HEV喫食歴問診調査

調査期間　2006/2/1 ～ 2/28
問診内容　『過去3ヶ月以内に生肉、レバー、ホルモンを食べましたか』

対象献血者数(札幌地区固定施設)	6,059	(100.0%)
喫食歴問診該当者数	1,690	(27.9%)
HEV-RNA陽性者	3	(0.05%)
HEV-RNA陽性問診該当者	1	(0.02%)

表1 HEV-RNA陽性問診該当者

No.	年齢	性別	献血日	ALT (IU/L)	HEVマーカー			喫食歴アンケート調査		問診該当
					RNA	IgM	IgG	肉の種類	食べ方	
1	39	F	02/02	35	+	-	+	ウシレバー	生	有
								ウシレバー	半生	
								ヒツジ精肉	十分加熱	
2	39	M	02/17	28	+	-	-	ブタホルモン、ブタレバー、ブタ胃、ヒツジ精肉	半生	無
								イノシシ精肉、ブタ精肉	十分加熱	
3	45	M	02/21	30	+	-	-	ウシ精肉	半生	無
								ブタ精肉、ブタレバー、ヒツジ精肉	十分加熱	

表2 年代別喫食歴問診該当者

年代	男	女	回答なし	総計	(%)
16 - 19	62	105	1	168	(10%)
20 - 29	238	293	5	536	(32%)
30 - 39	222	221	7	450	(27%)
40 - 49	183	131	5	319	(19%)
50 - 69	122	84	1	207	(12%)
回答なし	0	0	10	10	(1%)
総計	827	834	29	1690	(100%)

表3 問診該当食材内訳 (重複回答有)

動物種	肉の種類				総計
	ホルモン	レバー	精肉	不明	
ブタ	603	256	64	7	930
ウシ	283	279	160	6	728
ヒツジ	2	2	56	4	64
トリ	3	30	4	0	37
シカ	0	1	18	6	25
ウマ	0	2	3	0	5
イノシシ	1	1	0	0	2
不明	277	185	7	1	470
総計	1169	756	312	24	2261

表4 問診該当食材調理法内訳 (重複回答有)

肉の種類	調理法				総計
	十分加熱	半生	生	不明	
ホルモン	1140	15	4	10	1169
レバー	591	14	140	11	756
精肉	105	113	90	4	312
不明	12	5	5	2	24
総計	1848	147	239	27	2261

表5 問診該当食材・調理法内訳 (重複回答有)

動物種	肉の種類	調理法				総計
		十分加熱	半生	生	不明	
ブタ	ホルモン	593	5	0	5	603
	レバー	245	3	6	2	256
	精肉	35	26	3	0	64
	不明	5	1	0	1	7
	計	878	35	9	8	930
ウシ	ホルモン	269	7	4	3	283
	レバー	159	8	112	2	279
	精肉	33	58	66	3	160
	不明	2	0	3	1	6
	計	463	71	185	9	728
ヒツジ	ホルモン	2	0	0	0	2
	レバー	2	0	0	0	2
	精肉	31	20	5	0	56
	不明	1	3	0	0	4
	計	36	23	5	0	64
トリ	ホルモン	3	0	0	0	3
	レバー	28	0	2	0	30
	精肉	0	1	3	0	4
	計	31	1	5	0	37
シカ	レバー	1	0	0	0	1
	精肉	4	5	9	0	18
	不明	3	1	2	0	6
	計	8	6	11	0	25
ウマ	レバー	0	0	2	0	2
	精肉	0	0	3	0	3
	計	0	0	5	0	5
イノシシ	ホルモン	1	0	0	0	1
	レバー	1	0	0	0	1
	計	2	0	0	0	2
不明	ホルモン	272	3	0	2	277
	レバー	155	5	18	7	185
	精肉	2	3	1	1	7
	不明	1	0	0	0	1
	計	430	11	19	10	470
総計		1848	147	239	27	2261

別紙様式第2-1

No. 3

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 1. 24	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Fang CT, Chambers LA, Kennedy J, Strupp A, Fucci MC, Janas JA, Tang Y, Hapip CA, Lawrence TB, Dodd RY; American Red Cross Regional Blood Centers. Transfusion. 2005 Dec;45(12):1845-52.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			米国

