
**ASSESSMENT OF
MICROBIAL BIOBURDEN METHODOLOGIES
FOR TISSUE BANK SPECIMENS**

Kerry Varettas

**A thesis submitted in accordance with the
requirements for admission to the degree of
Doctor of Philosophy**

**University of Technology, Sydney
2014**

CERTIFICATE OF AUTHORSHIP / ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Production Note:
Signature removed prior to publication.

Kerry Varetas
2014

ACKNOWLEDGMENTS

I would like to thank Professor Sydney Bell, Associate Professor Peter Taylor and Chinmoy Mukerjee for providing me with the opportunity to undertake post-graduate research. Peter, I am especially grateful for your guidance, assistance and encouragement. Thank you to Associate Professor Chris McIver for your expertise and patient instruction in molecular bacteriology, to Professor Ruth Hall for always having the time to talk to me, and to my supervisor Associate Professor Cynthia Whitchurch for helping me through my PhD journey.

I could not have completed my research without the information provided by the Biotherapeutics Association of Australasia and the tissue banks of Australia, especially the NSW Bone Bank who also provided me with musculoskeletal tissue samples. Thank you to the Australian TGA-licensed bacteriology laboratories who shared their bioburden testing methods with me.

Finally, my thanks go to my amazing family for their support and understanding. Joanna thanks for all the proof reading and your constant encouragement.

PUBLICATIONS ARISING FROM THIS THESIS

Varettas K. (2014) RT-PCR testing of allograft musculoskeletal tissue – is it time for culture-based methods to move over? *Pathology. In press.*

Varettas K. (2014) Evaluation of two types of swabs for sampling allograft musculoskeletal tissue. *Aust NZ J Surg.* Doi: 10.1111/ans.12661

Varettas K. (2014) Swab or biopsy samples for bioburden testing of allograft musculoskeletal tissue? *Cell Tissue Bank.* 15:613-618

Varettas K. (2013) Broth versus solid agar culture of swab samples of cadaveric allograft musculoskeletal tissue. *Cell Tissue Bank.* 14:627-631

Varettas K. (2013) Micro-organisms Isolated from Cadaveric Samples of Allograft Musculoskeletal Tissue. *Cell Tissue Bank.* 14:621-625

Varettas K. (2013) Culture Methods of Allograft Musculoskeletal Tissue Samples in Australian Bacteriology Laboratories. *Cell Tissue Bank.* 14:609-614

Varettas K. (2012) Bacteriology Laboratories and Musculoskeletal Tissue Banks in Australia. *ANZ J Surg.* 82:775-779

Varettas K & Taylor P. (2012) Fungal Culture of Musculoskeletal Tissue: What's the Point? *Cell Tissue Bank.* 13:415-420

Varettas K & Taylor P. (2011) Bioburden Assessment of Banked Bone Used for Allografts. *Cell Tissue Bank.* 12: 37-43

PRESENTATIONS AT SCIENTIFIC CONFERENCES

Varettas K. Bioburden of allograft musculoskeletal tissue from cadaveric donors. Poster Presentation. 21st Annual conference of the European Association of Tissue Banks (EATB). Vienna, Austria. November 2012.

Varettas K. Broth vs solid agar culture of cadaveric allograft musculoskeletal tissue samples. Poster Presentation. 21st Annual conference of the European Association of Tissue Banks (EATB). Vienna, Austria. November 2012.

TABLE OF CONTENTS

CERTIFICATE OF AUTHORSHIP / ORIGINALITY	II
ACKNOWLEDGMENTS	III
PUBLICATIONS ARISING FROM THIS THESIS.....	IV
PRESENTATIONS AT SCIENTIFIC CONFERENCES	V
TABLE OF CONTENTS.....	VI
LIST OF TABLES	XI
ABSTRACT	XIII
CHAPTER 1: GENERAL INTRODUCTION.....	1
1.1 Musculoskeletal tissue transplantation.....	2
1.2 History of musculoskeletal tissue transplant	3
1.3 Source of allograft musculoskeletal tissue for transplant.....	3
1.4 Clinical use of allograft musculoskeletal tissue	4
1.5 Musculoskeletal tissue banks	5
1.6 Musculoskeletal tissue banks in Australia	6
1.7 National transplantation reforms in Australia.....	7
1.8 Therapeutic Goods Administration.....	9
1.9 Donor assessment.....	9
1.10 Allograft musculoskeletal tissue retrieval	11
1.11 Allograft musculoskeletal tissue processing.....	12
1.12 Allograft musculoskeletal tissue storage and distribution	12
1.13 Musculoskeletal tissue infection.....	12
1.14 What is bioburden?.....	13
1.15 Bioburden reduction processes	14
1.15.1 Autoclaving.....	14
1.15.2 Antibiotics	14
1.15.3 Freezing	15
1.15.4 Supercritical carbon dioxide.....	15

