



## Evaluation of the efficiency of Safetyhood™ as a barrier against microorganisms' crossing

### A) composition of Safetyhood filter

#### The filter of Safetyhood has the following characteristics:

1. An outer layer, which is composed by a medical, non woven tissue as a barrier for protecting the inner layers. It has the capacity for filtering substances larger than 2 microns (Figs. 1,2).
2. An active charcoal layer- 200 g/sq. M. - of which 114 g per sq/m is charcoal, which serves as a filter for smoke, odours, etc. Absorption capability of dust 14.6 g out of 16 g (91,25%) at airflow rate of 10 ft<sup>3</sup>/min(Figs. 3, 4)
3. A layer that filters substances from microns of size 0,3 or more. Air permeability (cfm/sf) 55. 0,3-0,5 micron 38,6%, 0,5-0,7 micron 68,8%, 0,7-1,0 micron 78,6%, 1,0 -2,0 micron 83,7% (Figs. 5, 6).
4. An inner layer which has the same characteristics of the outer membrane (Figs. 7, 8). Layers 1 and 4 have antiseptic properties because they are also consisted of 0,3% Cetylpyridinium Chloride (CPC) Two different types of Safetyhood (A and B) have been examined. The layers are the above described, but Safetyhood A filter is thinner than Safetyhood B. Consequently breathing is easier with Safetyhood A.

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### B) Evaluation of the antiseptic properties of layers 1 and 4

0,1 ml of an 8 hours broth culture (Tryptone Soya Broth) of the different microorganism species tested ***Staphylococcus cohnii* ATCC 35662**, ***Staphylococcus aureus* ATCC 6538**, ***Streptococcus pyogenes* ATCC 19615**, ***Enterococcus hirae* ATCC 10541**, ***Pseudomonas fluorescens* ATCC 49838**, ***Escherichia coli* ATCC 4157**, ***Salmonella enteritidis* ATCC 13076**, ***Serratia marcescens* ATCC 8100**, ***Proteus mirabilis* ATCC 7002**, ***Bacillus subtilis* spores ATCC 6633**, ***Bacillus clausii* spores Sonafi-Syntelabo Oto S.p.a.- Milan**, ***Bacillus stearothermophilus* spores ATCC 10149**, were spread on the surface of Tryptone Soya Agar Petri plate surface using glass spreaders. A fragment of 1cm<sup>2</sup> of layers 1 and 4 was put subsequently on the agar inoculated surface and plates were incubated for 24 hours at 36 ± 1 °C for all the micro organisms tested except for the ***Bacillus stearothermophilus*** that had to be cultured under the temperature of 56 ± 1 °C . The halo of growth inhibition was then evaluated.

### C) Evaluation of the efficiency of Safetyhood as a barrier against microorganisms' crossing

A bacterial or spore suspension of 1,5 – 7x10<sup>8</sup> UFC/ml of each of the following micro organisms (***Streptococcus pyogenes* ATCC 19615**, ***Staphylococcus cohnii* ATCC 35662**, ***Serratia marcescens* ATCC 8100**, ***Pseudomonas fluorescens* 49838**, ***Bacillus clausii* spores Sonafi-Syntelabo Oto S.p.a.- Milan**, ***Bacillus stearothermophilus* spores ATCC 10149**, ***Bacillus subtilis* spores ATCC 6633**) was aerosolised by means of a medical device for aerosol therapy. Fragments of Safetyhood filter, previously sterilized by gamma rays, were put into a stainless steel Seitz filter holder, sterilized in steam and plugged in an Erlenmeyer vacuum flask, connected to a vacuum pump, in order to aspirate the microorganisms' aerosol through the Safetyhood. As a control the same described apparatus was used, without the Safetyhood filter. The distance between the aerosol generator and the Seitz filter was about 25 cm. After ten minutes, aerosolisation and vacuum aspirations (0,4m<sup>3</sup>) were stopped.

In order to evaluate microorganisms' crossing through the apparatus, in the Erlenmeyer flask, 50 ml of a diluent solution (Tryptone sodium chloride) was then introduced in the Erlenmeyer flask, which was then subjected to a mechanical shaker. Both the content of Erlenmeyer and the diluent solution (50 ml), used two times to rinse the Erlenmeyer flask, were filtered through a membrane filtration apparatus. The filtration membrane is transferred to the surface of a Petri dish containing a suitable culture medium according to the species of microorganisms tested. The culture mediums used were:

1. Pseudomonas isolation agar (Difco) for *Pseudomonas fluorescens*
2. Chapman mannitol salt agar (Biomerieux) for *Staphylococcus cohnii*
3. Streptococcus selective medium (Oxoid) with 5% of defibrinated horse blood for *Streptococcus pyogenes*
4. Hektoen enteric agar (Biomerieux) for *Serratia marcescens*
5. Tryptone soya agar (Oxoid) for *Bacillus subtilis*, *Bacillus stearothermophilus* and *Bacillus clausii*. All Petri dishes were incubated at  $36 \pm 1^\circ\text{C}$  for 24-48 hours and the CFU development was evaluated. For *Bacillus stearothermophilus* the temperature of incubation was  $56 \pm 1^\circ\text{C}$ .

