On the Viral Safety of Plasma Pools and Plasma Derivatives

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Summary. In the industrialized world, a range of medicinal products are manufactured on a large scale from pools made of thousands of human blood and plasma donations. Policy makers as well as the general public are aware of the contamination hazards following accumulated risks of individual donations. Today, the manufacturing process must therefore consider a complex sequence of risk reducing interventions, including screening tests and quarantine periods on pools and individual donations. In this paper, we estimate the residual risk of Hepatitis C infection following such sequence of events. This is the most common bloodborne infection in the Western World, which affects an estimated 170 million persons worldwide. We investigate the costs and benefits of each intervention and study at several stages the dependence of the screening process on operational parameters that can be optimized, such as the pool size and the length of the quarantine period. This leads to alternative pooling strategies that may be more (cost-)effective or optimal under specific criteria.

Keywords: Dilution; Group Test; Plasma Derivatives; Pooling; Residual Risk; Transmission

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1 Introduction

Hepatitis C virus (HCV) infection is the most common chronic bloodborne infection in the Western World, which affects an estimated 170 million persons worldwide (Lauer and Walker, 2001). It may lead to chronic liver disease and has become the major cause of liver transplantation (Schafer and Sorrell, 2000). The virus is transmitted primarily through large or

repeated direct exposures to blood. This raises concern about the safety of plasma derivatives, i.e. stable medicinal products derived from blood and plasma, such as coagulation factors for treatment of hemophilia, immunoglobulines for treatment of an affected immuno-response and albumine solutions for treatment of burns, shock and trauma.

Plasma derivatives are produced on an industrial scale from plasma pools consisting of thousands of donations. In Belgium, approximately 5,000 donations are mixed into such pools. In Germany, pools containing up to 60,000 donations are considered. In the United States, some donor pool sizes are in excess of several hundred thousands (NIH, 1998). The risk of contamination of these pools increases rapidly with the pool size because one infected plasma may contaminate the whole pool. Furthermore, since large amounts of drugs are derived from a single pool, the product originating from an infected pool could infect many patients. Evaluating and improving the safety of plasma derivatives is hence a challenging objective of great public health concern (Thomas, Mathys and Gerard, 2002).

Several safety measures are currently imposed on individual donations and plasma pools in order to minimize the contamination hazards following the use of plasma derivatives. The applied rules differ from country to country, although common safety measures exist in most European countries. In Belgium, high risk donors are avoided by considering voluntary unpaid repeat donors and through interviews and medical examinations. Highly sensitive diagnostic tests are used on their samples and quarantine periods or inventory holds allow to exclude for seroconverted donors all donations that were collected during a prespecified period prior to the first detected seropositive donation. These interventions are not totally effective at reducing risks to negligible levels, primarily due to the existence of a window period between the onset of infectiousness (i.e. the time at which the virus can be transmitted through exposures to blood) and the development of sufficient antibodies or viral genomes in all parts of the body (Schreiber *et al.*, 1996). Contaminated samples collected during this period will go undetected. The length of the window period is variable, mainly due to differences in viral load between donors and differences in performance between test kits. The average length is approximately 66 days for third generation HCV antibody assays and 23 days for molecular biology-based HCV Polymerase Chain Reaction (PCR) tests (Barrera *et al.*, 1995; Schreiber *et al.*, 1996).

To further increase the safety of blood products, HCV PCR tests are now required by European regulations. These are highly sensitive because they test directly for the presence of the virus. PCR tests are currently applied on plasma pools and not on individual donations because they are costly and time consuming. However, little is known about their ability to detect contamination in large pools in which a single contaminated donation is present and diluted many times (Lefrère *et al.*, 1998; Flanagan, 1999).

In the context of blood donations destined for transfusion, the literature describes that HCV PCR on individual donations yields low benefits at a significant cost. This is because HCV infection is a slowly progressing disease and because many blood recipients have an advanced mortality risk even in the absence of HCV, due to their advanced age (Pereira and Sanz, 2000; Saura *et al.*, 1999). In the context of plasma pools destined for plasma derivatives, the costs and benefits of PCR screening plasma pools are not well understood (Flanagan, 1999). This is primarily due to the potential poor performance of PCR tests on large pools and because their potential benefits must be weighed against alternative strategies that could avoid the possible loss of many usable non-infectious donations in PCR-positive pools (Thomas, Mathys and Gerard, 2002). The goal of this paper is to develop greater insight in the risks and opportunities for improvement in this procedure. This can help patients, clinicians and public health officials decide on the efficacy of the safety techniques currently in use.

In Section 2, we present the sequence of screening procedures currently in place for HCV testing of plasma pools in Belgium. In Section 3, we model the effect of individual screening tests and inventory holds by adapting work by Satten (1997) on the risk of individual window period donations and by Kaplan and Satten (1999) on the efficacy of inventory holds on individual donations. We extend this work to accommodate the European situation and to evaluate these procedures applied on pools. We use the methodological results to quantify the contamination hazards of HCV via plasma derivatives in Belgium. In Section 4, we model and evaluate the ability of PCR tests to detect viral contamination in large pools. We balance the potential cost benefits of testing large pools against the severity of the loss of such a pool in case of a positive result. This leads to alternative pooling strategies derived to be more (cost-)effective or optimal under specific criteria. We end with a discussion.

2 Safety filters

The sequence of risk reducing interventions is typically complex. To focus attention, we will present results for the Belgian situation. Similar safety measures exist however across most European countries, but the operational parameters vary between countries and are not so well understood. In particular, the European regulations for pool testing apply to all European countries, but the length of the inventory hold, the pool size, the number and type of test kits, ... are variable.

In Belgium the plasma pool draws from individual blood and plasma donations collected by the Red Cross in several centers spread around the country. The target donor population consists of voluntary, unpaid, repeat donors. This is a relatively safe population because repeat donors have been screened on previous occasions. For instance, when HIV testing first became available in 1985, the prevalence of HIV in the US was 1.8 times higher in first-time donors than in repeat donors (Lackritz et al., 1995); the incidence of HIV in the US has been estimated to be 20 times smaller in unpaid than in paid donors (Kaplan and Satten, 1999).

The risk of viral transmission through plasma derivatives is further reduced via a sequence of safety filters within which we consider 6 stages (see Figure 1). Donors are first screened via questionnaires, medical examinations and indirect generic liver function tests (ALAT), which are repeated approximately every 2 years (stage 0). Volunteers at high risk of viremia according to at least one of these filters, are interdicted at this stage. Each individual donation is subsequently serologically screened for different viral markers (HBsAg, anti-HCV, anti-HIV, Syphilis) (stage 1). As for HCV-screening, the samples are first subjected to a third generation ELISA (ORTHO HCV 3.0 ELISA Test System). Donors with a negative test result are labeled HCV negative but others are retested by two third generation ELISA's. When at least 2 out of 3 test results turn out positive, confirmation follows from a WB Riba assay (Chiron RIBA HCV 3.0 SIA). Donations are labeled HCV negative otherwise (confirmation criteria for indeterminate test results may vary among the testing laboratories).

