Development of an optical/electronic system for rapid detection of bacterial infection

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Abstract

Bloodstream infections (BSIs) cause serious illnesses that can lead to sepsis. Sepsis accounted for almost 20% of all global deaths in 2017 according to the World Health Organization [1]. The SIMBLE-team [2] has recently developed a low-cost diagnostic device to aid in the diagnosis of BSIs designed for use in low- and middle-income countries called the "turbidimeter". It operates based on the change in turbidity of the fluid inside the blood culture bottle. This work looked to further improve the device for faster detection of more types of bacterial species. The two ways of doing this were adding more sensors and looking at the effect of sample volume. To do this, new sensor boards were designed. Afterwards, in vitro testing with human blood spiked with bacteria was done to examine the performance of the new measuring setup. The results showed that the sensors should be placed near the top of the liquid to allow for the earliest possible detection of bacteria. Additionally, an increase in blood and broth volume led to a longer sedimentation phase during which bacterial growth cannot be detected.

Introduction

For a previous study, B. Barbé et al [3] developed a custom turbidimeter as a simple, low-cost but effective means of detecting blood stream infections in blood cultures in resource-limited settings. To see where performance of that turbidimeter could be improved, several tests were done in the current work. The turbidimeter operates based on the turbidity of the blood culture [3]. To measure this turbidity, a white LED (Luminus, Sunnyvale, United States of America) illuminates the blood culture bottle (BCB) and its contents [3]. One TCS3772 color sensor (ams-OSRAM, Premstaetten, Austria) is positioned on the opposite side of the BCB to detect the light transmitted through the sample [3]. A second sensor is mounted orthogonally to be able to detect the amount of light that is scattered by the sample [3]. The light intensities that these sensors detect will depend on the turbidity of the sample.

A new turbidimeter generation was developed to examine possible improvements that can be made to the already improved second generation that is currently being used in new studies. The second generation turbidimeter augmented with these new capabilities will be referred to in this work as the third generation turbidimeter. This new generation has 16 sensors and 8 LEDs to be able to measure at 8 different heights. The TCS3400 sensor was used in the third generation turbidimeter instead of the TCS3772 because of availability issues. The measuring locations are at 20mm, 25mm, 30mm, 37.5mm, 45mm, 55mm, 65mm and 75mm relative to the bottom of the blood culture bottle.

Method

The tests with the third generation turbidimeter were done at the Institute for Tropical Medicine (ITM) in Antwerp with spiked human blood. The setup was configured such that measurements at different heights were done one by one, from the bottom to the top. Measuring at the 8 heights took 12.8 seconds and a period of 26.4 seconds was left between two measurement cycles. Testing happened over a period of five weeks. The first part of the tests was focused on determining the effect of measuring height on the blood culture measurements. Eight blood cultures were set up with 30ml of tryptic soy broth and 0.3mg/ml of SPS. 2ml of human blood was used to inoculate the sample. The human blood was kindly donated by several volunteers at ITM (ethics approval was received for sampling of blood). The blood of the volunteers was spiked in 7 out of the 8 bottles with a bacterial strain (*Escherichia coli*, *Salmonella* Typhimurium, *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Burkholderia cepacia*), to mimic blood samples of infected patients. The second part of testing examined the effect of sample volume. Eight more BCBs were filled with 2 different blood-to-broth ratios (40ml broth + 2ml blood or 40ml broth + 10ml blood) and were spiked with 2 different bacterial strains (*Escherichia coli* or *Salmonella* Typhimurium). This together with two samples from the previous part gave three different blood-to-broth ratios and two different bacterial strains to compare against each other as well as one or two blanks for each blood-to-broth ratio. The labelling of a positive sample happened manually by inspecting the growth curves from the turbidimeter. The positivity of samples was confirmed by subculturing a drop of the BCB content on solid agar after the incubation.

Results

The tests used BCBs containing a mixture of broth and blood spiked with bacteria. The results will therefore be in the form of bacterial growth curves. To make growth curves, the concentration of bacteria inside the medium should be measured over time [4]. However, this is not what was measured here. The turbidimeter measures the turbidity of the medium and not the microbial concentration directly. The growth curves used in this analysis might therefore not exactly line up with the actual growth curves in time or intensity, but they should show the same phases. There will also be an additional phase before the lag phase (unrelated to bacterial growth), the sedimentation phase. During this phase, some particles within the blood will fall to the bottom of the BCB and remain there as sediment. All the spiked samples showed growth except for the 2 samples spiked with *Burkholderia cepacia*. These did not show a clear growth phase. Because of this, no conclusions on time to detection can be made for these samples. In these results, only measuring locations below the liquid surface in the BCB will be discussed.

Effect of Measuring Height

In the first couple of hours, the transmission and scatter intensities rose for both blank and inoculated samples. This was the result of sedimentation. There were less particles in front of the sensors at the end of this phase so more light could get through the liquid. The decrease in particles also led to an increase in scattering which is counterintuitive. This was because the decrease in absorption had a greater effect on the measurements than the decrease in scattering. When the two effects were combined this led to an increase in the scattering measurement. The progress of the sedimentation throughout the BCB could be clearly followed by the transmission and scatter peaks. For the transmission, the end of the sedimentation phase is indicated by the first sharp increase in transmitted intensity. After this, a small decrease was visible mainly for a height of

37.5mm. For the scattering, the sedimentation phase showed a sharp increase in intensity at first because the absorption of the sample was going down. This was followed by a sharp decrease in intensity due to the scattering particles in the liquid sinking down to the bottom. Since the sedimentation occurred from top to bottom, the sedimentation phase of a blood culture went by quicker when the sensors were placed higher in the liquid. If the sedimentation phase lasts longer than the incubation time of the bacteria, it can occlude the growth phase. Because of this, the height at which turbidity is measured should be as high as possible. In the results of the samples spiked with bacteria, three of the four microbial growth phases were visible. The mortality phase did not yet take place during this relatively short measurement period. The most important phase for determining bacterial growth is the growth phase. It is this phase that separates a spiked sample from a blank sample the quickest. The measuring height did not have a significant effect on the time at which the growth phase took place. This means that relocating the sensors would not improve the time to detection of the turbidimeter when the samples are incubated statically. Some bacterial species grow better when they are shaken during incubation [5, 6]. Different sensor placement might be advantageous in this scenario. The phenomena discussed so far were observed in all the samples used for this part of testing.

