a mean underestimates the incubation period because mice that do not become ill are excluded. Applying survival analysis methods overcomes these issues and also incorporates previously censored data on animals that die without showing clinical signs of prion disease (asymptomatic on the last inspection). We calculated median incubation periods and their standard error based on survival curves calculated by the method of Kaplan and Meier (34).

The effects of a treatment protocol on two different prion strains were analyzed by separate Cox models (19) with terms for $\log_{10}$ dilution and inactivation and compared by stratification (33). All calculations were performed with Stata 8 (Stata Corp., College Station, TX).

RESULTS

Prions in SHa brain homogenates were chosen for our initial inactivation studies for three reasons: (i) SHa prions are the most well characterized with respect to physical properties, (ii) the titers of prions in SHa brain are 10- to 100-fold higher than those found in the brains of other species, and (iii) high-titer samples produce disease in ~70 days in hamsters and 45 days in Tg7 mice. The high titers of prions in SHa brain homogenates and short incubation times create a large range over which measurements can be made, and thus, provide the most sensitive system available for evaluating low levels of infectivity.

Acidic SDS denatures PrP^{Sc}. To examine whether solutions of 1 to 4% SDS could denature PrP^{Sc} more effectively at different pH values, we used 50 mM sodium acetate and Tris acetate buffers to maintain the pH between 3.5 and 10.0 for homogenates prepared from the brains of Syrian hamsters infected with Sc237 prions. Our studies demonstrated that aqueous solutions of ≥1% SDS could denature PrP^{Sc} completely at pH values of ≤4.5 or ≥10.0, as judged by immunoblotting for PrP after the samples were subjected to limited proteolysis (Fig. 1A). We also found that acidic solutions other than AcOH enable SDS to denature PrP^{Sc} in Sc237-infected brain homogenate: PrP^{Sc} was denatured in the presence of 1% SDS plus either 0.5% AcOH (pH 3.6), 50 mM glycine (pH 3.7), or 0.2% peracetic acid (pH 3.4) for 15 min at 37°C (Fig. 1B).

Acidic SDS denatured PrP^{Sc} in Sc237-infected brain homogenate in 30 min at room temperature, as judged by Western blotting (Fig. 1C, paired lanes 1). We next investigated whether dilute acid in the presence of detergents other than SDS could also denature PrP^{Sc}. We incubated Sc237-infected brain homogenates with various detergents, in the presence or absence of AcOH, for 30 min at room temperature. The detergents cholic acid, deoxycholic acid, Triton X-100, NP-40, Tween 20, CTAB (cetyltrimethylammonium bromide), Zwittergent, and CHAPS {3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate} failed to denature PrP^{Sc} at low pH (Fig. 1C, paired lanes 2 to 9, respectively). We next tested the ability of alkyl sulfates and alkyl sulfonates of various alkyl chain lengths to denature PrP^{Sc} in the presence of 0.5% AcOH. Alkyl sulfates with alkyl chains of 9 to 12 carbon atoms and alkyl sulfonates with alkyl chains of 10 to 13 carbon atoms denatured PrP^{Sc} substantially in the presence of dilute acid (Fig. 1D).

Physicochemical properties of PrP^{Sc} treated with acidic SDS. To investigate whether the inactivation of prions by acidic SDS is reversible, we added a neutralization buffer containing the nonionic detergent NP-40 to a sample of Sc237-infected brain homogenate initially exposed to 1% SDS and 0.5% AcOH for 15 min at 37°C. We then dialyzed the neutralized sample containing mixed micelles for 8 h at 4°C to remove the SDS. This