A randomly selected subset of donations is released for fractionnation into stable derivatives. They are held in quarantine for a period of about 3 months. This allows a look-back procedure whereby samples from returning seropositive donors are eliminated (stage 2). All remaining donations are pooled into batches of 1800 liters, containing about 5000 donations

each. These pools undergo the same screening process as the individual samples. The remaining risk of HCV transmission in the pools is thereafter reduced through PCR testing (HCV Cobas Amplicor, Roche Diagnostic Systems, USA V. 1.0 and V. 2.0) (Thomas *et al.*, 2000), via an additional holding period of about 2 months (i.e. the time needed for the manufacturing process) on the pools (stage 4) and virus inactivation techniques (stage 5). Finally, infectious released products are continuously traced and finally removed from the market.

Figure 1 about here.

3 Residual Risk Calculation

To evaluate the several safety filters, we calculate their impact on the residual risk of HCV after stage k, ρ_k ($\rho_k(c)$). This is defined as the probability that a donation (pool containing c independent donations) is contaminated when it has not been interdicted during or prior to that stage. The residual risk after stage 4 (i.e. $\rho_4(c)$) is of particular interest as it represents the proportion of HCV-infectious pools in the population of pools that are used for manufacturing plasma derivatives.

In this paper, we report results for blood donors who account for more than 75% of all donations (Rode Kruis Vlaanderen, 2000). We make 3 assumptions. First, we assume that each pools is composed of c *independent* donations. Knowing that on average 1 pool was constructed every 2 days (Rode Kruis Vlaanderen, 2000) by time of collection of the donations, this is considered a reasonable approximation for Belgian blood donors for whom a minimum interdonation interval of 2 months and a maximum of 4 donations per year is compulsory. Second, we assume that donors who become HCV infectious are unaware of their HCV status and, hence, do not alter their behaviour. Although HCV infection remains asymptomatic for quite some time, this assumption is somewhat questionable because exposure to HCV might result in some form of self-exclusion, thus increasing the interdonation interval (Thomas, Mathys and Gerard, 2002). If this is so, our analysis will yield overestimates for the residual HCV risk. Third, we assume that the event of seroconversion occurs independently of time over the considered period. The underlying steady-state model implies that a constant incidence rate (hazard) $\mathcal{I}_0$ acts on the repeat donor population which passed stage 0. Here, $\mathcal{I}_0$ is

defined as the likelihood (hazard) that a non-infectious repeat donor becomes infectious (at a given point in time). Our analysis considers the restricted time frame July 1997 - December 1999, suggesting this assumption holds approximately.

3.1 Risk reduction via diagnostic tests on donations (Stage 1)

3.1.1 The effect of a single diagnostic test

In steady state, the probability of collecting donations during the window period of the first serological test is obtained by multiplying the incidence $\mathcal{I}_0$ of HCV in the group that passed stage 0 with the average length $\mathrm{E}(W_1)$ of that (random) window period. This product

$$\rho_1 = \mathcal{I}_0 \mathrm{E}(W_1), \tag{1}$$

equals the residual risk of HCV after stage 1 when all false negative tests are due to window period donations (Satten, 1997). This is a reasonable asssumption because other sources of screening failure (e.g. immunologically variant viruses not consistently detected by current serologic assays, abnormal seroconversions and testing error) have only a minor contribution to the overall residual transmission risk (Saura *et al.*, 1999). Expression (1) assumes additionally that the incidence $\mathcal{I}_0$ and average window length $\mathrm{E}(W_1)$ for donors with a negative test result is the same as in the general donor population.

The average length of the window period of the considered anti-HCV third generation tests was taken from the literature as 66 days (Barrera *et al.*, 1995; Couroucé *et al.*, 1994). Estimates for the incidence of HCV in repeat donors among European Plasma Fractionation Association (EPFA) members were obtained as 2.43 per 100 million person-days. Accurate estimates specific to the Belgian situation are not readily available, but considered to be similar in magnitude (personal communication with Ruth Laub). From (1), we estimate that the residual risk ρ_1 of HCV after a single ELISA test is $1.60\ 10^{-6}$.

3.1.2 The effect of multiple diagnostic tests

In the current Belgian setting, the decision to interdict donations depends on a sequence of simultaneous diagnostic tests and expression (1) must be adapted accordingly. In particular, W_1 is now the window period of the adopted test strategy. When seropositive (seronegative) samples are only called positive when retested as positive, this window period W_1 is expected to be larger (smaller) than each of the average window periods of the 2 separate tests. For

instance, let W_{1k} $(k = 1, \ldots, 4)$ be the window period of the kth test in the first stage. Under the screening procedure of Section 2, a positive test then results if and only if the time between the onset of infectiousness and the next donation exceeds W_{11} to yield a positive result for the first test. It must in addition exceed $\min(W_{12}, W_{13})$ to yield a positive result for at least one of the subsequent two anti-HCV tests, and exceed W_{14} to yield a positive Western Blot result. This multiple testing procedure is thus equivalent to using a single diagnostic test with window period $W_1 = \max\{W_{11}, \min(W_{12}, W_{13}), W_{14}\}$.

The residual HCV risk after the first stage involves the average window length $\mathrm{E}(W_1)$. This is difficult to calculate because it requires information about the joint distribution of window periods W_{1k} $(k = 1, \ldots, 4)$. To the best of our knowledge, such information is not available in the literature. Dodd *et al.* (2002) report estimates for the average length of the window period of the multiple test sequence described in Section 2 and find an average of 70 days. Using this value, the residual risk of HCV is estimated at 1.70 10^{-6} after individual screening.

3.2 Risk reduction via a holding policy on donations (Stage 2)

Holding policies or inventory holds allow to exclude for seroconverted donors all negative donations that were collected in the last τ months prior to the first detected seropositive donation. This is a relatively cheap safety strategy that turns out very effective so long as the risk of rejecting previously non-infectious donations from seroconverted donors remains sufficiently small. Below, we evaluate the effectiveness of inventory holds by contrasting p_+ and p_-, the risks of excluding seropositive and seronegative donations, respectively. Next, we estimate the residual risk after the holding policy via Bayes theorem as:

$$\rho_2 \quad = \quad \frac{(1-p_+)\rho_1}{(1-p_+)\rho_1 + (1-p_-)(1-\rho_1)}.$$

When the minimum interdonation interval τ_0 is 2 months - as for blood donors in Belgium - there can be at most a single donation during a holding period of $\tau = 3$ months.

