

# **Linen disinfection by OptiMaser Microwave System**

**Interim Report**

**Work Package  
WP3.1-WP3.3**

**Period of Report**

**1<sup>st</sup> July 2017 to 31<sup>st</sup> July 2017**

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# Interim Report

## Module-A. Disinfection efficacy of OptiMaser Microwave System.

### Objective-3. Linen disinfection by OptiMaser Microwave System.

#### Work Package- WP3.1-WP3.3

Linen soiled with body fluids is a possible source of infection. Contaminated linen is generated by hospitals, care homes, nursing homes and similar facilities, as well as in the home care setting; or at places where care of the sick and infirm is undertaken. Infected linen is defined as linen derived from known infectious patients, including those with HIV, hepatitis B, C and other infectious agents. The nature of laundry soiling depends on the source, eg in hospital and nursing home environments, is likely to include blood, wound exudates, sputum, saliva, sweat and urine, as well as vomit and faeces. It is also important to recognise that blood, body wastes such as urine may also serve as a potential source of infection. The nature of the soiling will determine how contaminated items are sorted and processed

The Recommended treatment applicable to the healthcare setting is:

- Linen in this category should not be sorted, other than in a red, water-soluble bag, which is then placed in an outer polyester or nylon carriage bag. Infected linen may be stored in different coloured bags.
- Inner bag should be removed from the outer bag only at the point of transfer to the washer-extractor, followed by the outer bag.

However, there are snags in the existing recommended procedures:

- The same wash temperature profile as used for used (soiled and foul) linen is thought sufficient to inactivate HIV, but the evidence is less certain for hepatitis B and other pathogens.
- The wash temperature, coupled with the dilution factor, should render linen safe to handle upon cycle completion but risk factor always remains.
- Current recommended treatment to ensure disinfection of heat labile linen at ~40°C, is insufficient to disinfect, and thus chemical alternatives are required. Addition of hypochlorite may be possible, but efficacy may be reduced by the presence of soiling, detergents and alkalis in the main wash. Disinfection with hypochlorite is only reliable if the linen can tolerate its addition and if sodium hypochlorite is added during the

penultimate rinse of the cycle. A final concentration of 150 ppm available chlorine must be achieved for a minimum of 5 minutes exposure time.

Thus, there is urgent need to develop methods for low temperature disinfection/decontamination of Linen for safe reuse or disposal. Microwave technology provides an efficient and quick solution to the linen disinfection process.

### **A. Microorganisms used in the study**

- Bacteria
  - *Staphylococcus aureus*
  - *Escherichia coli*
- Spores (*Bacillus subtilis*)
- Fungi
  - *Candida albicans*
  - *Candida glabrata*

### **B. Sample preparation**

1. Bacterial culture were grown in LB broth.
2. Fungal culture were grown in YPD.
3. *Bacillus subtilis* sporulation was performed in sporulation media (SM). All cultures were maintained at 37°C .
4. Viable cell density of at least 10<sup>10</sup> cells was maintained for bacteria and fungus.

### **C. Methodology**

1. 1ml aliquot of each culture harbouring 10<sup>10</sup> cells (as determined by total aerobic plate count or spectrophotometrically at 600 nm wavelength) was dispensed separately, in sterile 10 ml screw capped glass tubes. 100µl of this culture was plated in triplicates on suitable medium as untreated control.
2. For calculating the disinfection efficacy (Log reduction) total aerobic plate count procedure was be followed.
3. For determining the disinfection efficacy, each organism was subjected to OptiMaser treatment at three different temperatures (70°C, 85°C and 100°C) for three different time points (5 min, 10 min and 20 min) (**Fig.1**).
4. For statistical significance disinfection efficacy of OptiMaser for each time point was determined using three biological replicates for each organism.

