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## **Research Article**

# A Procedure to Investigate the Efficacy of the Blood Donor Arm Disinfection Procedure for the Prevention of Blood Contamination

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#### Abstract

The safety of blood products has improved drastically over the years especially when it comes to the detection of viral pathogens. Contamination by bacteria and fungi remains a concern since these lead to transfusion transmitted infections. This project aims to provide a protocol to determine the efficacy of the disinfection procedure used by Blood Establishments. This method is based on collecting swab samples from the antecubital fossa of donors before and after the application of the disinfectant and by culturing of blood collected from the diversion pouch. The colony counts obtained before and after disinfection from the selected Blood Bank were analysed statistically and it was found that the disinfectant regimen currently used was more than sufficient in obtaining the required bacterial reduction and that it eliminated any fungal organisms. From the blood cultures performed, three blood cultures resulted as positive. Upon further investigation using a MALDI-TOF MS based identification technique, the contaminant bacteria were identified. None of these organisms were deemed to have originated because of improper disinfection upon reviewing the identification results obtained from the after-disinfection swabs. This implies that the current disinfection procedure is performing as expected.

Keywords: Bacteria; Fungi; Blood; Sterile; Disinfection

## Introduction

A good disinfection procedure is critical for the prevention of contamination within blood products. Contamination can arise from sources other than the skin of which most notably relate to asymptomatic bacteraemia within donors and the possibility of contaminated equipment used during the collection or processing of blood [1]. In addition to these, contamination may also be due to environmental conditions or the personnel involved not following the established protocols, both internal and external, for the sterile preparation, handling and storage of the desired blood products [2]. The protocol described in this study may help determine whether further actions should be taken to reduce the instances of contamination from the donor. Such actions would include alterations to the formula of the disinfectant such as the increase in the concentration of chlorhexidine, whether a change in the application of said disinfectant protocol is needed and the implementation of wider screening for blood products as well as the use of more advanced blood culture monitoring systems.

### **Methods**

This study was ethically approved by the Faculty Research Ethical Committee, Faculty of Health Sciences, University of Malta.

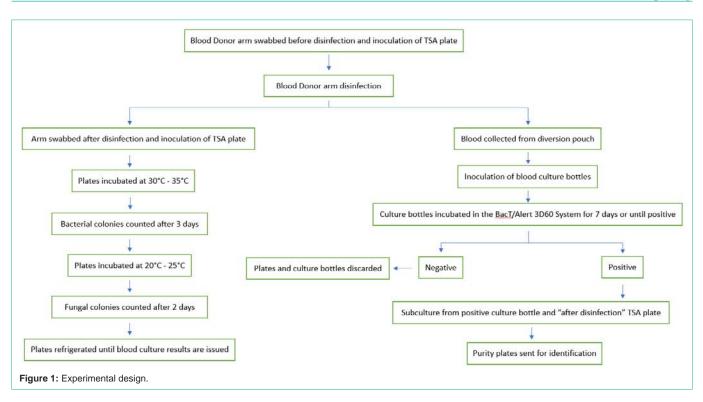
Each Blood Establishment needs to determine its own acceptable bacterial reduction range. This can be done by performing the swabbing exercise described hereunder and averaging the difference between the pre and post disinfection swab counts. However, to be accurate the exercise needs to be performed more than once and on a sample size that captures a good representation of the amount of blood donors.

A summary of the protocol used for this study can be found at Figure 1.

#### Blood donor arm swabbing and collection of samples

A swab from the antecubital fossa of the chosen arm was taken before any disinfectant was applied. This procedure involved wetting a sterile cotton swab (Biolab, Cat No.: CTA90004) with sterile saline (Fresenius Kabi, Cat No.: B230541) and passing the swab on the antecubital fossa several times. This was then used to inoculate an irradiated Tryptone Soya Agar (TSA) plate (BioMérieux, Cat No.: 43711). The antecubital fossa was then disinfected by the nurse in charge of the donation. Disinfection was performed using a sterile gauze wetted using nexCHLOREX 0.5% (Nex Medical Antiseptics Srl, Cat No.: FNEXC2EN09), which is a 0.5% Chlorhexidine (CHX) disinfectant containing 70% isopropyl alcohol. Arm disinfection was performed by rubbing the gauze on the skin with concentric outward circular movements. Once disinfection was carried out, another sterile saline-soaked sterile swab was then used to sample the antecubital fossa after disinfection using the same swabbing technique which was described previously. Following this procedure, venepuncture took place, and the blood was donated. As part of the blood donation procedure, the first 20 mL of the collected blood was diverted into a diversion pouch from which the 4 sodium citrate vacutainers (Vacuette<sup>®</sup> Blood Collection Tube, Cat No. 454322), used in this study were filled. After the first 20 mLs of blood from the donation have been diverted within the sample pouch, the vacutainers were attached