- An undetected infectious donation is then rejected when the time T to the next donation exceeds the remaining duration W^* of the window period and is smaller than τ and the remaining duration L^* of the donation career (see Figure 2a). Assuming that (T, W^*, L^*)

are mutually independent, this occurs with probability:

$$p_+ = \text{pr}(\min\{\tau, L^*\} > T > W^*) = \int_{\tau_0}^{\tau} \int_0^t f_T(t) f_{W^*}(w) \text{pr}(L^* > t) dw dt. \qquad (2)$$

- A non-infectious donation is rejected when T exceeds the time H^* until the donor becomes infectious plus the length W of the window period and is smaller than τ and L^* (see Figure 2b). Assuming that (T, H^*, W, L^*) are mutually independent and no false positives occur, this happens with probability:

$$\begin{aligned} p_- &= \text{pr}(\min\{\tau, L^*\} > T > H^* + W) \\ &= \int_{\tau_0}^{\tau} \int_0^t \int_0^{t-h} f_T(t) f_{H^*}(h) f_W(w) \text{pr}(L^* > t) dw dh dt. \end{aligned} \qquad (3)$$

In Appendix A, we derive more general expressions that are valid when the same donor can return several times during the holding period.

Estimating the distribution of W^* directly based on the distribution of W would likely be misleading because the distribution of W^* conditional on the sampling scheme is length biased. This is because window periods need to be long enough to observe a negative test for an infectious donation so that the longer window periods are likely overrepresented in our study. Following Cox (1962), the distribution of W^* can be obtained from knowledge of the window length distribution via the identity $f_{W^*}(x) = \text{pr}(W > x)/\text{E}(W)$. Likewise, $f_{L^*}(x) = \text{pr}(L > x)/\text{E}(L)$ and $f_{H^*}(x) = \text{pr}(H > x)/\text{E}(H)$, where L is the total duration of the donation career and H is the time from the second qualifying donation until the onset of infectiousness. In steady state, H is exponentially distributed with mean $1/\mathcal{I}_0$.

Figure 2 about here.

In Belgium, the interdonation interval T is on average 166 days (Rode Kruis Vlaanderen, 2000). To make computations feasible, we assume exponential distributions for W and L, a shifted exponential distribution for T, starting at $\tau_0 = 60$ days, and an average length of 10 years for the donation career. For τ equal to 90 days, the probability of rejecting a non-infectious donation during the holding period is $1.69\ 10^{-7}$, which is nicely small. The probability of rejecting an infectious donation during that period is estimated at 0.157. Our

calculations so far assume that no false positive tests occur. This assumption is likely invalid but does not affect the probability p_+ and modifies the probability p_- only slightly. Several empirical investigations have found a specificity Sp of at least 0.99 for individual third generation HCV tests. Assuming conditionally independent test errors, this amounts to a specificity of at least $1 - 1.99\ 10^{-6}$ for the multiple test sequence. We thus find that uncontaminated donations are rejected with probability $Sp\ q + (1 - Sp)p_- = 6.50\ 10^{-7}$, where q is the probability that non-infectious donors return during the holding period and prior to infection:

$$q = \int_{\tau_0}^{\tau} f_T(t)\mathrm{pr}(L^* > t)\mathrm{pr}(H^* > t)dt = 0.244.$$

Our calculations below acknowledge such false positivity. As such, we estimate that the residual risk of contamination equals $1.43\ 10^{-6}$ after the inventory hold.

Figure 3 (top) shows that longer holding periods would be more effective. Lengthening the holding period up to 6 months would allow to stop an estimated $p_+ = 52.7\%$ of all infectious donations, but only $p_- = 2.35\ 10^{-4}\%$ of all non-infectious donations. This would amount to more than halving the residual risk to $8.05\ 10^{-7}$ at the cost of merely $2.35\ 10^{-4}\%$ of usable donations. Because the cost of storing donations longer is relatively small compared to the cost of additional diagnostic tests (Kaplan and Satten, 1999), future practice could benefit from longer holding periods.

Figure 3 about here.

3.3 Risk reduction via screening tests on pools (Stage 3)

Donations that are not excluded after the second stage are pooled into batches of $c = 5000$. Because one infectious plasma may contaminate the whole pool, the risk of contamination increases approximately proportionally to the pool size, following $\rho_2(c) = 1 - (1 - \rho_2)^c \approx c\rho_2$. In Belgium, this corresponds to residual risks as high as 0.00714. The possible high rate of contamination in such large plasma pools has been one of the basic arguments of the Working Party of the European Committee on Proprietary Medicinal Products (EC CPMP) to require that all fractionated plasma products released for clinical use should test negative in validated HCV PCR (Polymerase Chain Reaction) assays (Flanagan, 1999). The benefits of PCR tests on large pools are however ill understood (Flanagan, 1999). This is mainly because PCR tests

may have little sensitivity to detect a single contaminated sample in a large pool and because their potential benefits must be weighed against alternative strategies that could avoid the possible loss of many usable non-infectious donations in PCR-positive pools.

To evaluate the performance of PCR tests on large pools, let us define the sensitivity $Se(c)$ (specificity $Sp(c)$) of a group test (i.e. a test performed on pools) as the probability of a positive (negative) test for a pool of size c that contains at least 1 (no) infectious donation. Little is known about these measures for pools of 5000 donations, but it seems reasonable to assume that $Sp(5000) = 1$. This is based on the observation that the specificity of currently used PCR tests on individual donations is already estimated as high as 0.98 (Albadalejo *et al.*, 1998) and that dilution effects in large pools make $Sp(c)$ increase with the pool size (Behets *et al.*, 1992). Some information on the sensitivity $Se(c)$ for pools of $c = 5000$ donations can be acquired by comparing the theoretical probability of a positive test, i.e. $Se(c)\rho_2(c) + \{1 - Sp(c)\}\{1 - \rho_2(c)\}$, with the empirical observation that only 2 in 447 plasma pools of size 5000 have tested HCV PCR-positive in Belgium over the considered study period from July 1997 to December 1999. With $c = 5000$ and $Sp(c) = 1$, we thus find an estimated sensitivity $Se(c)$ as low as 0.626.

