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Draft proposal:

Opinion of ISBT Working Party Transfusion-Transmitted Infectious Diseases, Subgroup Bacteria. It is intended to publish the opinion in Vox Sanguinis after discussion and release by the Working Party.

"Efficacy and Limitations of Methods for Platelet Bacteria Screening – Sense and Non-sense in Application of Screening Methods"

Since the impressive reduction of transfusion-transmitted virus infections, bacterial infections by blood transfusion represent the major infection risk. Platelet concentrates are the main source of bacterial transfusion transmission as they are stored under temperature conditions which allow growth of contaminating bacteria up to levels of 10^{10} microbes per platelet bag. In addition to bacteria cells themselves, endotoxins and/or exotoxins are often present, depending on bacteria species and strain. Transfusion of highly contaminated blood components will in general lead to immediate septic shock and potential death of the patient. Assigning the contaminating bacterium to pathogenic, apathogenic or facultative-pathogenic species, as defined in the criteria of Clinical Microbiology, is of secondary importance only in this context. If blood components contain large amounts of usually apathogenic bacteria, these can also cause therefore, fatal infections in the recipient after transfusion. The mortality rate of platelet-transmitted sepsis ranges from 1 in 20,000 to 1 in 100,000 transfusions. Therefore, there is a necessity for improvement of bacteria safety in blood transfusion. This paper does not consider the Pathogen Reduction Methods but will assess suitable Screening Methods available on the market.

Beside conventional microbiological approaches (like Gram's staining) or surrogate markers (like strips or dip sticks to assess pH or glucose), several efficient methods able to detect bacterial contamination in platelets are available on the market. They have to be divided into two different methodological principles:

1. **Detection of bacteria by incubation or cultivation in automated or technical systems (Incubation Methods)**

   The first principle is characterised by methods using cultivation/incubation of contaminating bacteria. They possess high sensitivity (theoretically 1 bacterium/cfu per sample), but they do need some time for signal production (usually at least one day). Considering the potential very low bacterial number introduced in the collection process (typically 10 – 100 bacteria per bag corresponding to 0.03 to 0.3 bacteria per millilitre), samples to be tested in a cultivation/incubation method must not be drawn to early. Otherwise, the sample could be free of bacteria leading to a false negative result in analysis whereas the

bag is contaminated. It is therefore prudent before sampling, that platelet concentrates are stored for at least one day (24 hours) after donation. During this time, multiplication of contaminating bacteria in the bag can take place leading to an increase in bacterial number and, therefore, to a higher probability for collecting bacteria into the diagnostic sample.

Regardless of postponing the sample drawing, there remains a residual small sampling error that cannot be avoided. The reason for this sampling error is low residual bacterial count, dependent on slow growth or a prolonged lag phase of the contaminating bacterial strain. It is difficult to calculate the dimension of this sampling error as little information is currently available. One paper reports on two overlooked bacterially contaminated platelet concentrates, leading both to life-threatening sepsis by *Bacillus cereus*, among 28,104 tested units (sampling error: 1 in ~ 14,000). Sampling had been done between 14 to 24 hours after donation. In another study, 1061 platelet concentrates were, following testing at day one, analysed for the second time after 7 days of which 2 were positive (sampling error: 1 in ~ 500). A third incident concerns two severe cases of sepsis (at least one which fatal) by transfusion of apheresis platelets (2 therapeutic units from one donation), in which a sample had been collected 20 hours post donation producing negative results both in BacT/Alert and in eBDS. In this study, all together 50,000 platelet concentrates have been tested (sampling error: 1 to 2 in 50,000).

Most experiences have been collected with the automated microbiological culture system BacT/ALERT (BioMerieux) that monitors continuously carbon dioxide production of bacteria. The BACTEC (Becton Dickinson) system uses the same detection system as BacT/ALERT, and is also used for platelet concentrate screening. Samples from platelet concentrate bags are inoculated into ready-to-use cultivation bottles. In general, aerobic as well as anaerobic bacteria can be detected. Several authors recommend continuing the automated culture after issue of the platelet concentrates in order to detect slowly growing bacteria. In case of positive signal after issuing, the hospital is informed in order to recall the product or to monitor the recipient if the platelet concentrates have already been transfused.

There are several reports on failures in detection of certain bacteria by the automated microbiological culture systems. For instance, the BacT/Alert system did not indicate growth of the non-fermentative Gram-negative species *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, whereas the BACTEC system did not found the Gram-positive species *Streptococcus mitis* if the culture bottles had been spiked with less than 61 cfu/ml. Detection of low bacterial number is required if a sample is taken early in blood component shelf life.