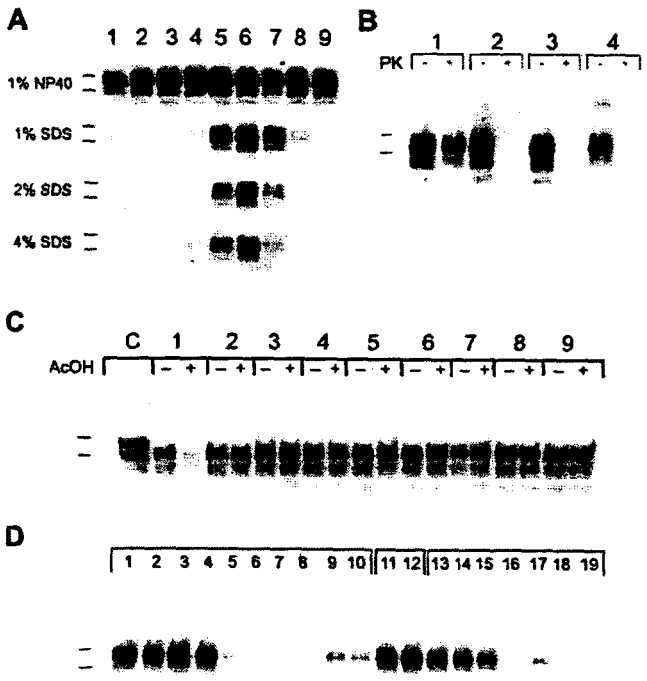

FIG. 1. Western blots of prion-infected brain homogenates treated with different detergents and at different pH values. For all panels, apparent molecular masses based on the migration of protein standards are 30 and 27 kDa (top and bottom markers, respectively). (A) Samples of 1% brain homogenate containing Sc237 prions were incubated for 15 min at 37°C with the indicated detergent at the following pH values: 3.5 (lane 1), 4.0 (lane 2), 4.5 (lane 3), 5.0 (lane 4), 6.0 (lane 5), 7.0 (lane 6), 8.0 (lane 7), 9.0 (lane 8), and 10.0 (lane 9). Sodium acetate buffers (50 mM) were used to maintain pH values 3 to 6 (lanes 1 to 5), and 50 mM Tris acetate buffers were used to maintain pH values 7 to 10 (lanes 6 to 9). After incubation, all samples were neutralized by the addition of an equal volume of 4% Sarkosyl–100 mM HEPES (pH 7.5)–200 mM NaCl and subjected to limited PK digestion (20 μg/ml for 1 h at 37°C). (B) Samples of 1% brain homogenate containing Sc237 prions were incubated for 15 min at 37°C in 1% SDS plus 50 mM Tris acetate (pH 7.0) (paired lane 1), 0.5% AcOH (pH 3.6) (paired lane 2), 50 mM glycine (pH 3.7) (paired lane 3), or 0.2% peracetic acid (pH 3.4) (paired lane 4). After incubations, samples were neutralized and then left undigested (−) or digested with PK (+) as described in panel A. (C) Samples of 2.5% brain homogenate containing Sc237 prions were incubated with 2% detergent either alone (−) or with (+) 1% AcOH at room temperature for 30 min. Anionic (paired lanes 1 to 3), nonionic (paired lanes 4 to 6), cationic (paired lane 7), and zitterionic (paired lanes 8 to 9) detergents were used. Lane assignments are as follows: lane 1, SDS; lane 2, cholic acid; lane 3, deoxycholic acid; lane 4, Triton X-100; lane 5, NP-40; lane 6, Tween 20; lane 7, CTAB; lane 8, Zwittergent; lane 9, CHAPS. All paired samples (lanes 1 to 9) were neutralized by the addition of 280 μl of 2% Sarkosyl–200 mM HEPES (pH 7.5)–100 mM NaCl and subjected to limited PK digestion (4 μg for 1 h at 37°C). C, undigested control. (D) Samples of 1% brain homogenate containing Sc237 prions were incubated with 0.5% AcOH (pH 3.6) and either 1% alkyl sulfates (lanes 1 to 10), NP-40 (lanes 11 and 12), or alkyl sulfonates (lanes 13 to 19) for 2 h at 37°C. Alkyl sulfate detergents with backbones of 5 to 14 carbon atoms (lanes 1 to 10, respectively) were tested. Alkyl sulfonate detergents with backbones of 6 (lane 13), 7 (lane 14), and 9 to 13 carbon atoms (lanes 15 to 19, respectively) were used. All samples were subjected to limited PK digestion.

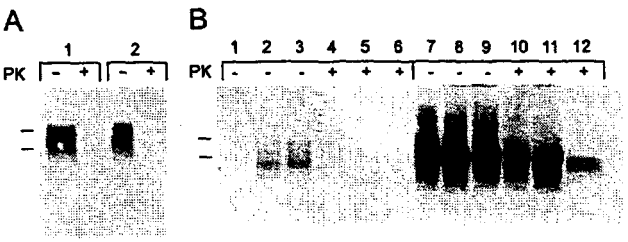

FIG. 2. Physical characterization of Sc237 prions denatured by acidic SDS. (A) A 0.5-ml sample of 1% prion-infected brain homogenate was incubated in 1% SDS–0.5% AcOH (pH 3.6) for 15 min at 37°C and then neutralized by the addition of 0.5 ml of 2% NP-40–100 mM HEPES (pH 7.5)–200 mM NaCl. A 50-μl aliquot was removed for analysis, and the remainder of the sample was dialyzed two times against 2 liters of 1% NP-40–50 mM HEPES (pH 7.5)–100 mM NaCl for 8 h at 4°C (molecular mass cutoff of 10 kDa). Sample before (paired lane 1) and after (paired lane 2) dialysis. Samples were undigested (−) or subjected (+) to limited PK digestion (20 μg/ml for 1 h at 37°C). The apparent molecular masses based on the migration of protein standards are 30 and 27 kDa. (B) Samples of 1% prion-infected brain homogenate were incubated at room temperature for 15 min with PBS (lanes 1, 4, 7, and 10), 50 mM Tris (pH 7.0) with 1% SDS (lanes 2, 5, 8, and 11), or 1% SDS–0.5% AcOH (lanes 3, 6, 9, and 12). After incubation, samples were centrifuged at 100,000 × *g* for 1 h at 4°C. Supernatant fractions (lanes 1 to 6) were removed and neutralized with an equal volume of 2× neutralization buffer (200 mM NaCl, 4% Sarkosyl, 400 mM HEPES [pH 7.5]) and subjected to limited PK digestion (PK-protein [1:50], for 1 h at 37°C) as indicated. Pellet fractions (lanes 7 to 12) were resuspended in 1× neutralization buffer and subjected to limited PK digestion as indicated. All samples were mixed with an equal volume of 2× SDS sample buffer. The apparent molecular masses based on the migration of protein standards are 36 and 29 kDa.