The residual risk $\rho_3(c)$ after the PCR test is estimated to be

$$\frac{\{1 - Se(c)\}\rho_2(c)}{\{1 - Se(c)\}\rho_2(c) + Sp(c)\{1 - \rho_2(c)\}} = 0.00268.$$

3.4 Risk reduction via a holding policy on pools (Stage 4)

The manufacturing process of plasma derivatives takes approximately 2 months. In several countries, the current policy is to exclude plasma pools that contain previously negative donations from seroconverted donors during this period. The effectiveness of this strategy is questionable because a false alarm for a single donation may imply the loss of a whole plasma pool. In Appendix B, we evaluate the effectiveness of such inventory hold by contrasting $p_+(c)$ and $p_-(c)$, the risks of excluding seropositive and seronegative plasma pools of c donations, respectively. Next, we estimate the residual risk after this holding policy via Bayes theorem as:

$$\rho_4(c) = \frac{\{1 - p_+(c)\}\rho_3(c)}{\{1 - p_+(c)\}\rho_3(c) + \{1 - p_-(c)\}\{1 - \rho_3(c)\}}.$$

Assuming exponential distributions for H, L, T (starting at τ_0) and W, we find that the

probability of rejecting an infectious pool in Belgium is 0.315 and that the probability of rejecting a non-infectious pool is 0.00565. The residual risk equals 0.00185 after the fourth stage. While the probability to reject infectious pools is nicely high, Figure 3 (bottom) shows that it can be further improved by making the holding period longer. Doing this would however imply an unaffordable loss of uncontaminated pools: after a holding period of 4 months, more than 1.1% of non-infectious pools would be rejected. We further find that $p_+(c)$ is hardly affected by the pool size, while $p_-(c)$ changes substantially (not displayed). In particular, $p_+(1) = 0.311$ approximates $p_+(5000) = 0.315$, but $p_-(1) = 1.13\ 10^{-6}$ is much smaller than $p_-(5000) = 0.00565$. As rejecting a pool of 5000 donations means wasting approximately 5000 donations, smaller pool sizes could be more cost-efficient. This is further investigated in Section 4. Alternatively, confirmatory PCR testing of donations that are picked up by the inventory hold, but have been pooled, can severely lower the risk of a false alarm.

4 Costs and benefits

Having quantified the contamination hazards in large plasma pools up to the point where viral inactiviation steps are commenced, we focus more deeply into the major open problems that formed the incentive for this work. In Section 4.1, we evaluate the ability of PCR tests to detect viral contamination in large pools of plasma that contain a single contaminated donation diluted by many uncontaminated ones. In Section 4.2, we study the increased severity of losing usable donations in large pools.

4.1 Dilution and diagnostic sensitivity

For many diagnostic tests - including PCR tests - false negatives occur when the antibody level or viral load drops below a detection limit κ (Lelie *et al.*, 1997). When this happens with probability 1, the probability $Se(c,d)$ of a positive test for a pool of size c that contains d infectious donations, is:

$$Se(c,d) = \text{pr}\left(\sum_{l=1}^{d} X_l > c\kappa | X_l > 0\ \forall l \in \{1,\ldots,d\}\right),$$

where X_l is the viral load in the lth infectious donation ($l = 1, ..., d$). As group tests are applied after the second stage, their sensitivity, i.e. the probability of a positive test for a

contaminated pool, is

$$Se(c) = \sum_{d=1}^{c} \left(\begin{array}{c} c \\ d \end{array} \right) \frac{Se(c,d)}{\rho_2(c)} \rho_2^d (1-\rho_2)^{c-d}.$$

When aspecific reactions in pools occur with probability 0, the residual risk after PCR screening follows from Bayes rule as:

$$\rho_3(c) = \frac{\{1 - Se(c)\}\rho_2(c)}{\{1 - Se(c)\}\rho_2(c) + 1 - \rho_2(c)}. \tag{4}$$

Evaluating $Se(c,d)$ requires specific knowledge about the HCV viral load distribution. To allow for flexibility and computational feasibility, we approximated $X_l|X_l > 0$ by the gamma distribution $\Gamma(\alpha,\beta)$ and used that $\sum_{l=1}^{d} X_l|(X_l > 0\ \forall l \in \{1,\ldots,d\}) \overset{d}{\approx} \Gamma(d \times \alpha, \beta)$. Estimates for α and β are obtained in Appendix C. With $\kappa = 30$ IU/ml, as for PCR tests currently used in Belgium, we find an estimate for the median viral load in a single undetected infectious donation before PCR screening equal to 6.58 10^7 IU/ml.

Hung and Swallow (1999) model dilution effects alternatively by assuming that each pool is diluted by a constant fraction f of all non-infectious units in the pool:

$$Se(c,d) = Se\frac{d}{(c-d)f + d}, \tag{5}$$

where Se is the sensitivity of the individual test ($Se = Se(1,1)$). This is equivalent to conceiving of a pool as a set of $(c-d)f$ diluting and d contaminating units and the diagnostic test result being obtained by individually testing a random unit from this pool. In Appendix C, we estimate that Se equals 0.750 and f equals 0.0000396, suggesting that pools of 5000 non-infectious donations contain merely 0.2 diluting units.

Figure 4 (top, left) displays the estimated sensitivity of PCR tests over varying pool sizes for the detection limit model and the Hung and Swallow model. We find that pooling reduces the diagnostic sensitivity of screening tests, but the effect is smaller than initially anticipated. This is due to (i) the small detection limit of PCR tests, (ii) the fact that the sensitivity is already low for individual donations. The latter is true because we are effectively screening donations conditional on having tested negative in all previous stages. We estimate that the sensitivity of PCR tests is 0.750 on anti-HCV negative donations and 0.625 on pools of 5000. Dilution effects are already observed for very small pools according to the dilution effect model, but not following the Hung and Swallow model. For instance, the sensitivity is just

0.70 for pools of 48 donations in the detection limit model, but still 0.75 in the Hung and Swallow model. As we find the dilution model the more plausible one, we will work with it below.

Figure 4 about here.

4.2 Risk of wasting usable donations

Because contaminated pools are likely to carry few infectious donations, rejecting them may imply a huge loss of initially usable donations. Non-infectious donations are excluded when they:

- are rejected during the individual antibody screening, which occurs with probability $1 - Sp$.
- are rejected during the first inventory hold, which occurs with probability $Sp \times p_-$.
- enter an infectious pool that is rejected via PCR tests or a holding policy on pools. This occurs with probability $Sp(1-p_-)\{1-(1-\rho_2)^{c-1}\}[Se(c)+\{1-Se(c)\}p_+(c)]$.
- enter a non-infectious pool that is rejected via an inventory hold on pools. This occurs with probability $Sp(1-p_-)(1-\rho_2)^{c-1}p_-(c)$.

The probability to reject a non-infectious donation is the sum of these 4 probabilities.

For pools of 5000 donations, we expect that 1.09 % of all non-infectious donations are rejected during one of the risk reducing interventions. This primarily due to the second inventory hold. Indeed, eliminating it (but keeping the pool size fixed at 5000 and the length of the first holding period at 90 days) would severely lower the risk of such a loss to 0.448%, but increase the residual risk with almost 45% at the same time (see Figure 4; bottom, right). Doubling the length of the first holding period (but keeping the pool size fixed at 5000) would lower the risk of losing uncontaminated donations to 0.869% (see Figure 4; bottom, left). Lengthening the first holding period to 6 months and shortening the second to 1 month would decrease the residual risk with 34% and the risk of losing uncontaminated donations with 48%. Halving the pool size would almost halve this risk to 0.551% (see Figure 4; top, right). The extra cost needed for doubling the number of PCR tests would not weigh

against this, because PCR screening is relatively inexpensive (approximately 25$ per test) when performed on pools. We conclude that smaller pool sizes and a shorter length of the second inventory hold could be much more cost-efficient.

