight donations (0.9%) had a VL <2 log IU/mL, including the 4 NAT-negative/CTM positive samples, and 4 NAT-positives showing a very low VL (2 <LOQ VL <1.39 UI/mL, including one Ab negative and 2 with a VL of 1.8 log IU/mL) (Figure 2).

Table II - Results of HCV-RNA detection with an alternative PCR assay in HCV positive blood donations according to NAT screening results

Period	NAT screening ¹	HCV-PCR assay ²	NAT positive								NAT negative									
			Total	HCV-PCR assay result								Total	HCV-PCR assay result							
				Tested		Negative		positive		Tested			Negative		Positive					
				N.	%	N.	%	N.	%	N.	%		N.	%	N.	%				
2001-2006	MP	Amplicor	1,048	816	77.9%	3	0.4%	813	99.6%	411	216	52.6%	216	100%	0	0%				
2007-2010	MP	CTM	454	436	96%	0	0%	436	100%	251	200	79.7%	197	98.5%	3	1.5%				
2011-2018	ID	CTM	483	461	95.5%	0	0%	461	100%	411	362	88.1%	361	99.7%	1 ³	0.3%				
Total			1,985	1,713	86.3%	3	0.2%	1,710	99.8%	1,073	778	72.5%	774	99.5%	4	0.5%				

¹MP: mini pools of 24 donations (Nuclisens Extractor/Ampliscreen Cobas Roche, LOD 95%: 13 IU/mL per donation) or mini pools of 8 donations (Procleix HIV-1/HCV assay, Chiron Blood Testing, LOD 95%: 15 IU/mL per donation); ID: individual testing (Procleix Ultrio, LOD95 7.5 IU/mL). ²Amplicor HCV Roche, LOD 95 50 IU/mL, Cobas Taq Man HCV Roche (CTM), LOD 95 9.3 IU/mL - LOQ 95 25 IU/mL. ³Three times negative when retested individually with Procleix Ultrio. HCV: hepatitis C virus; NAT: nucleic acid testing; PCR: polymerase chain reaction.

Table III - Anti-HCV positive blood donations with discrepant results between NAT screening and HCV PCR assay

Sample	Year of screening	NAT testing	Result	Anti-HCV	HCV-RNA assay	Result	Viral load (IU/mL)	Procleix Ultrio Tigris (ID-NAT)
1	2004	Individual	Positive	Positive	Amplicor	Negative	Not applicable	Not tested
2	2006	Individual	Positive	Positive	Amplicor	Negative	Not applicable	Not tested
3	2006	Pool ×24	Positive	Positive	Amplicor	Negative	<25 ¹	Not tested
4	2007	Pool ×24	Negative	Positive	CTM	+/+	<25	-/-/-
5	2008	Pool ×24	Negative	Positive	CTM	+/+/-	<25	Not tested
6	2008	Pool ×24	Negative	Positive	CTM	+/+	<25	-/-/-
7	2015	Individual	Negative	Positive	CTM	+/+/-	<25 ²	-/-/-

¹Positive when retested with CobasTaqMan (CTM; Roche). ²Donor treated in 2014. ID: individual testing. HCV: hepatitis C virus; NAT: nucleic acid testing; PCR: polymerase chain reaction.

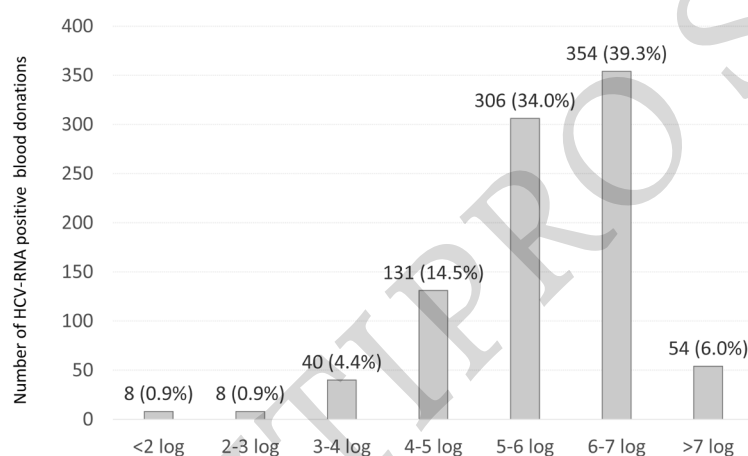


Figure 2 - Number and percentage of HCV-RNA positive donations according to the viral load (log IU/mL) among 901 viremic blood donors from 2007 to 2018
HCV: hepatitis C virus

DISCUSSION

In France, the overall HCV positivity among blood donations in 2018 (0.26/10,000) was 5 times lower than in 2001, with a rate 42 times higher in first-time donors than in repeat donors. This rate is one of the lowest reported in high-income countries over the same period¹⁸⁻²⁰. Since the implementation of NAT screening in July 2001, 0.3 per million donations (15 in 48.8 million) have been found HCV-NAT-only positive. However, in spite of the introduction of a highly sensitive individual NAT procedure in 2010, NAT yield was 14 times lower in the period 2011-18 (1 in 23.5 million donations, i.e. 0.04 per million) than in 2001-2010 (14 in 25.3 million donations, i.e. 0.55 per million). Of note, the rate of HCV-NAT only positive is very low in France when compared to other