研究報告の概要

○成分採血由来の血小板製剤における細菌汚染の検出:2004年、アメリカ赤十字の経験

背景:2004年3月、アメリカ赤十字の36の地域血液センター全てにおいて、成分採血由来の血小板製剤における細菌汚染に対するルーチンの品質管理試験を実施した。

実験デザイン及び方法:製剤の有効期間終了時まで、あるいは陽性反応が示されるまで、好気条件下で血小板検体を培養した。初期検査陽性反応の確認を行うため、再培養のために製剤から新たな検体を採取した。培養陽性であったボトルすべてについて、細菌分離と識別のため、検査を行った。解析のため、成分採血由来の血小板の採血情報とともに細菌検査データを収集した。成分採血由来の血小板による敗血症性副作用と考えられるものについては、報告及び調査のレビューを行った。

結果:細菌検査の最初の10ヶ月で、350,658検体中226検体が初期検査陽性であった。再度検体採取を行ったところ68件で細菌汚染が確認され、陽性率は全体で0.019%、5157件当たり1件であった。最も多く分離された細菌はブドウ球菌属(47.1%)及びレンサ球菌属(26.5%)で、陽性が確認された製剤の17.6%はグラム陰性細菌であった。初期検査陽性であった226例由来の354件の成分採血由来の血小板製剤中38件(10.7%)が、初期検査陽性反応が示されるまでに輸血されていた。しかし、これらの輸血された血小板製剤はいずれも細菌スクリーニングで陽性が確認されず、未確認の陽性製剤を輸血された患者で敗血症性輸血副作用の徴候を示した者はいなかった。スクリーニング陰性の血小板製剤による敗血症性輸血副作用の可能性が高いとされる3例が特定された。3例すべてで、コアグラーゼ陰性ブドウ球菌が原因とされた。

結論:著者らの経験から、品質管理手段として成分採血由来の血小板製剤の細菌検査がアメリカ赤十字のシステム全体で効果的に実施されたこと、また、全てではないが多くの細菌汚染血小板製剤を特定し、その輸血を防止するためにこの新たな手順が有効であることが示される。

報告企業の意見	今後の対応
2004年3月、アメリカ赤十字の36の地域血液センターにおいて、品質管理手段として成分採血由来血小板製剤のルーチンの細菌検査が実施され、全てではないが多くの細菌汚染血小板製剤を特定したとの報告である。	日本赤十字社では、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)における「本ガイドライン対象以外の病原体の取扱い イ. 細菌」に準じ細菌感染が疑われる場合の対応を医療機関に周知している。 今後も情報の収集に努める。採血時の初流血除去、白血球除去の導入とともに細菌を不活化する方策についても検討を進める。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Detection of bacterial contamination in apheresis platelet products: American Red Cross experience, 2004

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BACKGROUND: Routine quality control (QC) testing for bacterial contamination in apheresis platelet (PLT) products was implemented in all 36 regional blood centers of the American Red Cross in March 2004.

STUDY DESIGN AND METHODS: PLT samples were cultured under aerobic conditions until the end of the product shelf life or when a positive reaction was indicated. To confirm the initial positive reaction, a new sample was taken from the unit for reculturing. All positive culture bottles were referred for bacterial isolation and identification. Bacterial testing data along with apheresis PLT collection information were collected for analysis. Reports and investigations of potential septic reactions to apheresis PLTs were reviewed.

RESULTS: In the first 10 months of bacterial testing, 226 of 350,658 collections tested initially positive. Sixty-eight were confirmed on resampling to be bacterially contaminated for an overall confirmed-positive rate of 0.019 percent or 1 in 5157. *Staphylococcus* spp. (47.1%) and *Streptococcus* spp. (26.5%) were the most frequently isolated bacteria; Gram-negative bacteria accounted for 17.6 percent of the confirmed-positive products. Of the 354 apheresis PLT products derived from all 226 initial test–positive cases, 38 (10.7%) were transfused by the time the initial positive reaction was indicated. None of these transfused products, however, had a confirmed-positive bacterial screen and no patient who had been transfused with an unconfirmed-positive product had evidence of a septic transfusion reaction. Three high-probability septic transfusion reactions to screened, negative components were identified. In all three cases, a coagulase-negative *Staphylococcus* was implicated.

CONCLUSION: Our experience demonstrates that bacterial testing of apheresis PLT products as a QC measure was efficiently implemented throughout the American Red Cross system and that this new procedure has been effective in identifying and preventing the transfusion of many, although not all, bacterially contaminated PLT units.