Changing the pool size is currently prohibiting because it requires substantial modifications of the current infrastructure and pool containers. Furthermore, shortages of a number of plasma products raise concerns that steps to limit the size of plasma pools might further reduce the availability of important products (NIH, 1998). Some of these practical problems can be avoided by implementing an intermediate system of minipool testing between the first and second stage. In these systems, individual samples are organized on microplates with for instance 6 rows of 8 wells and pooling algorithms allow identification of the HCV positive samples in pools with a positive test result (Mortimer, 1997). This saves the identity of the separate donations and thus further reduces the risk of wasting usable donations. Intermediate pooling is also important from an ethical point of view for the release of blood components and to permit notification of the donor.

Figure 5 about here.

In Belgium, there are plans to screen intermediate pools of $c' = 48$ donations for HCV after individual screening. When such pool is contaminated, it contains just a single infectious donation with probability $48\rho_1(1-\rho_1)^{c'-1}/\{1-(1-\rho_1)^{c'}\} = 0.99996$. In that case, additional testing according to pooling algorithms would allow to identify this single donation with probability 1. In the absence of aspecific reactions, the residual risk of infection after intermediate pooling is then approximately:

$$\rho_1' = \frac{\{1-Se(c')\}\rho_1}{\{1-Se(c')\}\rho_1 + 1 - \rho_1},$$

where $Se(c')$, the diagnostic sensitivity of HCV PCR on pools of c' donations, follows from the dilution-effect model.

From Figure 5 (top) we see that the benefit from intermediate pooling is most pronounced for small intermediate pools. For $c' = 48$, we estimate that the residual risk is 0.000580 after all stages. The risk of wasting usable donations stays high (0.00724), primarily because of the relatively high probability to reject non-infectious pools during the fourth stage. These

results are approximate as there are currently no data to evaluate the impact of PCR tests and holding periods conditional on being undetected with intermediate pooling. This could lead to slightly underestimated residual risks, as PCR tests and holding policies are likely less effective when applied to donations that are not detected by intermediate pooling.

5 Discussion

Manufacturing processes for plasma derivatives are in general highly effective for removing or inactivating enveloped viruses like HCV (Willkommen *et al.*, 1999). Pooling lowers the viral load, which is important to guarantee a high effectiveness of these procedures, but it increases the risk of an infectious plasma pool at the same time. We have estimated that the risk of infectious pools is around 0.185% in Belgium before the application of virus inactivation steps. Those are claimed to be highly effective. The risk of *transmission* via plasma derivatives is thus much smaller, as it depends on the effectiveness of virus inactivation techniques, the susceptibility of the target population and the titer of the infectious agent in the final product. Nevertheless, it is important to improve the safety of plasma products before the application of these techniques, for the following reasons. First, the FDA has reported numerous quality control lapses in their implementation, ranging from failure to properly sterilize containers, use of invalidated software and delays in reviewing problems that were reported with released products (Kaplan and Satten, 1999). Second, their safety profile with respect to non-enveloped viruses like HAV and parvovirus B19 is not very high (Willkommen *et al.*, 1999). Third, their effectiveness is not yet fully understood as validation studies are based on a model virus of HCV which could have a different behaviour than the real virus (Willkommen *et al.*, 1999).

In response to these problems, we have investigated the effectiveness of the various safety interventions that preceed the application of virus elimination techniques. Their benefits are most pronounced when applied prior to large scale pooling: as Figure 5 (bottom) shows, omitting diagnostic tests on individual donations would inflate both the residual risk and the probability to reject uncontaminated donations with a sizeable amount. Important improvements are promised by better diagnostic tests or longer holding periods on the individual samples. The latter intervention would reduce the residual risk and the risk of wasting usable donations at a relatively small extra cost. Great benefits are also expected from a stage of

intermediate minipools. Their extra cost is (partly) compensated by a smaller loss of non-infectious donations. Interventions are less effective when applied on large plasma pools, mainly because they may inflate the number of falsely rejected donations too much.

An added advantage of PCR tests is that they enable to reduce the viral load below some detection limit (e.g. 30 IU/ml) at which virus inactivation techniques are more effective. For instance, albumin and immunoglobulin products are mostly manufactured on the basis of cold ethanol fractionation (Willkommen *et al.*, 1999), which removes HCV at a level of at least $8.3 \log_{10}$ IU/ml in the CAF-DCF plant in Belgium (personal communication with Ruth Laub). Based on results in Appendix C, we estimate that concentrations as high as this (or higher) occur with 21% chance in pools of 5000 donations, prior to PCR testing (note that this estimate is very imprecise). Coagulation factors are manufactured on the basis of less effective chromatographic methods, heat treatment and virus retention filters. The latter remove the virus at a level of at least $2.5 \log_{10}$ IU/ml. We estimate that concentrations of this magnitude (or higher) occur with 58% chance in pools of 5000 donations (note that this estimate is very imprecise). The considered safety filters are especially important for non-enveloped viruses, like HAV, which are more difficult to remove via current virus inactivation steps.

Our decision analysis was hampered by the non-availability of central databases that record the exact timings of each donation and the results of all safety filters. This made it impossible to account for possible correlations between HCV and other blood-borne infections such as HIV and HBV that are also tested in stage 1. Such correlations in disease incidence are expected, given the similar transmission patterns of these diseases and, if present, would further lower the residual risk of HCV. For similar reasons, it was impossible to quantify imprecision through confidence intervals and to evaluate the effectiveness of viral inactivation techniques based on the available data. Future studies on the safety profile of plasma derivatives would greatly benefit from such central databases. As decisions on the appropriateness of the current interventions should be made now, the current evaluations emerge as the most suitable tool for such endeavor.

