Rapid Separation of Very Low Concentrations of Bacteria from Blood
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Introduction: A rapid and accurate diagnosis of the extremely low levels of bacteria (1 to 100 cells/mL) found in a septic patient’s blood is vital to increase survival rates of those with bloodstream infections, particularly to help those with carbapenem-resistant enterobacteriaceae (CRE) infections. Very few techniques have been proven to inexpensively and effectively recover low numbers of bacteria from whole blood. In this presentation, very low concentrations of bacteria (6 to 200 CFU/mL) were separated from 7 mL of whole blood using sedimentation in a spinning hollow disk.

Materials and Methods: 1.5 mL PBS was added to 7 mL of human blood, and this mixture was spiked with various low concentrations of E. Coli and spun in a 12-cm hollow disk for 1 minute at 3000 rpm. During spinning, the red blood cells and white blood cells were sedimented towards the outer wall, and when the disk decelerated, the plasma along with the bacteria pooled down to the center of the disk. Following spinning, the plasma samples were vacuum-filtered, and colony counting was used to determine the percent recovery of bacteria. Experiments were done both without lysis and with lysis of red blood cells (RBCs). The lysis experiments included an extra step of adding SDS and double-distilled water to the samples before filtering in order to lyse the remaining RBCs.

Results and Discussion: We obtained an average of 69% recovery of bacteria from concentrations between 6-200 CFU/mL, as shown in Figure 1. There was no statistical difference between female lysis vs female non-lysis-treated blood samples, nor was there a difference between blood obtained from male vs female volunteers in the lysis experiments. While there is much scatter, there is not any trend in bacterial recovery as the initial bacterial concentration is varied.

Conclusion: We have shown a remarkably high recovery of low concentrations of bacteria from whole blood using a spinning hollow disk compared to other techniques. These results show that this technique is reliable at clinically relevant levels of bacteria and could be used to diagnose sepsis in the future. We believe that our method of using a spinning hollow disk is the first step in the development of an assay for rapid isolation of bacteria from whole blood, and that subsequent molecular techniques can easily be employed for identification of bacterial species and their resistance profile.

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