Septic reactions in recipients of bacterially contaminated blood products, particularly platelets (PLTs), have been recognized as serious problems for decades.[1-3] It was reported that from 1990 to 1998, bacterial contamination of blood accounted for 17 percent of all reported transfusion fatalities and ranked second only to hemolytic complications in the United States.[4] Since the early 1970s, however, focus on blood microbial safety has been concentrated on viruses, that is, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. In 2002, five transfusion medicine physicians jointly signed and issued a public call for the blood collection community to adopt detection methods for bacterial contamination in PLTs.[5] In the same year, the College of American Pathologists' Laboratory Accreditation Program added a requirement for bacterial contamination testing to its transfusion medicine checklist.[6] Furthermore, in 2003, the AABB included a new standard in its 22nd edition of *Standards for Blood Banks and Transfusion Services*, which required that by March 1, 2004, the blood bank or transfusion service should implement methods to limit and detect bacterial contamination in all PLT components.[7] In the meantime, the U.S. Food and Drug Administration had cleared two commercial culture-based methods for quality control (QC) testing for bacterial contamination in leukoreduced PLTs. This report presents data of the first 10 months of bacterial testing on apheresis PLTs collected by the American Red Cross Blood Services.

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MATERIALS AND METHODS

Collection of apheresis PLTs

Units of PLTs were routinely collected from voluntary donors at 36 regional blood centers of the American Red Cross with one of the following three apheresis systems: Amicus separator (Baxter Healthcare, Deerfield, IL), Spectra (Gambro BCT, Lakewood, CO), or Trima system (Gambro BCT). The phlebotomy site(s) was prepared with a povidone-iodine scrub followed by povidone-iodine application. For donors who were allergic to iodine, a chlorhexidine scrub was substituted. During the first 10 months (March 1-December 31, 2004) of bacterial detection, a total of 350,658 apheresis PLT units were collected from 87,430 donors at 36 sites for a systemwide mean of 7992 ± 217 units per week. The mean number of weekly collections per site was 222.0 ± 161.4 (median, 158.0) and varied widely from 35.6 to 751.3 collections. Overall, 78.2 percent of the apheresis PLT products were collected alone while the remaining 21.8 percent were collected in conjunction with plasma and/or red blood cells. Each apheresis collection was transfused either as one single therapeutic dose or divided into two doses ("split") during manufacture.

Detection of bacterial contamination

An automated microbial detection system (BacT/ALERT 3D, bioMérieux, Durham, NC) was validated and installed at each center for detection of potential bacterial contamination in apheresis PLTs. At least 24 hours after collection and before splitting (if applicable), 4 to 5 mL of PLTs from each unit were transferred through sterile connection into a sampling device—either SampLok sampling kit (ITL, Herndon, VA) or PLT sampling devices (Charter Medical, Winston-Salem, NC). After the PLT unit bag was sealed and disconnected, 4 mL from the sampling device was inoculated into a BacT/ALERT BPA aerobic culture bottle through a sterile needle that was part of the sampling device. Inoculation was performed in a laminar flow hood. The culture bottles were then placed in the BacT/ALERT incubator (36 ± 2°C) and incubated until the end of PLT product shelf life (5 days) or when a positive reaction was indicated by the monitor unit of the BacT/ALERT system. Product could be released for distribution after the bottle was incubated for a minimum of 12 hours without a positive reaction.

Reculturing and bacterial identification

Positive culture bottles were sent to independent microbiology laboratories for bacterial isolation and identification. All products associated with a positive bottle were quarantined, or retrieved if already released, for further investigation. A new sample was taken and inoculated into a new BacT/ALERT aerobic culture bottle following the same procedure as for the initial testing. If it was found again to be positive, the second culture bottle was also sent for bacterial isolation and identification. When reculturing was positive and the same bacterium was isolated as in the original culture bottle, the result was defined as "confirmed-positive." If no bacteria could be isolated from the original culture bottle, the result was defined as "false-positive due to instrument error." If bacteria could be isolated from the original culture bottle but reculturing was either negative or a different type of bacterium was isolated from the reculture bottle, the result was considered to be "false-positive due to contamination" during the sampling process. Finally, when PLT components were not available for reculturing because they were either destroyed or transfused, the initial result was considered "indeterminate" because the reproducibility of the bacterial isolation could not be assessed.

