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White Paper

**Limitations of Proposed Culture-Based Strategies in
Detecting Bacterial Contamination of Platelets**

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Three additional approaches to mitigate bacterial risk in platelets were proposed at the November 30, 2017 meeting of the FDA's Blood Products Advisory Committee (BPAC). These approaches presumably were intended for inclusion in the next version of Draft Guidance on the topic. All three approaches involve the application of modified culture methodologies focusing on delayed and/or larger volume sampling strategies. We will evaluate each in turn and demonstrate that there is insufficient evidence that they provide a level of safety comparable to the mitigation measures proposed by the FDA in Draft Guidance in March 2016.[1]

Background

To address the risk of bacterial contamination of platelets, culture shortly after collection has been widely employed in the United States (US) since 2004. The usual practice is to culture an 8mL sample aerobically 24 hours after collection. This procedure is well known to have limited sensitivity (11-47%[2]). Therefore, FDA issued a Draft Guidance to further mitigate the threat. This translates to a typical detection rate for early culture of apheresis platelets (also referred to as single-donor platelets) as recently reported by Eder et al. of 1/3,965 (252/million) for platelets collected with the Amicus and 1/8,959 (112/million) for platelets collected with the Trima Accel® (Terumo BCT, Lakewood, CO).[3]

Two recent reports advocate the use of protocols that use an increased sample volume with or without delayed culture as a stand-alone option without further measures to mitigate the risk of sepsis from platelet transfusion through the storage life of a platelet (at either 5 or

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[1] US Food and Drug Administration. Bacterial risk control strategies for blood collection establishments and transfusion services to enhance the safety and availability of platelets for transfusion; draft guidance for industry. Rockville, MD; c2016 [cited 2018 05 January]. Available from: 2018] https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM425952.pdf.

[2] FDA Draft Guidance at 27

[3] Eder AF, Dy BA, DeMerse B et al. Apheresis technology correlates with bacterial contamination of platelets and reported septic transfusion reactions. Transfusion 2017; 57:2969-76.

7 days).[4,5] In the Draft Guidance issued March 14, 2016, the Agency considered published work addressing both the large-volume and delayed pre-release culture protocols and elected not to include these options for risk mitigation.[6] While delaying culture testing and sampling a greater volume should theoretically result in greater culture sensitivity, evidence in support of improved safety from these practices is lacking. This analysis reviews data from the recent publications of McDonald et al.[7] and Kamel et al.[8] and demonstrates that they do not provide evidence of increased safety, as compared to the standard primary culture protocol used at present in the US.

Large volume/delayed sampling (LVDS) culture for 7-day dating as a stand-alone test -- UK

McDonald et al. reported the United Kingdom (UK) National Health Service Blood and Transplant's (NHSBT) experience with septic platelet transfusion reactions before and after the introduction of platelet culture 36 to 48 hours after donation. Testing was performed utilizing 8mL platelet samples inoculated into each of BacT/ALERT® (BioMérieux, Hazlewood, MO) aerobic and anaerobic culture bottles.[9] This volume represents 7% of their average apheresis component's volume. After six hours, culture negative-to-date components were released to hospitals. Between February 2011 and September 2015, a total of 1,239,029 platelet components were screened using this technique, with an overall confirmed-positive rate of 0.02% (200/million) for apheresis platelets and 0.07% (700/million) for buffy coat pools. The study included 960,470 apheresis doses and 278,559 buffy coat doses (pooled from four donations). The authors discussed the protocol's effectiveness based on passive clinician surveillance monitoring for adverse events, comparison of adverse events in the study period to

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[4] McDonald C, Allen J, Brailsford S, et al. Bacterial screening of platelet components by National Health Service Blood and Transplant, an effective risk reduction measure. Transfusion 2017;57:1122-31.
[5] Kamel H, Townsend M, Bravo M, et al. Improved yield of minimal proportional sample volume platelet bacterial culture. Transfusion 2017;57:2413-9.
[6] Op. cit. FDA Draft Guidance at 3.
[7] Op. cit. McDonald et. al.
[8] Op. cit. Kamel et. al.
[9] Op. cit. McDonald et. al.

the prior period (in which no bacterial culture or other mitigation was practiced) and testing of 4,515 (0.36% of the total) doses at outdate. The Trima Accel system was used to collect the apheresis platelets.

Overview of the NHSBT experience with culture

The authors emphasized that their protocol reduced the number of clinically adverse transfusion transmissions from bacteria by 90%, compared to a similar time period before implementation, i.e., when neither culture nor other mitigation efforts were in place. Such a comparison is not applicable to US protocols in which primary culture is already performed. The reported decline in risk for adverse events is similar to the risk reduction noted upon implementation of pre-release culture in the US. [10,11]

Bacterial detection rates

A closer look at the results in the publication and at additional information provided by McDonald et al. and by the 2016 UK Serious Hazards of Transfusion report is useful. Table 6 of the McDonald et al. article lists the "clinically significant" organisms (authors' designation) detected during the study. See copy of Table 6, extracted and included, below. Thirty-four "clinically significant" organisms were detected in apheresis donations and 19 were detected in the pools, for detection rates of 1/28,249 (35/million) and 1/14,661 (68/million), respectively. The significantly higher confirmed-positive rate in the pooled platelet components (0.07%; 700/million), as compared to that in the apheresis components (0.02%; 200/million), may be attributable to the number of venipunctures performed (four vs. one, respectively).