In order to detect low growing microorganisms, the culture time was extended to a week. No significant differences in the number of CFU were observed between the 24-48 hours culture and the one week culture.

In all experiences an identification with the API system of the developed CFU was done. The developed microorganisms were always the same, which have been previously aerosolised.

To evaluate the possible correlation, between the size and the capacity of crossing through the filter of the different tested bacteria and spores, droplets of bacteria or spore suspensions were spread over the surface of a cover glass. The different samples were all fixed for 3 hours at  $4^\circ\text{C}$  in a 3% glutaraldehyde solution in a 0.1 M phosphate buffer at pH 7.4. After several washings in phosphate buffer, the material was dehydrated by passing it through solutions with increasing concentrations of ethyl alcohol, in a propylene oxide solution and finally subjected to critical point drying.

The samples were then sputter coated with gold palladium (Edwards sputter coater S150) and examined under a scanning electron microscope (Cambridge Stereoscan S360). The dimensions of 50 bacteria or spores of each species were then measured at SEM.

## Results

### 1. Antiseptic activity of layers 1 and 4

The halos of inhibition growth for the tested microorganisms are shown in Table 1.

At the concentration of 0,3%, *Cetylpyridinium chloride* (which is present in layers 1 and 4) demonstrates a high effectiveness against the growth of *Bacillus* tested species, *B. stearothermophilus* and *B. clausii* and of *Enterococcus hirae*, demonstrates a good inhibition against the growth of *B. subtilis*, *Staphylococcus cohnii*, *Staphylococcus aureus*, *Streptococcus pyogenes* and results to have moderate effectiveness against the growth of *Proteus mirabilis*. On the contrary *Cetylpyridinium chloride* at the concentrations present in layer 1-4, doesn't seem to have any effects on the growth of *Escherichia coli*, *Salmonella enteritidis*, *Serratia marcescens* and *Pseudomonas fluorescens*.

### 2. Efficiency of Safetyhood as a barrier against microorganisms crossing

Our results are resumed in table 3 - 4. In our experimental aspiration apparatus the presence of Safetyhood filter strongly reduced the crossing of microorganisms and spores aerosolised in the air. In the case of aerosolization of vegetative forms of microorganisms the percentage (%) of reduction varies between 98,30% and 100%, whereas by aerosolising *Bacillus* spores results a likely lower percentage of reduction (from 94,25% to 99,12%). No evident correlation seems to exist between microorganisms' or spores' size and the capacity of crossing the filter of Safetyhood (compare Table 2 with Table 3 - 4 and Figs. 9-15).

It also seems to exist no difference between the capacity of penetration through the Safetyhood filter of spherical microorganisms (*Staphylococcus cohnii* and *Streptococcus pyogenes*) and rod shaped bacteria like *Pseudomonas fluorescens* and *Serratia marcescens*. It seems also very difficult to correlate the sensibility against the antibiotic (*Cetylpyridinium Chloride*) of different microorganisms tested with their capacity of crossing alive the different layers of the Safetyhood filter. We consider that the good barrier effect of Safetyhood against microorganisms is correlated to both the structure of different layers and the antibiotic, which is present in the outer and in the inner layers of the Safetyhood filter. In conclusion our results demonstrate that Safetyhood filter is a very efficient barrier against bacteria or spores' crossing.

**Table 1**

Halos of growth inhibition produced by Safetyhood layers 1 and 4.

Type of microorganism	Layer 1	Layer 4
<i>Escherichia coli</i>	0mm	0mm
<i>Salmonella enteritidis</i>	0mm	0mm
<i>Serratia marcescens</i>	0mm	0mm
<i>Proteus mirabilis</i>	1mm	1mm
<i>Pseudomonas fluorescens</i>	0mm	0mm
<i>Staphylococcus cohnii</i>	2mm	2mm
<i>Staphylococcus aureus</i>	3mm	2.9mm
<i>Streptococcus pyogenes</i>	2mm	2mm
<i>Enterococcus hirae</i>	10mm	11mm
<i>Bacillus subtilis</i>	2mm	2mm
<i>Bacillus clausii</i>	8mm	8mm
<i>Bacillus stearothermophilus</i>	8mm	9mm

**TABLE 2**

Size (mean and standard deviation) of different microorganisms tested; diameter for spherical bacteria, length and breadth for rod shape bacteria or spores.

