Microbiological testing

I. Antibacterial activity

Testing of Anolyte against 2 bacterial isolates from the utarus of a mare **undertaken by Capital Diagnostics, Scotland, UK**.

S zooepidemicus

Bacterial counts

	Exposure time				
Dilution	**30 s**	**1 min**	**5 min**	**10min**	**20 min**
10^2	0	0	0	0	0
10^3	0	0	0	0	0
10^4	0	0	0	0	0
10^5	0	0	0	0	0

Total viable count of inoculum > 5000 cfu/ml

E coli

Bacterial counts

	Exposure time				
Dilution	**30 s**	**1 min**	**5 min**	**10min**	**20 min**
10^2	0	0	0	0	0
10^3	0	0	0	0	0
10^4	0	0	0	0	0
10^5	0	0	0	0	0

Total viable count of inoculum > 5000 cfu/ml

II. Antibacterial activity

Undertaken by Department of Laboratory Medicine and second department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan
Anolyte microbial activity was tested against methicillin-sensitive Staphylococcus aureus, Staphylococcus epermidis, Serratia marcencens, Escheria coli, Pseudomonas auruginosa and Burkholderia cepacia which are important pathagenes.
The bactericidal properties of Anolyte were evaluated with three conventional disinfectants, including 0.1% chlorhexidine (Herbitane solution, ICI-pharma, Osaka, Japan), 0.02% povidine iodine (Isodine solution, Meiji Seika, Tokyo) and 80% ethanol (ethanol for disinfection, Maruisha Pharmaseutical Co. Ltd, Osaka). The selected concentrations represent those commonly used in solutions prepared for handwashing. All disinfectant solutions were mixed with sterile distilled water at the time of their use. Sterile distilled water was used as a control. The results
are summarised in Table 1 and Table 2.

Table 1. Bactericidal effect of Anolyte inocolum 1.7 x 10^4 cfu/mL

Bacteria	Disinfectant	10 s	60 s	180 s
MSSA methicillin sensitive Staphylococcus aureus	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 >500 >500	0 0 0 >500 >500
MRSA methicillin resistant Staphylococcus aureus	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 >500 >500	0 0 0 >500 >500
Staphylococcus epedermis	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 >500 >500	0 0 0 0 >500
Pseudomonas aeruginosa	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 41 >500	0 0 0 0 >500	0 0 0 0 >500
Escheria coli	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 0 >500	0 0 0 0 >500	0 0 0 0 >500
Serratia marcencens	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 27 >500	0 0 0 0 >500
Burkholderia cepacia	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 >500 >500	0 0 0 >500 >500

Table 2. Bactericidal effect of Anolyte inocolum 1.7 x 10^6 cfu/mL

Bacteria	Disinfectant	10 s	60 s	180 s
MSSA methicillin sensitive Staphylococcus aureus	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 8 0 >500 >500	0 0 0 >500 >500	0 0 0 >500 >500
MRSA methicillin resistant Staphylococcus aureus	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 15 0 >500 >500	0 0 0 >500 >500	0 0 0 >500 >500
Staphylococcus epedermis	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 >500 >500	0 0 0 >500 >500
Pseudomonas aeruginosa	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 >500 >500	0 0 0 >500 >500
Escheria coli	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 71 0 >500 >500	0 0 0 1 >500	0 0 0 0 >500
Serratia marcencens	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	0 0 0 >500 >500	0 0 0 27 >500	0 0 0 0 >500
Burkholderia cepacia	Anolyte 0.02% Povidine iodine 80% Ethanol 0.1% chlorhexidine Control: distilled water	3 >500 0 >500 >500	0 237 0 >500 >500	0 0 0 >500 >500

Conclusion: The number of bacteria was reduced below detection limit following incubation in Anolyte for 10s. The bactericidal activity of Anolyte was similar to that of 80% ethanol, but superior to that of 0.1 chlorhexidine and 0.02% povidine iodine. We conclude that Anolyte is a low cost but powerful disinfectant.

III. Sporicidal activity

Undertaken by Hospital Infection Research Laboratory, City Hospital NHSTrust, Birmmingham, UK.

Log_{10} spores remaining after exposure to Anolyte or 2% glutaraldehyde

Contact time	Anolyte	2% glutaraldehyde
Pre disinfection challenge	7.76	7.76
1 min	4.84	7.63
2 min	2.34	7.60
5min	1.30	7.46
10 min	0	7.19
20 min	0	6.87
30 min	0	6.34
1 hour	0	2.75
2 hours	0	0

Conclusions

This study shows that Anolyte (ORP > 1100mV and pH 2.0-3.5) generated using equipment produced by the Envirolyte Industries International Ltd. was highly effective as a sporicidal agent. A 6 log_{10} reduction in test spores was achieved with freshly generated solution in 5 minutes. This is far more rapid than the widely used 2% glutaraldehyde.