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Evaluation of bacterial inactivation in random donor platelets and single-donor apheresis platelets by the INTERCEPT blood system

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Abstract:

BACKGROUND: Blood transfusion of contaminated components is a potential source of sepsis by a wide range of known and unknown pathogens. Collection mechanism and storage conditions of platelets make them vulnerable for bacterial contamination. Several interventions aim to reduce the transfusion of contaminated platelet units; however, data suggest that contaminated platelet transfusion remains very common.

AIM: A pathogen inactivation system, "INTERCEPT", to inactivate bacteria in deliberately contaminated platelet units was implemented and evaluated.

MATERIALS AND METHODS: Five single-donor platelets (SDP) and five random donor platelets (RDP) were prepared after prior consent of donors. Both SDP and RDP units were deliberately contaminated by stable stock ATCC *Staphylococcus aureus* and *Escherichia coli,* respectively, with a known concentration of stock culture. Control samples were taken from the infected units and bacterial concentrations were quantified. The units were treated for pathogen inactivation with the INTERCEPT (Cerus Corporation, Concord, CA) Blood system for platelets (Amotosalen/UVA), as per the manufacturer's instructions for use. Post illumination, test samples were analyzed for any bacterial growth.

RESULTS: Post-illumination test samples did not result in any bacterial growth. A complete reduction of >6 $\log_{10}$ *S. aureus* in SDP units and >6 $\log_{10}$ *Escherichia coli* in RDP units was achieved.

CONCLUSION: The INTERCEPT system has been shown to be very effective in our study for bacterial inactivation. Implementation of INTERCEPT may be used as a mitigation against any potential bacterial contamination in platelet components.

Keywords:

Amotosalen, bacterial contamination, pathogen inactivation, random donor platelets, sepsis, single-donor platelets, transfusion, ultraviolet A

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Introduction

Platelets play a major role in regulating hemostasis and thrombosis. The need of platelet transfusion in many medical conditions has increased the demand of platelets. In the past, platelet transfusion was limited to patients suffering from uncontrolled bleeding, thrombocytopenia, and defective platelet function. Recently, platelet use is extended as a source of growth factors needed for tissue repair as well as for skin rejuvenation. The demand for platelet transfusion has been increasing rapidly but their availability

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is limited because of their short storage lifespan. Platelets tend to lose their morphology and function over time because of the processes involved during their collection and manufacturing, as well as during room temperature storage and agitation. Over the past few decades, improvements in storage bags which allow better gaseous exchange as well as the use of platelet additive solutions (PAS) have provided medical professionals with higher quality platelets that can in principle be stored for up to 7 days.[1,2] However, platelets have limited shelf lives because of bacterial contamination risks. Storage temperature of platelet units between 20˚C to 24˚C facilitates bacterial growth in this rich medium. The source of bacterial contamination can more often be donor skin flora; bacteria present on the skin of the donor, which can be introduced during venipuncture, or less often bacteria present in circulation in weakly bacteremic, but otherwise healthy-appearing donors. Other factors can be contamination during whole blood collection procedure, contamination during blood processing, or contaminated collection bags.[3] Some of the bacteria commonly associated with platelet contamination are *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus spp., Escherichia coli* as well as other bacteria from the Enterobacteriaceae group, *Pseudomonas spp., Acinetobacter spp.,* and *Streptococci spp*.[4]