5. After OptiMaser treatment serial dilutions were prepared from each sample (up to  $10^{-5}$ ) and 100  $\mu$ l sample of  $10^{-1}$ ,  $10^{-3}$ , and  $10^{-5}$  dilutions were plated in triplicates on suitable medium and incubated at 37°C for 48 h followed by calculation of log reduction (**Fig.1**).
6. Following OptiMaser cycles were used for this analysis:
  - Sprinkle time- 5 sec
  - Filament time- 90 sec
  - Microwave on time- 300- sec
  - Microwave Off time- 120 sec
  - Temp hold time- **300- 1200** sec
  - Cooling mode time- 400 sec
  - Water draining time- 0
  - Temp. Thresh hold- **70** °C
  - Printing numbers- 6

## Results

### Disinfection efficacy of Bacteria:

1. OptiMaser bacterial disinfection efficacy in Linen matrix was tested using representative gram negative (*Escherichia coli* K12) and gram positive bacteria (*Staphylococcus aureus*).
2. Bacteria was exposed to OptiMaser at 70°C for 5, 10 and 20min. It was observed that both gram negative and gram positive bacteria could be efficiently destroyed in Linen matrix by OptiMaser at 70°C for 10 min (Fig. 3).
3. Total aerobic plate count showed that 10 log reduction in bacterial counts was achieved via OptiMaser treatment at 70°C (hold time 20 min).

### Disinfection efficacy of Fungi:

1. OptiMaser Fungal disinfection efficacy was tested in Linen matrix using representative pathogenic fungi *Candida albicans* and *Candida glabrata*.
2. Fungi was exposed to OptiMaser at 70°C for 5, 10 and 20min. It was observed that both *Candida* species could be efficiently destroyed by OptiMaser even at 70°C for 5 min (Fig. 2). No fungal growth was observed at OptiMaser exposure time points of 10 min (Fig.3) and 20 min (Fig.4).

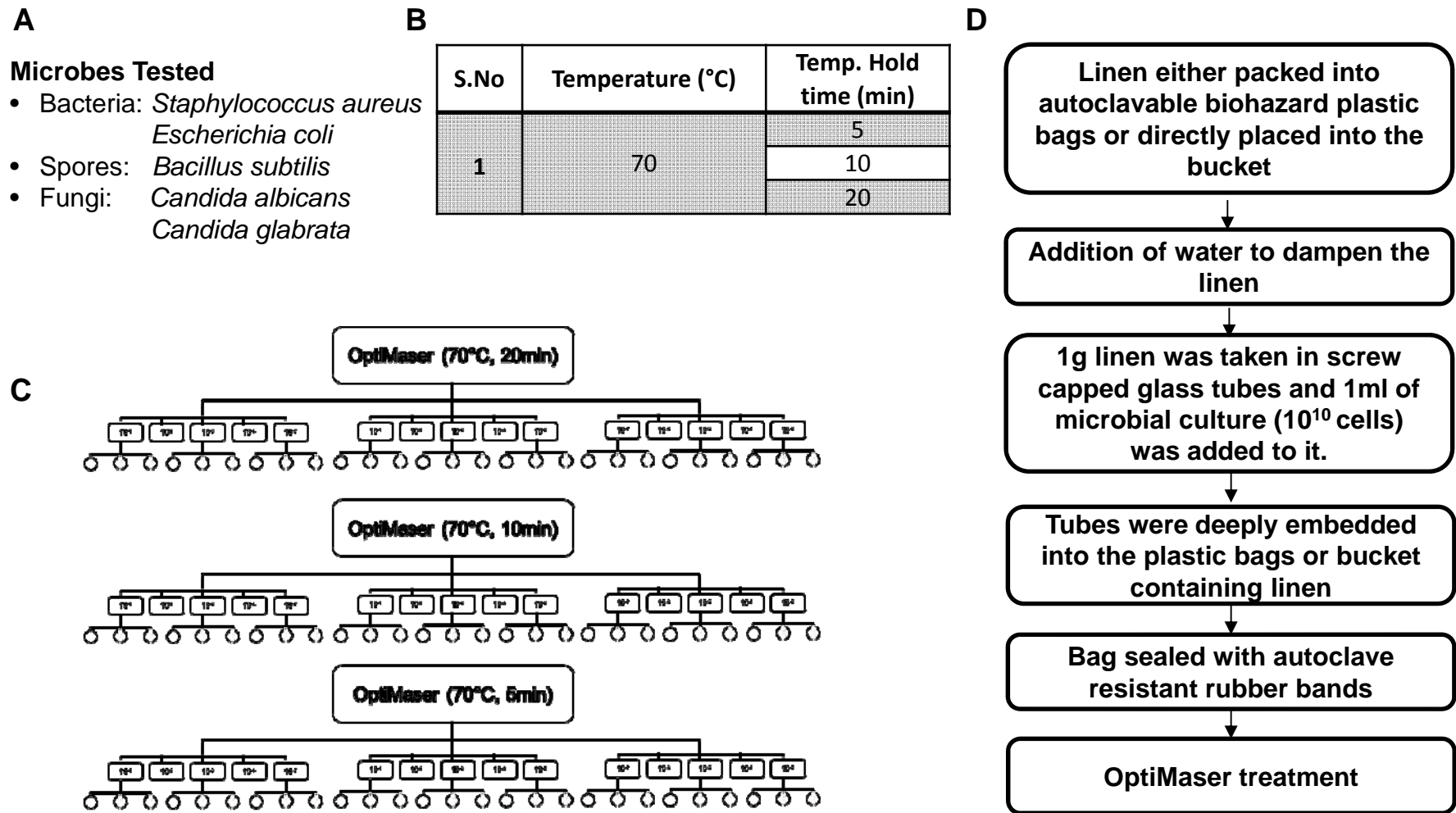
3. Total aerobic plate count showed that 10 log reduction in fungal counts was achieved via OptiMaser treatment at 70°C (hold time 5 min).

