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Original Article

Evaluation of Microbial Contamination Rates in Umbilical Cord Blood Collection for Biobanking in Vinmec Tissue Bank

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Abstract: The umbilical cord blood (CB) has recently been considered an abundant source of hematopoietic stem cells (HSCs) for transplantation compared to bone marrow. However, the collection and processing of CB have a high risk of microbial contamination. Hence, the procedures to collect and process the CBs are carefully considered. This study evaluates the microbial contamination rate to find the frequency and distribution of bacterial organisms among CB samplings in Vietnam. In addition, this study compares the contamination rates between two delivery methods: cesarean section and vaginal delivery. The results help create best practices to avoid a high level of contamination of CB during cord blood banking.

Keywords: Microbial contamination, umbilical cord blood, biobanking, umbilical cord blood bank.

1. Introduction

Hematopoietic stem cells (HSCs) transplantation is one of the main strategies to treat malignant or non-malignant blood diseases and immunodeficiency disorders. There are three main HSCs sources for stem cell regenerative medicine: bone marrow, peripheral blood, and cord blood (CB). Among them, CB has several advantages compared with the other sources such as: i) The proportion of highly proliferative HSCs is higher so that it enhances homing capacity and hematopoietic reconstitution; ii) The dominant characterization of CB suggests that they have remarkable self-renewal, proliferation, and expansion properties under optimal conditions; iii) The CD34+ expression on cells surface and longer telomere length on the chromosome; iv) The collection process is non-invasive and safety for both mother and baby; and v) The CB composites naive immune cells that result in a significantly lower incidence of graft vs. host disease (GVHD) and lower risk of blood-transmitted infectious diseases in CB recipients [1-6].

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Hence, since the 1990s, CB has been used as an alternative source of HSCs transplantation, and CB banking has existed to satisfy the human need for stem cell storage and preservation. To date, about 5 million CB units have been stored worldwide, and about 800,000 of these are in public banks. Over 4 million CB units are stored in private or family banks. It is easy to recognize that the field of CB banking and transplantation has increased noticeably in the past 25 years. The parents who are waiting for their baby have various options [1, 7]. Vietnam is not standing outside this common development trend. About six cord blood banks (CBBs) (including private and public banks) are operating in Vietnam.

There are many steps related to CB banking, including sample collection, centrifugation, plasma depletion, cryopreservation, storage, thawing, etc. Each step of stem cells collection and processing are burdened with a risk of microbial contamination. There was a range of CB contamination proportion reported vary from 0 to 48%. Cause of the CB unit contaminated with microorganisms leading to severe complications for recipients, including sepsis. To prevent these severe incidents, international accreditation organizations, including the American Association of Blood Banks (AABB), the Foundation for Accreditation of Cellular Therapy (FACT), and the Vietnam Ministry of Health mandate the sterility testing for all CB and cell therapy products [8-10].

Moreover, to reduce the contamination risk, people can use a sterile closed system for CB processing, and a good aseptic practice must be implemented according to the guidelines issued in the preparation and collection of CB samples [11, 12]. There are two main CB collection methods: in utero and ex utero in the different ways of delivery (vaginal delivery and cesarean section). The utero CB collection is generally performed immediately after the newborn is delivered, and the placenta may still be in the uterus. Meanwhile, in the ex utero collections, after the placenta detachment from the uterus, it will be removed from the delivery suite and transported to a nearby clean room for the collection. In both collection methods, disinfection is critical and affects the contamination of the sample [6, 8, 11].

In this study, the microbial contamination rate was evaluated to find the frequency and distribution of bacterial organisms among CB samplings in Vinmec Tissue Bank, Vietnam. In addition, this study correlates with contamination rates between the delivery methods, cesarean section, and vaginal delivery. The results help create best practices to avoid a high level of contamination of CB during collection for cord blood banking.

2. Methodology

This study consisted of 4,817 cord blood units (CBUs) that met the Cellular Therapy (CT) standard requirements (Standards for Cellular Therapy Services, 10th Edition 2021) for processing and storage at the Vinmec Tissue Bank (VTB) from 2014 to 2020. The CBUs without results for microbial screening were excluded from the analysis.