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to the tube holder until they were filled with a maximum of 2 mL of whole blood. Four vacutainers were filled up for each donor to collect a total of 8 mL of whole blood. To prevent and limit contamination, two precautions were taken: the first one was that the blood samples needed for this study were the first samples collected from the diversion pouch; the other measure taken was that of placing the plates in individual plastic bags to prevent exposure to the surrounding environment during transportation from the Blood Donation Area to the Quality Control Laboratory, where further testing was performed. Upon receipt of the plates in the Quality Control laboratory, these were immediately incubated at a temperature of 30°C-35°C (POL-EKO-APARATURA', Poland). The plates were incubated at this temperature for a total of 3 days. At the end of their incubation period, the plates were retrieved from the incubator and colonies were counted using a hand-held colony counter (Bel-Art' SP Scienceware', USA) and their number was recorded. Once all the counts were taken, the plates were then transferred to another incubator (Helmer, Inc, USA) set at a temperature of 20°C-25°C for 2 days, after which a fungal count was taken. The plates were then refrigerated (Fiochetti, Italy) at  $6^{\circ}C \pm 2^{\circ}C$  which prevented bacterial overgrowth in case a subsequent subculture was required. The blood samples were placed within a laminar flow cabinet and used to inoculate the Blood Culture bottles.

#### Calculating the bacterial reduction

This project requires the disinfection procedure to have bacterial reduction of 92.5%. From the colony counts obtained, the formula was used to calculate the bacterial reduction for each donor.

$$100 - \left| 100 \times \left( \frac{\text{Bacterial counts after disinfection}}{\text{Bacterial counts before disinfection}} \right) \right|$$

Formula used to calculate bacterial reduction. This was

implemented within the Excel raw data worksheet and the results

were produced for each valid blood donor.

## Inoculation of blood culture bottles

For each sample, BacT/ALERT' BPN (anaerobic) (BioMérieux, Cat No.:279045) and BacT/ALERT\* BPA (aerobic) (BioMérieux, Cat No.: 279044) blood culture bottles were inoculated. The blood samples were mixed well by inverting the vacutainers several times, after which the caps were removed. Using a sterile 20 mL syringe (Chirana T. Injecta AS, Cat No.: CH026L0840), the blood from all 4 tubes was aspirated thus obtaining a total of 8 mLs. The same syringe was then used to inoculate each culture bottle with 4 mLs of blood, starting by inoculating the BPN first. This was done to prevent the carryover of oxygen from the BPA to the BPN bottle, which could potentially inhibit the growth of strict anaerobes. The culture bottles were then loaded in the BacT/ALERT 3D 60 (BioMérieux, France) and left to incubate until a growth was detected or for a maximum of 7 days. On unloading the bottles, the sensor was visually checked to ensure that there was no colour change. Negative culture bottles were discarded. Positive culture bottles were subculture on Columbia Blood Agar medium (BioMérieux, Cat No.: 43050) plates in under both aerobic and anaerobic conditions until a purity plate was obtained and then sent to an external laboratory for identification. All positive blood culture bottles had their corresponding post disinfection plates subculture on Columbia Blood Agar Medium (COH) (BioMérieux, Cat No.: 43050) plates and sent for identification to an external laboratory.