#### **Disinfection efficacy of Spores:**

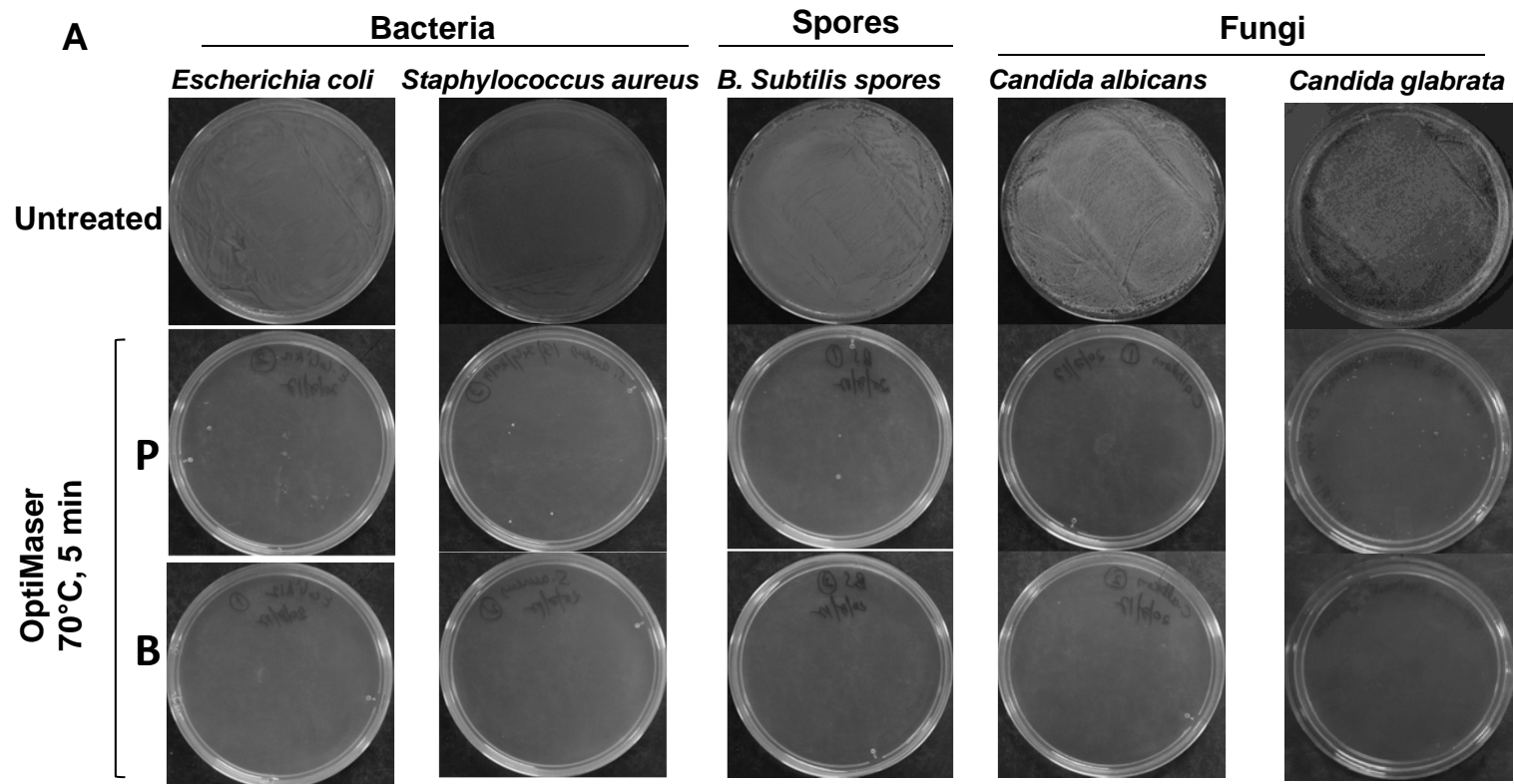
1. Optimiser's heat resistant bacteria spore disinfection efficacy was tested in Linen matrix using representative *Bacillus subtilis* spores.
2. It was observed that the disinfection efficacy of bacterial spores via OptiMaser was very good (8 log reduction) for 10 min exposure time at 70 °C (Fig. 3).
3. Total aerobic plate count method was used for the determination of disinfection efficacy.