Informed consent with the ethics committee approval was obtained before collection. CB was collected after the baby's birth by either vaginal delivery or cesarean section by dedicated VTBs staff and trained obstetric staff at the participating hospital. CB was collected using standard protocols [13]. Briefly, after cord antisepsis with Betadine (Povidone-iodine) solution, blood was drained into the collection bag by gravity with the placenta in situ (in utero) or after the placenta was delivered (ex utero). The collection bag contained 35 mL of citrate phosphate dextrose adenine (CPDA-1) anticoagulant (Teruflex, Terumo BCT, UK). CB was then stored at 15-25 °C for up to 36 hours before being processed.

The CB after the collection was transferred to the Automatic processing AXP system [13]. Then, two centrifugation steps at different speeds and times were used to remove red

blood cells, plasma, and other unnecessary components. The first was heavy spin with 1400 RCF in 20 minutes, then the second light spin at 80 RCF for 10 minutes. Approximately 20 - 22 mL of the final CB was collected by AutoXpress (CESCA Therapeutics, Thermogenesis). A volume of 3 - 5mL of whole cord blood before processing and plasma after processing was withdrawn for pre- and post-microbial testing, respectively.

The automated blood culture system evaluated in this study was carried out under aseptic conditions in a laminar flow cabinet of the BacT/ALERT 3D (BacT) (bioMérieux Canada, Inc.). Aerobic, anaerobic, and microbial media were evaluated in an automated blood culture system. Standard BacT/Alert culture bottles (Organan Teknika) were inoculated into BacT/ALERT FA (FAN aerobic) and BacT/ALERT FN (FAN anaerobic) "cord blood" culture bottles and inoculated into a single BacT/ALERT PF (FAN, low volume). The media used with the Bactec system were Plus Aerobic/F (aerobic), Plus Anaerobic/F (anaerobic), and Myco/F Lytic (mycology). In contrast, FA (aerobic), SN (anaerobic), and MB (mycology) were used with the BacT system. All anaerobic/microaerobic and fungal bottles were inoculated with 1-2 ml of blood for the Pre sample (CB after collection) and 3-5 ml of plasma for the Post sample (after processing). CBUs with pre- and post-plasma samples were inoculated into BacT/ALERT 3D aerobic and anaerobic culture bottles and incubated at 35-37 °C for a maximum of 14 days. The specificity of the positive culture bottles was verified by Gram staining. Positive results obtained pre- and post-CB was identified by VITEK 2 Automated Systems (bioMerieux, Hazelwood, MO).

A Chi-squared test was used to determine the relationship of methods of delivery. A p-value less than 0.05 was considered the threshold for significance. Data analyses were performed using GraphPad Prism software version 9.2.

3. Results and Discussion

The CB collection and processing data were reviewed and evaluated in the 2014-2020 period. The microbial contamination rate was analyzed based on the delivery methods, vaginal and Caesarean section (C-section), and their impacts. Overall, there were 28 samples among 4,817 umbilical CB units tested positive for microbial contamination in both pre and post samples, indicating the reason for contamination during collection, accounting for 0.58% (Table 1). Also, the proportion of contaminated CBs in vaginal collection (1.39%) was significantly higher than that of C-section collection (0.21%; $p<0.0001$). Among contaminated CBUs caused by collection, the rate of vaginal birth accounted for 75% (21 cases). In comparison, that of C-section was only 25% (7 cases) (Figure 1).

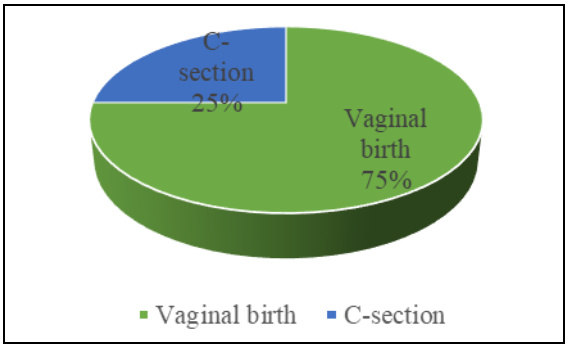

Figure 1. The proportion of contaminated-CBUs during collection by method of delivery (vaginal and C-section birth).