Investigation of clusters

Unusual but clinically significant bacteria of the same species isolated from multiple sites in a short period of time were sent to the Holland Laboratory (Rockville, MD) of the American Red Cross for investigation to determine if these isolates were identical, indicating a possible systematic contamination of a certain reagent or device being used at multiple centers. Both phenotyping, with the Apparaeils et Procédés d' Identification[8] (bioMérieux) system, and genotyping, with either the enterobacterial repetitive intergenic consensus (ERIC)[9] analysis for Gram-negative bacteria or the random amplification of polymorphic DNA[10] analysis for Gram-positive bacteria, were performed. When PLT products or culture bottles were not available for laboratory investigation, lot numbers of all materials used in the process of PLT collection and bacterial detection by the involved centers were examined to determine if any common factors were involved.

Review of septic reaction case reports

All reports of potential septic reactions to apheresis PLTs were documented and investigated. A case was categorized as high probability when the clinical signs and symptoms were typical of a septic reaction (e.g., fever, drop in blood pressure, chills, and/or rigors) and there was convincing evidence of pretransfusion bacterial contamination of the associated component (e.g., positive Gram stain of the residual component, positive culture of a component segment or cocomponent, and/or patient blood culture positive for the same organism isolated from the component). Records of reports and investigations of high probability cases since March 2003 were reviewed.

RESULTS

Positive rates in apheresis PLT products

For the first 10 months of bacterial testing on apheresis PLT products, a total of 226 positive reactions were initially identified from 350,658 apheresis PLT units for an overall positive rate of 0.064 percent or 1 in 1552 as shown in Table 1. Of these, 68 (30.1%) were confirmed as true-positive. Table 1 also shows the number and rate of false-positive samples due to instrument error, false-positive samples due to contamination, and positive but indeterminate samples. Figure 1 shows the systemwide monthly rates. During the first month (March) of implementation, the overall positive rate was significantly higher than the other 9 months combined ($p = 0.0025$), mainly due to higher numbers of indeterminate samples ($p < 0.0001$) and false-positive samples due to instrument error ($p = 0.0189$). December also had a higher number of false-positive samples due to instrument error ($p = 0.0227$). September had a slightly higher confirmed-positive rate (3.49 per 10,000) than the other 9 months combined ($p = 0.0487$). There was no trend in the confirmed-positive rate over the 10-month period.

TABLE 1. Numbers and frequencies of apheresis PLT collections with positive reactions in bacterial testing (total number of PLT collections, 350,658)

Sample result	Number (%)	Rate per 10,000
Confirmed-positive	68 (30.1)	1.94 (1 in 5,157)
False-positive due to instrument error	39 (17.2)	1.11 (1 in 8,991)
False-positive due to contamination	75 (33.2)	2.14 (1 in 4,675)
Indeterminate	44 (19.5)	1.25 (1 in 7,970)
Total positive	226 (100)	6.45 (1 in 1,552)

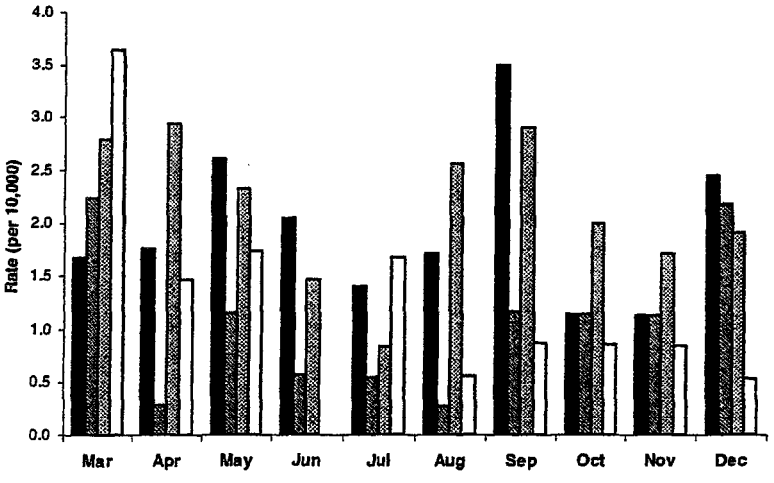

Fig. 1. 2004 monthly distribution of positive rates in bacterial testing on apheresis PLT products. (■) Confirmed-positive; (▨) false-positive (instrument); (▩) false-positive (contamination); (□) indeterminate.

