Peer Review File

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Reviewer 1:

Comments:

This manuscript is a timely narrative review of large volume delayed sampling (LVDS) for platelet bacterial culture and risk mitigation. The manuscript is generally well-written. The authors should qualify some of the statements about residual risk as noted below and, if possible, it would be helpful to discuss what might be considered acceptable residual risk.

1. "Derivation" should read "diversion"

Reply: we have substituted diversion for derivation in the text

2. 24 hour culture was performed in many countries, but it should be noted that some countries do not perform culture as a risk mitigation strategy.

Reply: we added the following comment at the end of the first paragraph of the introduction: "Not all countries introduced bacterial culture as a risk mitigation strategy."

3. When mentioning single culture bottle, it would be useful to specify the bottle type (i.e. aerobic).

Reply: we added the adjective "aerobic" in all instances when we described single bottle culture.

4. When discussing residual risk, the recent meta-analysis by Walker et al should be mentioned (Transfusion. 2020.60(9):2029-2037).

Reply: reference to this article was added to the text

Changes in text: added at the end of the second paragraph of the introduction: "Finally, Walker et al did a meta-analysis of studies that evaluated the detection rate of bacterial contamination of platelets whose 24-hour culture was negative, using either secondary culture or rapid testing: they

found that the rate of detection by culture was 0.94 per 1000 (95% CI 0.54-1.32) and 0.09 per 1000 (95% CI 0.01-0.25) by rapid testing8."

5. What is LVDS culture: when talking about volume, please make it more clear that the 16ml volume would be 8ml each for aerobic and anaerobic bottles. FDA also requires culturing of splits rather than just the mother bag.

Reply: The requirement that 8 mL of the sample needs to be inoculated in both the aerobic and anaerobic bottle was specified in the text. Concerning the FDA requirement that each daughter bag must be cultured, this was already specified in our text.

6. Page 3: Impact of sampling volume was analyzed using meta-analysis by White et al (Transfusion. 2020.60(5):986-996. It was statistically significant and the effect size was also included. This should be mentioned as well.

Reply: the following text was added to comply with this request

Change in text: Added in the first paragraph on Rationale: "As further support for increasing the sample volume in order to increase bacterial detection, a recent meta-analysis of studies reporting on bacterial contamination rates of cultured platelets found that there was a positive relationship between the sample volume and the rate of positivity13"

7. When discussing that "increasing volume does result in improved yield", it would be helpful to discuss the definitions used to determine a true positive.

Reply: we inserted a definition of true positive in the text.

Change in text: we inserted the following in the first paragraph of the section entitled evidence that LVDS results in enhanced product safety: "(defined as a positive culture confirmed by detection of the same organism upon reculture of the component or culture of a co-component)"

8. The authors mention "only 1 out of 4536 was found positive … after implementation of LVDS". Can the authors comment on the acceptability of that result from a safety standpoint? Do these data suggest LVDS is adequately safe, or just relatively safer than 24-hour culture using one bottle type?

Reply: To be able to state whether LVDS is adequately safe, we would need to arrive at a consensus as to the acceptable residual risk of bacterial contamination of platelets. As far as we know, that has not yet occurred. We believe that LVDS has led to greater safety than the previous approach of 24-hour single bottle culture. We have added text in the conclusion section to respond to this comment.

Change in text: Added in the conclusions section: "Is the enhanced safety sufficient? In a commentary published in the January 2013 edition of Transfusion, Brecher et al acknowledged that the 24-hour culture had greatly improved the safety of platelet transfusion in regard to bacterial contamination, at the same time recognising the need for further measures17. LVDS is regarded as an important step in that direction. The FDA acknowledged this since it included LVDS as one of the options available to make platelets safer in their recent guidance9. The approach is not 100% effective at eliminating bacterially contaminated platelets, as illustrated by the fact that there are breakthrough infections, albeit at a lesser rate than with the 24-hour aerobic bottle culture and a certain number of positive cultures at outdate, again at a lesser rate than previously in our experience. There is always room for improvement. That being said, LVDS, in conjunction with other measures, has decreased the residual risk of PTS following platelet transfusion to a level that now approaches what is reported with transfusion transmissible viral infections for which screening programs are in place18."

9. If the residual risk results are based on passive reporting, the true residual risk must be higher than that (possibly quite significantly). This context should be provided. The CBS paper that is cited (reference 10) also showed residual detection rates after secondary sampling and the authors should also comment on this from a safety standpoint.

Reply: Although it is true that the data in our paper concerning breakthrough infections is based on passive surveillance, it is important to note that the hemovigilance systems in the two countries from where the studies emanate are quite robust. As an example, in our jurisdiction (Québec), more than half of all febrile transfusion reactions undergo full microbiological investigation, based on guidelines published by a working group of the Public Health Agency of Canada. Therefore, we believe that most PTS cases are being detected, and certainly those of moderate or greater severity. This is also true for the rest of Canada and England.