All positive microbial contaminated CBUs were destroyed or separately stored after identifying microbial organisms and antibiotic sensitivity testing. Compared to previous reports (Table 2), this study's microbial contamination rate is very low. It can be seen that the techniques as well as protocols at VTB for CB collection are qualified and can be used as references for other CBB and medical facilities to collect CB.

The main contamination sources may occur at collection and processing phases from maternal vaginal, peri-anal flora, baby's intra-uterine or

peri-natal infection, skin contaminants at the time of collection, or laboratory processing and sampling [11]. Based on root cause analysis of 21 CBUs tested for positive bacterial contamination in the vaginal delivery, we recognized that most contamination derived from peri-anal flora, maternal vaginal, and skin contaminants at the time of collection. The main bacterial groups were identified, as shown in Table 3.

Diversity of microbes has been isolated from the contaminated CB samples, with the most frequent microorganisms including Escherichia coli (17.6%), *Streptococcus agalactiae* (17.6%), *Staphylococcus epidermidis* (8.8%), and *Corynebacterium spp* (11.8%). The main bacterial contamination sources are from four groups: i) Gastrointestinal tract flora (35.3%); ii) Skin flora (32.4%); iii) Vaginal flora (23.5%); and iv) Environmental contaminants (8.8%) (Table 3).

Improvement in the processes of the disinfecting, collecting, and processing of the CB and staff training can lead to a significant decrease in the rate of bacterial contamination. According to the previous reports, personnel with limited training conducted CB collection resulting in a 28% contamination rate [14, 15]; while well-trained personnel carried out the CB collection resulting in a less than 5% contamination rate [14-16]. Our finding shows that with the annual training schedule for collectors, the CB contamination rate in Vinmec Tissue Bank reduced significantly, down to 0.58%.

Table 1. Microbial contamination rate of umbilical CB collection

	Methods of delivery		Total of CBUs	p-value
	Vaginal (%)	C-section (%)		
Number of CBUs	1,515 (31.2%)	3,302 (68.8%)	4,817	<0.0001
Number of contaminated CBUs	21 (1.39%)	7 (0.21%)	28	

Table 2. Comparison of microbial contamination rates in the CB collection phase

CBB/Medical facilities	CB units	Bacterial contamination rate	References
Sanquin Blood Supply Foundation (The Netherlands)	740	13% (n=94)	[17]
Sydney Cord Blood Bank (Australia)	13,344	4% (n=537)	[11]
Chinese Cord Blood Bank (China)	7,032	1.98% (n=139)	[18]
National Center of Blood Transfusion (NCBT) (Mexico)	5,193	2.31% (n=120)	[19]
Public Cord Blood Banks (France)	338	3.5% (n=12)	[20]
Hospital Occidente de Kennedy (America)	3,105	5.1% (n=155)	[21]
CellSave (Arabia)	1,250	6.2% (n=78)	[22]
Vinmec Tissue Bank (Vietnam)	4,817	0.58% (n=28)	This study

Table 3. Occurrence frequency of bacterial organisms in Vaginal and C-section deliveries

No	Contamination sources	Bacterial organisms	Occurrence frequency (time)	
			Vaginal	C-section
1	*Gastrointestinal tract flora*	*Escherichia coli*	5	1
2		*Enterobacter aerogenes*	2	
3		*Klebsiella pneumoniae*	2	
4		*Enterococcus faecalis*	1	
5		*Lactococcus garvieae*	1	
6	*Skin flora*	*Staphylococcus epidermidis*	2	1
7		*Corynebacterium spp*	4	
8		*Staphylococcus coagulase*	1	
9		*Staphylococcus haemolyticus*	1	
10		*Staphylococcus hominis*	1	
11		*Staphylococcus carnosus*	1	
12	*Vaginal flora*	*Streptococcus agalactiae*	3	3
13		*Streptococcus anginosus*	1	
14		*Lactobacillus delbrueckii*	1	
15	*Environmental contaminants*	*Acinetobacter baumannii*		1
16		*Bacillus vietnamensis*	1	
17		*Burkholderia spp*		1

4. Conclusion

This study clearly shows that contamination rates can be substantially reduced by collecting CB following cesarean delivery. In addition, with the annual well-training plan for the collector, the contamination rate in Vinmec Tissue Bank reduced significantly.

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