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Version of Record: https://www.sciencedirect.com/science/article/pii/S0305417920304630
Manuscript_b65330b77797e40ee03adfa3dcb56a20

How to improve donor skin availability: Pragmatic procedures to minimize the discard rate of cryopreserved allografts in Skin Banking

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Abstract

Background
Microbial contamination of human skin allografts is a frequent cause of allograft discard. Our purpose was to evaluate the discard rate of skin bank contaminated allografts and specific procedures used to reduce allograft contamination without affecting safety.

Methods
We conducted at the Lille Tissue Bank a retrospective study of all deceased donors (n=104) harvested from January 2018 to December 2018. Skin procurement was split into 3 zones: the back of the body and the two legs that were processed separately. It represented 433 cryopreserved skin allograft pouches of approximatively 500 cm^2 each. Donors were almost equally split between brain-dead (53%, 55/104) and cadaveric (47%, 49/104) donors.

Results
Out of all donors, 42 (40,5%) had at least one sampling zone with a positive microbiological test resulting in 106 (24%) contaminated skin pouches. The contamination rate did not vary according to the harvested zone or type of donor. Traumatic deaths showed significantly less contamination rates than other death types ($p<0.05$). Contamination rate decreased with time spent in the antibiotic solution. The risk of having contaminated allografts was five-fold higher when the skin spent less than 96 hours in the antibiotic cocktail ($p<0.05$). According to our validation protocol, most donors (32/42, 76%) had skin allografts contaminated with bacteria (mainly *Staphylococcus spp*) compatible with clinical use. No recipient infection was recorded as a result of skin graft contaminated with saprophytic or non-pathogenic germs. By harvesting 3 separate zones per donor, the total surface area for clinical use increased by 53% for contaminated donors. Overall, the proportion of contamination-related discarded allografts was 3.2% (14/433 of pouches).

Conclusion
Few simple pragmatic measures (including skin incubation in the antibiotic bath for at least 96 hours at 4°c, splitting the skin harvesting areas to minimize the risk of cross-infection and clinical use of allografts contaminated with saprophytic and non-pathogenic germs) can reduce the discard rate of contaminated allografts without affecting clinical safety.

Highlights:

- Long-term incubation of skin into the antibiotic cocktail is crucial to reducing the rate of contaminated allograft
- Splitting the procurement into several anatomical zones from each skin donor reduces the risk of cross-infection
- Allografts contaminated with low bioburden of saprophytic and non-pathogenic germs can be accepted for safe clinical use

Keywords:
Tissue banking, Skin allograft, Cryopreservation, Bacterial and fungal contamination

Introduction

Deep burn wounds impede skin regeneration and healing. In the absence of sufficient autograft donor areas, the temporary use of skin substitutes may be required. Skin substitutes encompass a variety of materials including xenografts, biosynthetic skin substitutes, cultured epithelial cells and deceased donor skin allografts. Among them, skin allografts are one of the most commonly implemented skin substitutes in burn wound management in many hospital-based burn units. Skin allografts are successfully used to limit loss of bodily fluids, proteins and electrolytes, to promote wound healing as well as reducing pain and infection [1]. Skin allografts can also be used with autologous skin in a "sandwich"-grafting technique promoting a positive autograft take rate [2]. These indications explain the increasing demand from burn and reconstructive surgery units for skin allografts.

To meet the growing demands for high quality, safe skin allografts, skin banks are in charge of donor screening, skin processing and storage. The objective of skin bank procedures is to provide safe and qualitative skin allografts for clinical use. In term of quality, the allografts have to be uniformly thin with homogeneous surface area allowing skin expansion within meshers.

The main concern with the use of donor skin allograft is the risk of disease transmission, especially due to microbial contamination. This comes from two observations (i) the human skin is not sterile and normally contains microorganisms residing on its surface; (ii) major burn injuries lead to a state of immunosuppression and consequently, burn patients are particularly at risk of infection.

Strict protocols are needed to minimize contamination rate and avoid the risk of recipient infections. Frequently, skin allograft contamination occurs during recovery. There are various factors influencing microbial contamination risk during the retrieval process, these can be related to the cause of death, type of deceased donor (heart-beating or non-heart-beating donors), time between death and skin harvesting or number of people attending to the harvesting [3,4]. To minimize the risk of bacterial contamination, careful disinfection of the skin donor is required and skin should be harvested under aseptic procedures. Furthermore, cross-contamination can be limited with simple procedures consisting of harvesting and processing separately the different areas (*e.g*. back, right leg and left leg) for each donor. Moreover, containers and media for transporting the harvested skin to the skin bank must be sterile, stored at 4°C and antimicrobial drugs must be added to the transport medium. Unfortunately, the efficacy of the antibiotic cocktail is inconsistent. Studies showed that board-spectrum antibiotics were useless in approximatively 22 % of skin specimen[5]. Overall, the objective of these procedures is to decrease the contamination rate of skin allografts, even though they can never achieve a 0% contamination rate [6].

Sterile testing is a critical issue in human skin allografts. Commonly, it was admitted that contaminated allografts should be systematically discarded because of the potential risk of recipient infections. However, the systematic discard of all contaminated allografts increases costs and reduces allograft stocks. To avoid unnecessary discard rates that might deprive potential recipients of tolerable skin allografts, the risk/benefit ratio of contaminated allografts must be assessed carefully and evaluated for each patient. According to the EDQM 4th edition 2019 [7], allografts contaminated by low bioburden of non-pathogenic micro-organisms may be accepted for clinical use after a thorough risk assessment analysis and if the surgeon is informed before surgery.

