the data for the old methods have been pooled (with and without exclusion of the center with the diversion bag). Starting from October 2002, a uniform disinfection method was introduced nationwide with a 30-second spaced double-swab method with 70 percent IPA. Sanquin Blood Bank Region Southwest introduced this new disinfection method in October 2002, the Regions Northeast and Southeast did so in November 2002, and Region Northwest introduced it in January 2003.

Screening on bacterial contamination

For the screening on bacterial contamination, within 2 hours after preparation of a T5 (this is 16-22 hr after collection) or within 12 hours after collection of the apheresis product, both aerobic and anaerobic culture bottles were inoculated (approx. 7.5 mL per bottle; range, 5-10 mL). Inoculation was performed under aseptic conditions with a laminar airflow cabinet, as reported previously.[7]

Culture bottles were incubated at 35°C in the BacT/ALERT system until flagged positive or up to 7 days if negative (also in those cases were the corresponding bottle became positive). Positively flagged bottles were sent to regional reference laboratories for determination by plating the sample. PCs and RBCs related to an initially positive bottle were taken out of inventory or recalled. Corresponding RBCs, if available from stock or recall, were cultured in the BacT/ALERT (for 7 days; both aerobic and anaerobic bottle), and positively flagged bottles were also sent to the reference laboratories for determination.

Study design and statistical analysis

Both initial culture test results and data on the microbiologic determination have been included in analyses of the results over the complete calendar years 2002 and 2003. The results were used to test the effects of the new disinfection method and/or the diversion on the degree of contamination. The results of the center with the diversion bag were compared to the pooled results of all other centers not with the diversion bag.

Data showing a positive initial test, but without determination results, have been labeled as missing. If the determination test could not detect microorganisms, the initial test result has been labeled as negative determination culture.

Because the hypothesis was that the contamination degree would be decreased after introduction of diversion and/or new disinfection, differences in frequency of bacterial contamination between the (sub)groups were tested with a one-sided chi-square test unless indicated otherwise. Logistic regression was used to calculate the odds ratios (OR) for risk of bacterial contamination with the different methods (diversion and disinfection). Multivariate logistic regression was performed to correct for possible confounding effects, such as seasonal effects and AS.

All statistical analyses were performed with computer software (SPSS, version 11.0, SPSS Inc., Chicago, IL).

RESULTS

Number of contaminated products

In 2002 and 2003, a total of 122,907 PCs were tested in the BacT/ALERT system, with an aerobic and an anaerobic bottle per product, each inoculated with 7.5 mL of PC. The majority of these PCs were T5, but 1814 products were made by pooling three buffy coats, with an initially positive signal rate of 0.39 percent (n = 7). A total of 8000 apheresis PCs were tested, with 0.30 percent (n = 24) initially positive in the BacT/ALERT system. Because of their small numbers, the products made by pooling three buffy coats are left out of further analysis, and the apheresis products are presented as separate group, with limited data analyses, leaving 113,093 T5 products.

In approximately 10 percent of initially positive results, with some variation per region, no microorganism could be cultured from the positive bottle. Infrequently, however, a microorganism could be isolated from one of the associated RBCs (9 of 106). For a limited number of initially positive samples no information was reported regarding determination culture (these are labeled as missing), but for the large majority (98%) data on determination were available.

Effects of diversion and disinfection methods

In Table 1 the frequency of bacterial contamination for the different collection methods is shown. Diversion was associated with a significant reduction of bacterial contamination from 0.95 to 0.50 percent (chi-square test, $p = 0.002$) with the old skin disinfection method and from 0.85 to 0.37 percent (chi-square test, $p = 0.001$) when the new disinfection method was applied. For collections without diversion, the new skin disinfection method compared to the old methods resulted in a mild reduction in initially positive samples from 0.95 to 0.85 percent (chi-square test, $p = 0.049$). For collections with diversion, the new skin disinfection method compared to the old method also resulted in a reduction in initially positive samples from 0.50 to 0.37 percent (chi-square test, $p = 0.18$).