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[10] Fang CT, Chambers LA, Kennedy J, et al. Detection of bacterial contamination in apheresis platelet products: American Red Cross experience, 2004. Transfusion 2005;45:1845-52.
[11] Fuller AK, Uglik KM, Savage WJ, et al. Bacterial culture reduces but does not eliminate the risk of septic transfusion reactions to single-donor platelets. Transfusion 2009;49:2588-93.

Table 6 extracted from McDonald et al. article appears below.

TABLE 6. Confirmed-positive clinically significant species isolated from screened PLT components: time to detection and atmosphere of the initial-reactive screen bottle (February 2011-September 2015)

Bacterial species	PLT type	Number of donations contaminated	Number of contaminated components	Anaerobic Total	Anaerobic Detection time (hr), mean (95% CI)	Aerobic Total	Aerobic Detection time (hr), mean (95% CI)	Both aerobic and anaerobic Total	Both aerobic and anaerobic Detection time (hr), mean 95% CI
Streptococcus dysgalactiae (Group C/G)	Apheresis	2	4	0		0		2	10.5 (7.3-13.7)
	Pooled	8	8	2	11.0 (9.1-12.9)	0		6	9.0 (6.6-11.3)
Staphylococcus aureus	Apheresis	8	8	0		0		8	9.4 (8.2-10.6)
	Pooled	3	3	0		1	10.1	2	8.5 (5.2-11.8)
Streptococcus pneumoniae	Apheresis	8	13	0		0		8	11.8 (11.3-12.3)
	Pooled	3	3	0		0		3	12.1 (8.4-15.9)
Listeria monocytogenes	Apheresis	4	5	1	14.4	0		3	19.9 (15.5-24.3)
	Pooled	0	0	0		0		0	
Streptococcus agalactiae (Group B)	Apheresis	1	1	0		0		1	6.0
	Pooled	3	3	0		0		3	8.6 (7.5-9.7)
Bacillus cereus	Apheresis	0	0	0		0		0	
	Pooled	1	1	0		0		1	2.6
Escherichia coli	Apheresis	5	11	1	3.6	0		4	4.5 (3.6-5.4)
	Pooled	0	0	0		0		0	
Serratia marcescens	Apheresis	3	5	0		0		3	5.5 (3.4-7.5)
	Pooled	0	0	0		0		0	
Klebsiella pneumoniae	Apheresis	1	2	0		0		1	16.0
	Pooled	1	1	0		0		1	3.6
Campylobacter lari	Apheresis	1	1	1	32.0	0		0	
	Pooled	0	0	0		0		0	
Pseudomonas aeruginosa	Apheresis	1	1	0		0		1	14.6
	Pooled	0	0	0		0		0	

As buffy coat-derived platelets are not available in the US, it is most appropriate to review the authors' findings for apheresis platelets. Overall, the authors reported 208 confirmed-positive results (1/4,617; 217/million). Comparing McDonald et al.'s data to current US practice is complicated by performance of both aerobic and anaerobic culture in the UK. Use of anaerobic culture bottles results in detection of a large number of Propionibacterium species. Of the bacterial contaminants detected, 129 were skin flora, 79 of which were Propionibacteria (61% of the skin flora and 38% of all apheresis contaminants detected). Propionibacteria have never been reported to cause a fatal septic transfusion reaction in the US and have been considered to be of "questionable clinical significance" as platelet contaminants,[12] supported further by the omission of Propionibacteria in the 34 "clinically significant" organisms identified by McDonald et al. When the 79 Propionibacteria are removed from the total of 208, 129 confirmed positives remain (1/7,446; 134/million). This rate

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[12] Brecher ME, Hay SN. Bacterial contamination of blood components. Clin Microbiol Rev 2005;18:195-204.

compares to a current US detection rate for 8mL, 24-hour aerobic culture only of 1/3,965; 252/million for platelets collected with the Amicus and 1/8,959; 112/million for platelets collected with the Trima, as noted above (with no Propionibacterium species reported).[13] As stated previously, if only "clinically relevant" bacteria species are considered, the detection rate is 1/28,249 or 35/million.

In order to gain a fuller understanding of true clinical sensitivity, it is necessary to consider false negative culture results.

False negative culture results

There were four platelet doses from three donations (unit ages: 5, 4 and 5 days) that were false negative by culture. That is, they were culture-negative at screening but were identified as "near misses" after staff noticed visible clumping of material in the components prior to transfusion. As extremely high levels of bacteria are needed to cause visible contamination of a platelet unit, it can be safely assumed these were highly contaminated doses. All four of these false negative units were contaminated with *Staphylococcus aureus,* a well-documented bad actor among gram positive platelet contaminants. In spite of delayed sampling and a 16-20 mL culture sample per unit, prerelease culture results were negative on units that achieved high levels of contamination in 4 to 5 days. Additionally, one post-transfusion septic reaction occurred following administration of a pooled platelet contaminated with *Staphylococcus aureus*. Subsequent to publication, one positive culture was detected (*S. pneumoniae*) following testing of 6,015 outdated, previously screened components. [14] Given the positive-culture rates in the study, re-culturing only 6,015 outdated components (0.34%) of the updated total of 1,744,532 platelets (yield 166/million) does not provide enough statistical power to perform a meaningful analysis. A sufficiently large dataset with culture at issue or at outdate is needed to determine the true false-negative rate. McDonald et al.'s initial results

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[13] Op. cit. Eder.