## **Results**

#### Colony counts form swabbed plates

A total of 100 blood donors participated in this research. Samples with a before disinfection plate that resulted in a colony count of less than 50 colonies were excluded from this study. Furthermore

#### Zammit V

 Table 1: Descriptive statistics for the before and after disinfection bacterial and fungal counts.

|         | Bacterial<br>Counts Before<br>Disinfection | Bacterial<br>Counts After<br>Disinfection | Fungal<br>Counts Before<br>Disinfection | Fungal<br>Counts After<br>Disinfection |
|---------|--|---|---|--|
| Valid   | 91   | 91  | 91                                      | 91                                     |
| Missing | 0  | 0   | 0                                       | 0                                      |
| Mean    | 233.71                                     | 3.49                                      | 0.19                                    | 0                                      |
| Median  | 300.00                                     | 0.00                                      | 0.00                                    | 0.00                                   |
| Mode    | 300  | 0   | 0                                       | 0                                      |
| Minimum | 71   | 0   | 0                                       | 0                                      |
| Maximum | 300  | 35  | 8                                       | 0                                      |

Table 2: The organisms identified from the subcultures.

| Donor sample<br>number | Subculture              | Organism identified           |  |
|------------------------|-------------------------|-------------------------------|--|
|                        | Post-disinfection COH   | Moraxella osloensis           |  |
|                        | subculture              | Staphylococcus hominis        |  |
| 28                     | Aerobic subculture      | Staphylococcus<br>epidermidis |  |
|                        | Anaerobic subculture    | Staphylococcus<br>epidermidis |  |
| 66                     | 66 Anaerobic subculture |                               |  |
| 74                     | Aerobic subculture      | Staphylococcus capitis        |  |

 Table 3: The frequency of samples that passed and failed the criterium for successful disinfection.

|       |       | Frequency | Percent | Valid Percent | Cumulative Percent |
|-------|-------|-----------|---------|---------------|--------------------|
|       | Fail  | 7         | 7.7     | 7.7           | 7.7                |
| Valid | Pass  | 84        | 92.3    | 92.3          | 100                |
|       | Total | 91        | 100     | 100           |                    |

2 donors where disqualified because their after-disinfection count was too high meaning that arm swabbing post disinfection was done incorrectly. Therefore, analysis done from the results obtained from a total of 91 samples. The maximum number of bacterial colonies counted per plate was set as 300. All fungal colonies were counted. From the results obtained during this study, the before disinfection plates yielded the most colonies with an average of 234 bacterial colonies and 0.19 fungal colonies. The after-disinfection plates yielded much less colonies with an average of 3.49 bacterial colonies and no fungal colonies were observed. Table 1 collates the data for the before and after-disinfection plates for both bacterial and fungal colonies obtained during this project.

#### **Blood culture results**

Blood cultures were performed using blood samples from the 100 different donors during this study, after which 9 were excluded as explained before. From these, a total of 3 blood culture bottles resulted positive. These included the BPN bottle for donor number 28 and the BPA bottles for donors 66 and 74. Positive microbial growth was identified within the blood cultures for sample number 28, 66 and 74. The post-disinfection TSA plate corresponding to the BPN bottle of sample number 28 showed the presence of two colonies. The post-disinfection TSA plates corresponding to the positive samples 66 BPA and 74 BPA blood cultures could not be subcultured since no colonies were observed. After a 24-hour incubation period at 30°C - 35°C, the subcultures resulted in the growth of bacterial colonies. The

bottle subculture for donor number 28 resulted in growth within both the aerobic and anaerobic COH plates. The subcultures from sample 66 only resulted in bacterial growth within the anaerobic COH plate whilst those of sample 74 yielded bacterial colonies within the aerobic COH plate.

# Identification of bacteria isolated from the blood culture bottles

Table 2 details the organisms identified from the subcultures performed for each positive blood culture bottle.

### **Statistical Analysis**

#### Bacterial colony data analysis using the Wilcoxon test

The Wilcoxon test was applied to the data to determine if the bacterial colony count means as shown in Table 2, varied marginally or significantly between the before and after disinfection plates. The p-value obtained from the Wilcoxon test is 0.00, which is less than the required 0.05 level of significance. This indicated that there is a large difference between the bacterial colonies counted on the before disinfection plate versus the colonies counted on the after disinfection. Said result show that the disinfection using the current protocol resulted in a significant reduction of bacteria that were present on the antecubital fossa of the donors.

### Fungal colony data analysis using the Wilcoxon test

The Wilcoxon test was used to determine if there was a small or large difference in the mean number of fungal colonies shown in Table 2 between the before and after disinfection TSA plates. Since the p-value obtained from the Wilcoxon test is 0.026, which is less than 0.05 level of significance, a large difference between the fungal colonies counted on the before disinfection plate versus the colonies counted on the after disinfection was confirmed. This indicates that the disinfection using the current protocol resulted in a significant reduction of fungi that were present on the antecubital fossa of the donors.