Calculated on initially positive samples, diversion resulted in an OR of 0.47 (95% CI, 0.35-0.63; $p < 0.001$) with univariate logistic regression. Multivariate logistic regression, with correction for disinfection method, time of screening (season), and AS resulted in an OR of 0.49 (95% CI, 0.36-0.67; $p = 0.003$).

For the new disinfection, the OR was 0.88 (95% CI, 0.77-1.00; $p = 0.05$) in univariate analysis and 0.87 (95% CI,

TABLE 1. Degree of contamination in subsets of T5 products*

Collection method	No diversion	Diversion	Total
Old skin disinfections			
Total tested	42,582	4,362	46,944
Initially positive (%)	405 (0.95)	22 (0.50)	427 (0.91)
Positive determination culture (%)	373 (0.88)	18 (0.41)	391 (0.84)
Negative determination culture (%)	26 (0.06)	4 (0.09)	30 (0.06)
Missing subculture data	6	0	6
70 percent IPA skin disinfection			
Total tested	59,400	6,749	66,149
Initially positive (%)	505 (0.85)	25 (0.37)	530 (0.80)
Positive determination culture (%)	427 (0.72)	17 (0.25)	444 (0.67)
Negative determination culture (%)	68 (0.11)	8 (0.12)	76 (0.11)
Missing subculture data	10	0	10
Total test period			
Total tested	101,982	11,111	113,093
Initially positive (%)	910 (0.89)	47 (0.43)	957 (0.85)
Positive determination culture (%)	800 (0.78)	35 (0.32)	835 (0.74)
Negative determination culture (%)	94 (0.09)	12 (0.11)	106 (0.09)
Missing subculture data	16	0	16

* Absolute numbers, within parentheses: percentages of total tested.

0.76-0.99; p = 0.03) with multivariate analysis corrected for time of screening (season), AS, and diversion. Because of the study design, correction for the region was not possible, but no significant differences between the regions were found. Removing individual centers from the analysis did not alter any of the conclusions and only marginally affected estimated effect sizes and CIs.

The effects of the various interventions have been evaluated, either with the percentages of initially positive samples or with the percentage of positive samples resulting in a positive determination culture. The percentage of initially positive samples determines the effect of the interventions on the numbers of PCs and related RBCs blocked by the screening and thus the consequences for blood banking logistics. The positive determination cultures reflect the effect of the interventions on the final degree of bacterially contaminated PCs. The ORs were slightly lower (with lower p values) when calculated on the number of positive determination cultures, owing to the increase in negative determination cultures after introduction of diversion and/or the new disinfection method.

Plasma compared to AS

At three collection centers (Rotterdam in Region Southwest, Utrecht in Region Northwest, and Nijmegen in Region Southeast), T5 products were prepared in a mixture of PLT AS (PAS II, Baxter) and plasma (65:35 ratio, vol/vol; n = 35 812). We have compared the contamination in PAS II products without diversion with plasma products without diversion to evaluate the effect of AS on the degree of contamination.

The frequency of bacterial contamination was slightly reduced in PAS II PLT products (0.86%) compared to plasma products (0.91%; chi-square test, p = 0.44, not significant). This difference did not change after the introduction of the 70 percent IPA.

Determination results

Table 2 summarizes the different species found in the contaminated T5 products, with classification in some origin-related groups. The majority of detected microorganisms were skin bacteria, mainly diphtheroids or coagulase-negative staphylococci (CNS; and other Gram-positive cocci). The other bacteria belong either to the transient skin bacteria or to the gastrointestinal tract.

After diversion, contamination with skin flora (Table 2; both diphtheroids [chi-square test, $p < 0.001$] and the CNS plus group [chi-square test, p = 0.001]) was significantly reduced, whereas the other groups were not significantly reduced. The fact that no bacteria belonging to the gastrointestinal tract were found after diversion is probably due to their low frequency in combination with the relatively small numbers tested. The fraction of initially positive bottles with a negative determination culture was higher with diversion, although not significantly so (chi-square test, p = 0.36).

When comparing the old disinfection method with the new disinfection method (without diversion), a significant reduction of the CNS plus group was found (Table 2; chi-square test, $p < 0.001$), but no reduction of diphtheroids. Contamination with other bacteria was slightly reduced; however, this difference was significant only for *Staphylococcus aureus* (chi-square test, p = 0.02). As for diversion, an increase in the fraction with a negative determination culture was observed (chi-square test, p = 0.002).