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Appendix A: Calculation of p_+ and p_-

In this appendix, we show how to calculate p_+ and p_- when the holding period is so long that each donor can donate multiple times during that period. We explain the principle for the case where there can be 2 donations during the holding period. Let T_1 be the time to the first donation and T_2 be the time from T_1 to the second donation. Then p_+ becomes:

$$\begin{aligned} p_+ &= \int_{\tau_0}^{\tau}\int_0^{t_1} f_{T_1}(t_1) f_{W^*}(w)\mathrm{pr}(L^* > t_1)dwdt_1 \\ &\quad + \int_{\tau_0}^{\tau-\tau_0}\int_{\tau_0}^{\tau-t_1}\int_{t_1}^{t_1+t_2} f_{T_2}(t_2) f_{T_1}(t_1) f_{W^*}(w)\mathrm{pr}(L^* > t_1+t_2)dwdt_2dt_1, \end{aligned} \tag{6}$$

where the first term relates to the case where the first donation is observed after the end of the window period, and the second term to the case where the second, but not the first donation, is observed after that time. Likewise,

$$\begin{aligned} p_- &= \int_{\tau_0}^{\tau}\int_0^{t_1}\int_0^{t_1-h} f_{T_1}(t_1) f_{H^*}(h) f_W(w)\mathrm{pr}(L^* > t_1)dwdhdt_1 \\ &+ \int_{\tau_0}^{\tau-\tau_0}\int_{\tau_0}^{\tau-t_1}\int_0^{t_1}\int_{t_1-h}^{t_1+t_2-h} f_{T_2}(t_2) f_{T_1}(t_1) f_{H^*}(h) f_W(w)\mathrm{pr}(L^* > t_1+t_2)dwdhdt_2dt_1 \end{aligned} \tag{7}$$

$$+\int_{\tau_0}^{\tau-\tau_0}\int_{\tau_0}^{\tau-t_1}\int_{t_1}^{t_1+t_2}\int_{0}^{t_1+t_2-h} f_{T_2}(t_2)f_{T_1}(t_1)f_{H^*}(h)f_W(w)\mathrm{pr}(L^*>t_1+t_2)dwdhdt_2dt_1,$$

where the first term relates to the case where the first donation is observed after infection and the end of the window period, and the second 2 terms to the case where the second, but not the first donation, is observed after that time. In the first (second) expression, it is implicitly assumed that (T_1, T_2, W^*, L^*) $((T_1, T_2, W, H^*, L^*))$ are mutually independent. When many donations can be collected during the window period, as is the case for paid plasma donors in the U.S., one can use the expressions derived by Kaplan and Satten (1999).

Appendix B: Calculation of $p_+(c)$ and $p_-(c)$

Define $T_{ij(c)}$ as the set of test results that were obtained during the jth stage ($j = 1, 3$) for all samples in the ith pool of size c. It indicates 1 if the pool is interdicted and 0 otherwise. Let D_i denote the number of independent infectious donations in the ith pool and p'_+ (p'_-) be the probability of rejecting a single (non-) infectious donation during the second holding period conditional on not being rejected until the fourth stage.

To calculate $p_+(c)$ and $p_-(c)$, first note that a pool is rejected during the holding period when at least one of its members is rejected. Thus, an undetected non-infectious pool is rejected with probability:

$$p_-(c) \quad = \quad 1-(1-p'_-)^c.$$

Similarly, $p_+(c)$ equals 1 minus the probability of not interdicting any sample in the considered infectious pool, the latter being

$$\sum_{d=1}^{c}(1-p'_+)^d(1-p'_-)^{c-d}\mathrm{pr}(D_i=d|c, D_i\geq 1, \overline{T}_{i3(c)}=0).$$

Using Bayes theorem, the probability $\mathrm{pr}(D_i = d|c, D_i \geq 1, \overline{T}_{i3(c)} = 0)$ equals $\mathrm{pr}(D_i = d|c, \overline{T}_{i3(c)} = 0)/\mathrm{pr}(D_i \geq 1|c, \overline{T}_{i3(c)} = 0)$ when $d > 0$, with

$$\mathrm{pr}(D_i=d|c,\overline{T}_{i3(c)}=0)=\left(\begin{array}{c} c \\ d \end{array}\right)\frac{\mathrm{pr}(T_{i3(c)}=0|c, D_i=d, \overline{T}_{i2(c)}=0)\rho_2^d(1-\rho_2)^{c-d}}{\mathrm{pr}(T_{i3(c)}=0|c,\overline{T}_{i2(c)}=0)},$$

and $\mathrm{pr}(D_i \geq 1|c, \overline{T}_{i3(c)} = 0) = \sum_{d=1}^{c}\mathrm{pr}(D_i = d|c, \overline{T}_{i3(c)} = 0)$. The probability $\mathrm{pr}(T_{i3(c)} = 0|c, D_i = d, \overline{T}_{i2(c)} = 0) = 1-Se(c, d)$ follows immediately from the dilution-effect or Hung and Swallow model of Section 4.1. Expressions for p'_+ (p'_-) are obtained under the assumption that

having obtained a negative PCR group test result carries essentially no information about the remaining window length for infectious donations in the pool and the remaining time to infection for non-infectious donations. Let $p_+(\tau)$ ($p_-(\tau)$) be the probability to reject an infectious (non-infectious) donation in the time interval $[0, \tau]$. Let the second holding period stretch from τ to $\tau + \tau'$. Then, $p'_+ = \{p_+(\tau + \tau') - p_+(\tau)\}/\{1 - p_+(\tau)\}$, where $p_+(\tau + \tau')$ and $p_+(\tau)$ follow from expression (2). Likewise, $p'_- = \{p_-(\tau + \tau') - p_-(\tau)\}/\{1 - p_-(\tau)\}$.

Appendix C: Estimation of dilution effects

We first introduce some notation. Let T_{ik} be the result of the kth diagnostic test, which indicates 1 if the ith donation is interdicted and 0 otherwise. Let $\overline{T}_{ik} = (T_{i1}, \ldots, T_{ik})$ be the test result history for the ith donation. Let D_i indicate 1 for infectious donations and 0 otherwise. Then we derive an expression for the residual risk ρ_3 of HCV that would remain if the PCR test were performed on individual donations. We find that

$$\begin{aligned} \rho_3 &= \mathrm{pr}(D_i = 1 | \overline{T}_{i3} = 0) \\ &= \frac{\mathrm{pr}(T_{i3} = 0 | \overline{T}_{i2} = 0, D_i = 1)\rho_2}{\mathrm{pr}(T_{i3} = 0 | \overline{T}_{i2} = 0, D_i = 1)\rho_2 + 1 - \rho_2}, \end{aligned}$$

when we assume as before that $\mathrm{pr}(T_{i3} = 0 | \overline{T}_{i2} = 0, D_i = 0) = 1$. To derive an expression for $\mathrm{pr}(T_{i3} = 0 | \overline{T}_{i2} = 0, D_i = 1)$, we conceive of 3 co-existing test results T_{i1}, T_{i2} and T_{i3} for each donation (even if not all of them are effectively executed and observed). We then decompose $\mathrm{pr}(T_{i3} = 0 | \overline{T}_{i2} = 0, D_i = 1)$ into the quotient of $\mathrm{pr}(T_{i2} = 0 | T_{i1} = T_{i3} = 0, D_i = 1)\mathrm{pr}(T_{i3} = 0 | T_{i1} = 0, D_i = 1)$ and $\mathrm{pr}(T_{i2} = 0 | T_{i1} = 0, D_i = 1) = 1 - p_+$. Below, we first calculate $\mathrm{pr}(T_{i2} = 0 | T_{i1} = T_{i3} = 0, D_i = 1)$ and then $\mathrm{pr}(T_{i3} = 0 | T_{i1} = 0, D_i = 1)$.