Another cultivation approach is represented by the eBDS (enhanced Bacteria Detection System, Pall Corp.). This system uses the oxygen consumption by bacteria as a marker for detection. A sample from the platelets is transferred into a satellite bag that has to be incubated for 18 – 24 hours at 35°C. Thereafter, the oxygen level is measured in the headspace. One measurement can only be made with the Pall eBDS. The eBDS does not detect strictly anaerobic bacteria like

Clostridium perfringens or potentially very slowly growing bacteria.

2. Direct detection of bacteria without incubation or cultivation (Rapid Methods)

The second principle is represented by rapid methods capable of bacterial detection without an incubation step. Systems have been developed that are based on labelling bacterial DNA with a fluorescent stain and detection using laser technology. Furthermore, several authors reported on universal bacterial detection by NAT/PCR targeting evolutionary highly conserved nucleic acid sequences encoding for ribosomal RNAs. Rapid methods require only a short time for diagnosis (minutes up to a few hours), which enables a later sample draw in comparison to that of incubation methods. Considering the initially low bacterial load of blood components mentioned above, contaminating bacteria can multiply to a higher count which reduces the sampling error. Given ideal circumstances, the sample could be drawn immediately before issuing the platelet concentrate to the hospital in a bed-side-like manner. The overall sensitivity of rapid methods depends (a) on the analytical sensitivity of the method itself and (b) on the volume analysed. Thus, the diagnostic statement cannot be "the component is sterile" but "the component contains not more than …". On the other hand, late sampling combined with a rapid method would prevent transfusions of platelets containing very high numbers of bacteria leading to acute life-threatening situations like septic/pyrogenic shock as mentioned above.

The Scansystem (Hemosystems) detects bacteria by automated laser scanning with a sensitivity of approximately 10^2 to 10^4 bacteria per millilitre. The platelets have to be removed by a filtration step after aggregation of the cells. Thereafter, the bacteria are fluorescently labelled, fixed on a membrane, and analysed. The whole procedure requires 60 to 90 minutes.

Detection of bacteria by flow cytometry (BD Biosciences) can be performed in around 15 minutes. In a one-step procedure, platelets are lysed, and bacteria are fluorescence labelled. Thereafter, the analysis is performed in a standard flow cytometer. The sensitivity of the methods ranges currently from 10^3 to 10^5 cfu per millilitre. Applying a pre-incubation step of the samples prior to the analysis (one to two hours), the sensitivity can be improved up to 10^1 to 10^2 cfu per millilitre.

Universal bacteria PCR/NAT is the most sensitive rapid method; detection limits up to 1 to 10 cfu per millilitre have been shown in several papers. The most interesting variant would be a Reverse Transcriptase (RT) PCR starting from the ribosomal RNA itself, since up 20,000 ribosomes occur in one bacterial cell, thus helping to increase sensitivity of such assays substantially. Therefore, a routine detection limit of one cfu per sample is very likely. Currently, there are two restrictions in application of this approach in routine bacteria screening of platelets: (a) no manufacturer is offering a validated test and (b) a period of approximately 4 hours is required to obtain the result, at least for the RT-PCR,

which is relatively long for a real rapid method.

In general, the rapid methods can be applied as incubation methods, too. In this case, a sample can be drawn from the platelet bag into a satellite bag, which is incubated at 37°C to support microbial growth. The analysis is made using a sample from the satellite bag after incubation. This procedure improves the sensitivity of the given method and enables the blood transfusion facility to potentially perform in parallel with a culture method if so desired.

Is there any information about the use of "Bac-Detect" and of Verax machine (bacterial cell wall detection) in platelets?

Conclusions

Applying methods for platelet bacteria screening, two paradigms should be considered, which are summarised in the table. Considering the sum of aspects mentioned above, the potential improvement of bacteria safety of platelet concentrates is comparable in both strategies.

Paradigm 1	Paradigm 2
"early sampling" 24 h after donation combined with **incubation method**	**"late sampling"** before transfusion, ideally bed-side combined with **rapid method**
"early" sampling error bacteria count (still) to low, sample sterile, PC bacterially contaminated	**"late" sampling error** depends on detection limit and analysed volume result: PC contain less than …
relevant advantage: logistics, implementation into routine uncomplicated	**relevant advantage:** prevention of transfusion of highly contaminated platelets
relevant disadvantage: sampling error, false negative results	**relevant disadvantage:** logistics complicated

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