Some significant differences in the type of microorganisms were detected between plasma and PAS II PLT

TABLE 2. Determination of initially positive samples*

Variable	Old disinfection, no diversion	New disinfection, no diversion	Diversion (both disinfections)	Totals
Number of PCs tested	42,582	59,400	11,111	113,093
Initially positive bottles	405	505	47	957
Missing determination	6	10	0	16
Skin flora				
Diphtheroids†	160 (0.38)	230 (0.39)	18 (0.16)	408
CNS plus‡	153 (0.36)	119 (0.20)	12 (0.11)	284
S. aureus§	9 (0.02)	3 (0.005)	1 (0.01)	13
Transient skin flora				
Bacillus spp.‖	25 (0.06)	35 (0.06)	4 (0.04)	64
Gastrointestinal tract flora				
Gram-negative rods¶	8 (0.02)	9 (0.02)	0	17
Streptococci	5 (0.01)	1 (0.002)	0	6
Peptostreptococci	9 (0.02)	15 (0.03)	0	24
Residual group				
Others	4 (0.01)	15 (0.03)	0	19
No microorganism	26 (0.06)	68 (0.11)	12 (0.11)	106

* Values given as absolute numbers (percentage of total tested samples), with an additional column adding up absolute numbers.
† Diphtheroids include: *Propionibacterium* spp. (mainly acnes), *Corynebacterium* spp., and unspecified diphtheroid rods or Gram-positive rods (not spore forming).
‡ CNS plus include all coagulase-negative *Staphylococcus* subspecies (mainly epidermidis) and Gram-positive cocci (less than 10% of the total; aerococci [2]; micrococci [5], and unspecified [16]).
§ Although there are limited reports on *S. aureus* contamination originating from endogenous bacteremia in the donor.
‖ *Bacillus* spp. (roughly 50% *B. cereus*), including some unspecified spore formers (12).
¶ Various species, i.e., pseudomonas (1), brevundimonas (1), flavomonas (2), bacterioides spp. (4), salmonella (1), proteus vulgaris (1), and some unspecified (6 aerobic, 1 anaerobic).

products. Both *S. aureus* and Gram-negative rods had a significantly lower frequency in PAS II products (chi-square test, $p = 0.01$, two-sided). Moreover, the frequency of initially positive bottles with a negative determination culture was lower in PAS II (chi-square test, $p = 0.01$, two-sided). These differences might be explained by a lower initial load with bacteria, because less potentially contaminated whole blood–derived material is used.

Aerobic and anaerobic bottles

Table 3 shows the mean time until the aerobic and anaerobic bottles became positive, in relation to the determination result. In general, aerobic bottles became positive sooner than anaerobic bottles. From the positive aerobic bottles only 35 (11%) turned positive after 5 days, whereas from the positive anaerobic bottles 221 (35%) turned positive after 5 days. Except for diphtheroid (more particularly the *Propionibacterium* spp.) and peptostreptococci species, most bacteria did not show a preference for either the aerobic or the anaerobic bottles (Table 3). This is in agreement with the results of spiking studies by Brecher and colleagues[14] showing for most bacteria growth in both the aerobic and the anaerobic bottle. During the test period, only 46 positively flagged units were reported to become positive in both the aerobic and the anaerobic bottle. It must be kept in mind that every negative bottle (also in case the corresponding bottle became positive) was cultured for 7 days.

Apheresis products

Table 4 shows some details for the 8000 apheresis PCs, all collected with an apheresis set including a diversion pouch. Owing to small numbers, the 95 percent CIs overlap, and no significant differences were found between the old and new disinfection methods. As for the T5, the percentage of positively flagged bottles without microorganism in the determination culture was higher after introduction of the new disinfection method (chi-square test, $p = 0.26$). In the apheresis PCs in two cases a Gram-negative rod was detected (0.03%; an *Escherichia coli* and a *Bacteroides* spp.), in one case a *Bacillus* spp. (0.01%), and in two cases a *Streptococcus* (0.03%). In eight cases the bacteria belonged to the CNS plus group (0.1%), in three cases the bacteria belonged to the diphtheroids group (0.04%), and in seven cases a negative determination culture was obtained (0.09%). These frequencies were comparable to those found in the T5 products after diversion (Table 2), except for diphtheroids (0.04% vs. 0.16% in T5).