#### **Conclusions**

**At least 8 log disinfection efficacy of representative bacteria, fungi and spores (in Linen Matrix) was achieved via OptiMaser treatment at 70°C with a hold time of 10 min.**



**Figure 1: Plan of study for determining the Linen disinfection efficacy of OptiMaser-30 (2.45 GHz and 1.5 kW).** (A) Disinfection efficacy of OptiMaser was determined in linen matrix using representative bacteria, fungi and spores as indicated in the figure. (B) As per recommendations disinfection efficacy was calculated at 70°C (three hold times). (C) For statistical significance three biological replicates of each samples were exposed to OptiMaser, following microwave exposure, samples were serially diluted and  $10^{-1}$ ,  $10^{-3}$  and  $10^{-5}$  dilutions were plated in triplicates on LB agar plates for total aerobic plate. (D) Brief methodology. Bacteria was grown in LB media at 37°C and  $10^{10}$  cells were exposed to OptiMaser. Fungus was grown in YPD (Yeast Extract-1%, Dextrose-2%, Peptone- 2%) broth at 37°C and  $10^{10}$  cells were exposed to OptiMaser. *Bacillus subtilis* spores were generated via magnesium sulphate method.

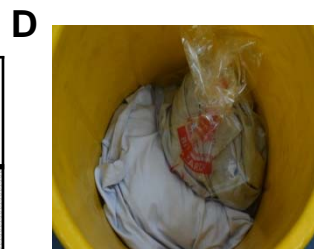


