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Large-Scale HCV RNA Screening in First-Time Blood Donors: The First Step Towards Genomic Screening of Blood Donations

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on behalf of the HCV RNA Screening Study Group[1]

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Abstract

Background and Objectives: Individual genomic screening for viruses in blood donations is becoming increasingly pressing as an alternative to pool testing to improve the safety of the blood supply. ***Materials and Methods:*** To determine the feasibility and, possibly, efficacy of genomic screening for hepatitis C virus (HCV) in the blood service setting, a representative population of first-time blood donors was screened individually with a semi-automated genomic amplification assay for HCV RNA. First-time blood donors in two blood centres in the United Kingdom were screened in parallel for anti-HCV and HCV RNA by RT-PCR. ***Results:*** 8,417 serum samples were screened. A 99.95% specificity was observed and one anti-HCV-positive, HCV-RNA-positive donation was found. No seronegative HCV-RNA-positive donations were detected. ***Conclusions:*** These results are consistent with the low prevalence of HCV infection in blood donors from the London area and demonstrate the high level of performance of the individual genomic screening method used in this study. When fully automated, such a method would be a highly suitable candidate for routine, automated genomic screening of HCV and, subsequently, of other pathogenic blood-borne viruses.

Introduction

Genomic screening for infectious agents, particularly viruses, became possible with the availability of several amplification techniques such as the polymerase chain reaction (PCR), ligase chain reaction nucleic acid sequence-based amplification and others. Such techniques have been applied to research, then to diagnosis of genetic and infectious disease [1]. Technologies for large throughput screening, such as that required for blood donation screening, are not yet available, although they are being rapidly developed. However, recent regulations originating from plasma derivative control agencies in the European Union will mandate that, by the 1st July 1999, all final pools of plasma for fractionated therapeutic products should be devoid of detectable hepatitis C virus (HCV) RNA.

This regulation has generated the development of various strategies to meet the deadline. Plasma fractionators as well as transfusion organisations developed genomic detection of HCV in pools of plasma samples [2]. Unpublished results show that a large proportion of positive results can not be resolved by the identification of an implicated individual donor, suggesting that poor specificity can be additional to the decreased sensitivity inherent to pooling [2–4].

Genomic detection in individual donations has received little attention. A few groups, however, developed techniques that reduced the testing time to less than 8 h in a format compatible with the current workflow of blood screening and with automation [5, 6]. In the first phase of our

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developmental studies, we wished to test the practical feasibility of rapid throughput HCV RNA detection as a preliminary step to an automated detection in routine blood screening and an alternative to the pool-testing approach.

Materials and Methods

Study Design

HCV RNA screening of random first-time blood donors was performed in parallel with anti-HCV screening in two blood centres which form part of the London and South East Zone of the National Blood Service. One collecting centre was based at the East Anglia Blood Centre, Cambridge; the other at the North London Blood Centre, Colindale, London. During the study period (June 1996 to August 1997), a representative sample of the total first-time donor population in each blood centre received an information sheet concerning the study. Upon verbal agreement, each donor had a special blood sample drawn at the end of the regular donation. Within 12 h, samples kept at 4 °C arrived at the HCV RNA testing site (East Anglia Blood Centre) and were processed. Blood components derived from the donations were quarantined for a maximum of 24 h from the time of donation, until the initial HCV RNA result was added electronically to the result of the mandatory anti-HCV screening assay. Components derived from units negative for both assays were released; components from units initially positive with the HCV RNA assay were maintained under quarantine for another 24 h until the result of the repeat HCV RNA testing was available. Components from units with non-repeatable results were released, and those repeatably positive with the HCV RNA assay were discarded.

Sample Collection

For the purpose of the study, an additional 6 ml dry vacutainer tube was collected from donors identified as first-time blood donors by standard interview. Depending on the collecting blood centre, random first-time donors were included in the study during either morning or evening collection sessions.

In the first collection site (Cambridge), 87% of eligible donors were selected during the 3 days per week allocated to the study for sample collection, representing approximately 50% of the total population of first-time donors. In the second collection site (North London), these percentages were approximately 20 and 12%, respectively. The number of samples meeting the selection criteria ranged between 40 and 90 per day (average 49).

HCV Antibody Screening

HCV antibodies were screened with routine third-generation enzyme immuno-assays using the Abbott (anti-HCV 3.0, Abbott Laboratories, North Chicago, Ill., USA) and Ortho (anti-HCV enzyme-linked immunosorbent assay, Ortho Diagnostics, Raritan, N.J., USA) in Cambridge and North London, respectively. Repeatably reactive sera were tested with RIBA 3 (Ortho Diagnostics) for confirmation. All assays were used according to the manufacturer's instructions.

HCV RNA Screening

All samples were tested with a previously described semi-automated method [5]. Briefly, HCV RNA was captured from 105 µl of serum through the poly U tract present at the 3′ end of the HCV genome by hybridisation to a 40-nucleotide biotinylated poly A sequence bound to streptavidin-coated polystyrene magnetic microparticles (SPMP, Promega Corporation, Madison, Wisc., USA). The captured RNA was eluted with a mixture of water and DMSO and added to a predispensed mix containing reverse transcriptase, *Taq* polymerase and the external pair of primers for the first step of the nested PCR. One microlitre of the first amplification product was used for the second amplification which included the doubly fluorophor-labelled Taqman probes (Perkin-Elmer). Fluorescence was read on the LS 50B fluorimeter (Perkin-Elmer). One blank (PCR buffer) and three no-template controls were included in each run in order to automatically calculate results with the provided software. Two positive and two negative controls were also included in each run to monitor the assay performance. Results were available within 6.5 h of sample reception in the laboratory.