## Determining the average bacterial reduction obtained for each donor

A bacterial reduction of 92.5% is set as the requirement to be met in this study. The Binomial test was used to determine if the results obtained indicate whether the average bacterial reduction obtained is of 92.5% or not. The p-value obtained from this test is of 0.00 which is less than 0.05 level of significance. Therefore, the average bacterial reduction obtained for this study was not 92.5%. The mean bacterial reduction obtained was in fact of 98.4%. The bacterial reduction percentage results obtained have been converted into an ordinal variable to determine the outcome of disinfection for each donor. This sorts the percentage results into two groups, either pass (result is equal to or above 92.5%) or fail (result is less than 92.5%). From this, it was determined that out of 91 samples analysed, 84 samples or 92.3% of the total) passed the disinfection percentage criteria, whilst another 7 samples or 7.7% of the total, failed to meet the required disinfection target. These results are illustrated in Table 3.

#### Analysis of the outcome of disinfection

To determine if the outcome of disinfection results obtained where affected by the bacterial counts from samples obtained before and after disinfection, the mean data from each variable was analysed using the Mann Whitney test. The p-value for the before disinfection group of variables was found to be 0.478 obtained from Table 3 which is larger than 0.05 level of significance. Therefore, the average bacterial colony count obtained before disinfection, did not affect the outcome of disinfection. The p-value obtained for the after-disinfection colony count variable was 0.00, which is less than the 0.05 level of significance. This indicates that the average after disinfection colony counts affected the outcome of disinfection. From these results, it can be concluded that the outcome of disinfection depends solely on the number of bacterial colonies counted after disinfection.

## **Discussion**

From the Binomial test results as illustrated in table 3.8 within the previous chapter, the average bacterial reduction from the donors analysed was of 98.4%, indicating that the protocol using the alcohol based 0.5% CHG solution was sufficient to reach the pre-set target of 92.5%. This indicates that the current disinfection protocol is adequate in significantly reducing the number of bacteria present on the donor's antecubital fossa prior to donating blood. The efficacy of disinfection was also noted by the difference in colony counts between the before and after disinfection TSA plates using the results from the Wilcoxon test. Fungal colonies were rarely noted and only 6 plates before disinfection resulted in fungal growth, all of which showed no growth within the after-disinfection plates done in parallel. One should also note the fact that none of the positive blood cultures resulted from the presence of fungi, reinforcing the belief that the disinfection protocol will prevent the contamination from said organisms. When looking at the overall frequency of passed or failed disinfection rate, 84 out of 91 valid donors reach the required target of a minimum 92.5% disinfection whilst the remaining seven donors did not. This is a more ambitious target when compared to other studies performed in the past, most notably by McDonald et al, which aimed for a 90% elimination of bacteria when testing different skin disinfection methodologies. Some of the methods tested in said study exceeded said target most notably by the single application of an in-house chlorhexidine and 70% alcohol wipe and the use of a single or double swab of a commercially available alcohol and iodine sponge applicator. It was noted that the single application using an up and down motion of the iodine and alcohol wetted sponge performed the best, resulting in an overall 99.8% reduction, followed by the same method done twice with a spiral motion resulting in a 98.2% reduction and finally the in-house chlorhexidine and alcohol swab which resulted in a 78.52% reduction. This project's results are comparable to the in-house method used in the aforementioned study both in the concentrations of the disinfectant ingredients used as well as the number of participants which included the results of 90 donors in the study [3] and this project's results included 91 participants. Nonetheless there was still a 7.69% failure rate in the disinfection protocol of this research project, which may seem high. This may have been due to the sample size of just 91 valid donors is quite small, and therefore a small variation in the results will skew the results negatively. One study conducted by Ramirez-Arcos and Goldman to determine the efficacy of different variations for disinfection in which they included a two-step CHG-alcohol based disinfectant protocol similar to the one used in this project. A single step modification of this protocol involving the use of a single use disinfectant applicator was deemed to be the most suitable even if the results obtained from both methods were comparable. This boiled down to the increased convenience when using the applicator instead of having to break ampules of the pre-mixed solution before each skin scrub [4].

