

Independent Laboratory Test Report

ROSS Efficacy on Specific Bacteria

Background

One of the most important items of any prosthetic device for those with limb loss is the sleeve and or liner. It acts as an interface between the amputees skin and prosthetic to enhance comfort and fit. Due to the contact between bare skin and the inside layer of the liner an optimal environment is created that allows bacteria to quickly grow exponentially causing most notably mal odor.

Most skin irritation and odor is due to poor hygiene, local infections and the liner being over-worn and or in need of replacement. In cases where the risk of infection is high i.e. diabetes, a clean sleeve is critical. If the residual limb has lesions and the liner is not clean, the user is highly susceptible to infection. In cases such as these the prosthetic device cannot be worn.

Cleaning regimes differ from patient to patient and the practice is often too laborious to be performed adequately. With that in mind, TriOx Innovations has designed and developed the world's first automated prosthetic liner sanitizer. The following is an independent laboratory test to validate the biocidal efficacy of ROSS.



ROSS Unit

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TriOx Innovations approached Assure Cloud Laboratories in Cape Town, South Africa to run testing with the ROSS device and its efficacy in eliminating bacteria from prosthetic sleeves.

Assure Cloud is Southern Africa's largest and most comprehensive food safety, environmental, veterinary, and occupational hygiene laboratory group. Through the strategic acquisition of innovative companies such as Deltamune Laboratories, and SAI Global Africa Assurance (QPRO and SAIGAS), Assure Cloud is a global leader in Food Safety and Occupational Health and Safety.

The below tests to verify the efficacy of ROSS were carried out at Assure Cloud's Paarden Island facility in Cape Town, South Africa.



Date of Laboratory tests – April, March 2022

Date of Report – July 2022

Written by – Barry Geer & JC Visser

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Test Objectives



To test and record the efficacy of ROSS in the elimination of viable bacteria in set time frames.

The equipment used in these lab tests were the following:

1. ROSS prototype
2. Prosthetic liner samples
3. Ozone measuring devices
4. Stop watch
5. Assure Cloud resources, specialised laboratory equipment and personnel

Preparation of prosthetic liner samples and petri dishes



Method

Bacterial swab samples were taken from multiple amputee liners after common day to day activities e.g. gym, work, and sleep in order to come up with an average bacterial count on given measured surface. The average count determined was $10E4$ (10,000 bacterium). The bacteria grown at Assure Cloud and used in testing the ROSS was *Staphylococcus aureus* or *S. aureus* for short.

“S. aureus has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most *S. aureus* infections are not serious, *S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections.” **Minnesota Department of Health Fact Sheet, February, 2010**

Two designated ozone settings were used to emulate the ROSS device, a 10 minute cycle and 20 minute cycle. Assure Cloud personnel were responsible for loading the prosthetic liner samples with bacteria, swabbing surfaces pre and post the ROSS process and performing Colony Forming Unit (CFU) bacterial counts.

Method continued:

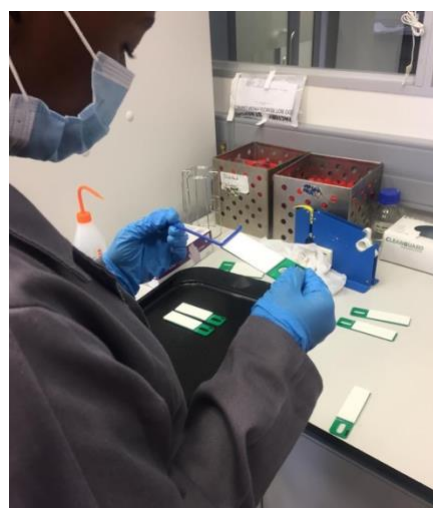
Bacteria used: *Staphylococcus aureus*

Bacterial load used:

1. 2×10^4 (20,000 CFU)
2. 10^5 (100,000 CFU)

To challenge the ROSS process bacterial loads were applied to liner samples at both 2x and 10x the average load found on an untreated amputee liner.