The Lille University Hospital Tissue Bank is a multi-tissue bank processing more than 300,000 cm^2 of cryopreserved skin allografts a year. Fourteen procurement teams from Northern France and Normandy harvest skin specimen for the Lille Tissue Bank. In this retrospective study we analyzed the skin allograft activity of our Tissue Bank for the year 2018, focusing on microbial contaminations, and the evaluation of specific microbiological procedures to reduce allograft contamination and the discard rate of contaminated allografts.

Methods

Donors and data collection

We reviewed all procurement donor files (n=104) from the Lille tissue bank between January 2018 and December 2018. The following data were collected and analyzed:

- Donor characteristics (type of donation, age, gender, cause of death, virologic testing number of specimens harvested, season (potentially more risks in the summer), presence of a controlled infection and antibiotic treatment at time of procurement),
- Skin allograft characteristics (size, bacteriological, mycological results, time spent in antibiotic and glycerol solution)

Procurement of skin samples

The overall skin procurement process is summarized in a timeline in Figure 1. Skins are harvested under aseptic conditions in the operating room after organ donation from multi-organ heart-beating donors (MOHBD), i.e., brain-dead donors or in a specific recovery room for cadaveric non heart-beating donors (NHBD). According to the European guidelines [7], the skin can be harvested up to 24 hours after death if the body is refrigerated within 4 hours of death. If the body is not refrigerated, skin must be harvested within 12 hours.
Blood samples for virologic testing (serology/NAT) were collected in order to screen for Hepatitis B, and C, Syphilis, HIV-1 and 2, and HTLV. The skin sampling medium consisted of 1 liter of saline solution with 500 mg of amikacin (effective on gram-negative bacteria), 1 gram of amoxicillin (broad-spectrum antibiotic), 500 mg of vancomycin (effective on gram-positive bacteria) and 50 mg of amphotericin B (effective on fungal infection) diluted with 20 ml of Glucose 5% w/v solution to avoid precipitation of the drug. The donor was positioned in a ventral position. Shaving and the first preoperative body scrubbing and disinfection with povidone-iodine 4% (foaming solution for skin application) were carried out. A second whole-body cleansing was done with the same povidone-iodine solution followed by an abundant rinsing with sterile water. The body was dried with sterile gauze and povidone-iodine 10% (solution for skin application) was applied. After sterile draping, povidone-iodine 10% was applied again. The skin was then harvested with an electric or pneumatic dermatome (according to the material used by the different procurement teams). Three different areas were harvested: back, left leg and right leg. The legs were generally harvested from buttocks to ankles for men and from buttocks to knees for women. For each harvested area, the dermatome blades were changed, and separate sterile containers (Cryokit, Verreries Talençonnaises, Reyrieux, France) containing the sampling medium were used to reduce cross-contamination. Skin samples were then kept at +4°C until processing at the Tissue Bank ordinarily within 5 days.

Processing and cryopreservation of skin allografts

Processing of skin samples was performed under sterile conditions in the clean room (grade A) of the Lille Tissue Bank. The three different areas were processed, evaluated and packaged separately. Firstly, the skin was taken out of the cryokit and incubated for 1 hour at room temperature in a cryoprotectant solution consisting of 17% glycerol (S.A.L.F. SpA,

Cenate Sotto, Italy) solution with saline solution (Fresenius Kabi, Bad Homburg Vor der Höhe, Germany). Size, shape and thickness of skin strips were macroscopically evaluated, edges were smoothed out and the surface area was recorded. Approximatively 500cm^2 of skin strips were then transferred into each sterile pouch (Agricons Ricerche, Piazzola sul Brenta, Italy) containing 60 ml of cryoprotectant medium. Afterwards, pouches were sealed and placed in a second sterile pouch, then sealed again. Sealed pouches were then transferred to a -80°C freezer and could be stored for up to two years until graft. Two samples containing a small piece of skin (2 to 5 cm^2) with 20 ml of cryoprotectant medium were kept for mycological and bacteriological banking as well as tissue banking up to 6 months post graft.

Bacteriological and mycological analyses

All microbiological analyses were performed at the microbiology department of the Lille Hospital. Microbial (bacteriological and mycological) analyses were conducted during allograft processing: one when the sterile containers were opened, and the other at the end of processing (indicated in the Figure 1 timeline). A third one was performed during skin grafting. The first two microbiological analyses took place during processing. In each analysis both skin remnants (approximately 1cm^2) and skin medium samples were collected and sent to the microbiology lab. Skin in medium was grinded (with a Potter-Elvehjem tissue grinder followed by vortex) and a drop of this suspension was inoculated into the generic medium for aerobes (Chocolate agar + PolyViteX -BioMerieux, Marcy-l'Étoile, France) and incubated for 18 hours at 35°C with 5% CO_2, 100µl were inoculated in a Brain-Heart infusion broth and incubated at 35°C for 48 hours and finally 100µl were inoculated in a Rosenow Cysteine broth paraffined and incubated at 35°C for 15 days. Samples were considered negative when bacteria did not grow for 14 days in aerobic and/or anaerobic cultures. Cultures were scored positive even if a single bacterial colony was identified at species level using MALDI-TOF mass spectrometry (Microflex, Brucker, Billerica, Massachusetts, USA). An antibiogram by the agar diffusion or automated method (Vitek2, BioMérieux) was performed only for saprophytic or non-pathogenic germs. Otherwise, the antibiogram was unnecessary since the nature of contaminants led to tissue discard.
The bioburden was evaluated by a simple procedure based on 14-day microbial cultures modified according to previously published data[8]. If microbial growth was detected during the first 7 days of incubation, it corresponded to a high bioburden and the skin batch was discarded. If growth was detected after 7 days (d8-d14), a low bioburden was suspected and the tissue was screened for micro-organisms. According to the nature of the germ found (pathogenic or commensal see Table 1), skin batches were released or not.
For the mycological analysis, 100µl and a tissue sample were inoculated in two Sabouraud-Chloramphenicol agar tubes, one was incubated at 37°C and the second one at 24°C for 14 days. Samples were considered negative if the fungi/yeast did not grow for 14 days. The identification was based on MALDI-TOF mass spectrometry (Microflex, Brucker) for yeasts, macro- and microscopic characteristics for filamentous fungi.