Implementation of improved phlebotomy practices has reduced bacterial contamination,[5] but the residual risk still needs attention. The organization formerly known as the American Association of Blood Banks (AABB) has reported bacterial contamination of platelets as the second most common problem. One in every 1000–2000 platelet unit is contaminated by bacteria in the USA and other parts of the world.[6] Data on platelet contamination and bacterial sepsis after contaminated platelet transfusion are underreported in India. A study in the year 2011 reported five cases of bacterial sepsis after transfusion of bacterial-contaminated platelet units. Three out of five patients died because of bacterial sepsis.[7] Viral transfusion-transmitted infections (TTIs) have been reduced remarkably in India with a prevalence of 0.4% for HCV, 0.5% for HIV, and 1.4% for HBV.[8] There is no doubt that implementation of nucleic acid testing for viral pathogens has provided safer blood, but a study reporting one in 825 platelet units to be bacterially contaminated drew attention toward unsafe platelet transfusions.[9] Considering that platelet contamination is a fatal threat, bacterial culture techniques are commonly implemented to detect contamination in platelets. For example, in the US, all platelet products are tested with an approved bacterial detection system. There are also rapid bacterial detection systems as well, that work based on pH changes, oxygen consumption, fluorescence or based on glucose estimation, as well as Gram staining. Sometimes, the quantity of bacteria is very low at the time of donation and testing and goes undetected by these bacterial detection methods. The bacteria then eventually grow to a large number during the storage period. These detection techniques are also prone to instrumental as well as sampling errors. Also, in emergencies, 12–30 h long delay for product release as required to increase ability to detect is not feasible, while the loss of product during sampling is undesirable.[10,11] Even in the US, where all platelet units are tested before release, there are still concerns about bacterial contamination for 1 in 1500–2000 units.[6]

Pathogen inactivation technologies promise a new and efficient way of dealing with platelet contamination. Pathogen inactivation is based on the idea of stopping the replication of pathogens and thus eliminating the contamination before it causes disease. In this study, a system called the INTERCEPT Blood System for Platelets [Figure 1] by Cerus Corporation, Concord, CA, was implemented and evaluated for bacterial contamination. The INTERCEPT system for platelets is based on the addition of the photochemically active compound, amotosalen, to platelet components followed by illumination with ultraviolet A (UVA) light. Amotosalen diffuses through membranes and binds to nucleic acids of bacteria, viruses, and leukocytes present in the platelet components. Upon illumination with UVA, amotosalen makes adducts and cross-links on nucleic acids of bacteria and other species and inactivates them by abrogating their ability to replicate. A wide range of bacteria, protozoa, viruses, and leukocytes have been shown to be effectively inactivated using this system.[12,13]

In this study, single-donor platelet (SDP) units, collected by apheresis, were deliberately contaminated by *E. coli*, and random donor platelets (RDP) collected by the Buffy Coat method were contaminated by *S. aureus*. The contaminated platelet units were then processed by the INTERCEPT Blood System. An overnight bacterial culture (10^8 to 10^9 colony-forming units, CFU/mL) was used to infect the platelet units, and after the inactivation process, bacterial reduction was calculated.

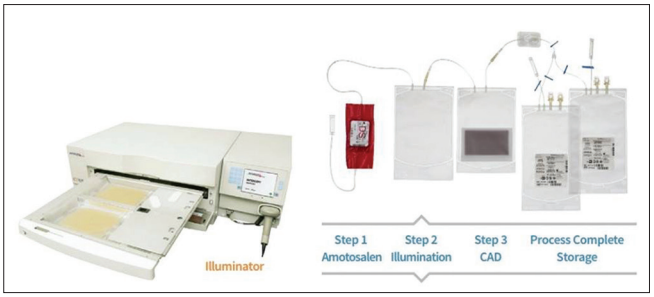

Figure 1: The INTERCEPT Blood System for Platelets (Package Insert). In our study, compound adsorption device and storage bag were not used

Materials and Methods

This study was performed at the Department of Transfusion Medicine, Indraprastha Apollo Hospital, New Delhi. All the microbiological work was carried out at the Department of Microbiology, Indraprastha Apollo Hospital, New Delhi. The protocols followed were based on departmental standard operating procedures (SOPs). Prior consent was obtained from individual donors.

Preparation of platelet concentrates

According to the INTERCEPT processing specifications, the processing volume of a platelet unit should be between 300 mL to 420 mL, with a platelet count of 2.5×10^{11} to 7.0×10^{11} while the RBC content should be no more than 4×10^{6} cells/mL. Whole blood was collected from eligible donors, processed for the separation of blood components, and RDP were prepared by Buffy-Coat (BC) method. Five RDP units of compatible ABO blood group were pooled and five such pooled RDP units were made. Five SDP (apheresis platelet) units were also used in this study. A single unit of SDP was collected from each donor using apheresis and then suspended in 65% of PAS and 35% of plasma. The gross weights of the finished five RDP and five SDP units were recorded and 1 mL of platelet sample was withdrawn from all ten units to evaluate swirling, pH, RBC, WBC, and platelet count using a Beckman Coulter cell counter. After meeting the manufacturer's processing criteria, all the platelet concentrates were stored at 22°C with continuous agitation until processed further.