Applying Staphylococcus aureus on liner samples



Tests were repeated to increase the data collected and confirm results

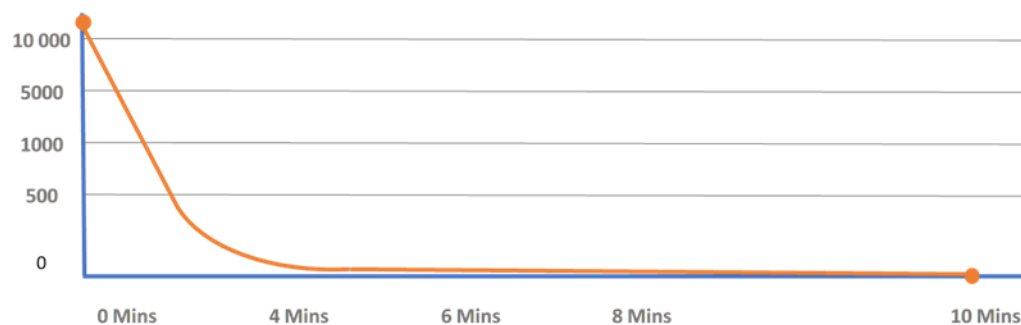
Total Swabs taken: 68 x 2

Total test times in hours: 7h x 2

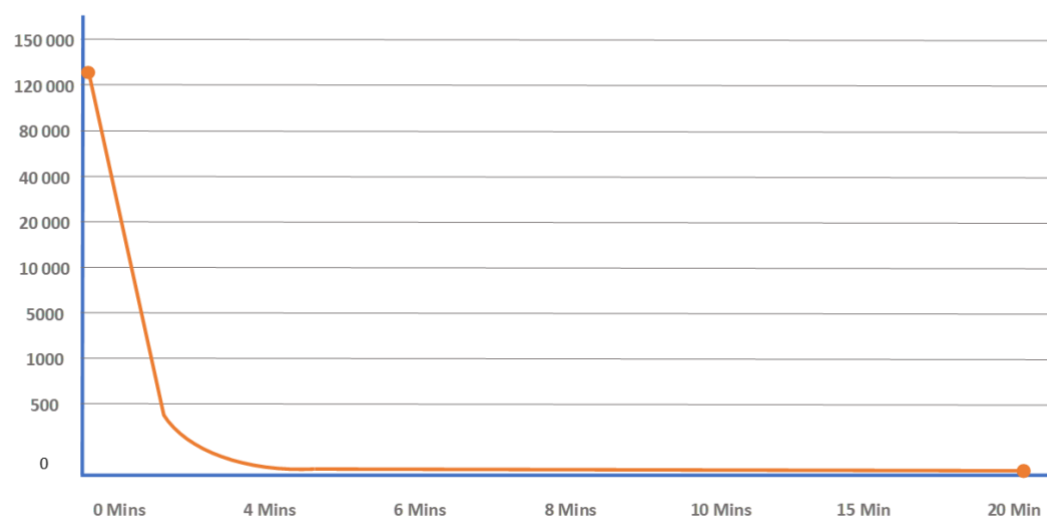
Total test time: 2 working days

Summary of results

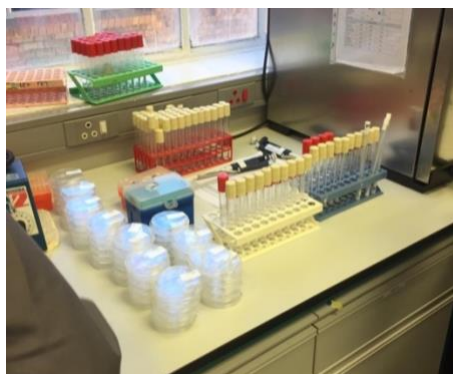
10 Minute Cycle in ROSS



20 Minute Cycle in ROSS



Samples being prepared for CFU counts



Conclusion

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Remaining *Staphylococcus aureus* found on test samples after the ROSS process were < 10 CFU and or no growth. Both the 10 and 20 minute ROSS cycle eliminated 99.9% of the bacteria load applied. The analysis also concludes that there was < 10 CFU and or no growth at both 4 minutes and two minutes regardless of the bacterial load at 2x and 10x the average load found on untreated liners worn by the amputee pool tested.

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Certificate Of Analysis

NO:	MC2022-03-24-007	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	5
	South Africa	Sample Identification:	RUN 5
Contact:	Barry Geer	Date Received:	24-Mar-2022
Order No:	Barry Geer	Date Issued:	29-Mar-2022

		Analysis	Swab/Total Plate Count (CFU/area swabbed)
		Method	MFC 8
		Unit	CFU/area swabbed
Lab No	Sample ID	Test Date	Result
MCG9574	81 Sterile	2022-03-24	<10
MCG9575	82 0 mins 10k	2022-03-24	2900
MCG9576	83 4mins 10k	2022-03-24	<10
MCG9577	84 8mins 10k	2022-03-24	<10
MCG9578	85 10mins 10k	2022-03-24	<10

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CFU = Colony Forming Unit
TNTC = Too Numerous to Count
ND = Not Detected
<10 = No Growth



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NO:	MC2022-03-24-008	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	5
	South Africa	Sample Identification:	RUN 6
Contact:	Barry Geer	Date Received:	24-Mar-2022
Order No:	Barry Geer	Date Issued:	29-Mar-2022

		Analysis	Swab/Total Plate Count (CFU/area swabbed)
		Method	MFC 8
		Unit	CFU/area swabbed
Lab No	Sample ID	Test Date	Result
MCG9579	86 Sterile	2022-03-24	<10
MCG9580	87 0 mins 10k	2022-03-24	11 500
MCG9581	88 4mins 10k	2022-03-24	<10
MCG9582	89 8 mins 10k	2022-03-24	<10
MCG9583	90 10 mins 10k	2022-03-24	<10

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NO:	MC2022-03-24-010	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	5
	South Africa	Sample Identification:	RUN 9
Contact:	Barry Geer	Date Received:	24-Mar-2022
Order No:	Barry Geer	Date Issued:	29-Mar-2022