Acceptance criteria of contaminated skin allografts

In agreement with the EDQM 4th edition 2019, we conducted a risk-assessment analysis with bacteriologists and anesthesiologists to assess the suitability of skin allografts taking into

account the nature of micro-organisms detected at the first or second stage of the skin processing (Figure 1). Accordingly, skin allografts were accepted for clinical use when microbiological analyses revealed only a low bioburden (see above) of saprophytic or non-pathogenic germs listed in Table 1. The surgeon was always informed of the nature of the micro-organism as well as the result of the antibiogram before using the allograft. Recipients were monitored more intensively after transplantation.

Statistical methods

Statistics and graphs were produced using GraphPad Prism version 8.2.0 for Mac (GraphPad Software, La Jolla California, USA). The Chi-square test or Fisher's exact test was used to identify associations between donor contamination rate, rate of microbial contamination and age, gender, season, type of donation, number of tissues harvested, presence of an infection, course of antibiotics and cause of death. A Student's t test was used to identify relationships between microbial contamination and donor age or mean time until body refrigeration. Statistical significance was set at $p<0.05$.

Results

Skin donor population

Our retrospective study was conducted in 2018, the database comprised 104 deceased skin donors including 52 males and 52 females. Ages ranged from 22 to 95 years, with mean age at 68.36 +/- 16.93 years and median age at 70.5 years. 49 donors (47%) were MOHBD, and 55 (53%) NHBD. For NHBD, mean body refrigeration time was 149 minutes (+/- 101 minutes). Stroke was the principal cause of death (35.6%) followed by heart attack (34.6%) as described in Table 2.

Harvested skin characteristics

Skin harvesting was undertaken by 14 different procurement teams disseminated throughout the North of France and Normandy. The total surface area of harvested skin was 231,164 cm^2 conditioned in 433 skin pouches of approximatively 500 cm^2 each. Mean surface area per donor was 2244.3 cm^2 (median: 2025.5 cm^2 and min 481-max 6166 cm^2). 187 pouches (43.2%) contained skin from donors' back, 111 pouches (25.6%) from the right legs, 112 (25.9%) from the left legs and 23 pouches (5.3%) with a combination of the 3 different zones. There were no differences in the distribution of sampling zones between MOHBD and NHBD (Figure 2). 46 skin pouches coming from 20 donors were discarded. The discard reasons were positive serology (30 pouches from 8 donors; 6 donors were positive for HVB, 1 for HCV and 1 for Syphilis), microbial contamination (14 skin pouches discarded from 10 donors) and procurement quality (2 pouches from 2 donors) (Figure 3).

Factors affecting the rate of skin allograft contamination

For all donors, 42 (40.5%) had at least one sampling area with positive microbiological testing resulting in 106 (24%) contaminated skin pouches (Figure 3). The contamination rate did not vary according to the harvesting area (back (n=50, 47%) and legs (n=51, 48%, respectively n=21, 20% for the right leg and n=30, 28% for the left leg).
High contamination rates were not significantly associated with key demographic donor criteria, such as age or gender (Table 3). The contamination rate from MOHBD (n=20/49, 40.5%) and NHBD (n=22/55, 40%) was not significantly different (Table 3). There were no substantial differences in skin contamination regarding body refrigeration time for NHBD (mean+/- SD, 154.64 minutes (+/-79.37) for contaminated donors vs. 145.25 minutes (+/-114.88) for non-contaminated donors, $p>0.05$). Similarly, our study did not unveil the influence of criteria commonly considered as potential risk factors for contamination such as number of specimens harvested per donor, season, presence of prophylactic or curative antibiotic course as well as presence of controlled infection (Table 3). Conversely, we found that the cause of death affected the positive rate of skin allografts since traumatic deaths showed significantly less contamination rates than other types of death ($p<0,05$) (Table 3).
We also studied the influence of processing on the contamination rate. Regarding time spent in the antibiotic cocktail (time between harvesting and processing at our tissue bank), contaminated allografts spent significantly less time in the antibiotic cocktail (mean 65.5 hours (+/- 28.6)) than non-contaminated allografts (mean 80.4 hours (+/-29.3); $p<0.05$)

(Figure 4). The risk of having contaminated allografts was five-fold higher when the skin spent less than 96 hours in the antibiotic cocktail ($p<0.05$) (Table 5).