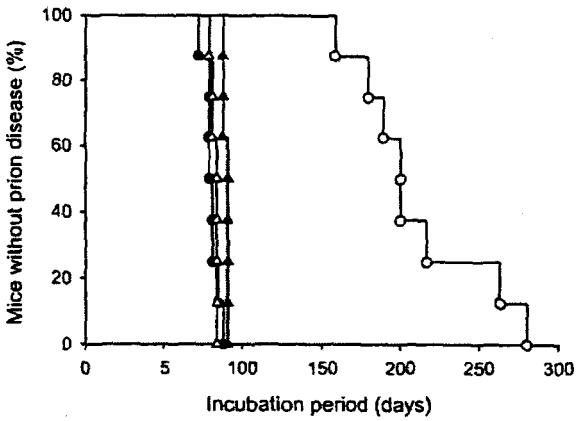

FIG. 3. Effect of acidic SDS and temperature on prion infectivity. Samples of 1% brain homogenate infected with Sc237 prions containing ~10^9 ID_{50} units/ml were incubated with continuous shaking for 2 h in the specified solution at room temperature. After incubation, samples were diluted 1:10 into calcium- and magnesium-free PBS plus 5 mg of bovine serum albumin/ml, and 50-μl aliquots were inoculated into eight Syrian hamsters each, and survival curves were calculated. Symbols: △, 50 mM sodium acetate (pH 7.0); ●, 0.5% AcOH (pH 3.6); ▲, 1% SDS and 50 mM sodium acetate (pH 7.0); ○, 1% SDS and 0.5% AcOH (pH 3.6).

procedure did not restore the protease-resistance of PrP^{Sc} that had been denatured by acidic SDS (Fig. 2A, paired lanes 2).

To investigate further the biophysical changes in PrP^{Sc} molecules induced by exposure to acidic SDS, we performed ultracentrifugation. We found that PrP^{Sc} molecules become soluble in acidic SDS (Fig. 2B).

Inactivation of Syrian hamster prions by acidic SDS. To determine whether denaturation of PrP^{Sc} caused by acidic SDS correlates with a reduction in prion infectivity, we incubated Sc237-infected brain homogenates for 2 h with different buffers at various temperatures and inoculated the treated samples intracerebrally into Syrian hamsters. Inoculation of a control brain homogenate containing ~10^9 ID_{50} units/ml (one ID_{50} unit is the infectious dose causing illness in 50% of inoculated animals) at neutral pH without detergent caused disease in all animals in 84 ± 0.4 days (Fig. 3). Animals inoculated with samples exposed to either 0.5% AcOH or 1% SDS alone for 2 h at room temperature developed disease in 79 ± 0.9 days and 91 ± 0.4 days, respectively (Fig. 3). In contrast, exposure of the inoculum to a solution of 1% SDS and 0.5% AcOH for 2 h at room temperature prolonged the median incubation time to 200 ± 2.3 days (Fig. 3). Similar results were obtained when samples were incubated at 37°C (data not shown).

The prolonged incubation times found after acidic SDS treatment of hamster brain homogenates indicate that the prion titers were reduced from ~10^9 ID_{50} units/ml to <100 ID_{50} units/ml. These titers were calculated from standard curves that relate the incubation time to the dose of prions in the inoculum (61, 62). That the prions were not completely inactivated by the procedure used is clear from the survival curves: all of the hamsters used for bioassay eventually developed disease (Fig. 3). Although the data argue that SDS and dilute acid act synergistically to diminish prion infectivity, complete inactivation necessitated modification of the protocol.

Bioassays in transgenic mice. Although these initial studies proved promising, we were concerned that extensive bioassays in hamsters would be complicated by the relatively short life span of these animals (69); we therefore turned to Tg mice expressing high levels of SHaPrP, designated Tg7 mice (70, 78). Uninoculated Tg7 mice remain healthy for more than 500 days, while those inoculated with ~10^9 ID_{50} units/ml of prions develop neurologic dysfunction in approximately 45 days. To make our studies more clinically relevant, we used crude brain homogenates, rather than homogenates precleared by low-speed centrifugation.

A 10% (wt/vol) Sc237-infected brain homogenate was serially diluted, and each dilution was inoculated into Tg7 mice. As the infected brain homogenate is diluted, the percentage of mice succumbing to prion disease decreases (Fig. 4A) and the median incubation period lengthens. These median time points were interpolated with an exponential decay curve to highlight this relationship (Fig. 4A). A similar curve was developed relating the length of the incubation time and the size of the inoculum dose for bioassays of human sCJD prions with Tg22372 mice (Fig. 4B).