1.15.5	Microwaving	15
1.15.6	Ethylene oxide	16
1.15.7	Gamma irradiation	16
1.16	Issues associated with bioburden reduction methods of allograft musculoskeletal tissue	17
1.17	Thesis overview.....	18
1.18	Thesis aims	18
1.19	Thesis format.....	18
CHAPTER 2: BACTERIOLOGY LABORATORIES AND MUSCULOSKELETAL TISSUE BANKS IN AUSTRALIA		20
2.1	Abstract	21
2.2	Introduction.....	22
2.3	Bacteriology laboratories and musculoskeletal tissue banks.....	22
2.4	Regulatory requirements	24
2.4.1	Australian Code of Good Manufacturing Practice – Human Blood and Tissues	24
2.4.2	AS ISO 15189:2009 medical laboratories – particular requirements for quality and competence	25
2.4.3	AS/NZS ISO 9001:2008 quality management systems – requirements	26
2.5	Musculoskeletal tissue samples.....	26
2.5.1	Swab samples	27
2.5.2	Biopsy samples	27
2.6	Swab versus biopsy samples.....	28
2.7	Tissue exclusion criteria based on organism recovery	28
2.8	Conclusion.....	29
2.9	Acknowledgements	29
CHAPTER 3: BIOBURDEN ASSESSMENT OF BANKED BONE USED FOR ALLOGRAFTS		30
3.1	Abstract	31
3.2	Introduction.....	32

3.3	Method	33
3.4	Results	34
3.5	Discussion	35
3.6	Conclusion.....	40
CHAPTER 4: MICRO-ORGANISMS ISOLATED FROM CADAVERIC SAMPLES OF ALLOGRAFT MUSCULOSKELETAL TISSUE		
		41
4.1	Abstract	42
4.2	Introduction.....	43
4.3	Method	44
4.4	Results	44
4.5	Discussion	45
4.6	Conclusion.....	49
CHAPTER 5: FUNGAL CULTURE OF MUSCULOSKELETAL TISSUE: WHAT'S THE POINT? 50		
5.1	Abstract	51
5.2	Introduction.....	52
5.3	Method	52
5.4	Results	54
5.5	Discussion	54
5.6	Conclusion.....	57
CHAPTER 6: CULTURE METHODS OF ALLOGRAFT MUSCULOSKELETAL TISSUE SAMPLES IN AUSTRALIAN BACTERIOLOGY LABORATORIES		
		60
6.1	Abstract	61
6.2	Introduction.....	62
6.3	Bacteriological Media Used in Culture Methods.....	62
6.3.1	Agar Culture	62
6.3.2	Broth Culture	63
6.4	Culture Methods Used by Australian Laboratories	64
6.5	International Culture Methods.....	64
6.6	Method Validation.....	65

6.7	Conclusion.....	65
6.8	Acknowledgments	65
CHAPTER 7: BROTH VERSUS SOLID AGAR CULTURE OF SWAB SAMPLES OF CADAVERIC ALLOGRAFT MUSCULOSKELETAL TISSUE.....		68
7.1	Abstract	69
7.2	Introduction.....	70
7.3	Method	70
7.4	Results	71
7.5	Discussion	71
7.6	Conclusion.....	73
CHAPTER 8: SWAB OR BIOPSY SAMPLES FOR BIOBURDEN TESTING OF ALLOGRAFT MUSCULOSKELETAL TISSUE?.....		75
8.1	Abstract	76
8.2	Introduction.....	77
8.3	Method	77
8.4	Results	79
8.5	Discussion	81
8.6	Conclusion.....	83
CHAPTER 9: EVALUATION OF TWO TYPES OF SWABS FOR SAMPLING ALLOGRAFT MUSCULOSKELETAL TISSUE.....		85
9.1	Abstract	86
9.2	Introduction.....	87
9.3	Method	88
9.4	Results	89
9.5	Discussion	91
9.6	Conclusion.....	96
9.7	Acknowledgment	96
CHAPTER 10: RT-PCR TESTING OF ALLOGRAFT MUSCULOSKELETAL TISSUE – IS IT TIME FOR CULTURE-BASED METHODS TO MOVE OVER?		97
10.1	Abstract	98

10.2	Introduction.....	99
10.3	Materials and Methods	100
10.4	Results	102
10.5	Discussion	102
10.6	Conclusion.....	105
10.7	Acknowledgments	105
CHAPTER 11:	GENERAL SUMMARY & CONCLUSIONS	107
CHAPTER 12:	BIBLIOGRAPHY.....	113
CHAPTER 13:	APPENDICES	138
13.1	Appendix 1: Tissue Bank Questionnaire	139
13.2	Appendix 2: Bacteriology Laboratory Questionnaire	141

LIST OF TABLES

Chapter 2

Table 2.1: Musculoskeletal Tissue Banks in Australia, 2012

Table 2.2: Bacteriology Laboratories Providing Bioburden Testing of Samples from Musculoskeletal Tissue Banks in Australia, 2012¹