Initially positive samples were retested in the assay run performed the following day in two separate aliquots, while the corresponding blood components remained quarantined. If at least one of the retested aliquots was positive, an additional aliquot was tested with the commercially available HCV Amplicor (Roche Molecular System, Branchburg, N.J., USA). To limit-cross-contamination at every step of the procedure, we strictly observed the precautionary measures recommended by Kwok and Higuchi [7]. The main steps of the procedure were performed in three separate rooms.

Table 1. HCV RNA screening in 8,417 samples from first-time blood donors

	Number of positive samples		
	Cambridge site	North London site	total
Initially positive	7 (0.250)	27 (0.481)	34 (0.40)
Repeatably positive	1 (0.036)	3 (0.053)	4 (0.048)
Confirmed positive[a]	0	1 (0.018)	1 (0.012)
Specificity (%)	99.96	99.95	99.95

Figures in parentheses are percentages.

[a]Confirmation was performed by an alternative in-house HCV RNA amplification method and by Amplicor HCV on both the initial sample and a second sample drawn from the recalled donors.

Results

The semi-automated screening assay for HCV RNA by genomic amplification used in this study of 8,417 first-time blood donors, yielded 0.40% initially positive and 0.047% repeatedly positive serum samples (table 1). Three of the four repeatedly positive samples could not be confirmed with two in-house assays [5], nor with the commercial HCV Amplicor kit applied to the initial sample and to a separate blood sample drawn when the donors were recalled. These three samples were therefore considered false-positive reactions. The fourth HCV-RNA-positive sample was con-

Table 2. Predicted and actual HCV RNA in sera from first-time blood donors

	Estimated number of HCV-RNA-positive donations detected by PCR testing per 10^4 donations			
	predicted[a]	95% confidence interval	observed	95% CI
HCV antibody positive	6.4	5.5–7.3	1.0	0.031–6.9
HCV antibody negative	0.021	0.0045–0.143	0	0–4.6

[a] Data provided by Barbara and Soldan [8] from the National Registry of the National Blood Authority.

firmed with Amplicor. It was also the only anti-HCV confirmed positive sample in the study. The specificity of the HCV RNA screening assay was 99.95%, irrespective of the blood centre in which the samples were collected. The prevalence of HCV antibody or RNA confirmed positive was lower than expected (table 2). This low prevalence explains why the pre-study estimate of 1–5 samples HCV RNA positive and antibody negative was not corroborated by the clinical data. The observed 0.01% prevalence of HCV infection in first-time donors lies well below the observed rate in England and Wales during 1996 (0.064%, 95% confidence interval: 0.055–0.073) [8].

Discussion

HCV transmission constitutes one of the main theoretical residual risk of posttransfusion viral infections. It is to a large extent related to the 10-week window period separating infection from HCV antibody detection with current screening enzyme immuno-assays and the presence of rare cases of viraemia without detectable antibodies [10, 11]. The risk of a repeat donor giving blood during an HCV infectious window period in the USA has been estimated as 1 in 103,000 [12]. The risk of an HCV infectious donation entering the blood supply in England has been estimated as 1 in more than 200,000 [13] (confidence interval 43,000–470,000).

When dealing with an event of very low frequency, predicted outcomes of proposed new assays, such as reverse transcription and amplification of HCV cDNA, are difficult to verify with clinical trial data because of the magnitude of the necessary studies. As demonstrated by the US experience of introduction of HIV antigen screening, large discrepancies between projected and actual figures may be obtained [14].

To reduce the size of our study, we chose to sample from first-time donors because of the expectation that such donors would have a higher prevalence of blood-borne infections than repeat donors, and because the prevalence of anti-HCV, and therefore the probability of detecting an HCV infection missed by donation antibody testing, is over five times higher in first-time donors than in repeat donors [Barbara and Soldan, unpubl. obs.]. Although this selection increased the likelihood of detecting individuals in the window period, it may not have affected the frequency of chronic carriers of HCV without anti-HCV in the population tested. At the time of the study design, a range of 1–5 in 10,000 donations with HCV RNA but without HCV antibody was projected on the basis of sparse published data [10, 15]. Results shown in table 2 indicate that this was clearly an overestimate. Recent estimates based on HCV infection rates in blood donors suggest that only one in 470,000 first-time donors donate in the infectious window period [Barbara and Soldan, unpubl. obs.].

The sensitivity of the assay could not be determined because of the unexpectedly small number of positive HCV RNA results. The specificity, which is traditionally considered the most critical parameter in large-scale screening for rare events, was even better than the best enzyme immunoassay used to currently screen viral markers in blood donations [16]. As shown in table 1, 23 of 27 of the initial reactive results did not repeat. This was due to samples whose fluorescence reading was just above the cut-off. Upon repeat testing, a slight change in the cut-off classified them as negative. Only three samples repeated at a low sample/cut-off ratio, and these were considered false positive. The confirmed positive sample was over three times the cut-off value and clearly distinguished itself from the other three positive samples.

Developments to adapt the method used here to a completely automated system with a 750 sample/8 h throughput are currently in progress. Such methods, when further developed, would offer an alternative to the implementation of pool testing for HCV RNA, where specificity is at best 50%. This poor performance observed by most investiga-

tors could potentially create serious disruptions in the blood supply, and could increase the cost of the strategy in massive proportion due to the discarding of many blood units included into pools found positive for HCV RNA but where no responsible donor could be identified [17]. It is anticipated that a fully automated system based on the method described here will be initially used to screen individual blood donations for HCV RNA and will later be extended to the detection of other RNA viruses, primarily HIV, in a multiplex format, an approach which will considerably reduce the cost of the screening procedure for viral RNA in individual donations.

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