Preparation of bacterial inoculums

An overnight culture of *E. coli* ATCC-25922 and *S. aureus* ATCC-25923 were prepared in 250 mL of Luria–Bertani (LB) broth. One isolated colony of each bacterium was used to prepare overnight culture. Sterility controls were maintained at every point based on departmental protocols. Further steps were followed only if sterility control tubes were contamination free. The overnight cultures of *E. coli* and *S. aureus* were approximately 1×10^{9} CFU/mL ($9 \log_{10}$). Adjusted stocks were prepared by diluting 1×10^{9} CFU/mL culture in LB broth. Both overnight/stock cultures and adjusted stocks were serially diluted and plated to confirm bacterial concentration.

Bacterial inoculation

Adjusted stock cultures (1×10^{8} CFU/mL) of $8 \log_{10}$ *E. coli* were used to spike SDP units, while a similar stock of *S. aureus* was used to spike RDP units. 30 mL of platelets from each platelet unit was withdrawn through a luer lock for rinsing. Volumes of 4.2 mL of adjusted bacterial stock for *S. aureus* and 3.0 mL of *E. coli* were then added to the respective units and withdrawn platelets were added back to rinse for the tubing for any residual bacteria. The infected platelet units were mixed gently and stored at 22°C until processed. Each spiked SDP and RDP unit had approximately 1×10^{6} CFU/mL *E. coli* and 1×10^{6} CFU/mL *S. aureus*, respectively.

INTERCEPT processing

In this study, the INTERCEPT SV set was used. The INTERCEPT system SV set [Figure 1] comes with a set of 15.0 mL of 3 mM amotosalen solution, a 1 L PL 2410 illumination container, a 1 L PL 2410 container with a Compound Adsorption Device (CAD), an in-line filter, and a 1.3 L PL 2410 final storage container. The final storage container and CAD were not used in this study. The inactivation process [Figure 2] includes two steps; addition of amotosalen and UVA illumination. Each platelet unit was attached to the tubing of the disposable kit using a sterile connecting device (SCD). The entire content of the platelet unit was passed through the amotosalen container and collected in the illumination container. The illumination container was given a gentle mix and a sample was withdrawn to determine pre-UVA bacterial titer (Control) and amotosalen concentration by HPLC. It was then illuminated with a single 3.0 J/cm^2 UVA light for approximately 3–4 min. After illumination, 11 mL of platelet concentrate was withdrawn from the illumination container and used to determine post-UVA bacterial titer (test) and amotosalen concentration. The same process was used for all the ten SDP and RDP contaminated units.

Bacterial titer analysis

Samples collected to determine amotosalen concentration were stored frozen at ≤30°C, and samples collected for bacterial analysis were processed right after the illumination process. Samples were serially diluted

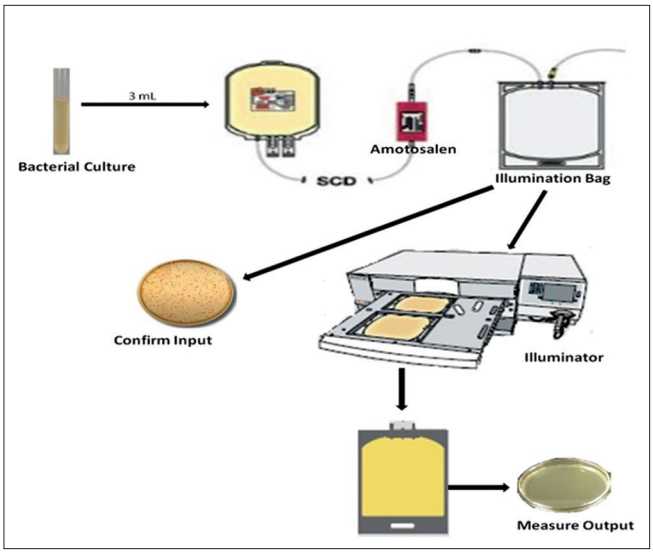

Figure 2: Experimental design for bacterial inactivation. Random donor platelet units (Buffy-coat) were infected by *Staphylococcus aureus* and single-donor platelet units (Apheresis) were infected by *Escherichia coli*

(10^{-1} to 10^{-8}) and selected concentrations were pour plated. 0.5 mL of sample was added to 4.5 mL of blood bank saline (BBS), which is 0.9% w/v sodium chloride solution (normal saline), for dilutions. 0.1 mL of each dilution was plated. Pre-UVA bacterial concentration was compared to post-UVA bacterial concentration and log reduction of bacteria was calculated.