Evaluation of microbial contamination

Microbial contaminants were mainly bacteria (Table 4). No fungal contamination was detected and only 2 pouches from the same donor were contaminated with *Candida albicans.* Isolated bacteria were preferentially gram-positive bacteria (n=39, 93%), with a high frequency of *Staphyloccocus spp* (n=35, 83%). The nature of bacteria (*e.g*. enterobacteria) did not vary according to the harvesting zone. According to our microbial validation process (see Material and Methods and table 1), bacteria incompatible with clinical use were gram positive for 5 donors (2 *Staphylococcus aureus*, 2 *Corynebacterium tuberculostearicum* and 1 *Enterococcus faecalis*), and gram negative for 4 donors (2 *Serratia marcescens*, 1 *Escherichia coli* and 1 *Proteus mirabilis*) plus 1 donor contaminated with yeast (*C. Albicans*)(Table 4). One should note that all microbial analyses were performed after exposure to the antimicrobial cocktail. All isolated bacteria were sensitive to antibiotics contained in the antimicrobial cocktail but almost all (except the *S. Marcescens*) were incubated for less than 96 hours.

Twelve donors were contaminated with multiple bacterial species. The rate of multi-contamination did not vary significantly according to the procurement zone.

According to our acceptance criteria (Table 1), most donors' (32/42, 76%) skin allografts were contaminated with bacteria compatible with clinical use. Only 10/42 donors' (24%) skin allografts were contaminated with germs incompatible with allograft transplantation. Overall, the proportion of allografts discarded due to contamination was 3.2% (14/433 of pouches.)

After grafting, none of the bacterial species isolated from allograft sites matched microorganisms found on the contaminated skin allografts. To date, the follow-up of the 31 recipients grafted with skin allografts contaminated with saprophytic or non-pathogenic germs did not reveal any clinical infections related to the allografts.

Discussion

A recent study by YH Choi et al. in 2018 showed significantly less mortality in burn patients who received skin allografts compared to those who did not [9]. This study, among others, demonstrates the importance of skin allografts for severe burn management and underlines the crucial role of the tissue bank in processing and preserving skin allografts [1,10,11]. Nowadays, according to the preservation methods, we can differentiate two types of skin allografts: cryopreserved or glycerol-preserved allografts. The choice of the preservation process impacts the main characteristics of final products. Skin allografts preserved in glycerol *a.k.a*. glycerol preserved allografts (GPA) were introduced for the first time in 1984 by the Euro Skin Bank. The major advantage of GPA is the intrinsic antimicrobial effect of glycerolization. The antibacterial effect of glycerol depends on its concentration, temperature and time of incubation. Almost all bacteria are killed with 85% glycerol for 14 days at 36°C [12]. De facto, highly-concentrated glycerol destroys all vital structures, including skin cells, thus reducing the antigenic potential of allografts [13]. Furthermore, the easy, cost-effective production and storage of GPA represents an interesting alternative for temporary wound coverage[14].
Unlike glycerolization, the freezing process preserves vital components and maintains the mechanical and chemical features of fresh human skin. Thus, cryopreserved allografts promote cell viability maintenance above 40%, a major factor for graft quality and performance [15,16]. Compared to GPA, the clinical use of cryopreserved allografts is associated with a significantly lower mortality rate [16]. Furthermore, much like fresh skin, cryopreserved allografts are less rigid than GPA and appear to be easily expanded and meshed [16–18]. For all these reasons, burn surgeons at the Lille University Hospital privilege the use of cryopreserved skin allografts.

A major concern for skin allografts is the risk of contamination. The use of contaminated skin allografts exposes recipients to wound infection risk, particularly bacterial infection. Therefore, skin allografts have to be controlled for bacterial and fungal contamination before storage. Multiple contamination sources exist. First, microorganisms can be naturally present on donor skin or can be introduced at harvesting. Especially for hospitalized patients, skin carries commensal skin flora but is also colonized with pathogenic microorganisms. A meta-analysis indicated that the donor skin contamination rate before processing varied and ranged from 10% to more than 95% of the harvested skin [6]. The initial contamination rate depends on different factors. The risk of contamination is highly influenced by the practice of procurement teams in particular regarding the skin disinfection protocol prior to harvesting. In our study, there were fourteen procurement teams. This may have led to a disparity in skin procurement protocols and could have increased the contamination rate. The risk of contamination also depended on external factors. The season, harvesting site, number of body areas sampled, presence of blood infection for example were reported as factors influencing the contamination rate [19–22]. These factors varied widely, depending on procurement centers and tissue banks. This is due to heterogeneous practices, which are difficult to compare. In our study, traumatic death was found to be a predictive factor of low bacterial contamination rate. This could be explained by the fact that in trauma patients hospital stay duration before death was generally short, and therefore less prone to colonization of skin surface.