**B**

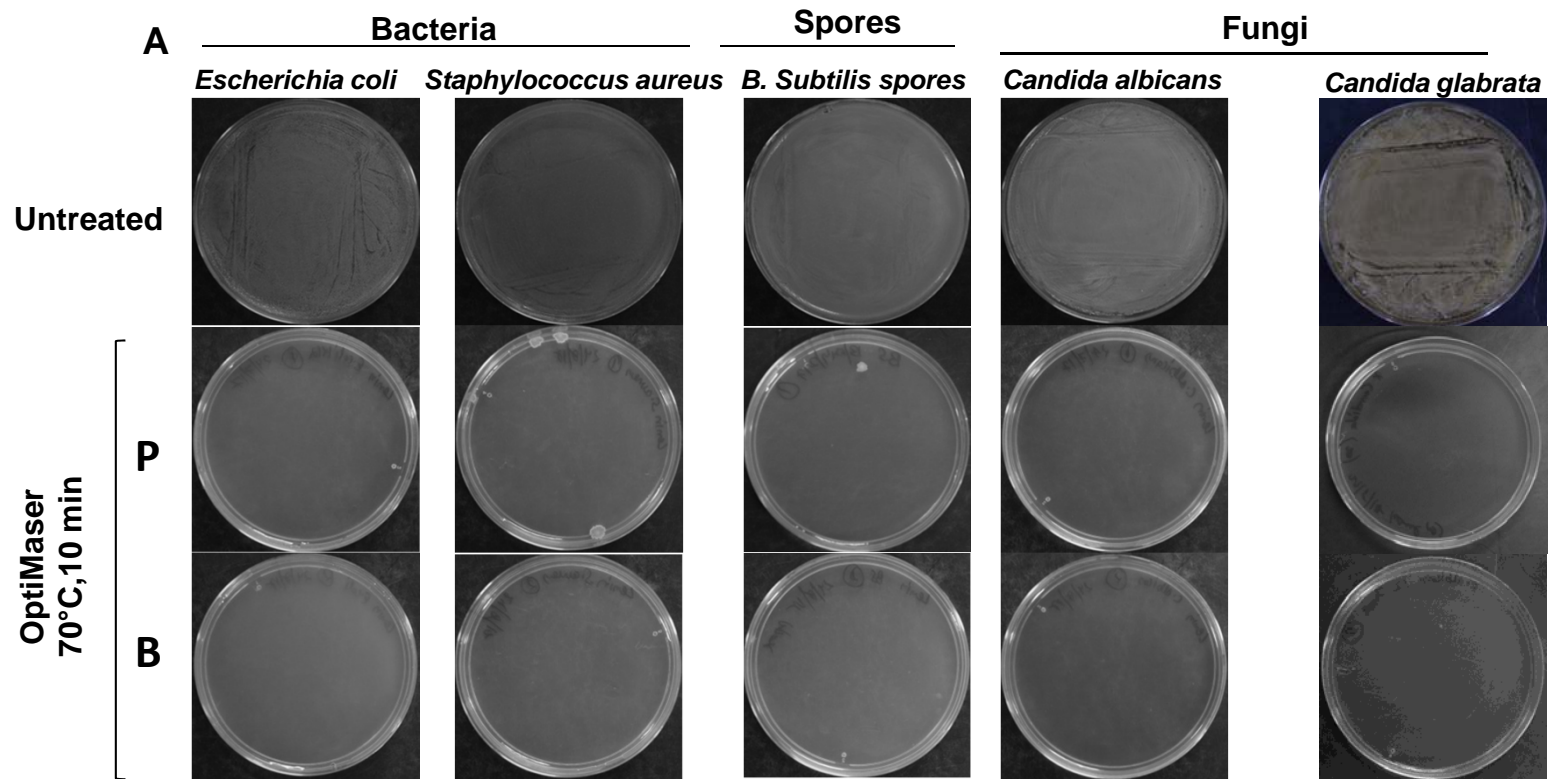
Temp. hold mode	Max . Temp. achieve	Total run time	Log reduction					
			Bacteria		Fungi		Spores	
70°C for 5 min	73°C	21 min	P	B	P	B	P	B
			8	8	10	10	6	8

**C**

Log Reduction	% Reduction of bacteria
10	99.99999999



**Figure 2. Linen Disinfection efficacy of OptiMaser at 100°C.** (A) Representative plates for total aerobic plate count. (B) OptiMaser parameter at a hold time of 5 min at 70°C and Log reduction in bacteria, Fungi and spores upon OptiMaser treatment. (C) Relation between log reduction and % reduction. (D) Arrangement of linen bags in OptiMaser bucket. Bags were arranged in two rows namely P(Polybag)and B(Direct Bucket).

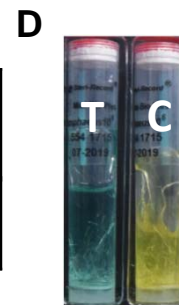


**B**

Temp. hold mode	Max . Temp. achieve	Total run time	Log reduction					
			Bacteria		Fungi		Spores	
			P	B	P	B	P	B
70°C for 10 min	82°C	24 min	10	10	10	10	8	10