$\mathrm{pr}(T_{i2} = 0 | T_{i1} = T_{i3} = 0, D_i = 1)$ is found by replacing the distribution of W_i^* in the expression for p_+ by the conditional distribution of the remaining lenght of the window period of the first-stage test, given that $T_{i1} = T_{i3} = 0$. Let X_1 and X_3 denote the lengths of the window periods of the stage 1 and 3 tests in which the considered donation falls, then X_1 and X_3 have the length-biased density functions $x_1 f_{W_1}(x_1)/\mathrm{E}(W_1)$ and $x_3 f_{W_3}(x_3)/\mathrm{E}(W_3)$ respectively (Cox, 1962). Given $X_1 = x_1$ and $X_3 = x_3$, the remaining length X of the window period of the first-stage test is uniformly distributed following:

$$f_X(x) = \begin{cases} 1/x_3 & \text{if} \quad x_1 - x_3 \le x \le x_1 \quad \text{and} \quad x_3 \le x_1 \\ 1/x_1 & \text{if} \quad 0 \le x \le x_1 \quad \text{and} \quad x_3 \ge x_1 \\ 0 & \text{otherwise} \end{cases}.$$

Assuming independent window lengths, the unconditional density function is

$$f_{W^*}(x) = \int_x^\infty \int_{x_1-x}^{x_1} \frac{x_1 f_{W_1}(x_1) f_{W_3}(x_3)}{\mathrm{E}(W_1)\mathrm{E}(W_3)} dx_3 dx_1 + \int_x^\infty \int_{x_1}^\infty \frac{x_3 f_{W_1}(x_1) f_{W_3}(x_3)}{\mathrm{E}(W_1)\mathrm{E}(W_3)} dx_3 dx_1.$$

The latter assumption appears plausible as the first and third-stage test use different markers (i.e. antibodies versus viral genomes) to detect infectiousness.

$\mathrm{pr}(T_{i3} = 0|T_{i1} = 0, D_i = 1)$ can be written as $\mathrm{pr}(T_{i3} = 0|D_i = 1)/\mathrm{pr}(T_{i1} = 0|D_i = 1)$ when we assume that $\mathrm{pr}(T_{i1} = 0|T_{i3} = 0, D_i = 1) = 1$. The latter assumption is plausible for PCR tests, which have a much smaller window period than third generation serological tests. When furthermore $\mathrm{pr}(T_{ik} = 0|D_i = 0) = 1$ for arbitrary k, then $\mathrm{pr}(T_{ik} = 0|D_i = 1) = \mathcal{I}_0\mathrm{E}(W_{ik})\mathrm{pr}(T_{ik} = 0)/\{\mathcal{I}_0\mathrm{E}(W_{ik})\mathrm{pr}(T_{ik} = 0) + \mathrm{pr}(T_{ik} = 1)\}$ and $\mathrm{pr}(T_{ik} = 1) = \mathrm{pr}(T_{ik} = 1|D_i = 1)\rho_0$, where $\rho_0 = \mathrm{pr}(D_i = 1)$. Combining both expressions yields

$$\mathrm{pr}(T_{ik} = 0|D_i = 1) = \mathcal{I}_0\mathrm{E}(W_{ik})(1 - \rho_0)/[\rho_0\{1 - \mathcal{I}_0\mathrm{E}(W_{ik})\}] \tag{8}$$

for $k = 1, 3$, after some algebra. We conclude that

$$\mathrm{pr}(T_{i3} = 0|T_{i1} = 0, D_i = 1) = \frac{\mathrm{E}(W_{i3})\{1 - \mathcal{I}_0\mathrm{E}(W_{i1})\}}{\mathrm{E}(W_{i1})\{1 - \mathcal{I}_0\mathrm{E}(W_{i3})\}}.$$

An alternative expression for $\mathrm{pr}(T_{i3} = 0|T_{i1} = 0, D_i = 1)$ can be found as follows. Given the current duration $Y = y$ of the window period of the first-stage test, the probability $\mathrm{pr}(T_{i3} = 0|T_{i1} = 0, D_i = 1, Y = y)$ equals $\mathrm{pr}(W_{i3} > y)$. To find the density of the random variable Y, we first treat the length W_1 of the window period of the first-stage test as fixed, i.e. $W_1 = w_1$. Then, Y is uniformly distributed between 0 and w_1, conditional on $T_{i1} = 0$ and $D_i = 1$. The unconditional density of Y is obtained by integrating over the distribution of W_1, which is length-biased. Like in Cox (1962), it follows that Y has the density function $\{1 - F_{W_1}(y)\}/\mathrm{E}(W_1)$, where F_{W_1} is the cumulative density function of W_1. Thus, the unconditional density is:

$$\mathrm{pr}(T_{i3} = 0|T_{i1} = 0, D_i = 1) = \int_0^\infty \frac{\{1 - F_{W_1}(y)\}\{1 - F_{W_3}(y)\}}{\mathrm{E}(W_1)} dy.$$

The previous results enable us to estimate $\rho_3(1) = \rho_3$, the residual risk for pools of size 1, based on knowledge of the window length. Combining with (4) yields information about the diagnostic sensitivity of PCR tests for $c = 1$. Further, we know that the risk of a positive test result for pools of size c is $\sum_{d=0}^{c} \left(\begin{array}{c} c \\ d \end{array} \right) Se(c, d)\rho_2^d(1 - \rho_2)^{c-d}$. Comparing with the observed

risk 2/447 for $c = 5000$ yields further information from which we can estimate the effect of pool size on diagnostic sensitivity, under either the dilution effect model or the Hung and Swallow model.