Type of microorganism	Size in Micron
<i>Staphylococcus cohnii</i>	0.70 ± 0.08
<i>Streptococcus pyogenes</i>	0.80 ± 0.16
<i>Pseudomonas fluorescens</i>	1.50 ± 0.49 x 0.51 ± 0.03
<i>Serratia marcescens</i>	1.10 ± 0.60 x 0.37 ± 0.04
<i>Bacillus stearothermophilus</i> spores	0.86 ± 0.13 x 0.53 ± 0.04
<i>Bacillus clausii</i> spores	1.01 ± 0.13 x 0.57 ± 0.05
<i>Bacillus subtilis</i> spores	1.25 ± 0.15 x 0.58 ± 0.07

**TABLE 3**

Number of developed CFU, in the filtration apparatus, and % reduction with or without Safetyhood filters after aerosolization of different bacteria.

Test Microorganism	No of CFU		Type of Safetyhood	Reduction with SAFETYHOOD
	with SAFETYHOOD	without SAFETYHOOD		
<i>Staphylococcus cohnii</i>	with SAFETYHOOD	0	A	100%
	without SAFETYHOOD	516		
	with SAFETYHOOD	0	B	100%
	without SAFETYHOOD	8000		
	with SAFETYHOOD	15	A	99.770%
	without SAFETYHOOD	6300		

Test Microorganism	No of CFU		Type of Safetyhood	Reduction with SAFETYHOOD
	with SAFETYHOOD	without SAFETYHOOD		
<i>Streptococcus pyogenes</i>	with SAFETYHOOD	0	B	100%
	without SAFETYHOOD	233		
	with SAFETYHOOD	0	B	100%
	without SAFETYHOOD	840		
	with SAFETYHOOD	2	A	98.88%
	without SAFETYHOOD	1650		

Test Microorganism	No of CFU		Type of Safetyhood	Reduction with SAFETYHOOD
	with SAFETYHOOD	without SAFETYHOOD		
<i>Serratia marcescens</i>	with SAFETYHOOD	5	A	99.4%
	without SAFETYHOOD	8004		
	with SAFETYHOOD	0	B	100%
	without SAFETYHOOD	304		
	with SAFETYHOOD	2	A	99.95%
	without SAFETYHOOD	4000		

Test Microorganism	No of CFU		Type of Safetyhood	Reduction with SAFETYHOOD
	with SAFETYHOOD	without SAFETYHOOD		
<i>Pseudomonas fluorescens</i>	with SAFETYHOOD	3	A	98.3%
	without SAFETYHOOD	176		
	with SAFETYHOOD	9	B	98.33%
	without SAFETYHOOD	538		
	with SAFETYHOOD	3	A	99,65 %
	without SAFETYHOOD	850		

**TABLE 4**

Number of developed CFU, in the filtration apparatus, and % reduction with or without Safetyhood filters after aerosolization of different bacteria

Test Microorganism	No of CFU		Type of Safetyhood	Reduction with SAFETYHOOD
	with SAFETYHOOD	without SAFETYHOOD		
<i>Bacillus clausii</i> (spores)	with SAFETYHOOD	95	A	97.93%
	without SAFETYHOOD	4440		
	with SAFETYHOOD	95	B	97.64%
	without SAFETYHOOD	4025		
	with SAFETYHOOD	17	B	97,50 %
	without SAFETYHOOD	680		
	with SAFETYHOOD	46	B	96,00 %
	without SAFETYHOOD	1148		

Test Microorganism	No of CFU		Type of Safetyhood	Reduction with SAFETYHOOD
	with SAFETYHOOD	without SAFETYHOOD		
<i>Bacillus stearothermophilus</i> (spores)	with SAFETYHOOD	3	A	99.12%
	without SAFETYHOOD	340		
	with SAFETYHOOD	9	B	97.84%
	without SAFETYHOOD	411		

Test Microorganism	No of CFU		Type of Safetyhood	Reduction with SAFETYHOOD
	with SAFETYHOOD	without SAFETYHOOD		
<i>Bacillus subtilis</i> (spores)	with SAFETYHOOD	30	B	94.25%
	without SAFETYHOOD	523		
	with SAFETYHOOD	4	B	97.45%
	without SAFETYHOOD	157		
	with SAFETYHOOD	18	A	96.37 %
without SAFETYHOOD				

	without SAFETYHOOD	496		
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## Pictures

**Figs 1-2** - Outer layer of the Safetyhood filter.

**Figs 3-4** - Active charcoal layer of Safetyhood filter. In fig 3 some micro fibres of the outer layer are sticking to the charcoal layer.