Several measures should be implemented to reduce the risk of contamination. The most common antimicrobial intervention was the use of antibiotics and antifungals immediately after procurement. Broad-spectrum antibiotics and antifungals were often added to the transport medium and might have contribute to decreasing the contamination rate. For instance, the study by Mathur et al. in 2008 showed a reduction of contamination rate from 70% at procurement to 16.7% after treatment with the antibiotic cocktail [23]. According to our procedures, microbial tests were only carried out after incubation in the antibiotic medium making it impossible to know the initial contamination rate of donors at the time of harvesting. In our study, the contamination rate after incubation with the antibiotic cocktail, was 40.5% (42/104 donors who had at least one contaminated zone). The contamination was dominated by coagulase-negative staphylococci as observed in other studies [4,21,22,24,25]. The antibiotic cocktail we used contained amikacin, amoxicillin, vancomycin and the antifungal agent amphotericin B. The latter was always successful as no sample was contaminated with fungi. As observed, the efficacy of the antibiotic cocktail was highly variable [6]. It depended on the nature and concentration of the antibiotics used, incubation temperature and time spent in the antibiotic cocktail. Antibiotic effect was optimal when skin was incubated for 3 hours at 37° - 38°C [26,27]. Like many other authors [3,8,23,28], we chose to incubate the skin at lower temperatures for an extended period of time. Importantly, we observed that skin incubation in the antibiotic cocktail at +4°C for ≥ 96 hours, reduced the contamination rate to ≤ 16% (Figure 4). This result could be explained because bacteria are often hidden in the depth of the skin hair follicles where antibiotics have a difficult time reaching a short-time incubation. This is supported by the demonstration that vancomycin solution is stable at 4°C for over 10 days[29]. This result is in agreement with previous studies indicating that incubation of skin at 4°C for 1 to 6 days led to contamination rates around 23 % [8,23,30]. Longer incubation time, up to 4 weeks, also decreases the contamination rate down to 1%[3]. However, one should note that long-term incubation in the antibiotic bath may reduce skin integrity [31]. Thus, it is essential to find a balance between maintaining cell viability and reducing the contamination rate for each decontamination protocol.

Another way to reduce skin allografts' contamination rate is to harvest and process separately several areas from each donor to avoid cross-contamination. This way we avoid throwing away the entire skin procurement when only one area is contaminated. It has been observed that splitting the skin harvesting in 8 to 10 areas could save 68 % of the total harvested surface in contaminated donors [21]. In this study, we split the skin procurement into 3 areas: back and the two legs that were processed separately. This procedure allowed to retain 53 % (16/30) of skin pouches from donors contaminated with germs incompatible with a skin graft.

Commonly, all skin allografts contaminated with microorganisms are discarded and not used in clinical settings. Indeed, there have been discussions on the possibility of using allografts contaminated with saprophytic and non-pathogenic bacteria because of the immunosuppressed status of patients with major burns. As reported by Mathur et al. in 2009, we consider that contaminated donor skin should not systematically be discarded and we established with microbiologists and burn surgeons a list of germs compatible with a skin graft [23](Table 1). As previously reported by Neely et al. in 2008, no evidence of bacteria

transferred from contaminated allografts to the recipient was shown [28]. This protocol allowed to save 21% (92/433) of skin pouches representing more than 52,000 cm^2 of skin saved. Up to now, more than 120 skin pouches contaminated with saprophytic and non-pathogenic germs have been released and no infection related to these germs was reported in the 42 recipients followed at the hospital until discharge, validating the safety of our procedure. Furthermore, within 120 pouches 78 were controlled sterile at the time of the graft, which could raise the question of long-term bacterial survival in cryopreserved skin.

Conclusion

Microbial contamination represents one of the major causes of skin allograft discard. We recommend few simple pragmatic measures to reduce the discard rate without affecting safety:

- To incubate skin in the antibiotic bath for at least 96 hours at +4°C
- To harvest and process separately several areas from each skin donor (zone splitting) to minimize the cross-contamination risk
- To use allografts contaminated with low bioburden of saprophytic and non-pathogenic germs.

Implementing the above procedures allowed us to dramatically reduce the discard rate. Thus, in our study, only 3.2 % (14/433) of skin allografts were discarded because of contamination. This rate is one of the lowest as evidenced by the systematic review of the literature [6].

Figures legends

Figure 1: Timeline of skin harvesting and processing.
Two microbiological analyses were conducted during the process: one at the opening of the jars, one after the glycerol bath and conditioning. A third microbiological analysis was done after grafting. RT: room temperature.

Figure 2: Distribution of sampling area according to type of organ donation. N = 258 skin bags were prepared from 55 NHBD and 175 bags were prepared from 49 MOHBD. Distribution of skin harvesting zones did not differ between two types of deceased donors ($p<0,05$).

Figure 3: Flowchart of Lille Tissue Bank procedure for skin banking. 104 skin donors have been studied during 2018 allowing 433 skin pouches preparation. Microbiological testing resulted in discarding 14 pouches, virological and quality analysis in discarding 30 pouches. In total 387 skin pouches were released for graft of which 92 with saprophytic and non-pathogenic germs.

Figure 4: Contamination rate and number of allografts regarding time spent in the antibiotic cocktail. Mean contamination rate ranging from 51% (+/- 6.77) for 24-96 hours to 15.38% (+/- 11.31) for >96 (mean +/- SD, $p<0.05$, n=102).

Authors contribution
Acquisition, analysis and interpretation of data were done by NG, ASH, LP, PG and PM. Article drafting and critical revision was done by NG, PMD, OG. Final approval of the version was done by PM and NG.

Conflict of interest
There are no conflicts of interest.