Results

The volume and platelet content of all five RDP and five SDP units met the processing requirements [Table 1]. The overnight bacterial culture was found to be 9 log copies/mL for *E. coli* and *S. aureus*. An adjusted 8 log copies/mL culture of *E. coli* was used to spike SDP platelet units and an adjusted 8 log copies/mL culture of *S. aureus* was used to spike RDP units. In our experiment, a high concentration of bacteria used as platelet units were not stored for a long time and there was no more than 10 min time between inoculation and the inactivation process.

Control and test samples were diluted and pour plated for colony counts. The control samples taken (pre-illumination) from the illumination bag were found to have approximately 6 log copies/mL bacterial titer in all the platelet units. Test samples resulted in no growth when pour plated. A complete > 6 log reduction of bacteria was established in all the platelet units regardless of the method used for platelet extraction or pathogen tested [Table 2]. Pre-UVA and post-UVA amotosalen concentration for all the ten contaminated units was measured by HPLC [Table 3]. The mean concentration of amotosalen pre-UVA in RDP units was 112.49 μM and 154.56 μM in SDP units. Post-UVA, the mean percentage of amotosalen left in SDP units was 18% and 52% in RDP units, indicating that the illumination process had resulted in the proper amount of amotosalen photoconversion.

Discussion

One of the most serious transfusion-related threats is septic reactions after transfusion. The US Food and Drug Administration (US-FDA) mandated storage of platelets for no more than 5 days.[14] In the US, it is mandatory to perform a test to detect bacterial contamination in platelet concentrates before release. Bacterial culture is the most common method used, but that delays platelet unit release for transfusion for 24–48 h and further decreases usable shelf life from 5 to 2 or 3 days.

Table 1: Platelet unit characteristics: IDs, their volume, platelet concentration

Unit ID	Volume pool (postsampling) (mL)	Platelet concentration (postsampling) 10^{11}/unit	Treatment volume (mL)
RDP1	343.65	3.8	322.3
RDP2	364.55	3.6	344.7
RDP3	349.50	4.1	349.5
RDP4	383.00	4.0	376.7
RDP5	393.70	3.8	377.2
SDP1	268.81	3.4	279.2
SDP2	271.30	4.3	280.0
SDP3	270.30	3.7	280.0
SDP4	264.80	3.5	269.3
SDP5	276.00	2.7	278.2

RDP = Random donor platelets, SDP = Single donor platelets

Table 2: Pre- and post-illumination log values of control and test samples

Test unit	Control specimen (initial log)	Test specimen (log after inactivation)	Log reduction
Pathogen: *Staphylococcus aureus*			
RDP1	6.11	0	>6.11
RDP2	6.08	0	>6.08
RDP3	6.07	0	>6.07
RDP4	6.04	0	>6.04
RDP5	6.04	0	>6.04
Mean±SD			>6.07±0.04
Pathogen: *Escherichia coli*			
SDP1	6.17	0	>6.17
SDP2	6.17	0	>6.17
SDP3	6.17	0	>6.17
SDP4	6.18	0	>6.18
SDP5	6.17	0	>6.17
Mean±SD			>6.17±0.01

RDP = Random donor platelets, SDP = Single donor platelets, SD = Standard deviation

Table 3: Concentration of amotosalen in post- and pre-ultraviolet A random donor platelet and single-donor platelet units