Since the samples were processed from October to December, it is important to keep in mind the potential for seasonal variation on the impact of skin flora. Studies have shown that there is considerable variation between summer and winter when it comes to bacterial counts obtained. In a study conducted by [5], it was mentioned that the proliferation of certain bacteria may change depending on the season especially due to temperature changes leading to changes in sweat excretion and changes in the garments worn and the production of protective lipids from the skin. The antecubital fossa was described as being non-exposed part of the skin, usually composed of normal skin that is not dry but usually scaled [5]. Antimicrobial peptides from the skin play a role in the selection of certain bacteria that can live on the skin. Such regions are neutral and allow for the cohabitation of different pairs or multiple types of bacteria within the same area unlike dry or oily regions of the skin where specific bacterial species may have a preference. In view of this, the protocol shown in this project should be repeated in the summer months to see if the protocol has the same effectiveness [6]. Nonetheless one should also keep in mind that the Maltese climate overall is generally warmer than the locations were some of these studies may have taken place and the climate change between seasons is not extreme.

From the blood culture results, out of 100 blood culture bottle pairs, 3 bottles of donors, a BPN culture for donor 28 and two BPA cultures for donors 66 and 74, gave a positive result. For donor number 28, the organism identified in the blood sample was Staphylococcus epidermidis whilst the two colonies analysed within the after-disinfection plate were identified to be Moraxella osloensis and Staphylococcus hominis. The contamination of the blood sample with S. epidermidis, one of the more established skin commensals [7] is indicative of a failed disinfection for this particular donor as it is known to be readily eliminated using the combination of chlorhexidine and alcohol [8]. This may be due to the heavy bioburden within the donor's arm upon applying the disinfectant which may have not been able to neutralise this organism as a result. With regards to the after disinfection plate, M. osloensis has been recently identified as a skin commensal trough genomic sequencing [9]. This organism was also reported to cause osteomyelitis and therefore, bacteraemia from this organism is also possible [10]. S. hominis was also identified from the same plate. This organism is a known skin commensal that plays an active role in warding off potential pathogens [7]. In this case, since these grew within the after-disinfection plate and was not identified within the blood sample, their presence is likely due to improper skin disinfection rather than failure of the disinfectant. From Donor number 66, Bacillus species was identified. Bacillus species especially B. subtilis are common transient skin commensals that compete with other constituents of the flora for colonisation and is mainly found within the plantar skin, or foot soles of humans. This species is also known for its ability to form biofilms and are motile due to possessing flagella. It directly competes with the commensal S. epidermidis and is capable of engulfing said bacterial cells [11]. Its contamination of whole blood could lead to it surviving within the plasma and red cell subunits [12]. The ability of this bacterium to survive and also during the whole blood holding period, is highly dependent on the

donor's repertoire of antibodies and other immune factors which can neutralise it during the component's storage period [13]. The main issue with this particular organism is that it can form spores and therefore disinfection with alcohol-based solutions such as with CHG may be sufficient to eliminate the viable bacteria on the skin, but it is unable to kill the spores which can then proliferate during the storage of blood components mainly platelet units [14]. Finally, the blood sample from donor number 74 was found to be contaminated with *Staphylococcus capitis*. This bacterium is one of the most frequently isolated *Staphylococcal bacteria* from the skin as confirmed by 16rs gene identification techniques [7]. It is generally a commensal organism although it has been linked to pathogenic cases of skin, urinary tract and respiratory infection and more severely to cases of neonatal sepsis.