European countries. The most recent data show highest numbers of new HCV infections in Germany (1.3 per million)²¹, in Italy (1.5 per million)¹⁸, and in Poland (7.2 per million)²². In the first years of NAT testing, our results are in the same order of magnitude as observed in the UK in the 1996-2003 survey period (0.8 per million)²³. Given that NAT performance is currently similar between countries, the reason for these differences seems most likely related to the differences in donor epidemiology itself. The low incidence of HCV in blood donors and, therefore, the extremely low residual risk of HCV transmission by blood (estimated in France at 1 in 34 million donations over the period 2016-2018)²⁴ limit the benefit associated with the additional implementation of donor screening by NAT. Conversely, the proportion of viremic anti-HCV

positive donors, which declined by 40% from 2001 to 2018, suggesting that an increasing number of blood donors spontaneously resolved a past infection, raises the issue of maintaining anti-HCV screening. This issue is of particular concern in blood settings where maintaining an adequate blood supply is challenging.

In our study, we observed that 0.5% (4/778) of blood donations which were confirmed positive for anti-HCV and initially non-reactive with NAT were found RNA positive with an extremely low VL. Unsurprisingly, the proportion of missing samples was the highest (1.5%, 0.26 per million donations) when NAT testing was carried out in mini pools (vs 0.3%, 0.04 per million donations in the ID NAT screening period) and when the PCR assay was the most sensitive (Table II). Moreover, the Amplicor assay, used between 2001 and 2006 probably failed to detect some NAT negative donations with low VLs due to its low sensitivity level in contrast to the most recently developed assays that have been improved to accurately quantify all HCV genotypes²⁵⁻²⁷. On the other hand, we cannot rule out the possibility that some ID-NAT tested negative donations which were further tested negative with CTM would have been positive if tested in multiple replicates with ID-NAT, as reported by Elkiaby *et al.*¹⁵. Accordingly, the number of donations with low-levels of HCV RNA going undetected with NAT was probably underestimated, with the highest frequency in the period 2001-2006. Conversely, it could be that the 4 NAT negative/CTM positive cases were HCV-RNA false positive, although this is unlikely since these samples were repeatedly positive with CTM.

Our study also showed that the vast majority of viremic blood donors had high HCV viral loads that cannot be missed by NAT screening assay even though performed in MP, and that <1% (n=8) had a HCV-RNA level <2 log IU/mL. In the worst-case scenario, in which anti-HCV testing would be replaced by ID-NAT alone, assuming that NAT could fail to detect these 8 donations, one RNA positive in 4.4 million tested donations (8/35 million donations in the 2007-2018 study period) would have entered the blood supply.

Although extremely low, the risk of missing donations with extremely low HCV-RNA level may occur, as observed here and as recently reported in the US by Proctor *et al.*²⁸ whose study showed 0.34% HCV-RNA reactivity in 1,161 anti-HCV positive/MP-NAT negative blood donations when retested in replicate with ID-NAT. Discontinuing anti-HCV

screening, if considered, would raise the question of the infectivity of such donations. Animal experiments have shown that a 1.2 copies/mL concentration from plasma collected at the very early phase of infection is infectious²⁹ whereas plasma from the chronic phase may be 100-fold less infectious than in acute phase^{30,31}, probably due to the presence of neutralising antibodies which may decrease infectivity. Retrospective studies carried out on patients who were transfused with anti-HCV positive components also showed that the virus transmission can occur below the detectability of even the most sensitive HCV RNA assays, but with a very low frequency³². Nowadays, the absence of infectivity of anti-HCV positive donations with very low HCV-RNA levels undetectable by current NAT assays has not been established with any certainty. Thus, it seems premature to modify the HCV screening strategy. Moreover, at least in France, the removal of anti-HCV testing would not have a substantial impact on the blood supply given that less than 40 donations per year would be saved compared to a total of about 3 million donations collected.

CONCLUSIONS

Through the systematic viral surveillance implemented in blood donors, we have estimated that approximately 0.5% of anti-HCV positive but NAT negative donations in France had low RNA levels. Even though this rate was probably underestimated, the occurrence of such a situation remains exceptional. Although the risk of HCV transmission through these donations is probably extremely low, the uncertainty of their infectivity and the small number of donations that would be saved if anti-HCV screening were to be discontinued, do not justify changing the current strategy in our country. Nevertheless, the question remains open in countries with high HCV prevalence and a shortage of blood products where replacing anti-HCV screening with highly sensitive HCV-NAT^{33,34} may be considered. However, further investigation to ensure the safety of anti-HCV positive/NAT negative donations and a cost-benefit assessment of such a measure would assist the decision making process.

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AUTHORSHIP CONTRIBUTIONS

SLa, DC and PC are responsible for study design and drafting the manuscript. EG, CM and SLe are responsible for data acquisition. PG and PM collected the data. JP, LB, RC and QL interpreted the data. EG, CM, PM, JP, LB, RC and QL revised and approved the manuscript.

The Authors declare no conflict of interests.

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