Follow-up procedures after positive flagging

PCs. For 790 (83% of total) positively flagged T5, data on recall procedures were available (Table 5). From these units, 386 (49%) units had to be recalled and 404 units were still at the blood bank. The majority (88%) of the recalled products had already been transfused and only 45 of the recalled products could be prevented from being transfused (Table 5). Positively flagged units, which were

TABLE 3. Growth characteristics for different bacteria species

Variable	Total number of bottles	No data on time until positive	Both bottles positive	Aerobic bottle		Anaerobic bottle	
				Time until positive*	Number	Time until positive*	Number
Total	957	38	46	1.8	330	4.0	635
Skin flora							
Diphtheroids†	408	5	4	3.7	45	5.0	362
CNS plus‡	284	2	26	1.1	165	2.6	143
S. aureus§	13	0	0	0.9	6	1.3	7
Transient skin flora							
Bacillus spp.‖	64	1	7	1.7	41	2.1	29
Gastrointestinal tract flora							
Gram-negative rods¶	17	0	0	1.9	10	2.9	7
Streptococci	6	0	3	2.8	4	3.0	5
Peptostreptococci	24	1	0		0	3.9	23
Residual group							
Others	19		3	1.3	9	4.0	13
No microorganism	106	13	3	2.3	50	2.4	46
Missing determination	16	16					

* Mean time in days until positive signal with BacT/ALERT.
† Diphtheroids include: *Propionibacterium* spp. (mainly acnes), *Corynebacterium* spp., and unspecified diphtheroid rods or Gram-positive rods (not spore forming).
‡ CNS plus include all coagulase-negative *Staphylococcus* subspecies (mainly epidermidis) and Gram-positive cocci (less than 10% of the total; aerococci [2]; micrococci [5], and unspecified [16]).
§ Although there are limited reports on *S. aureus* contamination originating from endogenous bacteremia in the donor.
‖ *Bacillus* spp. (roughly 50% *B. cereus*), including some unspecified spore formers (12).
¶ Various species, i.e., pseudomonas (1), brevundimonas (1), flavomonas (2), bacterioides spp. (4), salmonella (1), proteus vulgaris (1), and some unspecified (6 aerobic, 1 anaerobic).

TABLE 4. Degree of contamination in subsets of apheresis products*

Variable	Old skin disinfections	70% IPA skin disinfection	Total test period
Total number tested	3037	4963	8000
Initially positive (%)	*7 (0.23)*	*17 (0.34)*	*24 (0.30)*
Positive determination culture (%)	6 (0.20)	12 (0.24)	18 (0.23)
Negative determination culture (%)	1 (0.03)	5 (0.10)	6 (0.08)
Missing subculture	0	1	1

* Absolute numbers, within parentheses: percentages of total tested. All apheresis products were collected with a set with diversion pouch.

already transfused at the moment of detection, had a mean time until detection of 4.7 days, whereas for the units still in the blood bank the mean time until detection was 2.2 days. Of 345 units with a positive signal in the first 48 hours of culture, only 27 units had already been transfused.

Table 5 also shows the recall information in relation to the various microorganisms. For the rapidly growing bacteria, most of the PCs were still in the blood bank inventory or could successfully be recalled. The majority of already transfused PCs were contaminated with diphtheroids, mainly flagging positive between Day 4 and Day 7 of culture.

During this surveillance period, no severe clinical effects of units flagged positive after transfusion were reported to Sanquin or the Dutch hemovigilance system (Transfusion Reactions in Patients; TRIP). Over the entire period, there were 165 follow-up reports (for every case a report was asked, but only in 40% a response was received) of transfused products corresponding to cultures flagged positive after release. In two cases mild clinical symptoms (fever) were reported, but no direct relation with the PLT transfusion was established. During the same period, two cases of sepsis were reported after transfusion of PCs with a negative signal in the screening[15] (also after 7 days of culturing). In both cases *Bacillus cereus* was identified as the causative microorganism, but it remains unclear whether this microorganism could have entered the PC during preparation or storage, due to pinholes in the bag, or whether it was definitely a false-negative result of the screening system (present in the sample, but not resulting in a positive flagging of the culture).