Bacteria isolated from confirmed-positive products

Table 2 lists the identities of bacteria isolated from the 68 confirmed-positive cases. More than 80 percent of the isolates were Gram-positive bacteria, mainly *Staphylococcus* (47.1%) and *Streptococcus* (26.5%) spp. Of the Gram-negative bacteria, *Serratia marcescens* was isolated most frequently ($n = 4$). An unspecified *Bacillus* species was identified from one of the confirmed cases. A pin hole, however, was found in this particular PLT bag and it took 105.6 hours of incubation time before a positive reaction was indicated, which was much longer than the time required for the next longest confirmed-positive reaction (50.4 hr). Although this case was classified as confirmed-positive, it was suspected that the contamination was introduced during storage. Unlike the confirmed-positive group, *Bacillus* spp. accounted for a large proportion (>40%) of false-positive results due to contamination and indeterminate cases as shown in Table 3. All Gram-negative bacteria were associated with confirmed-positive cases.

The original PLT collection had been split in 35 confirmed-positive cases. The same microorganism was isolated from the second component in 31 cases. For the remaining 4, 2 were culture-negative and 2 were not available for culturing. As expected, when the collection associated with false-positive results due to sampling contamination were split and the split components were available for culture, the vast majority were negative (45/46). In one case, a different organism was isolated, indicating a second false-positive result.

Culturing time required for a positive reaction

Table 4 shows the mean detection time (time to positive) of positive reactions according to the type of result. The confirmed-positive group had the shortest mean incubation time whereas the indeterminate group had the longest. The overall difference of time to positive among these four groups was significant ($p < 0.0001$ by ANOVA). The difference between the confirmed-positive group and the false-positive due to instrument error group was not significant ($p = 0.2721$), even after the one confirmed-positive case due to *Bacillus* contamination mentioned above was excluded from analysis ($p = 0.0845$). Furthermore, the distribution of time to positive for these four groups was also

somewhat different as shown in Fig. 2. The distribution of time to positive for the indeterminate group was more similar to the group of false-positive samples due to contamination ($R^2 = 0.3074$) than to the confirmed-positive group ($R^2 = 0.0505$) or to the false-positive samples due to instrument error group ($R^2 = 0.0129$), indicating that cases in this group were also probably due to contamination during the sampling process rather than to the presence of bacteria introduced into the PLT unit at the time of collection.

Table 5 shows the required incubation time for positivity for different types of bacteria isolated on two or more occasions. For the confirmed-positive group, the Gram-negative bacteria (n = 12) required a significantly (p = 0.0005) shorter incubation time for triggering a positive signal than the Gram-positive bacteria even after the one with *Bacillus* spp. (time to positive 105.6 hr) was excluded from analysis.

Investigation of clusters

Among the 67 confirmed-positive cases, 5 were identified as being contaminated with *Streptococcus bovis*, which is known to be associated with colon cancer[11] and biliary tract disease[12] in humans. Samples from three of these five cases were available for further laboratory investigation.

TABLE 2. Bacterial species isolated from confirmed-positive apheresis PLT units

Bacterial strain	Number of cases (%)
Gram positive	56 (82.4)
Staphylococcus spp.	32 (47.1)
Streptococcus spp.	18 (26.5)
Enterococcus avium	2 (2.9)
Bacillus spp.	1 (1.5)
Lactobacillus spp.	1 (1.5)
Listeria monocytogenes	1 (1.5)
Micrococcus spp.	1 (1.5)
Gram negative	12 (17.6)
Serratia marcescens	4 (5.9)
Klebsiella spp.	3 (4.4)
Escherichia coli	3 (4.4)
Citrobacter diversus	1 (1.5)
Unspecified gram negative rod	1 (1.5)
Total	68 (100)

Results of phenotyping with the Apparaeils et Procédés d' Identification biochemical reagents indicated that the isolate from a PLT unit collected at Center A demonstrated hippuricase and β-galactosidase activities, which was not the case for the isolates from 2 units collected at Center B. The latter two isolates also differed from each other in β-glucoronidase activity. Genotyping patterns with the random amplification of polymorphic DNA amplification with a primer pair and procedure described by Torriani and colleages[10] also show that the one isolate (Fig. 3, Lane 4) from Center A was quite different and the two isolates (Fig. 3, Lanes 5 and 6) from Center B, which were also slightly different from each other.

In addition, there were four cases of *S. marcescens* contamination. Because materials were not available for further laboratory investigation for these four cases, extensive record review on reagents, supplies, instruments, procedures, and operations at these collection sites was conducted and no common factor could be identified to indicate a potential systematic problem.