[14] McDonald C. Bacterial interventions to increase blood safety. Presented at: British Blood Transfusion Society Annual Conference 2017; Sept 13-15, 2017; Glasgow. [cited 2018 05 Jan]. Available from: https://www.bbts.org.uk/bbts2017/programme1/microbialsafety/.

may lack statistical significance but they provide a worrying indication of what can legitimately be expected to be found with a larger n.

The 2016 UK Serious Hazards of Transfusion (SHOT) report (released July 2017) documents four additional false negative culture results (classified as "near miss" cases) of bacterial transmission by platelet components in the UK. [15] These four cases are in addition to the four previously reported doses described above since the practice of LVDS culture was implemented in 2011.

All four cases were identified by visual inspection of the components prior to transfusion, again indicating very high levels of bacterial contamination. Three of the four newly reported cases were reported by the Northern Ireland Blood Transfusion Service (NIBTS) and one was reported by the NHSBT. Northern Ireland cultures two 8mL samples from the mother bag 48 hours post-collection. The NHSBT cultures two 8mL samples from each split product 36 hours after collection according to the SHOT report and 36-48 hours after collection according to McDonald et al.'s publication. Platelets are released six hours after culture is initiated. Culture bottles remain on test for 7 days.

The three cases from the NIBTS included two from 2016 (Cases 1 and 2) and one from 2014 (Case 4) that had not been reported previously. *Staphylococcus aureus* was isolated in each instance from the platelet component. In the case reported by the NHSBT, from 2016, (Case 3), *Serratia marcescens* was isolated.

Benjamin et al.[16] have recently summarized and tabulated the SHOT reports from 2006 through 2016. From the introduction in the UK of LVDS in 2011 through the end of 2016, 1,652,761 platelet units were distributed. Among those 1,652,761, there were eight false-negative "near misses" detected in the transfusing facility prior to connecting the infusion set to the bag, one definite post-transfusion sepsis, one possible post-transfusion sepsis and 12 "indeterminate/undetermined" cases. The 12 "indeterminate/undetermined" cases included those in which the component bag was unavailable to culture or by the possibility of retrograde

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[15] Serious Hazards of Transfusion (SHOT). Annual SHOT Report 2016. Manchester, UK; c2017 [cited 2018 05 Jan]. Available from: https://www.shotuk.org/wp-content/uploads/SHOT-Report-2016_web_11th-July.pdf. pp 150-3.
[16] Benjamin RJ, Braschler T, Weingand T, et al. Hemovigilance monitoring of platelet septic reactions with effective bacterial protection systems. Transfusion 2017.

contamination of the bag. While there was a significant improvement compared to the period when the UK was not culturing platelet collections, it illustrates the continuing risk to recipients once the UK protocol was introduced. See Figures 1 and 2.
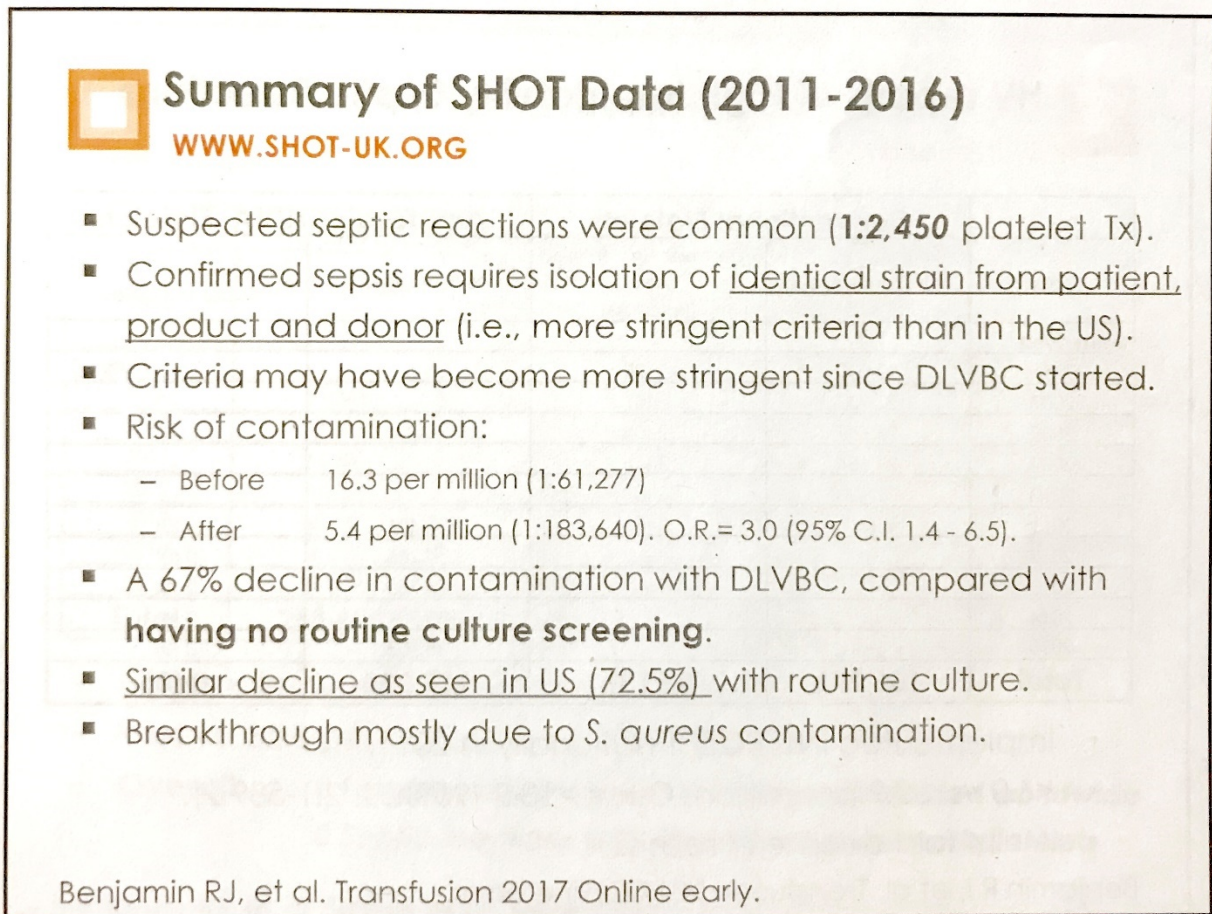
Figure 1: **Summary of SHOT Data**