Test unit	Pre-UVA amotosalen concentration (in μM)	Post-UVA amotosalen concentration (in μM)	Remaining amotosalen concentration (%)
RDP1	95.32	47.67	50.0
RDP2	117.72	61.78	52.5
RDP3	121.13	61.53	50.8
RDP4	114.76	61.85	53.9
RDP5	113.50	59.76	52.7
Average	112.49	58.52	52
SDP1	150.38	26.87	17.9
SDP2	169.98	30.94	18.2
SDP3	156.36	28.98	18.5
SDP4	154.29	26.62	17.3
SDP5	141.78	25.54	18.0
Average	154.56	27.79	18.0

RDP = Random donor platelets, SDP = Single-donor platelets, UVA = Ultraviolet A

Automated culture systems have been in use and provide safer platelets than conventional but result in 1–2% sample loss, higher cost, and sampling errors. The overall system efficacy is about 50%. There are other rapid methods available which are based on pH, glucose level, and oxygen levels, but their specificity is questionable.[10,11] Despite the use of culture to address the bacterial contamination risk, a recent study estimated that 1:2500 units may still be contaminated with bacteria, implicated in clinical sepsis for 1:10,700 units transfused.[15]

In Europe and areas where the CE mark is accepted, pathogen reduction has been used as a method to address bacterial contamination without the need of culture since 2003 and can be used for both apheresis and whole blood-derived PCs, including buffy coat pools. The AABB has also recognized pathogen reduction as an alternative to culture for the prevention of bacterial sepsis (Rule 4.1.4.1) since 2005, and accredited AABB blood centers outside the US have been using pathogen reduction instead of bacterial culture since then, even though the INTERCEPT system had not been approved for use in the US until the end of 2014. After that time, the system has been approved in the US and remains the only approved system for pathogen reduction of platelet concentrate (PC) in the US.

Pathogen inactivation can impact all issues from sample loss to contamination. The INTERCEPT Blood System (Cerus Corporation, Concord, CA) can reduce the risk of platelet and plasma contamination, including sepsis by inactivating pathogens present in the units.[6] The US FDA has published a draft guidance for the industry in March 2016 that aims to address the continuing concerns about bacterial contamination of transfused platelets, despite culture testing for all platelets in the US.[16] The draft guidance that is expected to be finalized in 2017 requires the retesting of previously cultured and released PC with a primary bacterial culture (PBC), if transfused after more than 4 days of storage.[16] The PBC assay qualifies the PC for use for 24 h and needs to be repeated for each day after. An alternative option to the PBC is the use of an approved system for pathogen reduction. Currently, the only system approved in the US is the INTERCEPT Blood system for apheresis platelets prepared in either 65% PAS or 100% plasma.

In our study, we prepared five RDP units by the buffy coat method and five SDP units by the apheresis method. RDP units were inoculated with *S. aureus*, while SDP units were inoculated with *E. coli*. *S. aureus* and *E. coli* are two of the most common bacteria found in contaminated platelet units.[4,5] A final concentration of approximately 1×10^6 CFU/unit was chosen as a robust challenge, modeling heavily contaminated PC units.[5] The titers of bacteria before and after illumination process were recorded and compared with nonilluminated control samples. A successful complete reduction of >6 $\log_{10}$ was established after the process. Control samples which were taken from infected RDP and SDP units post inoculation, but that were not treated with UVA showed no reduction of bacteria.

Data from routine use of the INTERCEPT system in Switzerland and France since 2006 have shown no instances of breakthrough infections after more than 380,000 inactivated platelet units were transfused, in contrast to untreated platelets transfused in the same countries that had fifty TTI diseases, including nine deaths.[17-19]

A broad-spectrum pathogen inactivation system like the INTERCEPT Blood system is effective not only against bacteria, but also against known, emerging, and unknown pathogens including fungi, viruses, and protozoans. Dengue and Chikungunya outbreaks