Transfusion of culture-positive PLT products

Of the 226 collections that tested initially positive, 128 units were split producing a total of 354 PLT products. Of these, 38 products (10.7%) from 25 collections had been transfused at the time the culture bottle turned positive. Sampling for reculturing could not be performed in 18 (72%) of these 25 cases either because the original collection was not split and it had been transfused or because it was split and both halves had been transfused. The original positive culture therefore fell into the indeterminate group. The remaining seven cases were all in the false-positive groups due to either contamination (n = 5) or instrument error (n = 2). None of these transfused units were from a collection with a confirmed-positive screening culture and no septic reactions were reported in the patients who had received these products. The incubation time until the bottle turned positive for these 25 collections tended to be long (mean, 72.3 ± 29.1; median, 76.8; range, 23.8-120.0 hr) and more typical of false-positive results.

TABLE 3. Bacteria isolated from apheresis PLT collections according to type of positive result

Bacteria isolated	Confirmed-positive (%)	False-positive (%)	Indeterminate (%)
Gram-positive	56 (82.4)	75 (100)	37 (100)
Staphylococcus spp.	32 (47.1)	22 (29.3)	10 (27.0)
Streptococcus spp.	18 (26.5)	2 (2.7)	3 (8.1)
Bacillus spp.	1 (1.5)	35 (46.7)	16 (43.2)
Corynebacterium spp.	0 (0)	5 (6.7)	4 (10.8)
Other Gram-positive organisms	5 (7.4)	11 (14.7)	4 (10.8)
Gram-negative	12 (17.6)	0 (0)	0 (0)
Total	68 (100)	75 (100)	37 (100)

Septic reaction case reports

From March 2003 through December 2003, before screening, 15 septic reactions involving apheresis PLTs were reported. Twelve were assessed as high probability, 2 of which were fatal. In the same period following screening, 8 septic reactions involving apheresis PLTs were investigated and 3 were assessed as high probability. The investigation of these 3 cases did not identify any irregularities in the donor physical exam, donor health history and record, phlebotomist arm preparation technique, component sampling for culturing, or incubation of the inoculated bottle.

Case 1. An elderly man received apheresis PLTs after a difficult coronary artery bypass redo. One hour later, his temperature increased from 37.6 to 39.4°C with rigors, tachycardia, shortness of breath, and hypotension. Gram stain of the PLT unit showed Gram-positive cocci in clusters. Cultures of both the unit and the patient's blood grew coagulase-negative staphylococcus, subsequently confirmed to be identical strains of *Staphylococcus lugdunensis*. The BacT/ALERT bottle had not alarmed and the bottle indicator remained negative. Gram stain and culture of the bottle were negative. The bottle's content was noted to be slightly cloudy, however, and PLTs were seen on the Gram stain, documenting that the bottle had been inoculated. The bag was examined at the hospital for any defects that may have allowed contamination; none were found. The patient died the day after his reaction when support was withdrawn. (Note: This case was previously published by the Centers for Disease Control and Prevention.[13])

Case 2. A 15-year-old patient was admitted with fever, neutropenia, and thrombocytopenia due to "lymphosarcoma thorax." Halfway through transfusion of an apheresis PLT unit, she developed a temperature elevation from 36.7 to 39.1°C with severe rigors. A Gram stain of the unit showed many Gram-positive cocci subsequently identified as *Staphylococcus epidermidis*. The patient's post-transfusion blood cultures were also positive for *S. epidermidis*. The BacT/ALERT bottle had not alarmed and the bottle indicator remained negative. Gram stain and culture of the bottle were both negative. The patient was treated and survived.

Case 3. A 49-year-old woman was admitted with acute coronary syndrome and gastrointestinal bleeding and found to have a drug-induced thrombocytopenia. While receiving an apheresis PLT, she developed a rise in temperature from 37.1 to 39.0°C, dyspnea, chills, and a mottled skin appearance. A Gram stain and culture on the residual bag contents showed Gram-positive cocci in clusters subsequently identified as coagulase-negative *Staphylococcus*. The patient was treated and survived. The other half of this split collection had been transfused uneventfully to a patient with acute myelogenous leukemia who was medicated with diphenhydramine and acetaminophen, and was receiving vancomycin, fluconazole, metronidazole, and ceftazadine.