Summary of SHOT Data (2011-2016)
WWW.SHOT-UK.ORG

- Suspected septic reactions were common (**1:2,450** platelet Tx).
- Confirmed sepsis requires isolation of <u>identical strain from patient, product and donor</u> (i.e., more stringent criteria than in the US).
- Criteria may have become more stringent since DLVBC started.
- Risk of contamination:
  - Before   16.3 per million (1:61,277)
  - After   5.4 per million (1:183,640). O.R.= 3.0 (95% C.I. 1.4 - 6.5).
- A 67% decline in contamination with DLVBC, compared with **having no routine culture screening**.
- <u>Similar decline as seen in US (72.5%)</u> with routine culture.
- Breakthrough mostly due to *S. aureus* contamination.

Benjamin RJ, et al. Transfusion 2017 Online early.

Figure 2: **SHOT-UK reports of Platelet Contamination & Sepsis**

SHOT-UK reports of Platelet Contamination & Sepsis
Benjamin RJ, et al. Transfusion 2017, Online Early

Year	Platelets	Excluded	Ruled out	Definite Sepsis	Possible Sepsis	Indeterminate/ Undetermined	Near Miss
2006	259,654		14	2	0	0	0
2007	255,474		3	1	0	0	1
2008	258,419		7	6	0	1	1
2009	266,312		13	4	0	3	3
2010	246,962		30	0	0	2	3
Total	1,286,821		67	13	0	6	8
2011	260,278	77	28	0	0	1	0
2012	268,565	70	27	0	0	2	0
2013	268,630	75	28	0	0	0	2
2014	274,623	56	36	0	0	1	2
2015	273,695	87	19	1	1	3	0
2016	306,970	85	14	0	0	5	4
Total	1,652,761	450	152	1	1	12	8

Strict imputability criteria underestimate the risk of break through units

No assessment of culture false negatives in the UK can be made without consideration of the very different system for assessing break-through contamination events compared to US practice. The UK passive hemovigilance protocol has very stringent criteria for diagnosing post-transfusion sepsis. Clinicians must report a reaction for further investigation. Many cases are investigated but are not considered septic reactions owing to failure to meet the "imputability" standards. The UK clinician reporting system criteria for imputing bacterial transmission from a blood component state that cultures from the patient's blood must match cultures from the

component bag and/or from the donor.[17] As in the US, the potentially contaminated component bag will not typically be available for culture confirmation and the source from the donor may well have been a transient skin contaminant or represent a low level, asymptomatic bacteremia. McDonald et al. do not state explicitly how many reactions were not investigated (or not confirmed) because of the imputability criteria. Attending physicians in the UK have reported many additional cases of suspected transfusion sepsis that have failed to meet the strict imputability criteria imposed in the UK system. In fact, 108 cases of suspected bacterial transmission by blood components were reported in 2016,[18] 111 cases reported in 2015,[19] 93 in 2014,[20] 103 in 2013,[21] and 99 in 2012.[22] Only one of the 514 cases was adjudicated as a definite platelet transfusion-transmitted bacterial septic reaction. These data and the known false-negative "near misses" suggest strongly that additional cases of bacterial sepsis occurred as the result of platelet transfusions but are not being accurately categorized due to the stringent imputability criteria. In fact, Benjamin et al.[23] concluded that UK investigations, though unstated in the SHOT report, require the same strain of bacteria to be isolated from the patient, the component bag and the donor "to impute a definite/confirmed septic transfusion reaction" following platelet transfusion and that this stringency results in an underestimation of risk.