Inactivation of Syrian hamster prions by acidic SDS at elevated temperatures. In an attempt to destroy the residual prion infectivity found by initial bioassay (Fig. 3), we increased the concentrations of SDS to 2% and of AcOH to 1% and raised the treatment temperature to 65°C (Table 1). A solution composed of 4% SDS and 2% AcOH was added directly to an equal volume of sample containing a 5% (wt/vol) prion-infected crude brain homogenate, mixed, and incubated without additional agitation.

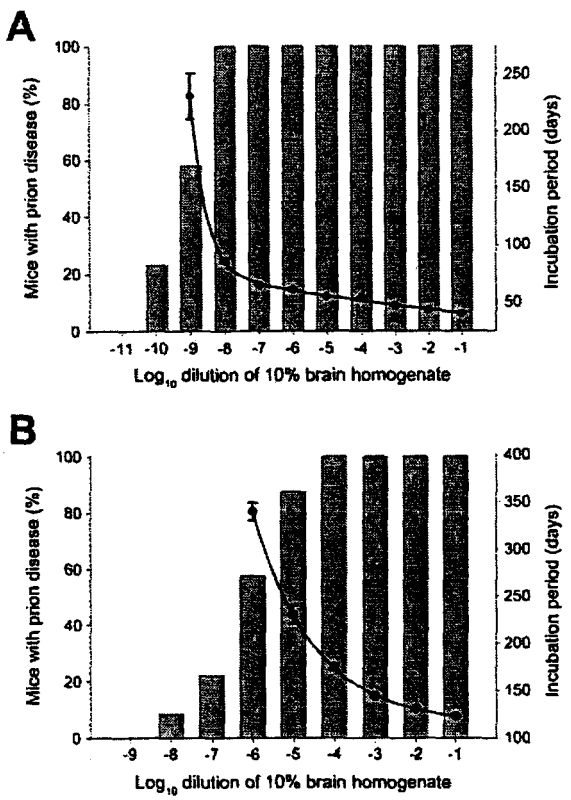

FIG. 4. Proportion and incubation period of transgenic mice succumbing to disease with each dilution of inoculum. Ten percent brain homogenates were serially diluted 10-fold, and each dilution was inoculated into 12 mice. Bars represent the percentage of mice that showed clinical signs of disease when inoculated with the indicated $\log_{10}$ dilution of brain homogenate. The incubation period, the number of days from inoculation to the manifestation of clinical symptoms, was also measured for these mice. When at least half of the animals became ill, the median incubation period was determined by Kaplan-Meier statistics and plotted against $\log_{10}$ dilution (datum point ± the standard error). Values were interpolated with the best fit to an exponential decay curve. (A) Sc237 prions bioassayed in Tg(SHaPrP)7 mice; (B) sCJD prions bioassayed in Tg(MHu2M, M165V, E167Q)22372 mice.

Inoculation of a control Sc237-infected brain homogenate at neutral pH without detergent produced disease in Tg7 mice with a median incubation time of 46 days (Table 1). Although uninoculated Tg7 mice remained healthy for 500 days, some animals can develop a nontransmissible neuromyopathy after this time (96). Since mice are inoculated at 8 to 10 weeks of age, bioassays in Tg7 mice were terminated after 400 days to exclude the possibility of illness not caused by prions. After exposure of Sc237-infected brain homogenates to 2% SDS and 1% AcOH at 65°C for 30 min, the inoculum produced prion disease in some of the mice, with significantly prolonged incubation times. Increasing the exposure time of the inoculum to 2 h or 18 h resulted in no animals becoming ill 400 days after inoculation.

Acidic SDS inactivates Syrian hamster prions bound to steel wire. To study the inactivation of prions on surfaces (100), we soaked stainless steel wires in 10% brain homogenates containing Sc237 prions for 16 h at room temperature. The prion-coated wires were implanted into the parietal lobes of Tg7 mice and produced disease in 52 ± 0.3 days. Treatment of the wires at 65°C for 30 min with 2% SDS and 1% AcOH increased the incubation time to 82 ± 0.7 days after implantation into the brains of Tg7 mice; treatment at 65°C for 2 h further lengthened the incubation time, but most of the animals still developed disease in fewer than 400 days. Only treatment at 65°C for 18 h resulted in no animals succumbing to disease before 400 days (Table 1).

Inactivation of human prions by acidic SDS. Because different prion strains exhibit distinct resistances to inactivation by chaotropic salts and heat (56, 57, 81, 89, 91), we investigated the inactivation of sCJD prions by acidic SDS. Brain homogenates were prepared from an sCJD patient homozygous for methionine at residue 129, who carried no mutations in the *PRNP* gene and whose unglycosylated PrP 27-30 migrated at 21 kDa on SDS-PAGE (53). These human prions, designated sCJD(MM1), are the most common strain found in sCJD patients (52, 53). We either inoculated brain homogenate containing sCJD(MM1) prions intracerebrally or implanted wires that were coated with the homogenate into Tg22372 mice (38). It is noteworthy that, like the human PrP^{Sc} inoculum, the PrP transgene in Tg22372 mice encodes methionine at position 129.