Acknowledgements
This study was supported by the CHU de Lille

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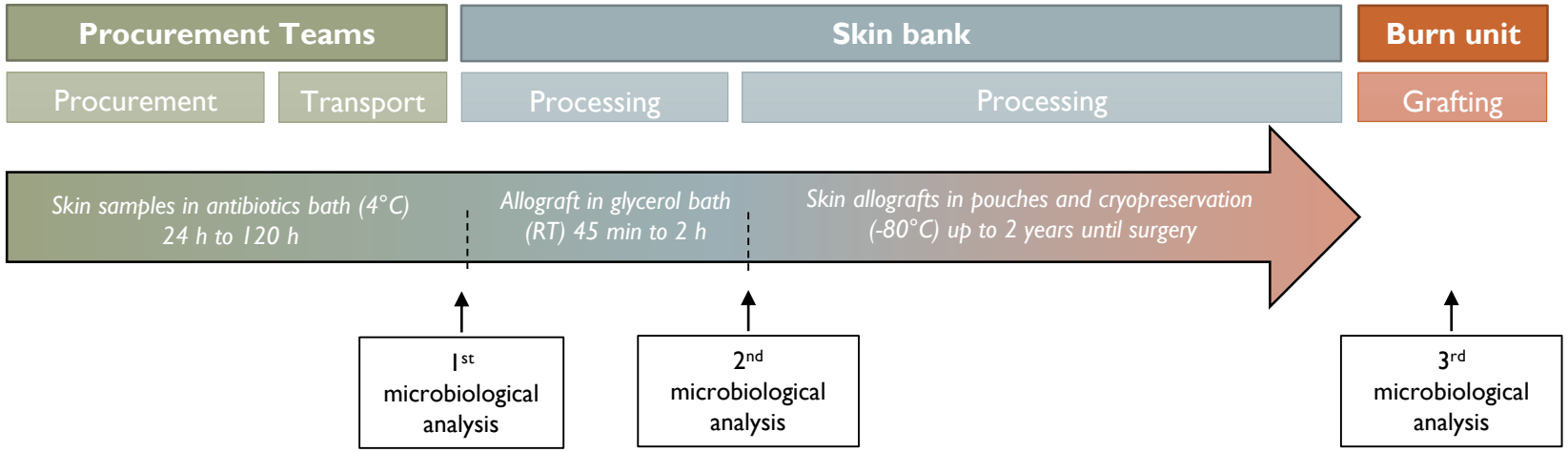
Procurement Teams
Skin bank
Burn unit
Procurement
Transport
Processing
Processing
Grafting
Skin samples in antibiotics bath (4°C)
24 h to 120 h
Allograft in glycerol bath
(RT) 45 min to 2 h
Skin allografts in pouches and cryopreservation
(-80°C) up to 2 years until surgery
1st microbiological analysis
2nd microbiological analysis
3rd microbiological analysis

Figure 1

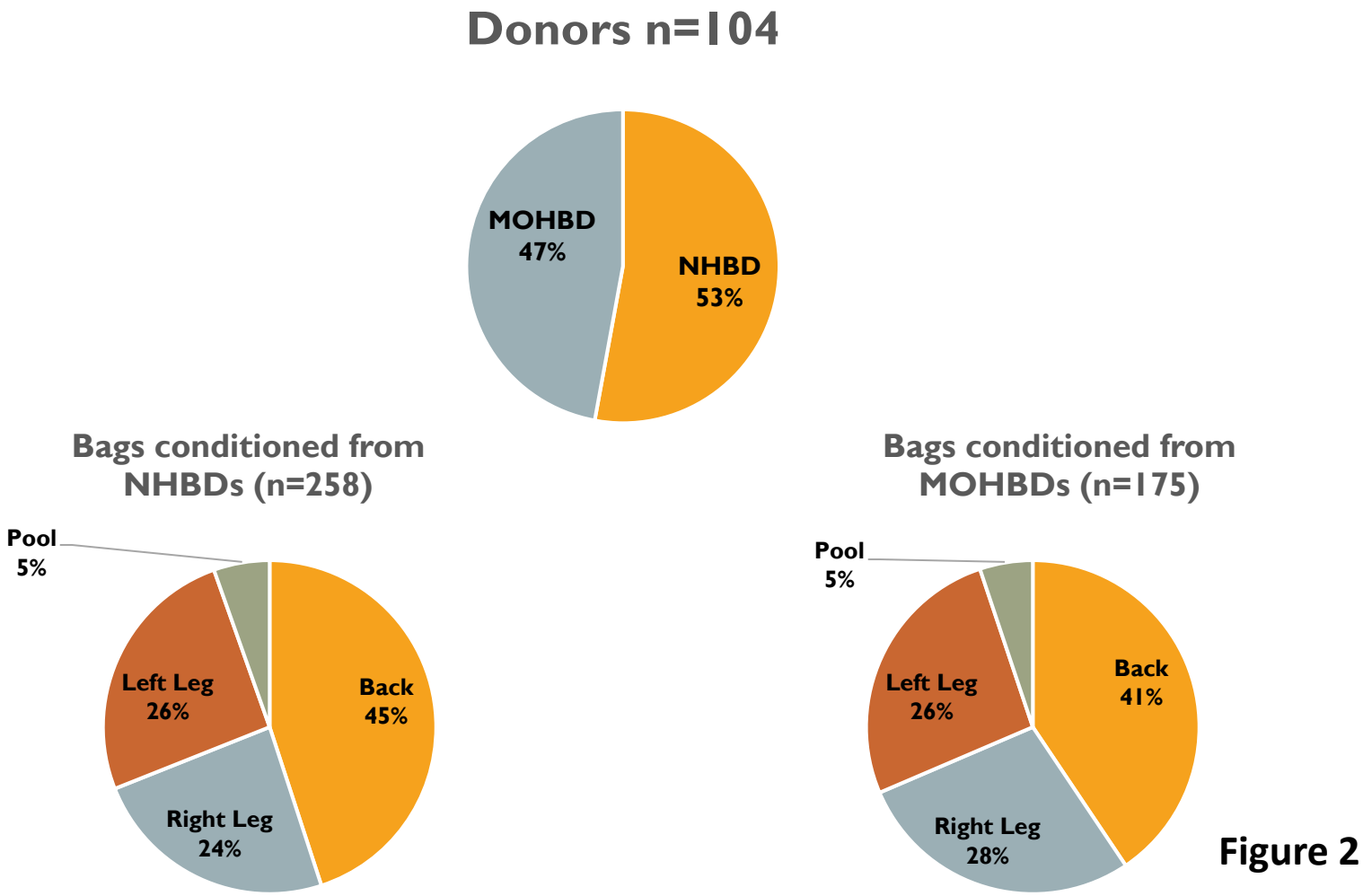
Donors n=104
MOHBD
47%
NHBD
53%
Bags conditioned from
NHBDs (n=258)
Pool
5%
Left Leg
26%
Back
45%
Right Leg
24%
Bags conditioned from
MOHBDs (n=175)
Pool
5%
Left Leg
26%
Back
41%
Right Leg
28%