Furthermore, passive surveillance has been documented to be inadequate for identifying septic transfusion reactions. Hong and colleagues[24] performed active and passive

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[17] Serious Hazards of Transfusion (SHOT). Definitions of current SHOT reporting categories & what to report. c2016 [cited 2018 05 Jan]. Available from: https://www.shotuk.org/wp-content/uploads/SHOT-Definitions-Jan-2016.pdf.

[18] Serious Hazards of Transfusion (SHOT). Annual SHOT Report 2016. Manchester, UK; c2017 [cited 2018 05 Jan]. Available from: https://www.shotuk.org/wp-content/uploads/SHOT-Report-2016_web_11th-July.pdf.

[19] Serious Hazards of Transfusion (SHOT). Annual SHOT Report 2015. Manchester, UK; c2016 [cited 2018 05 Jan]. Available from: https://www.shotuk.org/wp-content/uploads/SHOT-2015-Annual-Report-Web-Edition-Final-bookmarked.pdf.

[20] Serious Hazards of Transfusion (SHOT). Annual SHOT Report 2014. Manchester, UK; c2015 [cited 2018 05 Jan]. Available from: https://www.shotuk.org/wp-content/uploads/report-2014.pdf.

[21] Serious Hazards of Transfusion (SHOT). Annual SHOT Report 2013. Manchester, UK; c2014 [cited 2018 05 Jan]. Available from: https://www.shotuk.org/wp-content/uploads/2013.pdf.

[22] Serious Hazards of Transfusion (SHOT). Annual SHOT Report 2012. Manchester, UK; c2013 [cited 2018 05 January]. Available from: https://www.shotuk.org/wp-content/uploads/2013/08/SHOT-Annual-Report-2012.pdf.

[23] Op. cit. Benjamin RJ, et al.

[24] Hong H, Xiao W, Lazarus HM, et al. Detection of septic transfusion reactions to platelet transfusions by active and passive surveillance. Blood 2016;127:496-502.

surveillance for bacterially contaminated platelet components from 2007 through 2013 at one institution in the US by culture of platelet aliquots at the time of transfusion and reviewing reported transfusion reactions. Each platelet had been cultured 24 hours after collection and released for transfusion as negative. The authors found via active surveillance that 20 of the 51,440 platelet units that were thought to be safe (1 in 2,572; 389/million doses) and were transfused were actually bacterially contaminated. Five of these resulted in septic transfusion reactions, one of which was fatal, one life-threatening, one severe and two moderate. Four of the five reactions occurred in outpatients. None of these reactions had been reported to the transfusion service by passive surveillance. During this period, 284 transfusion reactions were reported by passive surveillance (e.g., nonhemolytic febrile and allergic reactions), although none of these patients had received bacterially contaminated platelets.

Analysis

The overall bacterial detection rate reported by McDonald et al. is not significantly different from the detection rate of single-bottle culture in the US. Eder at al.[25] originally reported the rates of confirmed bacterial contamination of apheresis platelet collections detected by bacterial culture according to the technology utilized for collection. Four mL of the platelet component was cultured in an aerobic bottle only, utilizing the BacT/ALERT system, at least 24 hours after collection, with product release at least 12 hours thereafter. Between March 1, 2004 and May 31, 2006, 186 of 1,004,206 donations were confirmed positive by bacterial culture testing, a rate of 1/5399 (185/million). The most informative data in this report are the results obtained with the 198,558 collections using the Trima device. Twenty-five collections were culture positive with no Propionibacteria detected (1/7,942; 126/million). This rate is almost identical to the rate reported by McDonald et al. without Propionibacteria for apheresis components (1/7,446; 134/million) collected on Trima devices. For the bacteria McDonald et al. characterize as "clinically significant," the contamination rate in apheresis components was 1/28,249 (35/million) and, if all skin contaminants (other than *S. Aureus*,

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[25] Eder AF, Kennedy JM, Dy BA, et al. Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006). Transfusion 2007;47:1134-42.

which McDonald et al. do identify as "clinically significant") are removed from the Eder et al. data, the rate was 1/19,855 (50/million). These results, using the same culture and collection devices, strongly suggest that the McDonald et al. protocol does not provide additional safety, as compared to the 4mL aerobic bottle-only protocol used by Eder et al. Subsequently, Eder et al.[26] reported results with the Trima device using an 8mL sample for culture instead of a 4mL sample on 118,014 collections. They found an overall contamination rate of 1/4,370 (228/million). This is very similar to McDonald et al.'s overall apheresis rate of 1/4,617 (217/million). When skin contaminants (excluding *S. aureus*) were removed from consideration, the contamination rate was 1/6,211 (161/million).[27] This rate is more than four times as high as McDonald et al.'s detection rate for "clinically significant" bacteria (1/28,249; 36/million). McDonald et al. note that their result "broadly reflects confirmed-positive rates reported by other blood services."[28] As stated previously, Eder et al. recently reported overall bacterial contamination rates with the current US practice of 1/3,965 (252/million) for Amicus and 1/8,959 (112/million) for Trima.