Detection of contamination within blood products using blood cultures is the current gold standard method employed at blood banks. Apart from the BacTALERT' system used in this study there are also others such as the BacTec' (Becton, Dickinson and Company, New Jersey USA) and VersaTREK<sup>\*</sup> (Thermo Fisher Scientific, Massachusetts, USA) systems which although differ in the way they detect bacterial proliferation, their end goal is the same and tend to perform similarly but are however dependent on certain circumstances such as the type of bacteria present within the culture. Although blood cultures stood the test of time, there are still problems one faces when using this method and it is mostly related to bacterial kinetics or the way the bacteria behave within the culturing medium upon inoculation and incubation. McDonald et al summarised its potential pitfalls due to: Auto-sterilisation, were bacteria die due to antibodies and other immune factors derived from the donors, poor bacterial propagation due to species growth characteristics with some being notorious for having a much longer lag phase and also due to a low initial bacterial inoculum [15]. These can cause false negative results leading to the reporting of blood cultures as being negative within the pre-determined detection window set for the system in use, whilst the corresponding units are issued for transfusion. This can in turn lead to potential for infection or more severe sequela within the recipient patient, especially when it comes to the transfusion of PCs due to their short shelf-life. In fact, certain jurisdictions have allowed for additional measures to combat the instances of missed contamination or false negatives. This includes measures such as delaying the sampling of PCs by 24 hours before inoculating the blood cultures, as well as the use of rapid point of care test using the Verax\* Platelet Pan Genera Detection test (Verax Biomedical, Massachusetts, USA.) to determine contamination within said component before initiating transfusion [16]. Such systems have their own drawbacks, namely an increase in cost when it comes to the addition of an extra test, poor sensitivity of rapid testing, as well as a reduced supply of PC's when delaying sampling [17]. In Northern Ireland, sampling of PCs for blood culture is done immediately after collection, followed by a secondary culture 24 hours after collection which allows for the extension of their shelf life for up to seven days if their respective culture is negative [18]. This is a much more expensive approach, but further improves safety by reducing the risk of false negatives. One often overlooked pitfall of detection is the fact that although bacteria may auto-sterilise, some might have still produced undetectable endotoxins within the component in question which can lead to septic shock if present in high enough concentration [15]. The Limulus Amebocyte Lysate gel clot assay may be used to detect and roughly quantify the presence of endotoxins produced by Gram negative organisms. Although potentially useful, it is difficult to implement such test within the busy routine environment of blood product manufacturers [19]. Some institutions have also experimented with different testing methodologies to improve detection, such as the Pall eBDS system which makes use of the fact that some bacteria consume oxygen from PCs during their proliferation. A major issue of this method is that it takes longer to detect contamination when compared to more established blood culture systems [20]. Another obvious drawback is that this system suffers when it comes to anaerobic bacteria since these do not proliferate well within the aerated environment of PCs. There is also the requirement of training staff as well as increased turnaround time since the system cannot process more than one sample at a time unless pooling is done [17].

In this project the sampling method utilising the saline dipped swab was determined to be ideal since one can precisely swab the area within the antecubital fossa from which the needle is inserted. If done correctly, the swab is also ideal for sampling skin with a rough topology due to scarring from repeated donations. A different approach is the use of contact plates consisting of a nutritious agar that facilitates microbial growth. This technique was used by Goldman et al. and was highly effective in sampling this area. It is simple to use as it samples a large area of the fossa for bacteria by just touching the plate to the skin. The plate is then incubated and colony counts are taken [21]. The main benefit of using such a technique is that they cover a standardised area of antecubital fossa skin. Swabs are not standardised, and sampling can vary from person to person. On the other hand, swabs can pick up bacteria from crevices or folds within the skin unlike the contact plates which only sample the superficial area. This makes swabbing advantageous especially in instances where the donor has developed a scar due to repeated donations. Scars are a common failing point of disinfection when the disinfectant is not applied thoroughly enough, the remaining bacteria may reside within the crypts [21]. The main drawback of using contact plates is that a larger area than the antecubital fossa would be sampled thus introducing a higher risk of contamination from the environment after the plate is opened, as well as an increased overgrowth risk from bacteria due to sampling from regions other than the puncture site within the antecubital fossa. In fact in a study with similar objectives, conducted by McDonald et al, none of the contact plates that were used to sample the site after disinfection resulted in a zero colony count, unlike the results obtained in this project [3]. On the other hand when research was performed for the collection of skin commensals for the genomic analysis of the skin microbiome by Ogai et al, saline solution collection hindered the collection of certain aerobic bacteria when compared to a tape based skin collection method [22]. The detection of contamination within the blood samples is a novel approach in evaluating disinfectant efficacy and very few studies incorporated said methods together. Some relied on bacterial detection using nutritious broth as a means to detect contamination by looking for turbidity and relying on conventional biochemical tests which are labour intensive [23].