Related RBCs. In case of a positive signal in the bacterial screening of a pooled PLT product, corresponding RBCs were cultured. Data on RBC recall and cultures were available for 94 percent (901/957) of the initially positive flagged T5 units. In 40 percent of these cases there was a positive signal from the BacT/ALERT for one of the RBC units. In 85 percent of positive RBC units, the same microorganism as in the corresponding PC was found (majority diphtheroids). In the majority of cases where the microorganism was of different species, it belonged to the same group. Only in 27 cases was the microorganism found in

TABLE 5. Recall and transfusion details with respect to corresponding PCs and RBC units

Variable	Corresponding PC units				Related RBC units			
	Total number of PCs	Unknown*	Recall†	Transfused‡	Total number of RBC units	Unknown*	Recall†	Transfused‡
Total	957	167	386	341	4785	375	741	138
Skin flora								
Diphtheroids§	408	57	256	234	2040	76	553	113
CNS plusǁ	284	54	57	42	1420	85	92	12
S. aureus¶	13	0	0	0	65	2	0	0
Transient skin flora								
Bacillus spp.**	64	10	16	12	320	15	28	2
Gastrointestinal tract flora								
Gram-negative rods††	17	0	3	3	85	25	2	1
Streptococci	6	1	1	1	30	0	1	0
Peptostreptococci	24	3	14	12	120	2	16	3
Residual group								
Others	19	3	11	9	95	0	6	1
No microorganism	106	23	28	28	530	90	43	6
Missing determination	16	16			80	80		

* No data on recall available.
† Recall of products already released to hospitals.
‡ Already transfused at time of recall.
§ Diphtheroids include: *Propionibacterium* spp. (mainly acnes), *Corynebacterium* spp., and unspecified diphtheroid rods or Gram-positive rods (not spore forming).
ǁ CNS plus include all coagulase-negative *Staphylococcus* subspecies (mainly epidermidis) and Gram-positive cocci (less than 10% of the total; aerococci [2]; micrococci [5], and unspecified [16]).
¶ Although there are limited reports on *S. aureus* contamination originating from endogenous bacteremia in the donor.
** *Bacillus* spp. (roughly 50% *B. cereus*), including some unspecified spore formers (12).
†† Various species, i.e., pseudomonas (1), brevundimonas (1), flavomonas (2), bacteroides spp. (4), salmonella (1), proteus vulgaris (1), and some unspecified (6 aerobic, 1 anaerobic).

the RBCs totally unrelated to the organism as found in the PCs. This number included the 9 cases with no determination culture for the PC, whereas a microorganism could be isolated from one of the associated RBC units. The bacterial species in these cases were CNS plus (4), diphtheroids (4), and *Bacillus* spp. (1).

Of a total of 4505 RBC units (901 PCs each with 5 related RBC units), 741 RBC units had already been released and had to be recalled for culturing. The recall was successful in 68 percent of the cases. A total of 234 units were not available for culture, including 138 RBC units (relating to 99 PC units) that had already been transfused. For 69 of these 138 cases, one of the other related RBC units contained the same bacterium as found in the culture from the PC. From this it might be concluded that only in the remaining 69 cases possibly contaminated RBCs were transfused. These were mainly related to positive PLT cultures in which diphtheroids found (66/69). Because RBCs are stored at 4°C, however, it is not likely that these transfused RBCs contained harmful quantities of bacteria. This is supported by the fact that no transfusion reactions were reported for these units.