have become common every year in India and other parts of the world, and deferral of all plasma or platelet units is not feasible in endemic areas.[20,21] Pathogen inactivation can be of great use during such outbreaks. Successful inactivation of Chikungunya virus with a high titer in both platelets and plasma by INTERCEPT pathogen inactivation (blood system) has already been established.[22] In a different study, plasma units were spiked with dengue virus (DENV), and then pathogen inactivation using INTERCEPT system was performed. A reduction of >5.61 log of DENV was achieved. Cell culture, when infected with the same inactivated plasma, did not result in infected cells establishing the efficacy of INTERCEPT system.[23] Dengue transmission has been demonstrated through blood transfusion and the same is true for other Arboviridae such as West Nile virus and Zika virus.[24] TTIs through protozoa have also been reported, and the successful use of INTERCEPT Blood system to inactivate *Plasmodium falciparum* and *Babesia microti* in platelets, as well as plasma, has been established. Mean inactivation of *B. microti* in platelets and plasma was found to be limiting detection (>5.3 log) and preventing infection in an animal model. Inactivation of *P. falciparum* in platelets was >6 log and >6.9 log in plasma units.[25] Pathogen inactivation by amotosalen and UVA has recently been established for Zika virus in plasma[26] and platelets.[27,28] A log reduction of >6 log was also reported.

A 6 log reduction of bacteria in our study is consistent with prior data, confirming that the INTERCEPT system for platelets is effective against representative bacteria. A pathogen inactivation system promises safer blood and blood components for transfusion, but no system inactivates all pathogens.

Pathogen reduction technology by INTERCEPT looks very promising, but there are concerns related to the safety of the procedure as well as of the end product. One is the possibility of mutagenic and carcinogenic effects of the residual amotosalen and photoproducts. Tice *et al.* have reviewed a comprehensive series of the experiments conducted; bacterial reverse mutation tests, *in vitro* mammalian cell gene mutation tests, *in vitro* mammalian chromosome aberration test, *in vivo* mammalian erythrocyte micronucleus test in mice, unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo,* and carcinogenicity test with $p53^{+/-}$, with amotosalen-treated platelets without CAD. They conclude in their study that risk of mutagenic adverse effects including carcinogenic effects on a recipient of amotosalen-treated platelets is negligible.[29]

In another study, which included carcinogenicity, single-dose and multiple-dose (up to 13-week duration) toxicity, safety pharmacology (CNS, renal, and cardiovascular), reproductive toxicity, genotoxicity, vein irritation, phototoxicity, and toxicokinetic testing using animal model (mice and dog), no specific target organ toxicity, reproductive toxicity, or carcinogenicity was demonstrated. The model did not record CNS toxicity and phototoxicity except when given in doses which are >30,000-fold the expected clinical exposure. The authors have concluded no toxicologically relevant effects of platelets and plasma when treated with the INTERCEPT Blood System.[30,31]

There have been concerns about adverse immunologic responses in recipients due to the presence of any potential neoantigens formed during the photochemical process. In a pivotal clinical study, 523 patients received INTERCEPT-treated platelet and plasma transfusion for more than 8000 times and no immune responses against neoantigens could be detected by ELISA after transfusion. This indicates that there is no neoantigen generation in recipients of photochemically treated plasma or platelets.[32]

Safety, as well as potency of treated blood components, should also be evaluated when using pathogen-inactivated blood components in hemorrhagic risk or trauma cases. A recent analysis of routine use from Austria provides some insights on the support for patients that have received massive transfusions with platelet support exclusively with INTERCEPT platelets.[33] The results are promising in that no additional bleeding was observed for these patients, as evidenced by the use of equivalent amounts of PC used in addition to equal or less plasma and RBCs components. The results obtained in this study are consistent with the routine use experience for INTERCEPT PC, that when examined at the level of a center,[34] a region,[17] or a full country implementation,[19] do not result in increased component use.

An effective, broad-spectrum pathogen inactivation system can offer advantages and compliment other safety measures addressing areas of weakness such as emerging viruses which are not possibly being tested and bacterial transmission in the current safety paradigm, while preserving the function and safety of the treated blood products.

Conclusion

Bacterial sepsis and wastage of platelet concentrates because of contamination is a common sight, but there is no nationwide reporting system to keep an account of such data. Under this scenario, our experiment is first in India to evaluate the efficacy of INTERCEPT Blood System for platelets and has been found to be 100% efficient for bacterial contamination by *S. aureus* and *E. coli* since the testing for the same is not in place.

Pathogen inactivation is a proactive approach that will keep up with a wide range of pathogens including emerging and unknown infections. The cost-effectiveness of such an advanced technique needs to be evaluated by considering the morbidity, mortality, treatment cost, and trauma associated with TTIs.

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Conflicts of interest

There are no conflicts of interest.

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