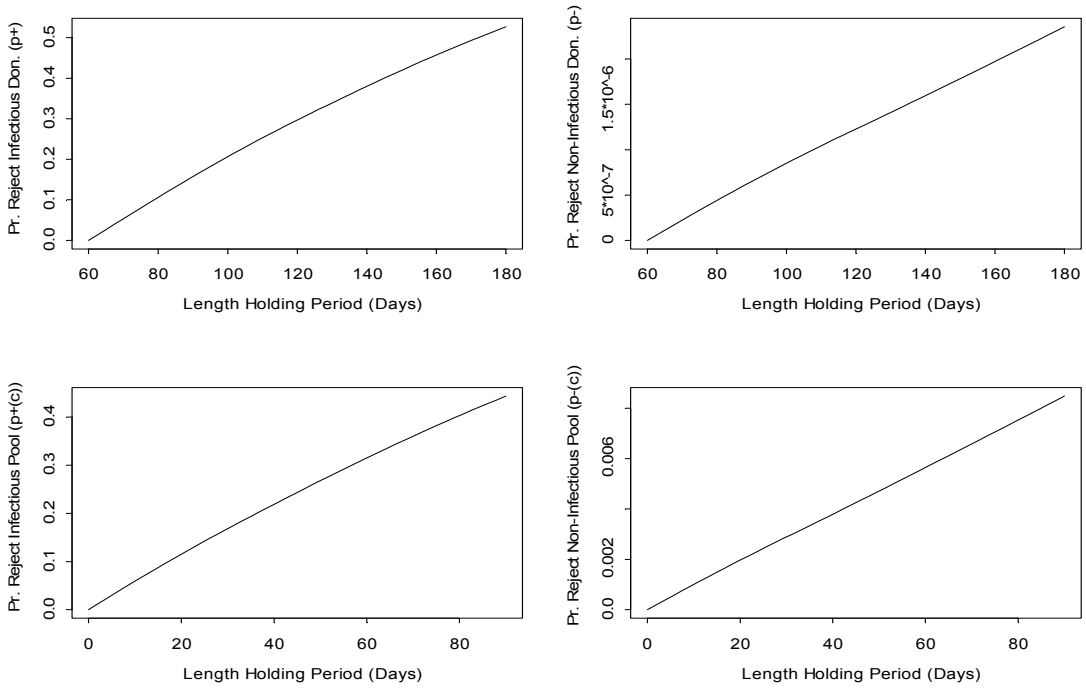

Fig. 3. Top: p_+ (left) and p_- (right) versus length τ of the first holding period; Bottom: $p_+(c)$ and $p_-(c)$ versus length τ of the second holding period.

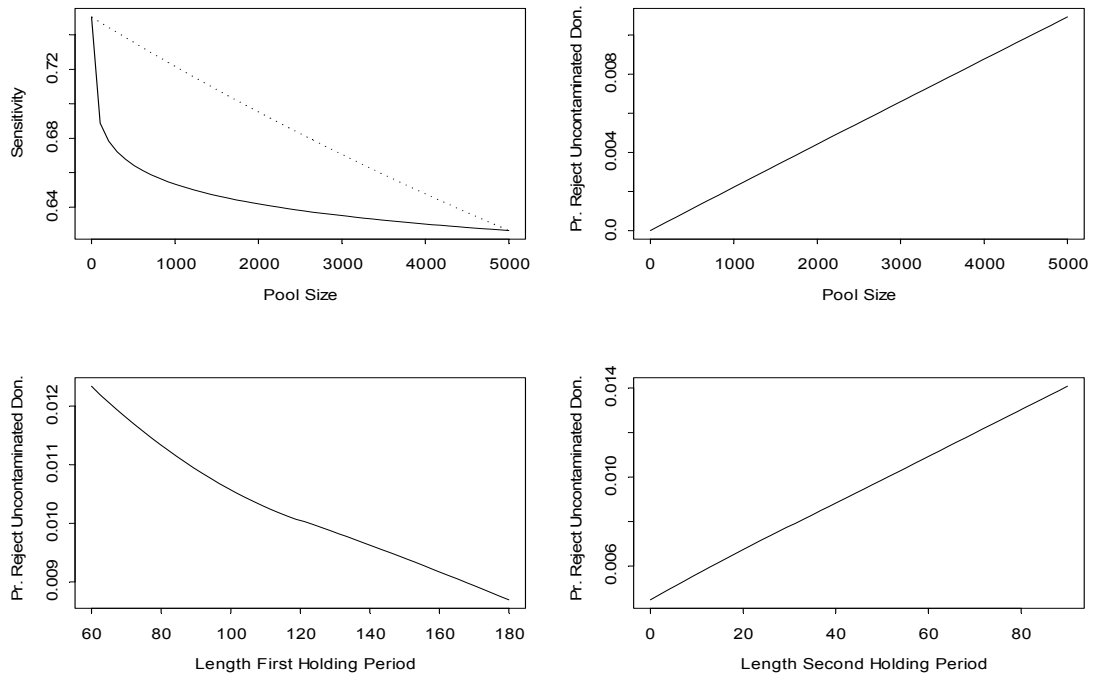

Fig. 4. Diagnostic sensitivity versus pool size c for dilution effect model (solid) and Hung and Swallow model (dashed) (top, left); Probability to reject uncontaminated donations versus pool size (top, right), length of the first holding period (bottom, left) and length of the second holding period (bottom, right).

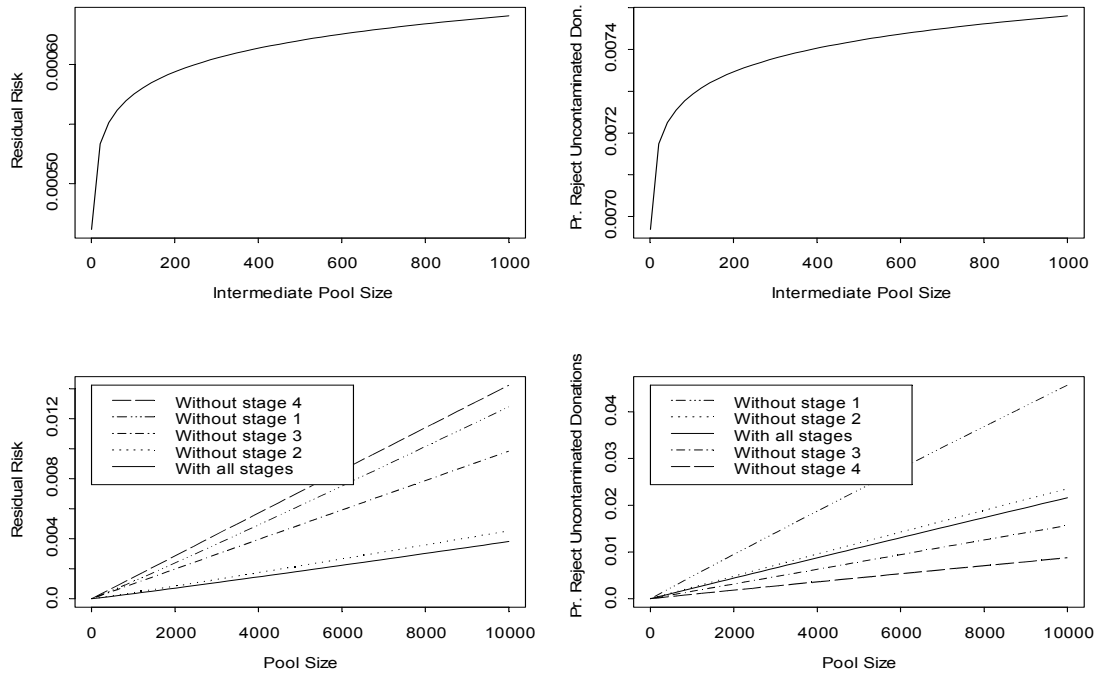

Fig. 5. Top: Residual risk (left) and probability to reject uncontaminated donations (right) versus intermediate pool size; Bottom: Residual risk (left) and probability to reject uncontaminated donations (right) versus pool size in the absence of safety filters.