Sporadic CJD(MM1) prions in brain homogenates or on steel wires were treated with 2% SDS and 1% AcOH at 65°C (Table 1). For brain homogenates, acidic SDS treatment for 30 min at 65°C prolonged the incubation times from 131 ± 0.7 days to 266 ± 8.5 days. Increasing the time of exposure (2 h or 18 h) to acidic SDS at 65°C lengthened the incubation time (>500 days) and decreased the proportion of Tg mice developing disease (~25%). Studies of sCJD-coated steel wires prolonged incubation times only slightly, and removal of infectivity on these surfaces was less efficient than in brain homogenates.

TABLE 1. Inactivation of prions by 2% SDS plus 1% AcOH at 65°C[a]

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg23372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)
Negative control	>400	0	>400	0	>500	0	>500	0
Positive control	46 ± 0.2	100	52 ± 0.3	100	131 ± 0.7	100	215 ± 0.9	100
2% SDS–1% AcOH, 30 min	>400	26	82 ± 0.7	100	266 ± 8.5	74	354 ± 1.6	86
2% SDS–1% AcOH, 2 h	>400	0	269 ± 3.2	68	>500	26	>500	44
2% SDS–1% AcOH, 18 h	>400	0	>400	0	>500	25	>500	25

[a] Median incubation period (IP) ± the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

TABLE 2. Inactivation of prions by 4% SDS plus 1% AcOH at 65°C[a]

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg23372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)
Negative control	>400	0	>400	0	>500	0	>500	0
Positive control	55 ± 0.0	100	63 ± 0.1	100	139 ± 0.4	100	181 ± 1.2	100
ddH_2O, 18 h	55 ± 0.1	100	67 ± 0.2	94	179 ± 4.5	62	208 ± 3.8	100
1% AcOH, 18 h	60 ± 0.4	100	63 ± 0.1	100	172 ± 0.8	100	196 ± 1.9	100
4% SDS, 18 h	77 ± 0.4	100	106 ± 3.1	62	>500	32	223 ± 1.8	79
4% SDS–1% AcOH, 30 min	>400	0	>400	14	>500	5	278 ± 15	71
4% SDS–1% AcOH, 2 h	>400	0	>400	25	>500	13	259 ± 6.4	64
4% SDS–1% AcOH, 18 h	>400	0	>400	0	>500	0	379 ± 30	60

[a] Median incubation period (IP) ± the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

The same treatment protocol (exposure to 2% SDS and 1% AcOH for 30 min at 65°C) resulted in large differences in the level of inactivation for the two prion strains studied (Table 1). Because the titer of Sc237 prions is 10- to 100-fold higher than sCJD prions in brain homogenates (Fig. 4), data were analyzed by a stratified Cox regression (33) in order to quantify differences between hamster and human prions. Taking the ratios of coefficients in the Cox model, it is possible to relate the effect of an inactivation procedure to an approximately equivalent dilution. Applying the stratified Cox regression to the 30-min exposure to acidic SDS, we estimate a 9.0 $\log_{10}$ reduction in infectivity for Sc237 prions and a 3.8 $\log_{10}$ reduction for sCJD prions. The difference in inactivation between the two prion strains is 5.2 $\log_{10}$ (95% confidence intervals, 3.7 to 6.8 $\log_{10}$). This analysis argues that sCJD prions in human brain homogenates are 10^5-fold more resistant to inactivation than Sc237 prions in SHa brain homogenates.

Two-step inactivation protocol. Concerned that exposure to acid results in the aggregation of proteins and that such aggregates might protect PrP^{Sc} from acidic SDS–mediated denaturation, we developed a two-step protocol: SDS at neutral pH is used to disperse proteins prior to exposure to AcOH. In these studies, exposure to 4% neutral SDS was performed at 65°C for 30 min, followed by acidic SDS (4% SDS and 1% AcOH) at 65°C for 30 min, 2 h, or 18 h (Table 2). All two-step inactivation studies were completed in three independent sets (n = 8) and, since no significant differences were observed, the sets were combined for analysis.

SHa brain homogenates (10% [wt/vol]) containing Sc237 prions were treated by using the two-step acidic SDS protocol. Untreated homogenates produced neurologic dysfunction after 55 days in Tg7 mice (Table 2), whereas the two-step procedure at 65°C for 30 min completely abolished prion infectivity. In experiments with Sc237 prion-coated wires, untreated control wires produced disease in 63 ± 0.1 days after implantation. In contrast to homogenates, exposure of prion-coated wires to the two-step procedure at 65°C for 30 min did not completely inactivate prion infectivity.