Change in text: the following text was inserted in the section entitled Evidence that LVDS results in enhanced product safety: "Although it is true that the data concerning breakthrough infections is based on passive surveillance, it is important to note that the hemovigilance systems in the two countries from where the studies emanate are quite robust. As an example, in our jurisdiction (Québec), more than half of all febrile transfusion reactions undergo full microbiological investigation, based on guidelines published by a working group of the Public Health Agency of Canada16. Therefore, we believe that most PTS cases are detected and reported, and certainly those of moderate or greater severity. This is also true for the rest of Canada and England."

10. Age of platelets at transfusion: the rates presented of platelet consumption per 1000 persons includes no statistical analysis to support the claim that rates decreased. Were these rates truly different? I realize the point is that rates did not increase, but statistical support should be provided if the claim is made that rates actually decreased.

Reply: we have changed the text to address the reviewer's concern.

Change in text: the text now reads: "Rate of platelet consumption per 1000 persons was monitored: the rate went from 4.6 per 1000 in 2012 to 4.1 per 1000 in 2017, suggesting that implementation of LVDS did not lead to increased need for platelet transfusions."

11.Conclusion: the authors state that LVDS has the advantage of relative simplicity when compared with multistep approaches. This statement should be balanced with any potential disadvantages of LVDS relative to multistep approaches. For example, Walker et al. Transfusion. 2020. 60(8):1723-1731 used simulation to compare safety of these strategies and found two step strategies are generally safer.

Reply: we did not include the Walker et al reference in our paper because we felt it suffered from a number of methodological flaws that did not allow a fair assessment of the safety of the various risk mitigation approaches evaluated. We do agree that LVDS can be inferior to 2-step approaches if the lag time is 48 hours or more. However, most strains do not have such a long lagtime. In a study of 20 strains of Staphylococcus epidermidis originally isolated from contaminated platelets, only 5 strains showed slow growth when inoculated in platelets, and even among these, there was detectible growth at 48 hours. That being said, we included text in our discussion to address the

reviewer's concern.

Change in text: The following text was added in the conclusion section: "Are other culture approaches superior in terms of safety? In a recent paper, Walker et al, with the use of modelisation to simulate various hypothetical contamination scenarios, compared the performance of the nine risk control strategies contained in the recent FDA guidance20. They concluded that two-step policies involving secondary culture were generally safer. The 11 by 10 matrices of scenarios in the paper compared various combinations of lag times (from 0 to 120 hours) and doubling times (from 1 to 10 hours). Most of the scenarios in these risk matrices had a lag time of 48 hours or more (63%), and all scenario in the matrices were considered to have an equal probability of occurrence. This methodological approach obviously disadvantaged LVDS, since in any scenario with a 48-hour or greater lag time LVDS will perform poorly. The equal weighing of scenarios in their modeling exercise is recognised as a limitation by the authors. In a paper on the growth characteristics of 20 strains of Staphylococcus epidermidis originally isolated from contaminated platelets, only 5 of the strains showed slow growth when inoculated into platelet products, and even in these five, there was evidence of growth, albeit reduced when compared to the other 15 strains, at 48 hours21. Furthermore, Walker et al used a single starting inoculum of 10 CFU, which is lower than the estimated mean level of 77 CFU in contaminated platelets at 24 hours in a ARC paper previously cited in this article5. We therefore feel that the data in the Walker et al paper, although interesting, do not allow a head-to-head comparison of the various culture strategies found in the FDA guidance. Only time, and ongoing evaluation of the various strategies, once implemented, will allow to eventually determine if one does have a more favorable performance than the others."

Reviewer 2:

Comments:

The authors provide a succinct overview of LVDS bacterial culture as a means of reducing the risk of bacterial sepsis. The report is novel, timely and of interest to the readers of the Annals.

1. In the discussion section, it would be appropriate to provide a very brief summary of the alternative methods suggested by the FDA to improve platelet bacterial safety and to discuss the

relative pros and cons of the LVDS method.

Reply: Since this paper is part of a special series on bacterial contamination of platelet, an accompanying article in the special issue will include a formal comparison of the pros and cons of the options in the FDA guidance. Knowing this, we limited our comments to a short enumeration of the advantages and disadvantages of LVDS at the beginning of the conclusion section. We consulted the guest editor on this issue and she agreed with our decision.

2. The authors mention a technical improvement that reduce the false positive rates with apheresis platelets. It would be useful to share the details of this improvement for others to emulate.

Reply: we have added text that describes in some detail the technical improvement.

Change in text: the text now reads: "Following the advice of our supplier of bacterial culture material, we implemented a technical improvement (installation of a software application called a compensation filter that leads to a reduction of the number of false positives due to the drop of temperature when loading new bottles) that dropped the false-positive rate to 0.075% in apheresis platelets."