Official journal of the Cord Blood Association

Stem Cells Translational Medicine

Cord Blood Collection, Maintenance, and Cell Engineering

Microbial Contamination in Umbilical Cord Blood: A Comparison Before and After Cryopreservation

Mingming Li, Daniel Lim, Kin Fai Tang, Sheena Lim, Kenny Boey

Cordlife Group Limited, Singapore, Singapore

Abstract 2

Introduction

Testing of microbial contamination is one of the major criteria for umbilical cord blood (UCB) applications. Due to limited collection and post-processing volume, a UCB contamination test is only conducted with a small volume of samples for incubation.

Objective

The primary objective of this study was to assess microbial viability in the donated UCB units after cryopreservation.

Methods

Microbial presence in the pre-freeze and the post-thaw UCB samples was compared. A total of 76 contaminated UCB samples donated to the bank were used for analysis. All samples were infused with dimethyl sulfoxide (DMSO) using an automated mixing and cooling device to achieve a final concentration of 10% DMSO. The samples were frozen with a controlled-rate freezer prior to cryogenic storage maintained at below –150°C from 2011 to 2016. Each individual sample was retrieved and thawed using a water bath set at 37°C with gentle agitation; 1 ml and 10 ml of the samples were then inoculated into BacT/ALERT BPA and BPN culture bottles directly for 14 days culture in the microbial detection system. Positive bottles were unloaded following alert from the detection system and sent to a referral laboratory for microbial identification.

Results

As shown in Table 1, of the 76 contaminated samples, 11 strains of microorganism were detected prior to cryopreservation but only 10 were detected post-thaw. Some of the *Streptococcus* species (i.e., *Peptostreptococcus*, *Streptococcus anginosus*) were unable to survive cryopreservation. Cryopreservation has no effect on the viability of *Escherichia coli* and *Enterococcus* species. Growth was observed in only 28% of the samples with *Bacteroides fragilis* contamination. Nonviable *Lactobacillus* species were detected in 75% of the post-thaw samples and 25% had no growth. In addition, the 10 ml sample inoculation showed better sensitivity in microbial detection than the 1 ml inoculation.

Discussion

Similar to a previous study in which 55% of the post-thaw cultures were positive [Ann Clin Microbiol 2013;16:8–12], the present results show that approximately 54% of cultures were positive, further supporting the conclusion that some bacteria strains do not survive after cryopreservation. Contrary to another study [Vox Sang 1998;74:88–94], no effect on the survival of *E. coli* was found post-cryopreservation.

Table 1. Presence of microbial strains in the pre-freeze and the post-thaw umbilical cord blood samples

Strain	Number of samples	Number of positive aerobic bottles		Number of positive anaerobic bottles	
		1 ml inoculation	10 ml inoculation	1 ml inoculation	10 ml inoculation
Coagulase-negative Staphylococcus	14	7	9	6	7
Propionibacterium acnes	12	1	2	3	7
Escherichia coli	11	10	11	11	11
Enterococcus species	8	7	8	7	8
Bacteroides fragilis	7	1	1	1	2
Delftia acidovorans	5	3	3	0	0
Lactobacillus species	4	1	2	1	3
Bifidobaterium breve	4	1	1	3	3
Lancefield Group B *Streptococcus*	4	4	2	3	2
Peptostreptococcus, Streptococcus anginosus, and non-groupable *streptococcus* species	4	0	0	0	0
Candida albicans	3	2	3	3	3
Total	76	37	42	38	46