

Test Conducted at the

THE UNIVERSITY OF NEW SOUTH WALES • SYDNEY • AUSTRALIA

UNSW

UNSW

By:  
Professor Brett Neilan

**“Oxygen Spray-O2” a product from “Reach for Life”**

**Phase 1: Antimicrobial activity of the liquid test product against common skin-colonizing microorganisms.**

#### **Aim**

To evaluate the inhibition of microbial growth and/or microbial death elicited by Oxygen Spray-O2 (OSO2) in its liquid form, against the common skin colonizing microorganisms (bacteria and fungi), *Staphylococcus aureus*, *Streptococcus pyogenes*, *Candida albicans* and *Micrococcus luteus*.

#### **Methods**

- 1) Ten millilitres of nutrient media were inoculated with a loop-full of each test organism and incubated overnight to obtain a dense culture (~ 1 million cells/mL).
- 2) Six, fifty millilitre sub-cultures of each organism were prepared (~ 5000 cells/mL).
- 3) The test product was added to the sub-cultures (in duplicate) at 0, 10 or 50% vol/vol.

4) One hundred microlitre aliquots were removed from each duplicate sub-culture following 5 and 30 minutes incubation with the test product. Aliquots were inoculated onto nutrient agar plates and incubated overnight.

5) Colony forming units (cfu) were recorded for each plate the following morning.

## Results

The results of the experiment are presented in Table 1. OSO2 inhibited most of the test organisms in a dose dependant fashion, with the higher dosage (50% OSO2) inhibiting all test organisms by >95%. The extended incubation time (30 min) did not significantly increase the inhibitory effects of the product.

*S. aureus* and *S. pyogenes* were not inhibited by the low dosage (10%) of OSO2, however, the higher dose (50%) resulted in 99-100% inhibition of *S. aureus*, and total inhibition of *S. pyogenes*.

Low dosage with OSO2 inhibited *C. albicans* by >97%, while the higher dose resulted in greater than 99% inhibition of fungal growth.

Low dosage with OSO2 inhibited *M. luteus* by ~54%, while the higher dose resulted in >95% inhibition of bacterial growth. The extended incubation time (30 min) combined with a high dosage of the product, resulted in total inhibition of *M. luteus* cultures.

**Table 1. Inhibition of microbial growth by OSO2\***

OSO2 dose	<i>S. aureus</i>		<i>S. pyogenes</i>		<i>C. albicans</i>		<i>M. luteus</i>	
	5 min	30 min	5 min	30 min	5 min	30 min	5 min	30 min
10%	0	0	0	0	97	>99	67	40
50%	100	>99	100	100	>99	>99	95	100

\*0 corresponds to no inhibition, 100 corresponds to complete inhibition.

## Conclusions

OSO<sub>2</sub> significantly inhibited the growth of all microbial organisms when administered to test cultures at 50% of the total culture volume. *M. luteus* displayed the highest sensitivity to low dosage with OSO<sub>2</sub>, while *S. aureus* and *S. pyogenes* were completely resistant. While the administered dose of OSO<sub>2</sub> appeared to be an important factor for determining the degree of microbial inhibition, extending the incubation period from 5 to 30 min had little effect in most cases.

## Additional Notes

*Staphylococcus aureus* (commonly called **staph infection**) is a spherical bacterium, frequently living on the skin or in the nose of a healthy person, that can cause illnesses ranging from minor skin infections (such as pimples, boils, and cellulitis) and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, Toxic shock syndrome (TSS), and septicemia. It was discovered in Aberdeen in 1880 by the Scottish surgeon Sir Alexander Ogston in pus from surgical abscesses<sup>[1]</sup>. Each year some 500,000 patients in American hospitals contract a staphylococcal infection. It is abbreviated to *S. aureus* or sometimes referred to as *Staph aureus* in medical literature, and should not be confused with the somewhat similarly named streptococci which are also medically important.

*Micrococcus luteus* is a gram positive spherical saprotrophic bacterium that belongs to the Micrococcaceae bacterial family. An obligate aerobe, *M. luteus* is found in soil, dust, water and air, and as part of the normal flora of the mammalian skin. The bacterium also colonizes the human mouth, mucosae, oropharynx and upper respiratory tract.

Although *M. luteus* is non-pathogenic and usually regarded as a contaminant, it should be considered as an emerging nosocomial pathogen in immuno compromised patients. *M. luteus* is relatively resistant to reduced water potential and can tolerate drying and high salt concentrations fairly well.

*M. luteus* forms yellow colonies on nutrient agar.

*Description and Significance* *M. luteus* can be found in many places such as the human skin, water, dust, and soil. *Micrococcus* is generally thought of as harmless bacterium,

but there have been rare cases of Micrococcus infections in people with compromised immune systems, as occurs with HIV patients.

*Candida albicans* is a diploid sexual fungus (a form of yeast), and a causal agent of opportunistic oral and vaginal infections in humans. Systemic fungal infections (fungemias) have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). In addition, hospital-related infections in patients not previously considered at risk (e.g. patients on an intensive care unit) have become a cause of major health concern.

*C. albicans* is among the gut flora, the many organisms that live in the human mouth and gastrointestinal tract. Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis. Candidiasis is often observed in immunocompromised individuals such as HIV-positive patients. Candidiasis also may occur in the blood and in the genital tract. Candidiasis, also known as "thrush", is a common condition that is usually easily cured in people who are not immunocompromised. To infect host tissue, the usual unicellular yeast-like form of *Candida albicans* reacts to environmental cues and switches into an invasive, multicellular filamentous form