The LVDS culture protocol used by the NHSBT was established with 7-day dating of platelets. Neither McDonald et al. nor the PASSPORT study investigators re-cultured the platelets at day 5 of storage. We are unaware of data to support the premise that the NHSBT protocol or a similar approach would provide safer platelets after five days of storage. Given that the NHSBT detection rates are so similar to those in the US and given the absence of sufficient data on residual risk at the time of transfusion, one may infer that the risk at transfusion on days 3, 4, or 5 would also be similar to that of the US under its current practice. The NHSBT data do not provide proof that their protocol provides improved patient safety from bacterial contamination inside a 5-day life without secondary testing (rapid or re-culture), let alone through day 7, where breakthrough contaminated units of clinical significance were shown to exist.

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[26] Eder AF, Kennedy JM, Dy BA, et al. Limiting and detecting bacterial contamination of apheresis platelets: inlet-line diversion and increased culture volume improve component safety. Transfusion 2009;49:1554-63.
[27] Id.
[28] Op. cit. McDonald et. al.

Conclusions

The UK reports of multiple false negative "near misses," the case of actual bacterial transmission and the end-of-storage positive culture; the actual bacterial detection rate; the use of a passive surveillance system; and use of very stringent imputability criteria strongly suggest that the UK protocols do not improve patient safety at five or seven days of platelet storage compared to the current US protocol of 8mL culture volume at 24 hours with no secondary testing. Data reported by McDonald et. al. prove only that the UK protocol is better than doing no culturing at all.

Minimal proportional sample volume (MPSV) culture for 5-day dating as a stand-alone test - BSI

The recent publication by Kamel et al.,[29] reported a two-period study performed at Blood Systems on a delayed large volume culture approach in which leukocyte reduced platelets were collected with the Trima device. In the first period (n=188,389), an 8mL sample from the mother bag was cultured aerobically 24 to 36 hours after collection. In the second period (n=159,098), at least 3.8% of the mother bag volume was cultured aerobically in one to three bottles (7-10 mL each) also at 24-36 hours after collection.

Bacterial detection rates

Kamel et al. reported true-positive culture rates of 0.9/10,000 (90/million) and 1.83/10,000 (183/million) in the first and second periods, respectively. While the detection rate in the second period was significantly higher than that in the first (p<0.05), the false-positive rate in the second period (15.05/10,000 [1,505/million]) was also significantly higher than that in the first period (3.66/10,000 [366/million]), p<0.0001. One septic transfusion reaction occurred in each period. While the higher culture volume resulted in a greater number of true-positive results, the contamination rate found in the second period (1/5,486; 182/million) was comparable to the rate of culture positivity reported by others with the current US practice

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[29] Op. cit. Kamel et al.

(i.e., Eder et al., as noted previously: 1/4,370; 228/million). The authors do not discuss why their results in the first period (1/11,081; 90/million) are so much lower than results reported by Eder et al. despite using virtually identical protocols. Thus, this report does not document improved safety, as compared to current US practice.

False negative culture rates

Dr. Ralph Vassallo, MD, FACP, Chief Medical and Scientific Officer, Blood Systems, Inc. was an invited speaker at the November 30, 2017 BPAC meeting.[30] He described four positive bacterial cultures on expired products (cultured on day 5 or day 7) that had tested falsely negative with the MPSV protocol.. Three were from double products and one from a triple product. Regarding the triple product, the platelet bag matching the culture bottle that indicated growth was discarded and no growth was obtained from the bottle that was cultured. This collection, therefore, was categorized as "indeterminate" despite the fact that the third unit from this collection had been transfused and the recipient had a septic transfusion reaction that had not been recognized by passive surveillance. Blood culture results determined that the platelet recipient had coagulase-negative staphylococcus in his blood.

In summary, Dr. Vassallo reported three true positives and one indeterminate among the expired units. In addition, he reported two patients who were presumably infected with the same organism that grew from the bottle and, in one case, from the bag.

Analysis

Dr. Vassallo concluded that from 3 to 6 of the 8,038 contaminated Trima units cultured in this study were MPSV false negatives. These numbers represent residual risk rates of 373/million to 746/million. Using the lowest number provided by Dr. Vassallo for false-negatives, 3/8038 (0.000373), and a rate of 1 in 5,000 for true positives (0.0002) results in an overall contamination rate of 1 in 1745 (0.000573). Therefore, the sensitivity of the method

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[30] BPAC Meeting Transcript, November 30, 2017, at 51-73. Available at:
https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/Blood
ProductsAdvisoryCommittee/ucm543914.htm   [cited 05 January 2018]

using a very conservative calculation was:

$$\text{Sensitivity} = \text{true positives} / (\text{true positives} + \text{false negatives}) \text{ or}$$

$$\text{Sensitivity} = 0.0002 / .000573 = 34.9\%$$

This number falls squarely within the range of 11-47% of the FDA-reported sensitivity of primary culture today as practiced in the US[31]. If the highest number of false negatives in the range reported by Dr. Vassallo (six (6)) is used in the sensitivity calculation, the sensitivity is 21.1%. In the March 2016 Draft Guidance, FDA deemed these sensitivities insufficient without additional mitigation strategies.

Conclusions

Dr. Vassallo compared his results to those of the PASSPORT study in which surveillance at eight days storage found a residual risk of 662/million,[32] which was deemed unacceptable. Dr. Vassallo pointed out that 662/million is not statistically significantly different from the rate of 746/million calculated from the BSI.[33] This residual risk is also unacceptable.

