

Time course of pH in platelet concentrates after bacterial contamination

Thomas Montag¹, Sven-Boris Nicol¹, Uta Schurig¹, Julia Brachert¹, Annette Sauer¹, Alexandra Friedrich¹, Thomas Müller², and Christian K. Schneider¹

¹ Paul Ehrlich Institute (PEI), Langen, Germany

² German Red Cross – Blood Transfusion Service NSTOB, Institute Springe, Germany

Purpose:

Among the methods for Platelet Bacteria Screening, lowering of pH in Platelet Concentrates (PCs) is considered as one indicator for bacterial contamination (e.g. recommendation of AABB in 2004). There are long lasting critical discussions regarding the sensitivity of this approach (e.g. Wagner and Robinette, 1996). Recently, the BCSI pH1000 system has been developed allowing non-invasive pH measurements in PCs at any point in time during storage until the moment of transfusion. This novel tool has been used in the current study for precise characterisation of pH time course in Pooled Platelet Concentrates (PPCs) after defined artificial bacterial contamination.

Material and Methods:

Platelet Concentrates: Plasma-reduced platelet concentrates were prepared from pools of four buffy coats. The residual plasma content was approximately 30 percent. The storage medium (SSP+, identical with PAS-III M) was obtained from MacoPharma, Tourcoing, France. The storage bags for the platelet concentrates (CLX; nominal volume, 1.5 L) were obtained from Pall, Dreieich, Germany. The volume of the platelet concentrates was approximately 300 ml. The platelet concentrates were stored on an agitator at 22 to 24°C.

Bacteria: All bacteria strains used were characterised prior to the study with respect to their ability to grow up in platelet concentrates obtained from a great number of different donors (at least 100 different donors). This was completed in order to exclude influences of host defence mechanisms on the growth behaviour of the respective strain in the given PC. Thereafter, these bacteria strains were prepared by a specifically developed procedure leading to deep-frozen cell suspensions consisting of living cells only in a defined count (so called PEI Bacteria Standards). This approach allows artificial contamination of PCs with a very low number of living bacteria (approximately 10 CFU per bag corresponding to 0.03 CFU/ml) simulating "real life" conditions.

Two PEI Bacteria Standards were used as models: PEI-B-20-06 (*Streptococcus pyogenes*) and PEI-B-08-09 (*Klebsiella pneumoniae*). All together, 20 PCs were contaminated (10 each for the respective bacteria species) with either 0.03 CFU (Colony Forming Units) per mL or 10 CFU per mL. Two sterile PCs served as controls. The PCs were stored under usual blood bank conditions; bacteria count and pH were monitored over up to 8 days in parallel.



Figure 1: BCSI pH measuring device

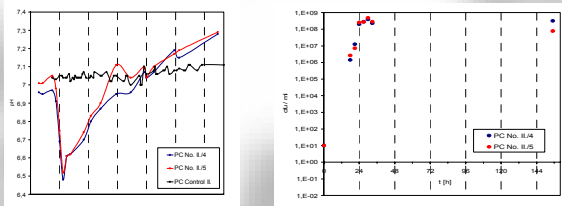


Figure 2: Left side: Time course of pH after contamination of two PPCs with 10 CFU per millilitre *Klebsiella pneumoniae* (PEI-B-08-09) each. For comparison, the pH time course of a sterile PPC is included. Right side: Time course of bacterial count (in CFU/ml) in the same contaminated platelet bags.

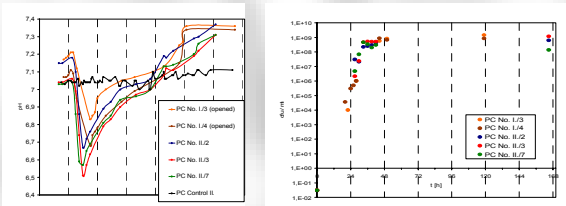


Figure 3: Left side: Time course of pH after contamination of five PPCs with 0.03 CFU per millilitre *Klebsiella pneumoniae* (PEI-B-08-09) each. For comparison, the pH time course of a sterile PPC is included. Right side: Time course of bacterial count (in CFU/ml) in the same contaminated platelet bags.

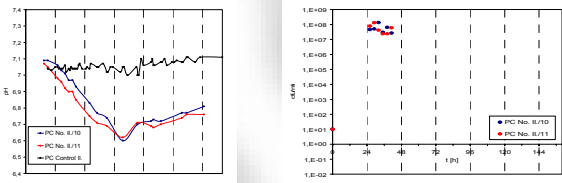


Figure 4: Left side: Time course of pH after contamination of two PPCs with 10 CFU per millilitre *Streptococcus pyogenes* (PEI-B-20-05) each. For comparison, the pH time course of a sterile PPC is included. Right side: Time course of bacterial count (in CFU/ml) in the same contaminated platelet bags.

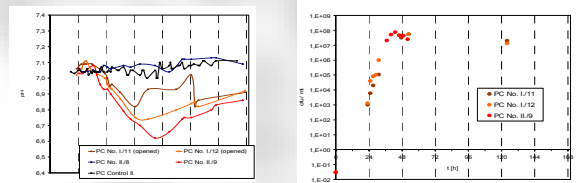


Figure 5: Left side: Time course of pH after contamination of three PPCs with 0.02 to 0.03 CFU per millilitre *Streptococcus pyogenes* (PEI-B-20-05) each. For comparison, the pH time course of two sterile PPC is included. Right side: Time course of bacterial count (in CFU/ml) in the same contaminated platelet bags.

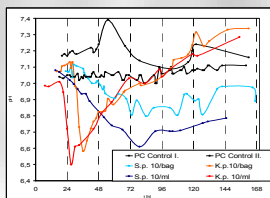


Figure 6: Time course of pH in PPCs in dependency on the way of contamination. Averages of the results obtained applying the different contamination protocols (see figures 2 – 5). Abbreviations: K.p.: *Klebsiella pneumoniae*; S.p.: *Streptococcus pyogenes*

Results:

As expected, the pH in contaminated PCs started to decrease after the bacteria had reached a count of 10^6 to 10^7 followed by a further lowering down to 6.7 to 6.5. Thereafter, a re-increase of pH values could be observed in all contaminated PCs. In case of PCs contaminated with *Klebsiella pneumoniae*, the pH returned to its initial value three days after contamination and increased further up to 7.3 to 7.4 on day 7.

Conclusions:

Generally, pH measurement in PCs is not acceptable to be a solitary approach for Platelet Bacteria Screening because of its low sensitivity. On the other hand, there are time points at which the pH provides a warning signal to prevent transfusion of bacterially contaminated PCs. Additionally, the BCSI pH 1000 System allows pH measuring immediately before transfusion without opening the bag which gives a clear effort in comparison with pH estimation by strips or dip sticks (as recommended, for instance, by AABB in 2004). There is a need for further studies regarding pH changes in bacterially contaminated PCs.