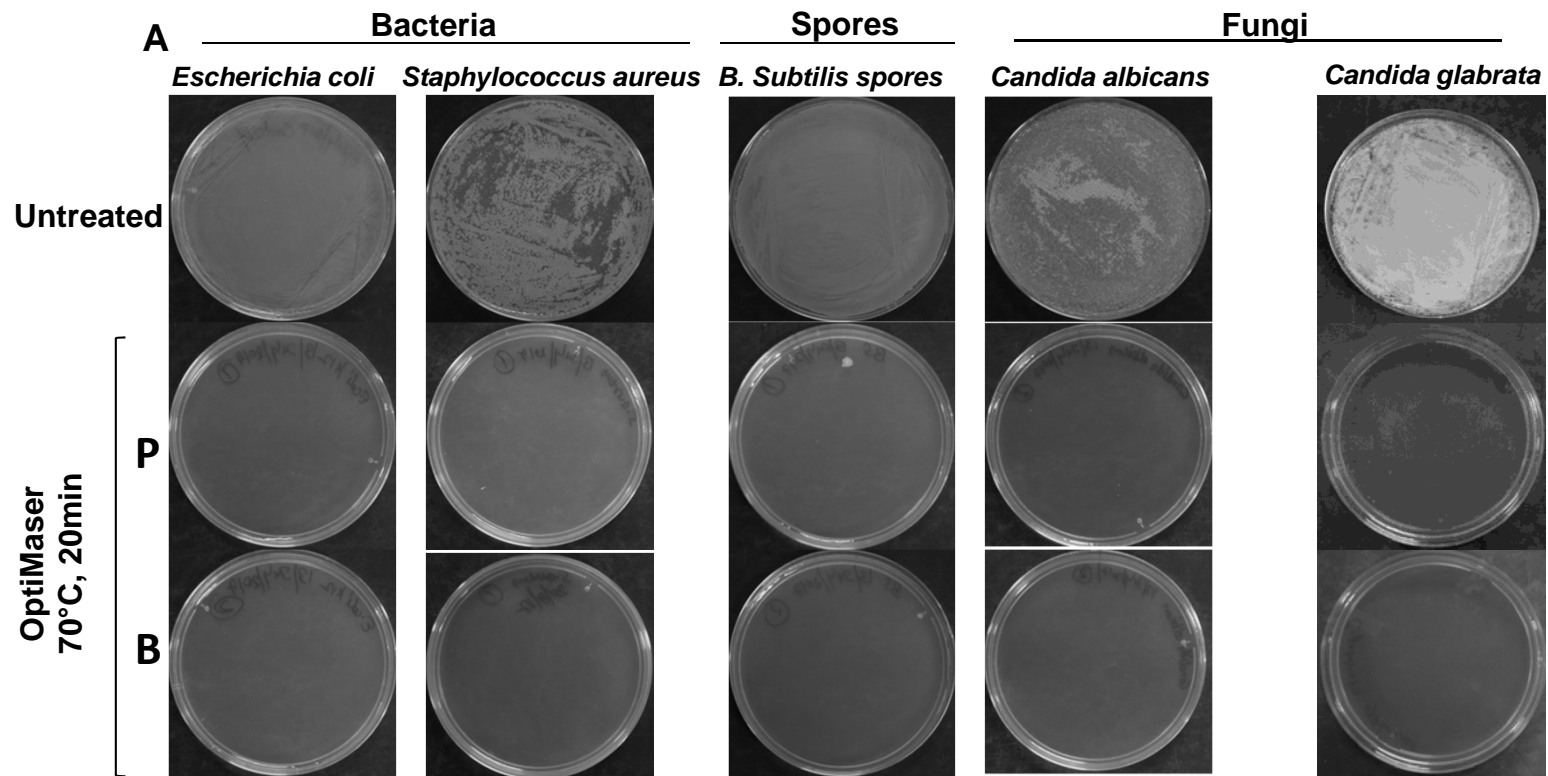
**C**

Log Reduction	% Reduction of bacteria
10	99.99999999



**Figure 3. Linen Disinfection efficacy of OptiMaser at 70°C.** (A) Representative plates for total aerobic plate count. (B) OptiMaser parameter at a hold time of 10 min at 70°C and Log reduction in bacteria, Fungi and spores upon OptiMaser treatment. (C) Relation between log reduction and % reduction. (D) Disinfection of  $10^6$  *B. atrophaeus* spores upon OptiMaser exposure at 70°C for 10min along with Lenin load. No change in colour of the medium in treated vial (T) after 48hrs of incubation at 37°C suggest complete disinfection. C, Untreated control. Lenin's were arranged in two rows namely P(Polybag) and B(Direct Bucket).





**B**

Temp. hold mode	Max . Temp. achieve	Total run time	Log reduction					
			Bacteria		Fungi		Spores	
			P	B	P	B	P	B
70°C for 20 min	96°C	31 min	10	10	10	10	10	10

**C**

Log Reduction	% Reduction of bacteria
10	99.99999999

**Figure 4. Linen Disinfection efficacy of OptiMaser at 100°C.** (A) Representative plates for total aerobic plate count. (B) OptiMaser parameter at a hold time of 20 min at 70°C and Log reduction in bacteria, Fungi and spores upon OptiMaser treatment. (C) Relation between log reduction and % reduction. Bags were arranged in two rows namely P(Polybag)and B(Direct Bucket).