TABLE 4. Incubation time (hr) required for initial positive result

Sample result	Number	Mean ± SD	Median	Range
Confirmed-positive	68	19.0 ± 13.8	16.2	5.9-105.6
(Confirmed-positive*)	(67)	(17.7 ± 8.9)	(16.0)	(5.9-50.4)
False-positive due to instrument error	39	22.6 ± 20.2	20.0	0.0-117.0
False-positive due to contamination	75	34.1 ± 22.6	25.6	6.2-110.0
Indeterminate	44	48.4 ± 34.1	35.3	4.1-120.0

* Excluding one *Bacillus* contaminated unit with a time to positive of 105.6 hours.

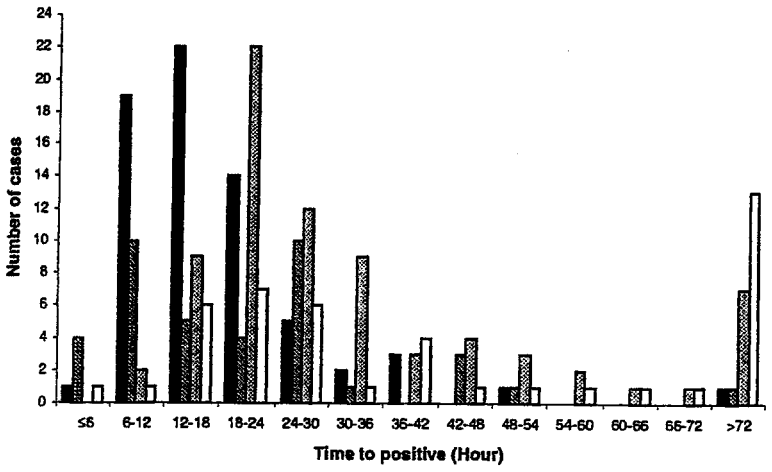

Fig. 2. Distribution of incubation time required for different types of positive reactions. (■) Confirmed-positive; (▩) false-positive (instrument); (▨) false-positive (contamination); (□) indeterminate.

DISCUSSION

Routine culturing for detection of bacterial contamination of apheresis PLTs was successfully implemented in every regional blood center of the American Red Cross. The positive rate was 1 in 1552 collections, of which 30.1 percent could be confirmed, based on reculturing and bacterial isolation, for a contaminated collection detection rate of 1 in 5157. This is significantly lower ($p = 0.0127$) than our previous report[14] of a contamination rate of 1 in 1249 PLTs collected at four regional blood centers also represented in this study. The earlier study methods were significantly

TABLE 5. Incubation time (hr) required for confirmed-positive reaction according to type of bacteria

Type of bacterium	Number	Mean ± SD	Median	Range
S. epidermidis	10	19.0 ± 2.4	19.2	16.4-22.9
Staphylococcus aureus	2	10.8 ± 1.6	10.8	9.7-11.9
Other coagulase-negative *Staphylococcus* spp.	19	23.9 ± 7.6	22.0	15.3-41.6
S. bovis	5	12.0 ± 1.5	12.2	9.8-13.7
Streptococcus viridans	6	16.5 ± 10.7	12.3	10.2-38.0
S. marcescens	4	9.9 ± 1.8	9.2	8.7-12.5
Klebsiella spp.	3	7.8 ± 1.8	7.7	6.1-9.7
E. coli	3	11.3 ± 5.8	10.6	5.9-17.5
All Gram-positive organisms*	55	19.4 ± 8.8†	16.5	8.5-50.4
All Gram-negative organisms	12	9.9 ± 3.3†	9.2	5.9-17.5

* Excluding one *Bacillus* contaminated unit with a time to positive of 105.6 hours.
† $t = 3.639$; $p = 0.0005$.

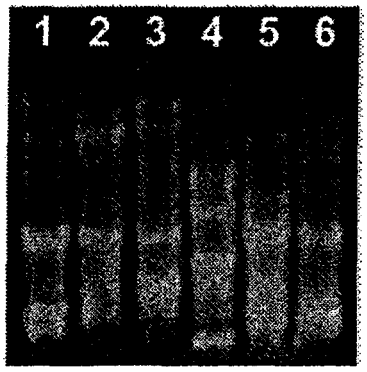

Fig. 3. Random amplified polymorphic DNA analysis on different *S. bovis* strains. (Lanes 1-3, three different strains obtained from the ATCC; Lanes 4-6, isolations from three apheresis PLT products).

different from the program designed here: whole blood–derived PLTs were tested, samples were collected 1 to 7 days after expiration, and cultures in both sheep blood agar plate and thioglycollate broth medium were performed. In the earlier study, one of the four positive cases was *Propionibacterium acnes*, which is an anaerobic organism that usually does not grow in the aerobic culture bottle of the BacT/ALERT system.[15] If this case is excluded from analysis, the difference between the pilot study and the 10-month experience is not significant ($p = 0.1295$).