Figure 2

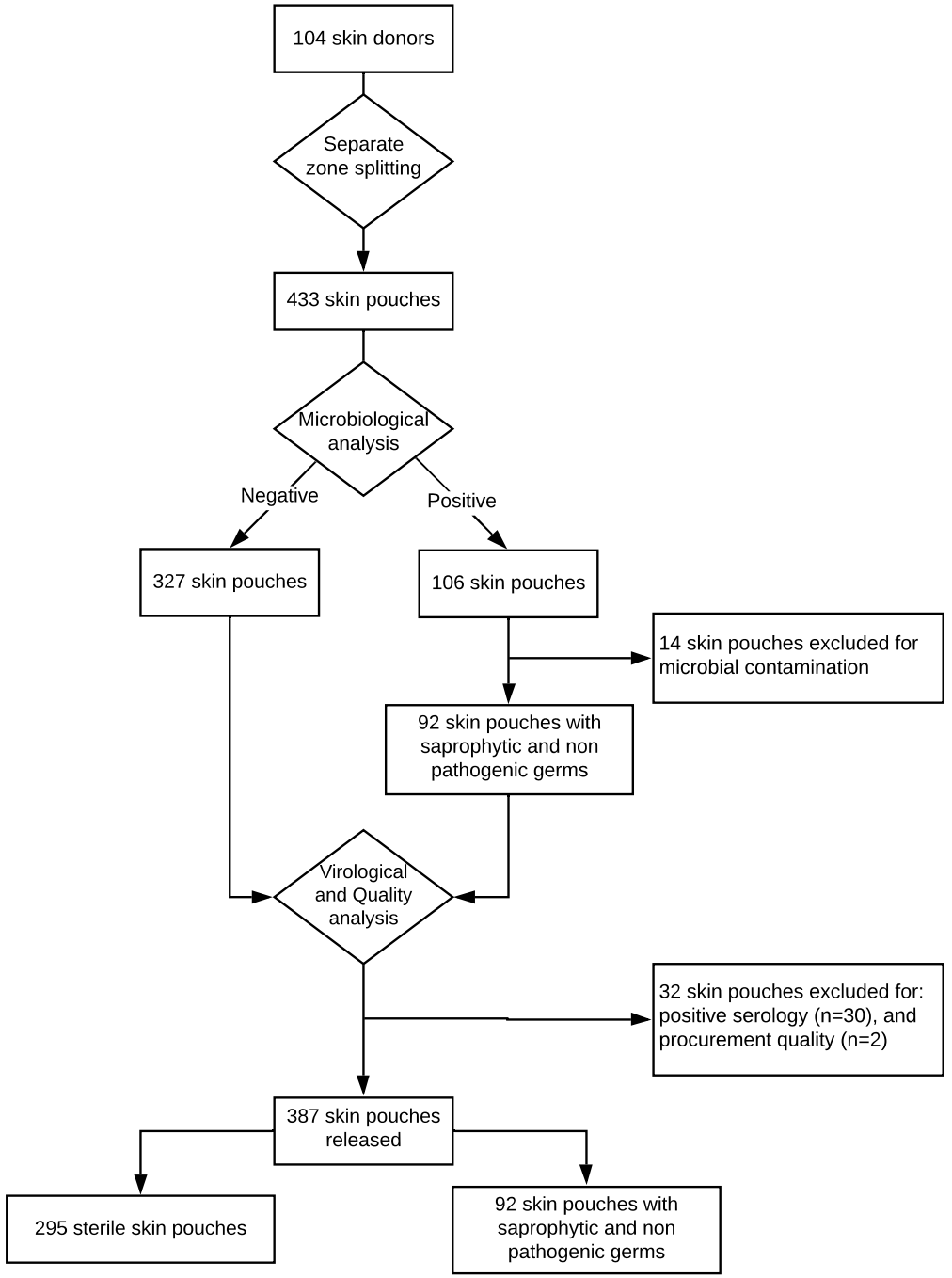
104 skin donors
Separate zone splitting
433 skin pouches
Microbiological analysis
Negative
Positive
327 skin pouches
106 skin pouches
14 skin pouches excluded for microbial contamination
92 skin pouches with saprophytic and non pathogenic germs
Virological and Quality analysis
32 skin pouches excluded for: positive serology (n=30), and procurement quality (n=2)
387 skin pouches released
295 sterile skin pouches
92 skin pouches with saprophytic and non pathogenic germs