Human brain homogenates (10% [wt/vol]) containing sCJD (MM1) prions were treated by using the two-step acidic SDS protocol as described above. Untreated homogenates produced neurologic dysfunction in 139 ± 0.4 days in Tg22372 mice (Table 2). Exposure of these homogenates to the two-step procedure at 65°C for 30 min substantially reduced prion infectivity, while extending the time to 18 h completely abolished infectivity. In three separate experiments with sCJD prion-coated wires, untreated control wires produced disease in 181 ± 1.2 days after implantation. In contrast to homogenates, exposure of prion-coated wires to the two-step procedure at 65°C, even for 18 h, did not inactivate prion infectivity, with the majority of Tg22372 mice still succumbing to disease.

Acidic SDS and autoclaving abolish prion infectivity. Although exposure of Sc237 and sCJD(MM1) prions to 2% SDS and 1% AcOH at 65°C for 2 h was sufficient to destroy more than 99.99% of the infectivity in brain homogenates, complete inactivation was not achieved (Table 1). Also, although the two-step procedure using 4% neutral SDS, followed by a combination of 4% SDS and 1% AcOH, further diminished infectivity levels, residual infectivity could still be measured, particularly on the surfaces of steel wires coated with sCJD(MM1) prions (Table 2).

To determine whether autoclaving for a brief time in the presence of acidic SDS could eliminate prion infectivity, we exposed Sc237 and sCJD(MM1) prions in brain homogenates or on wire surfaces to 121°C in the presence or absence of acidic SDS (Table 3). Neither Sc237 nor sCJD(MM1) prions in brain homogenates or on wire surfaces were detectable by bioassay in Tg mice after exposure to 121°C for 15 min in the presence of 2% SDS and 1% AcOH (Table 3). Similarly, samples first exposed to 4% neutral SDS followed by a combination of 4% SDS and 1% AcOH at 134°C were devoid of prion infectivity (Table 4). The efficacy of acidic SDS-mediated inactivation of prions is strikingly documented by how many prions survived autoclaving at 121°C or 134°C for 15 min, 30 min, or 2 h in the absence of acidic SDS (Tables 3 and 4). The survival of sCJD(MM1) prions bound to steel wires after autoclaving for 2 h at 134°C is especially alarming (Table 4).

To quantify the $\log_{10}$ reduction in prion titer from these procedures, we used a Cox proportional-hazards model based on the serial dilution data (Fig. 4) and derived partial-likelihood ratios based on 95% confidence intervals. Treatment of sCJD prions in brain homogenate for 30 min at 121°C (Table 3) results in a 6.8 $\log_{10}$ reduction (lower 95% confidence interval, 5.3 $\log_{10}$). If no animals succumb to disease, the extent of inactivation cannot be quantified. However, it is still possible to determine a lower 95% confidence interval. For example, treatment of sCJD prions in brain homogenate with the two-step protocol for 15 min at 134°C (Table 4) gives a

TABLE 3. Inactivation of prions by 2% SDS plus 1% AcOH at 121°C[a]

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg23372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)	IP (days)	% (n = 10)
Positive control	46 ± 0.2	100	62 ± 0.3	100	146 ± 0.4	100	207 ± 1.1	100
Untreated, 15 min	344 ± 20	73	160 ± 7.3	100	221 ± 1.0	100	>500	22
Untreated, 30 min	>400	12	>400	20	>500	0	>500	0
Untreated, 2 h	>400	12	>400	0	>500	0	414 ± 15	73
2% SDS–1% AcOH, 15 min	>400	0	>400	0	>500	0	>500	0
2% SDS–1% AcOH, 30 min	>400	0	>400	0	>500	12	>500	0
2% SDS–1% AcOH, 2 h	>400	0	>400	0	>500	0	>500	0

[a] Median incubation period (IP) ± the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

lower 95% confidence interval of 6.1 $\log_{10}$ reduction in infectivity.

The incubation periods for prion-coated wires cannot be converted into titers since the prions seem to be bound tightly to the surface of the wire (95) and it is not possible to remove them for measurement.

DISCUSSION

The differences in the inactivation profiles of prions and viruses provided the first clues that the scrapie agent was not a slow virus as had been widely thought (6, 23, 41, 48, 58, 59, 80).

Mechanism of acidic SDS inactivation. From the results of the studies described here, exposure to acidic SDS appears to denature PrP^{Sc} (Fig. 1). Although SDS between pH 4.5 and pH 10.0 at room temperature is a poor denaturant, it becomes an excellent denaturant at a pH of ≤4.5 and ≥10 (Fig. 1). Moreover, weak acids are poor denaturants for PrP^{Sc} except in the presence of SDS.

Denaturation of PrP^{Sc} by SDS at pH 4 was dependent on the concentration of SDS, the time of exposure, and the temperature. Equivalent levels of denaturation seemed to be achieved by inversely varying the time and temperature; the time of exposure for complete inactivation of prions was 15 min when a temperature of ≥121°C was used (Tables 3 and 4).

In our initial experiments, denaturation of PrP^{Sc} was measured by the loss of protease-resistance of PrP 27-30 and a decrement in prion infectivity. Immunodetection of PrP 27-30 by using Western blotting has a dynamic range of ~100-fold, whereas bioassays measure prions over an $\sim 10^9$-fold range as described above. In determining the mechanism of acidic SDS-mediated denaturation of PrP^{Sc}, immunoassays are useful but only prolonged bioassays are adequate to assess whether or not complete inactivation of prion infectivity has been achieved.