Effect of Volume

All samples in this part were tested according to the method described above except for one of the blank samples with 40ml broth and 2ml blood. Its subculture after the incubation period showed growth. This means that the sample was contaminated. This sample was not included in the results of any test in this work. All the spiked samples showed growth at the expected incubation time. When the sample volume was increased (either blood or broth), the sedimentation phase at every height took longer. This was expected because the sedimentation phase in a sample with more liquid lasts longer. This observation strengthens the previously made statement that the measuring locations should be as high as possible, but still under the liquid surface. In the results of the 40ml broth + 10ml blood samples, the bottom 3 sensors (20mm, 25mm and 30mm) had barely passed the sedimentation phase when the growth phase started. This means that bacteria that started growing before the 11-hour mark would not have been detectable by the bottom most sensor. When the broth volume alone was increased (from 30ml to 40ml), a decrease in transmitted intensity was seen for the measurement locations at 30mm and 37.5mm. This was true for the samples spiked with *E. coli* and *S.* Typhimurium. The scattering intensities showed the same phenomenon. For the blank samples, the decrease in transmitted intensity is visible at every location below the liquid (20mm, 25mm, 30mm,

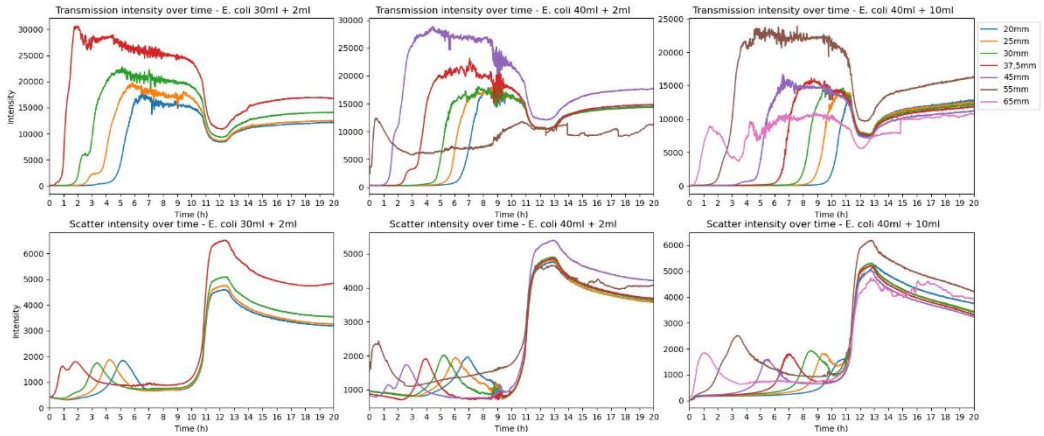

Figure 1: Transmission and scatter intensities at multiple heights of 3 samples spiked with E. coli and with different blood-to-broth ratios.

37.5mm). The scattering results here did not show the same pattern. Small variations might not have been detectable because the sensor resolution was too low. When comparing 10ml of blood with 2ml of blood in combination with 40ml of broth, another significant decrease in transmitted intensity across the whole sample was observed. This decrease was seen in all of the 10ml blood samples. This was not true for the scattering results. The visible variations were too small between the samples to draw an accurate conclusion there, like mentioned above. Finally, the highest sensor in the liquid, in both the 40ml broth + 2ml blood sample and 40ml broth + 10 blood did not have the maximum observed intensity, which was always the case in the previous measurements. Measurements from these sensors also fluctuated more frequently and intensely. This was especially true for the transmission sensors. These sensors were located 3mm and 4mm beneath the surface of the liquid. The lowest distance between the liquid surface and the sensor where a stable measurement was still observed, was 6.5mm. It is therefore recommended that a sensor is placed at least 6.5mm below the liquid-air interface.

CONCLUSION

The results obtained from testing the third generation turbidimeter show that the design works and that it can detect most of the relevant organisms in a blood culture. The ideal measurement location is near the top of the blood-broth mixture. This is where the sedimentation phase goes by the quickest which means that organisms can be detected there earlier. There is however no decrease in the time to detection at this location. If the sedimentation phase has passed throughout the whole BCB, all measurement locations will have a similar time to detection. However, a measuring distance of 6.5mm below the top of the liquid should be maintained to make sure the signal stays stable. When blood and/or broth volumes in the BCB were increased, the sedimentation phase took longer to end. This means that growth in the sample was undetectable for longer at every measuring height. When blood volume alone was increased from 2ml to 10ml, the transmitted and scattered intensity went down significantly across the whole sample period. This limited the part of the input range that the signal covered. A careful trade-off should therefore be made between maximizing blood volume, minimizing the non-functional time, and preventing the transmitted and scattered intensities from dropping too much. It should be noted that the tests in this work were performed on a small number of samples. Only six bacterial strains were tested over 22 samples (not equally distributed). Future studies, including field studies, will be performed with the second generation turbidimeter by the SIMBLE-team [2], while continuing to improve its performance.

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