## Limitations

Despite this project successfully confirmed that the current disinfection procedure is performing as expected, improvements

can still be made, especially to the methodology of this project. The use of mono-sterile saline vials could have further reduced potential environmental contamination of the before and after-disinfection plates. This comes down to the simple fact that the saline bottle used to moisten the swabs was used to swab all donors screened during one session. This saline could still harbour organisms which might end up on gloves or other surfaces or even contaminating the solution itself. The use of saline solution itself may impede the growth of certain bacteria and therefore it would have been ideal to use a transport medium or any other solution that has no action on the bacteria present upon collection from the skin [23]. When it came to the processing of samples, larger volume vacutainers would have been preferred. To obtain the required volume of 8mls for blood culturing, 4 vacutainers were used to collect blood from the diversion pouch. The length of the needles of the syringes were a limiting factor making the aspiration strenuous and difficult at times. Even though the processes took place within sterile environment of the laminar flow cabinet, there was still a risk of contamination as a single syringe was used to aspirate the fluid from the four different blood sample vacutainers for each donor. Nonetheless contamination was deemed a non-issue at this stage as stringent observation of aseptic technique was maintained every time this procedure was done.

## **Future Work**

For further investigations one may consider how to improve the disinfection or identification procedure. Without doubt the method of disinfection application plays a large part in antisepsis. Instead of using the swirling method as described in this protocol, an up and down movement may yield better results. As described by Rafiee et al., peeling off dead keratinised desquamated cells from the superficial layer of the skin improves disinfection outcomes as this allows for the disinfectant to penetrate easier within crypts of the skin [23]. The gold standard for bacterial detection has always been considered to be by means of a blood culture system. Another option for detection would be the use of molecular techniques such as the 23S rRNA Bacterial Identification. An adaption of the PCR assay utilising 23S rRNA gene primers can be used to specifically detect bacterial cells since these are the only kind that possess such genetic material. Such method proposed by Firoozeh et al, improves upon 16S DNA based PCR bacterial detection as it allows for the retention of bacterial cells since these are not destroyed and is less time consuming. This method is also useful for testing donors who have not disclosed prolonged antibiotic use, which can have asymptomatic bacteraemia [24]. Pathogen reduction techniques should also be considered. The basic principle of this methodology involves treating blood products to inactivate pathogens with minimal effect or added risks to the blood product and its recipient [25]. Although pathogen reduction technologies seem the way forward for a contamination free blood supply, most of the methodologies used do not offer any protection against endotoxin production [26]. Future studies may use this protocol to further consolidate the use of new disinfectants and disinfectant procedures while at the same time validate the potential of using delayed sampling to increase PCs storage times to 7-days. However, nowadays most blood banks are substituting Transfusion Transmitted Infections (TTBI) screening with pathogen reduction techniques.

## Conclusion

From the results obtained at the end of this research project, the disinfection protocol in use was deemed appropriate for its continued use at the selected Blood Donation Area. However, as the number of required transfusions increases, the risk of TTBI events increases as well, leading to the need of more vigilant procedures such as routine sampling and testing of all units. Rapid testing may also be implemented to allow for the extension of shelf life of platelet. Pathogen inactivation should also be taken in consideration and its potential for implementation should be evaluated.

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### References

- Heltberg O, Skov F, Gerner-Smidt P, Kolmos H, Dybkjaer E, Gutschik E, et al. Nosocomial epidemic of Serratia marcescens septicemia ascribed to contaminated blood transfusion bags. Transfusion. 1993; 33: 221-227.
- 2. Debrincat A, Gialanze J, Spiteri N, Zammit V. Blood Donor Arm Disinfection-Preventing the Contamination of Blood Components. 2020.
- McDonald CP, Lowe P, Roy A, Robbins S, Hartley S, Harrison JF, et al. Evaluation of donor arm disinfection techniques: Donor arm disinfection techniques. Vox Sanguinis. 2001; 80: 135-141.
- Ramirez-Arcos S, Goldman M. Skin disinfection methods: prospective evaluation and post implementation results. Transfusion. 2010; 50: 59-64.
- Li X, Yuan C, Xing L, Humbert P. Topographical diversity of common skin microflora and its association with skin environment type: An observational study in Chinese women. Scientific Reports. 2017; 7: 18046.
- Cid J, Ortin X, Ardanuy C, Contreras E, Elies E, Martin-Vega C. Bacterial persistence on blood donors' arms after phlebotomy site preparation: analysis of risk factors. Haematologica. 2003; 88: 839.
- O'Sullivan JN, Rea MC, O'Connor PM, Hill C, Ross RP. Human skin microbiota is a rich source of bacteriocin-producing staphylococci that kill human pathogens. FEMS Microbiology Ecology. 2019; 95: 241.
- Benjamin RJ, Dy B, Warren R, Lischka M, Eder AF. Skin disinfection with a single-step 2% chlorhexidine swab is more effective than a two-step povidone-iodine method in preventing bacterial contamination of apheresis platelets: Skin Preparation to Prevent Bacterial Contamination of Plts. Transfusion. 2011; 51: 531-538.
- Lim JY, Hwang I, Ganzorig M, Huang S-L, Cho G-S, Franz CMAP, et al. Complete Genome Sequences of Three *Moraxella osloensis* Strains Isolated from Human Skin. Genome Announc. 2018; 6: e01509-e01517.
- Alkhatib NJ, Younis MH, Alobaidi AS, Shaath NM. An unusual osteomyelitis caused by *Moraxella osloensis*: A case report. International Journal of Surgery Case Reports. 2017; 41: 146-149.
- 11. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2011; 9: 244-253.
- Damgaard C, Magnussen K, Enevold C, Nilsson M, Tolker-Nielsen T, Holmstrup P, et al. Viable Bacteria Associated with Red Blood Cells and Plasma in Freshly Drawn Blood Donations. Gregori L, editor. PLoS ONE. 2015; 10: e0120826.
- Mohr H, Lambrecht B, Bayer A, Spengler H-P, Nicol S-B, Montag T, et al. Basics of flow cytometry?based sterility testing of platelet concentrates. Transfusion. 2006; 46: 41-49.
- 14. de Korte D, Curvers J, de Kort WLAM, Hoekstra T, van der Poel CL, Beckers EAM, et al. Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands.