Chapter 3

Table 3.1: Summary of Number of Episodes Received, Bone Swabs and Fragments Received and Culture Results from January 2001 to December 2007

Table 3.2: Positive Culture Results in Bone Swabs and Fragments from January 2001 to December 2007

Chapter 4

Table 4.1: Summary of number of episodes, samples received and culture results, 2006-2011

Table 4.2: Micro-organisms isolated from samples of cadaveric allograft musculoskeletal tissue, January 2006 to December 2011

Chapter 5

Table 5.1: Fungal Isolates from Tissue Banks A, B & C from August 2008 – 2010

Table 5.2: Review of Musculoskeletal Tissue Bioburden Rates and the Number of Fungal Isolates

Table 5.3: Review of Musculoskeletal Tissue Post-Transplant Infection Rates and the Number of Fungal Isolates

Chapter 6

Table 6.1: Summary of Culture Methods of Allograft Musculoskeletal Tissue Samples by Six TGA-licenced Clinical Microbiology Laboratories in Australia

Table 6.2: International Literature Review of Samples and Culture Methods of Allograft Musculoskeletal Tissue

Chapter 7

Table 7.1: Micro-organisms isolated from swab samples of cadaveric allograft musculoskeletal tissue, January 2006 to December 2011

Chapter 8

Table 8.1: Amies swab: In-vitro colony forming unit (CFU) inoculations and percentage (%) recovery

Table 8.2: Allograft femoral head biopsies: In-vitro colony forming unit (CFU) inoculations and percentage (%) recovery

Table 8.3: Bioburden results from paired swab and biopsy samples of allograft femoral heads, 2001 - 2012

Chapter 9

Table 9.1: Percentage (%) recovery of in-vitro inoculated Amies and ESwabs

Table 9.2: Amies and ESwab % recovery after sampling inoculated allograft whole femoral heads

Table 9.3: Amies and ESwab culture results after sampling inoculated allograft whole femoral heads

Table 9.4: Bioburden results of cadaveric musculoskeletal allografts using Amies and ESwabs

Table 9.5: Prospective study: Amies or ESwab culture positive

Table 9.6: Prospective study: Amies and ESwab culture positive

Chapter 10

Table 10.1: Swab inoculation and method detection of challenge organisms

Table 10.2: Biopsy inoculation and method detection of challenge organisms

Table 10.3: Limit of detection of PCR methods

ABSTRACT

Musculoskeletal tissues form part of the skeletal and/or muscular system of the body, vital in providing support and mobility. Musculoskeletal tissue transplants outnumber all other organ and tissue transplants. The bioburden assessment of allograft musculoskeletal tissue must be performed as part of the assessment screening of living and cadaveric donors to minimise the potential risk of transmission of infectious diseases via the allograft to the recipient.

There are no guidelines or standard method for determining the bioburden assessment of allograft musculoskeletal tissue and microbiology laboratories may use different types of samples, culture media and methods. Determining the suitability of the allograft tissue sample and the sensitivity of the bioburden testing methods required investigation especially with the advent of nucleic-acid testing (NAT). Subsequently, this investigation highlighted the lack of information regarding microbiology laboratories and the tissue banking industry in Australia.

A questionnaire was sent to all Australian tissue banks to determine their current status and the types of allograft samples being collected for bioburden assessment. Another questionnaire was designed for Therapeutic Goods Administration (TGA) licensed clinical microbiology laboratories to establish what bioburden assessment methods were being used for allograft samples. The information obtained from these questionnaires guided the evaluations undertaken in this thesis to compare different allograft samples and methods for bioburden assessment.

The current practice of collecting a swab and biopsy sample of allograft musculoskeletal tissue appears optimal for bioburden assessment. Retrospective reviews of isolates recovered from allograft musculoskeletal tissue and from the literature found a wide range of aerobic and anaerobic micro-organisms with fungi infrequently isolated.

An evaluation of the Amies gel swab and the ESwab systems was performed to determine if bioburden recovery could be improved at the pre-analytical stage. Both swab systems were found to be suitable sampling devices for bioburden testing of allograft musculoskeletal tissue.

The most common bioburden assessment methods, agar and broth culture, were compared with a broad-range NAT method. Swab and biopsy samples were inoculated with known quantities of challenge organisms and the percentage recovery of the challenge organisms was compared. In this study, the NAT method was not more sensitive than the culture-based techniques evaluated with broth culture being the most sensitive.

Microbiology laboratories must continue to re-evaluate current methods and investigate new ones to improve sensitivity. Future directions must be cost-effective as the value of maintaining a TGA-licence has become uncertain for some laboratories. Ultimately, tissue banks, clinicians and, most importantly, the allograft recipient must have confidence in the pre-analytical sampling techniques and the testing methods used to determine the bioburden of allograft musculoskeletal tissue prior to transplant.