DISCUSSION

Based on the results of two calendar years (2002 and 2003) of bacterial screening of all PCs in the Netherlands, it can be concluded that interventions to reduce bacterial contamination were very effective, resulting in a reduction of initially positive cultures for pooled T5 products from 0.95 percent (no interventions) to 0.37 percent (two interventions: diversion and new skin disinfection). The 50 percent reduction in frequency upon diversion is very similar to that found in our previous whole-blood diversion study[10] and also corresponds with the reduction in contamination reported by McDonald and coworkers.[16] The double-swab 70 percent IPA disinfection method slightly enhanced the reducing effect on bacterial contamination of diversion, although this is of marginal significance and without synergy. The final frequency of 0.37 percent (95% CI, 0.24-0.55) positive cultures found for pooled T5 products after introduction of both diversion and new disinfection method is not significantly different from the 0.30 percent contamination rate for single donor apheresis PC (95% CI, 0.19-0.45), whereas the differentiation profile is also similar. This suggests that the relation between number of donor units and degree of bacterial contamination of the final PC, as described by Ness and colleagues,[1] no longer exists if special care is taken to avoid introduction of bacteria during collection. The finding that contamination is reduced to such a large extent by diversion has led to the introduction of the diversion pouch throughout the Netherlands, starting from July 2004.

Our current screening system for bacterial contamination of PC is highly sensitive by use of two bottles. The

fact that in less than 5 percent of positively flagged units both bottles were positive suggests that positive units have an initial bacterial contamination around the lower detection limit of the culture system. Owing to the high sensitivity, the percentage of initially positive units is relatively high in our screening system compared to other reported rates of bacterial contamination. Most likely, this is due to the fact that other studies use only one culture bottle, with lower inoculation volumes and shorter culture times. Wagner and Robinette[17] showed that higher inoculation volumes lead to shorter detection times. Our results are very similar to those in Belgium, where a similar screening approach is used.[18,19] When reanalyzing our results according to the system used in Denmark, that is, only the aerobic bottle and 5 days of culture, our result of 0.4 percent initially positive for T5 (with double-swab disinfections and without diversion) is very comparable to the Danish results.[20] Also for apheresis units, the recalculated initially positive rate of 0.1 percent (only aerobic bottle, 5 days culturing) resembles the values reported by other groups[3] taken into account that our inoculated volume is larger.

The selectivity of a screening system should be as high as possible, because false-positive samples result in unnecessary recalls and false-negative samples result in possible transfusion of positive units. For some as yet unknown reason, the rate of negative determination cultures is increased after the introduction of interventions for disinfection and diversion. The fraction of negative determination cultures is 0.047 percent of total tested bottles (two per unit), which is in agreement with data from Belgium and Denmark.[18,20] This would result in a false-positive rate by system failure of about 1 of 2000 cultures. Given that in 9 percent of cases with a negative determination culture for PCs, one of the RBC units contained a microorganism, it remains questionable whether a negative determination culture really should be flagged false-positive. Another possibility would be that in some cases the microorganism still has growth capacity (positive signal in BacT/ALERT), but no colony-forming properties (negative determination culture). This possibility is not hypothetical, because experiments from our group showed that after UV-C illumination the survival of bacteria is much higher (about 1.5 log) when measured in liquid culture, compared to measurement of colony formation on solid media (unpublished results). Based on these results, we intend to implement a follow-up procedure for negative determination cultures, with additional attempts to obtain a subculture.

The effect of introducing a standardized double-swab 70 percent IPA disinfection method showed borderline significance in both univariate and multivariate statistical testing with respect to reduction of bacterial contamination. This is in contrast to literature results. McDonald and colleagues[16] reported a possible reduction of approximately 50 percent by the introduction of double-swab disinfection. These results, however, were obtained with a first swab with IPA and a second swab with iodine tincture, which might explain differences in the results. Lee and coworkers[21] recently also reported a significant reduction of the degree of bacterial contamination of PCs prepared from whole blood by the PLT-rich plasma method, as a result of a changed disinfection method. With respect to skin flora, however, as expected, our new disinfection method has a highly significant reducing effect on the CNS plus group, but no effect on diphtheroids. This can be explained by the fact that diphtheroids especially colonize the interior of the sebaceous glands[22] whereas Gram-positive cocci, including CNS, are more on the skin surface. Surface disinfection will therefore be less adequate to remove diphtheroids, whereas diversion will reduce all kind of skin flora, as found in our study.