Transfusion. 2006; 46: 476-485.

- 15. McDonald CP. Transfusion risk reduction: Testing for bacteria. VOXS. 2013; 8: 73-79.
- Harm SK, Delaney M, Charapata M, AuBuchon JP, Triulzi DJ, Yazer MH. Routine use of a rapid test to detect bacteria at the time of issue for nonleukoreduced, whole blood-derived platelets: USE OF THE PGD TEST ON WBP. Transfusion. 2013; 53: 843-850.
- Debrincat A, Gialanze J, Spiteri N, Zammit V. Bacterial Screening of Blood Components: Past, Present and Possibly Future Methodologies for Improving Transfusion Safety. Austin Hematology. 2021; 6: 1033.
- Abela MA, Fenning S, Maguire KA, Morris KG. Bacterial contamination of platelet components not detected by BacT/ALERT ®: Bacterial contamination of platelet components. Transfusion Med. 2018; 28: 65-70.
- Ramirez-Arcos S, Perkins H, Kou Y, Mastronardi C, Kumaran D, Taha M, et al. Bacterial growth in red blood cell units exposed to uncontrolled temperatures: challenging the 30-minute rule. Vox Sang. 2013; 105: 100-107.
- Benjamin RJ, Kline L, Dy BA, Kennedy J, Pisciotto P, Sapatnekar S, et al. Bacterial contamination of whole blood-derived platelets: the introduction of sample diversion and prestorage pooling with culture testing in the American Red Cross. Transfusion. 2008; 48: 2348-2355.

- Coldman M, Roy G, Fréchette N, Décary F, Massicotte L, Delage G. Evaluation of donor skin disinfection methods. Transfusion. 1997; 37: 309-312.
- 22. Ogai K, Nagase S, Mukai K, Iuchi T, Mori Y, Matsue M, et al. A Comparison of Techniques for Collecting Skin Microbiome Samples: Swabbing Versus Tape-Stripping. Frontiers in Microbiology. 2018; 9: 2362.
- Rafiee MH, Kafiabad SA, Maghsudlu M, Moradi M, Jalili L. Chlorhexidine alcohol versus povidone-iodine: The comparative study of skin disinfectants at the blood transfusion centers of Iran. Transfusion Clinique et Biologique. 2020; 27: 78-82.
- 24. Firoozeh F, Shiralinezhad A, Momen-Heravi M, Aghadavod E, Zibaei M. Rapid Detection of Pathogenic Bacteria in Whole Blood Samples Using 23S rRNA PCR Assays. The Open Microbiology Journal. 2019; 13.
- 25. Solheim BG. Pathogen reduction of blood components. Transfus Apher Sci. 2008; 39: 75-82.
- Taha M, Kalab M, Yi Q-L, Landry C, Greco-Stewart V, Brassinga AK, et al. Biofilm-forming skin microflora bacteria are resistant to the bactericidal action of disinfectants used during blood donation. Transfusion. 2014; 54: 2974-2982.