In our experience, the proportion of Gram-negative bacteria (17.6%) in the confirmed-positive group is similar to what was reported by Goldman and Blajchman[16] where they compiled a list of contaminating organisms involved in PLT contamination cases from eight different reports. Although representing approximately one-fifth of contaminations, the BaCon study[17] reported that Gram-negative organisms accounted for 41 percent of deaths due to septic transfusion reactions. This is presumed to be because Gram-negative bacteria multiply rapidly, as reflected in the shorter incubation time to detection in our experience, and produce endotoxin, both of which mediate more severe clinical consequences.

Likewise, our experience and the Goldman and Blajchman[16] review show that close to 50 percent of PLT contaminations were of *Staphylococcus* spp. In our experience, *Streptococcus* spp. (26.9%) were the next most frequently implicated organisms whereas other studies have reported *Corynebacterium* species (diphtheroids). This may reflect the fact that we did not consider an organism to be confirmed as having caused contamination of the component unless it could be isolated from a second independent sample from either the original collection or at least one of the split products. Without this requirement, it would be possible for other programs to misinterpret a single positive culture for *Corynebacterium* spp., which are common sampling and handling contaminants. In our study, *Corynebacterium* spp. were isolated from none of the confirmed-positive culture bottles but were from 6.7 percent of false-positive samples (Table 3).

For our routine bacterial QC testing, only the aerobic culture bottles are used for three major reasons: 1) PLT products are stored under an aerobic condition and hence provide a poor environment for growth of strict anaerobic organisms; 2) many of the clinically significant facultative anaerobic bacteria grow under aerobic conditions; and 3) most anaerobic bacteria that would be encountered during PLT manufacture, for example, *Propionibacterium* spp., are rarely clinically significant. We also release the PLT components to transfusion as long as the culture has been negative for 12 hours, although the culture bottles are kept in the incubator until the end of product shelf life. This improves the availability of PLT products for patient use. Our internal data show that 42 percent of products are actually distributed 48 hours or more after collection. During this 10-month review, the earliest time a PLT unit was transfused before an initial positive signal by the BacT/ALERT system was 23 hours. In this case, the initial result was falsely positive owing to sampling; a repeat culture from the other half of the collection was negative. No PLT associated with a confirmed-positive screen was transfused during the period of the study. A total of 52.9 percent of confirmed-positive cases, however, did

require incubation of between 12 and 24 hours, and 17.6 percent more than 24 hours, before positivity. Therefore, it remains possible that transfusion of bacterially contaminated components may occur before contamination is detected.

Since implementation of bacterial testing the risk of septic reactions to apheresis PLT transfusions has declined, but not disappeared. It is notable that coagulase-negative *Staphylococcus* was involved in the high-probability cases from screened components, which suggests that the source of contamination was the donor's skin rather than asymptomatic donor bacteremia or environmental contamination. Coagulase-negative *Staphylococci* are known to grow more slowly than coagulase-positive *Staphylococci* or Gram-negative organisms in both blood components and the BacT/ALERT system.[15,18] In each of the reaction cases, the associated culture bottles remained negative for the full shelf life of the implicated component, and sterility of the bottle was confirmed by Gram stain and culture, although in the component itself bacterial proliferation was sufficient to cause a septic reaction. Our hypothesis is that the starting concentration of organisms was sufficiently low that the bottle inoculation sample was sterile although the component was contaminated. If so, a longer waiting time before sample collection would have allowed higher concentrations of bacteria to proliferate in the component and might have helped ensure that the sample contained bacteria in sufficient concentrations (i.e., >10 CFU/mL) to guarantee a positive BacT/ALERT culture.[19] Likewise, a larger sample volume may have increased the sensitivity for detecting very low contamination levels.

Bacterial screening does not appear to have resulted in a higher component outdate rate, as might have been expected given the longer manufacturing interval and shortened shelf life at the time of distribution for most components. Our internal data indicated that for the 6-month period of July through December 2003, the number and rate of outdated components at all Red Cross locations were virtually identical when compared to those for the same period in 2004 (data not shown). We do not have data to indicate, however, whether the outdate rate at hospitals increased.

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