The various bacteria species detected in 2 years of bacterial screening of PCs in the Netherlands are classified as skin flora, transient skin flora, or gastrointestinal tract flora, with some very rough subclassification. Within the skin flora, the relatively slowly growing diphtheroids (either anaerobic *Propionibacterium* or aerobic *Corynebacterium*) represent the largest group. These bacteria are usually not considered being a clinical hazard[14,23] but there are some reports describing transfusion-transmitted bacterial infection (TTBI) with *Propionibacterium*.[24,25] Diphtheroids also are among the Gram-positive bacteria that can colonize prosthetic valves and intravascular implants, causing infections that are difficult to treat.[26] Upon prolongation of storage time to 7 days or longer, and without a screening system able to detect these bacteria, one should be aware of transmission of slow-growing bacteria, such as *Propionibacterium* spp. This problem is currently not encountered, because PCs containing these bacteria are generally transfused before reaching the threshold for inducing TTBI in the recipient.

The second largest group are the Gram-positive cocci not being *S. aureus* (CNS plus). These commensal skin flora are not considered very pathogenic either. Gram-positive cocci, however, are a regular cause of TTBI[24,27] but seldom responsible for septic fatalities.[28] The small subgroup of *S. aureus* is the type of skin flora with a high clinical hazard profile[1] but all 13 cases found in our surveillance had a positive culture before the products were released.

The *Bacillus* spp. belongs to the transient skin flora and this group includes some not further determined spore formers. Owing to the etiology of infection (we are only considering transmission via transfusion), no further distinction was made between *B. cereus* (known from food poisoning) and other *Bacillus* spp. The new disinfection method has no effect on the frequency of this group, as expected, because spores are not inactivated by 70 percent IPA. Diversion is expected to result in a reduc-

tion for spore formers, but the observed reduction is not significant, likely because of the low numbers. Because spore formers remain a risk to cause TTBI even with bacterial culturing in place, further research should be focused on improved disinfection also effective in inactivating spores.

Approximately 5 percent of all initial positive samples were determined as normally belonging to the gastrointestinal tract flora, although these bacteria can also be encountered as transient skin flora. The largest subgroup are the anaerobic *Peptostreptococci* spp. (associated with abdominal wound infections) and some *Streptococcus* spp., which are often reported in transfusion-associated sepsis.[28]

The group of Gram-negative rods is very heterogeneous, with *Bacteroides* spp. and *Salmonella* spp. highly likely to have come from the donor's blood circulation. The aerobic Gram-negative rods, although we did not fully determine the species in all cases, are most probably nonfermentative bacilli and are highly likely to have been introduced as transient skin flora. The Gram-negative rods are well known as causative agents for TTBI, especially those cases related to septic fatalities.[1,2,24,25,28] In our study most products positive for the presence of Gram-negative rods were prevented from entering the transfusion chain. In the single case with a transfused RBC unit, the culture turned positive after 5 days.

Among the remaining group, one remarkable microorganism is found, a *Nocordia* spp., well known as transient skin flora present in soil and causing pneumonia, especially in immunocompromised patients or in chronic lung disease.

Despite the absence of a quarantine period, in the Dutch practice more than 90 percent of the units that flagged positive within 2 days could be prevented from being transfused, including the majority of those contaminated with the more dangerous bacteria like Gram-negative bacteria, *B. cereus* and *S. aureus*. Ninety percent of units flagged positive after being released had a positive signal after more than 2 days of culturing. These units were mainly found to be contaminated with diphtheroids, especially *Propionibacterium* spp. and the majority of these units had already been transfused. During the period of the study, two mild transfusion reactions were reported due to transfusion of such units. In general, however, underreporting is noticed in hemovigilance systems, emphasizing the need for improvement. Extension of the storage period of PCs from 5 to 7 days allows for an initial quarantine period as proposed by Munksgaard and colleagues[20] but this will have a very limited impact on the number of PCs already transfused at the time of a positive culture.

In conclusion, it is shown that introduction of diversion and improvement of skin disinfection are effective in reducing the frequency of contaminated blood products. Still, the remaining risk is high enough to warrant screening for bacterial contamination. The screening system in place in the Netherlands proves to be successful in preventing the seriously contaminated PCs from entering the transfusion chain, a similar conclusion to that reported for Belgium[19] with a similar screening system.

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