Day 4 retesting with culture to achieve 7-day dating

Dr. Stephen Field, MBChB, MA, MMed, FCPath(SA), Medical and Scientific Director, Irish Blood Transfusion Service (IBTS) was invited to present information from the IBTS experience at the November 30, 2017 BPAC meeting. While IBTS manufactures both apheresis and buffy coat platelet pools, which are not available in the US, we focus on the IBTS apheresis experiences. The IBTS recombines all members of a multi-dose collection prior to sampling 48 hours post collection and takes a single 16 mL sample, testing eight mL each in aerobic and anaerobic bottles. Platelets are released six hours after culture is initiated.[34] To extend outdate to seven days, platelet doses remaining in inventory on day four are retested; a 16 mL sample is removed

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[31] FDA Draft Guidance at 27
[32] Dumont LJ, Kleinman S, Murphy JR et al. Screening of single-donor apheresis platelets for bacterial contamination: the PASSPORT study results. Transfusion 2010;50:589-99.
[33] Op. cit. BPAC Meeting Transcript at 93-110
[34] Op. cit. SHOT 2016 at 153.

from each dose, with eight mL added to both aerobic and anaerobic bottles. Culture is continued for nine days.

Bacterial Detection Rates

Dr. Field presented summary data for an 11-year period. For apheresis platelets, the primary culture confirmed positive rate was 29/106,337 (1/3667; 273/million). Day 4 culture detected 5/51,041 (1/10,208; 98/million) confirmed positives. For the cohort of 5-day platelets, 2 of 6395 (1/3198) had positive cultures at expiration.

Analysis

Clinical sensitivity can be calculated for primary culture for five-day platelets for all detected bacteria at 47%. Although it is not possible to calculate sensitivity for only clinically significant bacteria based on the way data were reported, that value would most certainly be less than 47% after removal of *Propionibacterium acnes* detections.

Conclusions

This sensitivity again demonstrated that a sample volume that was twice as large as that currently obtained in the US, did not increase sensitivity above the reported sensitivity of primary culture today as practiced in the US (11 – 47%).[35] Although the cohort of 7-day platelets had no culture positives at expiration of 2,169 cultured, this very small number of end-of-storage cultures does not provide meaningful data.

Assessments of large volume culture strategies by speakers during the open public hearing

Richard Benjamin, MD, ChB, PhD, FRCP, Chief Medical Officer Cerus Corporation, spoke at the open public hearing.[36] Dr. Benjamin summarized data demonstrating that after introduction of culture screening in the UK there was a 67% reduction in bacterial

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[35] Op. cit. FDA Draft Guidance at 27.
[36] Op. cit. BPAC Transcript at 152-58.

contamination of platelets compared to a reduction of 72% in the US after primary culture was introduced. He stated, "I would venture they are not different." He concluded "that 7-day storage with delayed large volume culture [LVDS in this paper] is not superior to the current system in the US with 5 days when you take into account these near misses" and further stated, "I would venture, also, that there's little evidence that minimum proportional volume screening improves blood safety one iota."

Michael Jacobs, MD, PhD, Professor of Pathology, Case Western Reserve University, Director, Clinical Microbiology University Hospitals Case Medical Center also spoke during the open public hearing access portion of the meeting.[37] His institution performed culture testing of platelets at time of transfusion (active surveillance) before primary culture was introduced and for about 13 years after introduction of primary culture. He reported that the bacterial detection rate determined at his site by culture at the time of transfusion was the same before primary culture was introduced and after, whether his platelet suppliers used four or eight mL platelet samples for culture testing. He showed detection rates from a number of studies and showed they were similar. His presentation concluded that the outdate testing culture-positive rate reported by McDonald and colleagues at this BPAC meeting (1/6,015) was not statistically significantly different from outdate bacterial detection rates found in other studies. In a personal communication after the meeting, Dr. Jacobs also pointed out that Townsend et al. (BSI culture using 3.8% sample[38]) found a secondary testing rate after day 7 (Trima 2/7,382) that was similar to other studies using earlier time points and lower volume proportions for primary testing.

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[37] Op. cit. BPAC Transcript at 129-37.
[38] Townsend J, Bravo M, Vanderpool M. et al. Surveillance cultures on day-7 apheresis platelets which outdated on day-5. Vox Sanguinis 2015;109 (S1):232.

Conclusions

The UK Model

McDonald et al.'s data indicate that extending dating in the US to 7 days using the UK strategy, i.e., single culture test with no secondary testing, would likely result in a significant number of contaminated platelet doses not being detected and interdicted. These contaminated platelets would be made available for transfusion. The UK protocol would also allow contaminated doses to be transfused even later in unit life than at present, increasing the risk of transfusing highly contaminated doses, which pose the greatest clinical risk. The effectiveness of reducing the risk of bacterial contamination at the time of transfusion is not sufficiently evaluated by relying on very stringent diagnostic criteria, passive hemovigilance and the published data describing the retesting of only 6,015 (0.34%) units at outdate.

Using the UK protocol for 5- or 7-day platelet storage in the absence of secondary testing represents an ill-advised strategy that would put a large number of US transfusion recipients at risk of severe or fatal platelet transfusion-associated sepsis. The UK data demonstrated no improvement from current US primary culture protocols. The eight cases of false negative "near misses" reported from the UK indicate a continuing and unquantified risk. Relying on visual inspection to interdict contaminated platelet units should be relegated to a strategy of last resort given the potential for failure.