Stabilities of different prion strains. The strain phenotype of the prion is enciphered in the conformation of PrP^{Sc} (9, 43, 57, 94), and different strains display distinct conformational stabilities as reflected in their resistance to denaturation by chaotropes and heat (36, 43, 57, 77, 81, 91). Besides the strain of prion, the sequence of PrP, determined by the last host in which the prion was passaged, influences the conformation of PrP^{Sc} and hence its stability (79). The passage of prions through different species may lead to new prion strains with properties different from those of the parental strain (21, 35, 38, 54, 57, 72).

Conformational stability profiles, as measured by sensitivity to denaturation by GdnHCl gave half-maximal ($Gdn_{1/2}$) values of 1.5 and 1.8 M for Sc237 prions (56, 57) compared to a $Gdn_{1/2}$ value of 1.8 M for sCJD(MM1) prions (101). However, the resistances of these strains to denaturation by acidic SDS are significantly different (Table 1 to Table 4), implying that inactivation protocols validated on one strain cannot be extrapolated to another. Exploring the spectrum of strain conformations with respect to inactivation by acidic SDS should be facilitated by the availability of synthetic prions, especially since the MoSP1 strain is the most stable strain studied to date (42, 43).

Limitations on the measurement of prion infectivity. Bioassays for prions are most effective when high-titer samples are being assessed. Under these circumstances, the incubation times are the shortest and the variance among the animals is

TABLE 4. Inactivation of prions by 4% SDS plus 1% AcOH at 134°C[a]

Condition(s)	Hamster Sc237 prions in Tg7 mice				Human sCJD prions in Tg23372 mice			
	Homogenate		Wire		Homogenate		Wire	
	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)	IP (days)	% (n = 24)
Positive control	55 ± 0.0	100	63 ± 0.1	100	139 ± 0.5	100	181 ± 1.2	100
Untreated, 15 min	>400	5	96 ± 0.6	87	>500	0	218 ± 4.1	73
Untreated, 30 min	>400	0	262 ± 10	55	>500	0	242 ± 2.8	63
Untreated, 2 h	>400	0	>400	9	>500	0	>500	46
4% SDS–1% AcOH, 15 min	>400	0	>400	0	>500	0	>500	0
4% SDS–1% AcOH, 30 min	>400	0	>400	4	>500	0	>500	0
4% SDS–1% AcOH, 2 h	>400	0	>400	0	>500	0	>500	0

[a] Median incubation period (IP) ± the standard error and percentage of animals succumbing to prion disease were calculated by using Kaplan-Meier analysis.

the smallest. As the incubation times lengthen, the variance increases. In studies of prion inactivation, it is possible to measure a reduction in titer over a range of $\sim 10^9$-fold with Sc237 prions using either Syrian hamsters or Tg(SHaPrP) mice. Inactivation studies performed with sCJD(MM1) prions using Tg mice permit measurements of prion infectivity over a range of $\sim 10^6$-fold.

It is clear that the best measurements can be obtained when the incubation times are short, as is the case for high-titer samples. For example, Tg7 mice develop neurologic dysfunction ~45 days after inoculation with high-titer samples of Sc237 prions (Fig. 4A).

Prion inactivation with acidic SDS. From the foregoing studies, acidic SDS provides for the first time a means of completely inactivating prions under relatively gentle conditions. The need for noncorrosive inactivation of prions is clearly illustrated by cases of iatrogenic CJD in which prion-contaminated neurosurgical equipment seems to have spread prions from one patient to another (8, 11, 14, 15, 20, 31). This concern has been heightened in Britain, where the finding of high titers of vCJD prions in lymphoid tissues worries authorities that any surgical procedure could result in the spread of prions from one patient to another (18, 32). In Britain, 15 young people, all of whom later developed vCJD, donated blood that was transfused into 48 recipients. Recently, two of these recipients have been identified, in whom it is likely that vCJD prions were transmitted from the blood of the young donors (45, 55).

Conventional hospital disinfectants including ethylene oxide, propriolactone, hydrogen peroxide, iodophors, peracetic acid, chaotropes, and phenolics have little effect on prion infectivity (89), although some modified cleaning reagents have been suggested to have utility in diminishing prion titers (25, 71). In addition, prions are resistant to inactivation by UV irradiation, aldehyde fixation, boiling, standard gravity autoclaving at 121°C, and detergent solubilization (1, 5, 22, 24, 40, 64, 89).

Although prolonged proteolytic digestion diminishes prion titers (17, 47, 68), complete inactivation of prion infectivity is difficult to achieve enzymatically. As with any enzymatic reaction, protease-catalyzed hydrolysis of PrP^{Sc} becomes increasingly less efficient as the concentration of the substrate PrP^{Sc} decreases (44, 50). Moreover, one or more substances in crude suspensions may inhibit an enzyme or mixture of enzymes used to digest PrP^{Sc}.