Figure 3

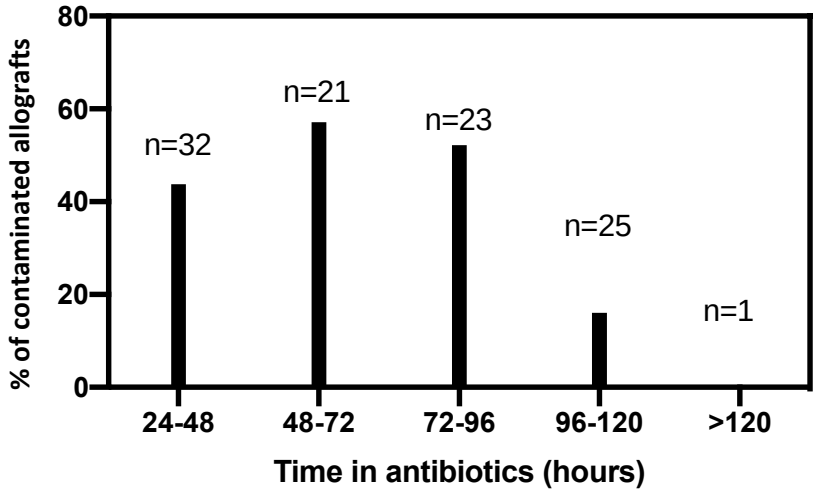
80
60
40
20
0
% of contaminated allografts
n=32
n=21
n=23
n=25
n=1
24-48
48-72
72-96
96-120
>120
Time in antibiotics (hours)

Figure 4

Table 1: Non-exhaustive list of bacteria that are compatible or not with the clinical use of skin allografts at the Lille Tissue Bank

Bacteria that is compatible with the clinical use of skin allografts*	**Bacteria that result in skin discard**
Coagulase negative staphylococcus	*S. lugdunensis* and *S. schleiferii*
Micrococcus and related (*Micrococcus*, *Kocuria*, *Arthrobacter*)	
Propionibacterium acnes or *granulosum*	
Bacillus	*B. cereus* and *B. anthracis*
Corynebacterium spp and related (*Microbacterium*, *Brevibacterium*, *Dermabacter*, *Curtobacterium*, *Cellulomonas*)	*Corynebacterium diphteriae*, *ulcerans*, *mcginleyi*, *jeikeium*, *urealyticum*, *tuberculostearicum* and *minutissimum*
Moraxella osloensis	
Actinomyces odontolyticus	

* only at low microbial load (see text for details)

Table 2. Causes of death of 104 donors

Causes of death	n	(%)
Stroke	37	(35,6)
Cardiac Arrest	36	(34,6)
Trauma	12	(11,5)
Respiratory	11	(10,6)
Others (coma, intoxication)	8	(7,7)

Table 3. Repartition of potential factors that can influence the skin contamination rate

Factors		Whole donor population N (%)	Contaminated donors N (%)	OR	*P*
Age	≦60 years	29 (27.9)	14 (13.5)	1.567 (0.6349;3.834)	*0.374*
	>60 years	75 (72.1)	28 (26,9)		
Sex	Men	51 (49.0)	22 (21,2)	1.252 (0.5618;2.834)	*0.90*
	Women	53 (51.0)	20 (19,2)		
Season of procurement	Spring/Summer	49 (47.1)	23 (22,1)	1.676 (0.7511;3.535)	*0.233*
	Fall/Winter	55 (52.9)	19 (18,3)		
Type of donation	NHBDs	55 (52.9)	29 (27,9)	0.9667(0.4317;2.178)	*>0.99*
	MOHBDs	49 (47.1)	22 (21,2)		
Infectious disease	Yes	25 (24.0)	7 (6,7)	0.4889 (0.1937;1.349)	*0.168*
	No	79 (76.0)	35 (33,7)		
Donors who received Antibiotics	Yes	45 (43.3)	14 (13,5)	0.3871 (0.1200;1.381)	*0.191*
	No	13 (12.5)	7 (6,7)		
Cause of death	Trauma	12 (11.5)	1 (1,0)	0.1131 (0.01026;0.7045)	*0.025**
	Others	92 (88.5)	41 (39,4)		
Number of tissue harvested	1	22 (21.2)	11 (10,6)	1.645(0.6227;4.352)	*0.335*
	>1	82 (78.8)	31 (29,8)		
Time spent in antibiotics	24-96h	76 (73.1)	38 (36,5)	5.00 (1.654;15.12)	*0.002**
	>96h	26 (25.0)	4 (3.8)		

* Statistical tests were done on contaminated donors

Table 4. List of different micro-organisms identified from 42 donors

Micro-organisms identified	Number of donors	(%)
*Compatible with clinical use of allografts**	38	(36,5)
Staphylococcus epidermidis	29	(69,0)
Staphylococcus haemolyticus	6	(14,3)
Staphylococcus warnerii	2	(4,8)
Staphylococcus capitis	2	(4,8)
Rothia dentacariosa	1	(2,4)
Propionibacterium acnes	2	(4,8)
Micrococcus luteus	1	(2,4)
Other *Coagulase-negative staphylococci*	2	(4,8)
*Incompatible with clinical use of allografts**	10	(9,6)
Serratia marcesens	2	(4,8)
Staphylococcus aureus	2	(4,8)
Escherischia coli	1	(2,4)
Corynebacterium tuberculostearicum	1	(2,4)
Proteus mirabilis	1	(2,4)
Candida albicans	1	(2,4)
Enterococcus faecalis	2	(4,8)

* According to Table 1