Lab No	Sample ID	Test Date	Result
MCG9589	101 Sterile	2022-03-24	<10
MCG9590	102 0min 10K	2022-03-24	162 000
MCG9591	103 4min 10K	2022-03-24	<10
MCG9592	104 8min 10K	2022-03-24	<10
MCG9593	105 10min 10K	2022-03-24	<10

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NO:	MC2022-03-24-011	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	5
	South Africa	Sample Identification:	RUN 8
Contact:	Barry Geer	Date Received:	24-Mar-2022
Order No:	Barry Geer	Date Issued:	29-Mar-2022

Lab No	Sample ID	Test Date	Result
MCG9594	96 Sterile	2022-03-24	<10
MCG9595	97 0 mins 100k	2022-03-24	110 000
MCG9596	98 4 mins 100k	2022-03-24	<10
MCG9597	99 8 mins 100k	2022-03-24	<10
MCG9598	100 10 mins 100k	2022-03-24	<10

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NO:	MC2022-03-24-012	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	5
	South Africa	Sample Identification:	RUN 10
Contact:	Barry Geer	Date Received:	24-Mar-2022
Order No:	Barry Geer	Date Issued:	29-Mar-2022

		Analysis	Swab/Total Plate Count (CFU/area swabbed)
		Method	MFC 8
		Unit	CFU/area swabbed
Lab No	Sample ID	Test Date	Result
MCG9599	106 Sterile	2022-03-24	<10
MCG9600	107 0min 10K	2022-03-24	12 400
MCG9601	108 4min 10K	2022-03-24	<10
MCG9602	109 8min 10K	2022-03-24	<10
MCG9603	110 10min 10K	2022-03-24	<10

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NO:	MC2022-03-31-004	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	7
	South Africa	Sample Identification:	Run 1A
Contact:	Barry Geer	Date Received:	31-Mar-2022
Order No:	Barry Geer 1	Date Issued:	05-Apr-2022

		Analysis	Swab/Total Plate Count (CFU/area swabbed)
		Method	MFC 8
		Unit	CFU/area swabbed
Lab No	Sample ID	Test Date	Result
MCH0118	1 Sterile	2022-03-31	<10
MCH0119	2 100k Load	2022-03-31	221 000
MCH0120	3 100k 2 mins	2022-03-31	<10
MCH0121	4 100k 4 mins	2022-03-31	<10
MCH0122	5 100k 6 mins	2022-03-31	<10
MCH0123	6 100k 8 mins	2022-03-31	<10
MCH0124	7 100k 10 mins	2022-03-31	<10

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NO:	MC2022-03-31-005	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	7
	South Africa	Sample Identification:	Various Swabs
Contact:	Barry Geer	Date Received:	31-Mar-2022
Order No:	Run 2A	Date Issued:	05-Apr-2022

Lab No	Sample ID	Test Date	Result
MCH0125	8 Sterile	2022-03-31	<10
MCH0126	9 100k Load	2022-03-31	247 000
MCH0127	10 100k 2 mins	2022-03-31	<10
MCH0128	11 100k 4 mins	2022-03-31	<10
MCH0129	12 100k 6 mins	2022-03-31	<10
MCH0130	13 100k 8 mins	2022-03-31	<10
MCH0131	14 100k 10 mins	2022-03-31	<10

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NO:	MC2022-03-31-006	Sample Description:	Various Swabs
Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	7
	South Africa	Sample Identification:	Run 3A
Contact:	Barry Geer	Date Received:	31-Mar-2022
Order No:	Barry Geer 1	Date Issued:	05-Apr-2022

		Analysis	Swab/Total Plate Count (CFU/area swabbed)
		Method	MFC 8
		Unit	CFU/area swabbed
Lab No	Sample ID	Test Date	Result
MCH0132	15 Sterile	2022-03-31	<10
MCH0133	16 100k Load	2022-03-31	219 000
MCH0134	17 100k 2 mins	2022-03-31	<10
MCH0135	18 100k 4 mins	2022-03-31	<10
MCH0136	19 100k 6 mins	2022-03-31	<10
MCH0137	20 100k 8 mins	2022-03-31	<10
MCH0138	21 100k 10 mins	2022-03-31	<10

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Customer:	TRIOX INNOVATION LLC	Sample Condition:	Chilled
Address:	5140 Moundview Drive Red Wing MN55066	No of Samples	7
	South Africa	Sample Identification:	Run 4A
Contact:	Barry Geer	Date Received:	31-Mar-2022
Order No:	Barry Geer 1	Date Issued:	05-Apr-2022

		Analysis	Swab/Total Plate Count (CFU/area swabbed)
		Method	MFC 8
		Unit	CFU/area swabbed
Lab No	Sample ID	Test Date	Result
MCH0139	22 Sterile	2022-03-31	<10
MCH0140	23 Load 100k	2022-03-31	197 000
MCH0141	24 100k 2 mins	2022-03-31	<10
MCH0142	25 100k 4 mins	2022-03-31	<10
MCH0143	26 100k 6 mins	2022-03-31	<10
MCH0144	27 100k 8 mins	2022-03-31	<10
MCH0145	28 100k 10 mins	2022-03-31	<10

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