Currently recommended protocols for prion decontamination include either (i) >2% available chlorine of sodium hypochlorite for 2 h, (ii) 2 M NaOH for 1 h, or (iii) autoclaving at 134°C for 4.5 h (4, 12, 63, 66, 73, 74, 76, 82, 92, 93). Each of these protocols has important limitations: sodium hypochlorite and NaOH are corrosive at the concentrations required to inactivate prions; NaOH did not inactivate CJD prions completely in some reports (85, 86); and extended autoclaving at high temperature is deleterious to many materials. Currently, some high-risk surgical instruments are soaked in 2 N NaOH for 1 h, rinsed with water, and autoclaved at 134°C for 1 h (75), while many other such instruments are discarded.

In the studies reported here, sCJD(MM1) prions on steel wires were not completely inactivated by autoclaving at either 121 or 134°C in the absence of acidic SDS (Tables 3 and 4); this contrasts with Sc237 prions that were completely inactivated by autoclaving alone (Tables 3 and 4). It is noteworthy that the prion-coated wires were not subjected to any procedures that might reduce the level of prions, such as washing, shaking, scrubbing, and sonicating. Such cleaning procedures are known to reduce substantially the titers of many different pathogens (74).

The data presented here document the efficacy of acidic SDS combined with autoclaving for complete inactivation of human and hamster prions in brain homogenates and on the surface of steel wires (Tables 3 and 4). Acidic SDS combined with autoclaving should supplant routine autoclaving used to sterilize medical and dental equipment. For equipment such as fiber optic instruments that cannot be autoclaved, submerging such equipment in acidic SDS at 65°C will substantially reduce prion infectivity but not completely eliminate it (Table 2). Acidic SDS at room temperature may also find application in the inactivation of equipment and surfaces. Sc237 prion infectivity was reduced more than 99.99% in studies conducted at room temperature (Fig. 3).

Concluding remarks. The results of inactivation studies described here argue that acidic SDS combined with autoclaving be applied immediately for sterilization of surgical and dental instruments. Because SDS is both a detergent and a protein denaturant, it should prove especially apt for sterilizing instruments with complex shapes, serrations, locks, bores, and crevices. Whether other compounds such as urea that are known to denature PrP^{Sc} (51, 63, 64) will prove useful in combination with a weak acid is unknown.

In addition to inactivating prions on the surfaces of surgical instruments and diagnostic equipment, acidic SDS may prove useful in the sterilization of dental and ophthalmologic instruments, as well as the cleaning of operating theaters and invasive diagnostic suites. Acidic SDS is likely to find use in the cleansing and sterilization of equipment used in the production of biopharmaceuticals. In addition, acidic SDS might be applied in the cleaning of abattoirs, meat-processing plants, butcher shops, kitchens, and wherever mammalian products are prepared for human consumption. Acidic SDS might also be used on equipment used in the rendering of offal. Whether acidic SDS or a biodegradable formulation such as acidic urea can be incorporated into some products derived from rendered offal remains to be determined.

In summary, it is important to recognize that procedures routinely used in medical and dental settings do not inactivate prions. That being the case, it may be prudent to institute acidic-SDS protocols as configured in the studies presented here. Acidic SDS combined with autoclaving completely inactivated prion infectivity, even on steel surfaces. Inactivation of human and hamster prions mediated by acidic SDS occurs rapidly and can be achieved without boiling or autoclaving. Acidic SDS is noncorrosive and offers other practical advantages that make it suitable for widespread use.

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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。

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

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。

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)

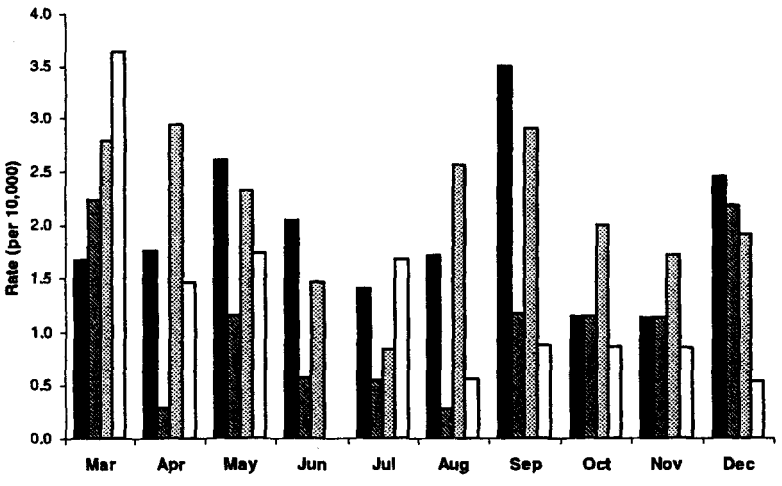

Fig. 1. 2004 monthly distribution of positive rates in bacterial testing on apheresis PLT products. (■) Confirmed-positive; (▩) false-positive (instrument); (▨) false-positive (contamination); (□) indeterminate.

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)