As previously noted, in the Draft Guidance issued March 14, 2016, the Agency considered published work that addressed both large-volume and delayed pre-release culture protocols and elected not to include these as options to mitigate risk.[39] The Draft Guidance made two references to the work of McDonald and colleagues, one of which presented in abstract form much of the data included in the May 2017 *Transfusion* publication.[40,41] The abstract publication described results from screening 822,603 apheresis platelet components or

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[39] Op. cit. FDA Draft Guidance at 3.

[40] McDonald CP, Allen J, Roy A, et al. One million and counting: bacterial screening of platelet components by NHSBT, is it an effective risk reduction measure? Vox Sang 2015;109:61.

[41] McDonald P, Ball J, Allen J, et al. The first year of bacterial screening of platelet components - the English experience. Vox Sang 2012;103:176.

86% of the components reported in the published manuscript.[42] The confirmed positive rate was 0.02%, which is identical to the rate reported for the 960,470 components included in the published manuscript.

The BSI and IBTS Models

Kamel et al. do not explain why their confirmed positive rate with the current protocol was significantly lower than that previously reported by Eder et al. Nor do they explain why the positive rate with the larger-volume culture is no higher than the rate observed using the current practice (Eder at al.). The data presented by Dr. Vassallo clearly demonstrate a rate of false-negative results that show no improvement over current US practice of performing aerobic culture using a single eight mL sample at 24 hours post-collection.

The report by the Irish Blood Transfusion Service demonstrates that the sensitivity of early culture with the addition of an anaerobic bottle is within the reported range for single bottle (aerobic only) culture using 8mL sample as is routinely practiced in the US. Data on contamination rates on Day 7 is insufficient to draw meaningful conclusions due to the small number of units recultured.

Summary

We recognize that there are challenges in comparing bacterial detection rates across different time periods and collection establishments. We also note that the results described by McDonald et al. and Kamel et al. do not provide convincing evidence that introducing large-volume/delayed culture protocols in any form as a policy in the US will improve transfusion safety, as compared to current practice.

Key characteristics, including platelet collection device, culture testing protocol, and test results reported for four studies are summarized in tabular form in this section. See table titled **Comparison of Studies: Collection Devices, Culture Protocol, and Results**.

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[42] Op. cit. McDonald C, Allen J, Brailsford S, et al.

Final Conclusion

Bacterial contamination of platelet components remains a significant risk of transfusion. In the Draft Guidance issued in March 2016, the FDA recommended measures to mitigate this risk in addition to the early bacterial culture that is universally employed in the US. The technologies recommended (pathogen reduction, rapid testing, repeat culture) are established means of reducing this risk and are being increasingly used. The alternative early bacterial culture practices discussed at the 30 November meeting of the BPAC (LVDS, MPSV) have not been demonstrated to detect more bacterial contaminations or more contaminations with bacteria considered clinically significant, than the current early culture procedure in use in the US today. McDonald et al. demonstrated that their LVDS protocol was better than doing nothing and both McDonald et al. and Kamel et al. demonstrated comparable bacterial detection to the early culture protocol employed in the US at present. Just as the early culture practiced today is not adequate as a standalone measure to reduce the risk of bacterial transmission by platelet components, neither LVDS nor MPSV should be implemented as a standalone measure to protect patients from the life-threatening risk of bacterial contamination of platelet components.

Table: Comparison of Studies: Collection Devices, Culture Protocol and Results

Study Publication	Collection Device	Culture Protocol	Culture Results	
			Primary	**Time of Transfusion/End of Storage**
Townsend et al. Vox Sang 2017;109:(S1)232	Trima (T) Amicus (A)	≥3.8% aerobic; 24-36 hours; Mother Bag	Not stated	Day 7 culture: T: 2/7,382 271/million (95% CI 84-978) A: 3/1,659 1,808/million (95% CI 657-5272)
Kamel et al. Transfusion 2017;57:2413-9	Trima	≥3.8% aerobic; 24-36 hours; Mother Bag	29/159,098 183/million No Propionibacterium species reported	Reported at BPAC Day 7 culture: "3-6"/8,038 373-746/million (95% CI for 3: 136-1090; 95% CI for 6: 350-1624) (sensitivity of MPSV* 21%-35%)
McDonald et al. Transfusion 2017;57:1122-31	Trima	8mL aerobic, 8mL anaerobic; 36-48 hours; Splits (Doses)	208/960,470 (apheresis) 216/million Without Propionibacterium species: 134/million	Reported at BPAC Day 7 culture: 1/6,015 166/million (95% CI 40-926) (sensitivity of LVDS**: 45%; including Propionibacterium species: 57%)
Dumont et al. Transfusion 2010:50:589-99	Trima and Amicus	4-5mL aerobic, 4-5mL anaerobic; 24-36 hours; >99% Mother Bag	90/388,903 231/million Without Propionibacterium species: 68/388,903 175/million	Day 8 culture 4/6039 662/million (95% CI 180-1695) (sensitivity of primary culture: 21%; including Propionibacterium species 26%)

*MPSV = minimal proportional sample volume

**LVDS = large volume/delayed sampling