**Figs 5-6** - Intermediate layer of Safetyhood filter.

**Figs 7-8** - Inner layer of Safetyhood filter.

**Fig 9** - Scanning electron micrograph of *Staphilococcus cohni*.

**Fig 10** - Scanning electron micrograph of *Streptococcus pyogenes*.

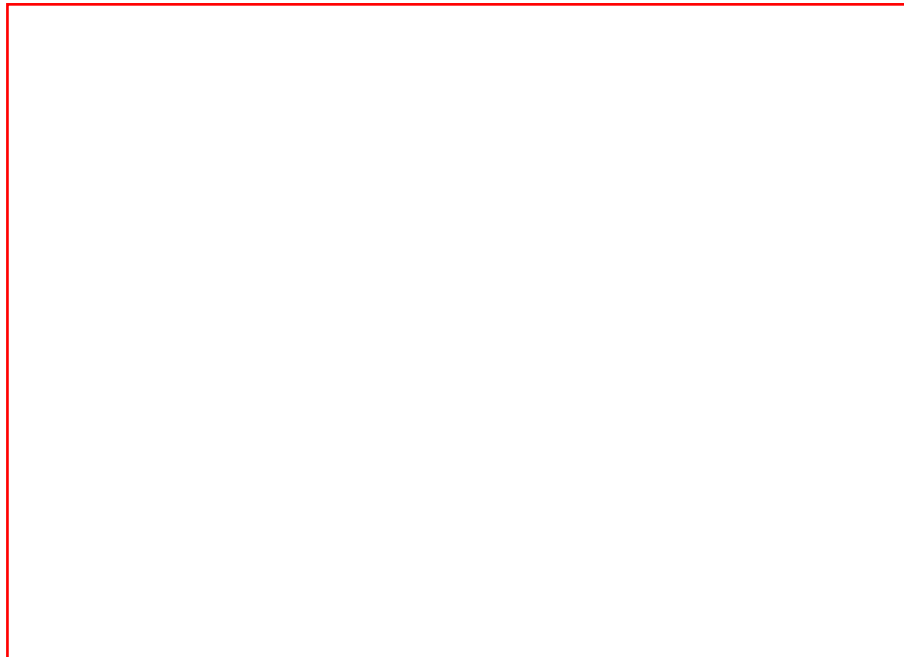
**Fig 11** - Scanning electron micrograph of *Serratia marcescens*.

**Fig 12** - Scanning electron micrograph of *Pseudomonas fluorescens*.

**Fig 13** - Scanning electron micrograph of *Bacillus stearothermophilus* spores.

**Fig 14** - Scanning electron micrograph of *Bacillus clausii* spores.

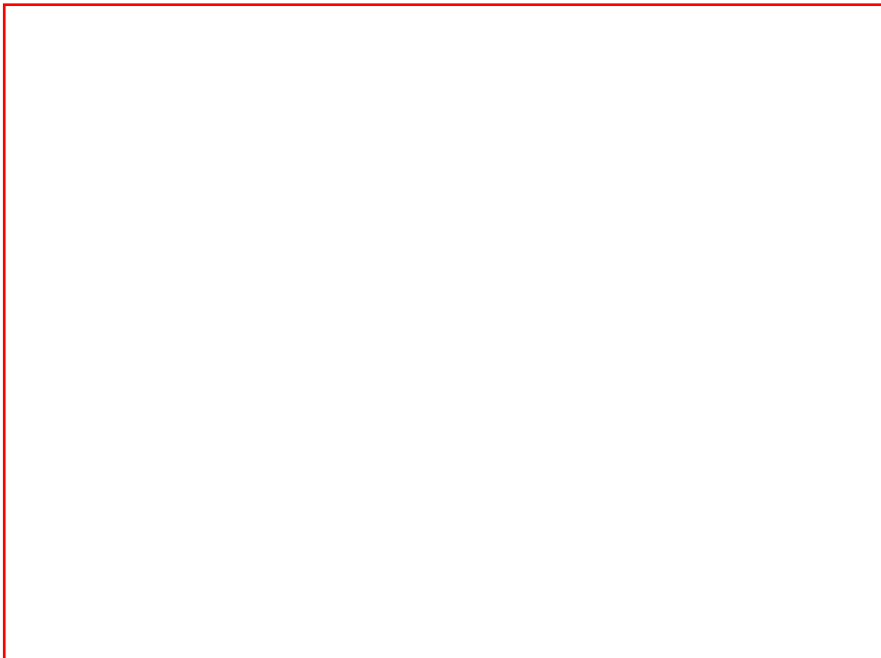
**Fig 15** - Scanning electron micrograph of *Bacillus subtilis* spores



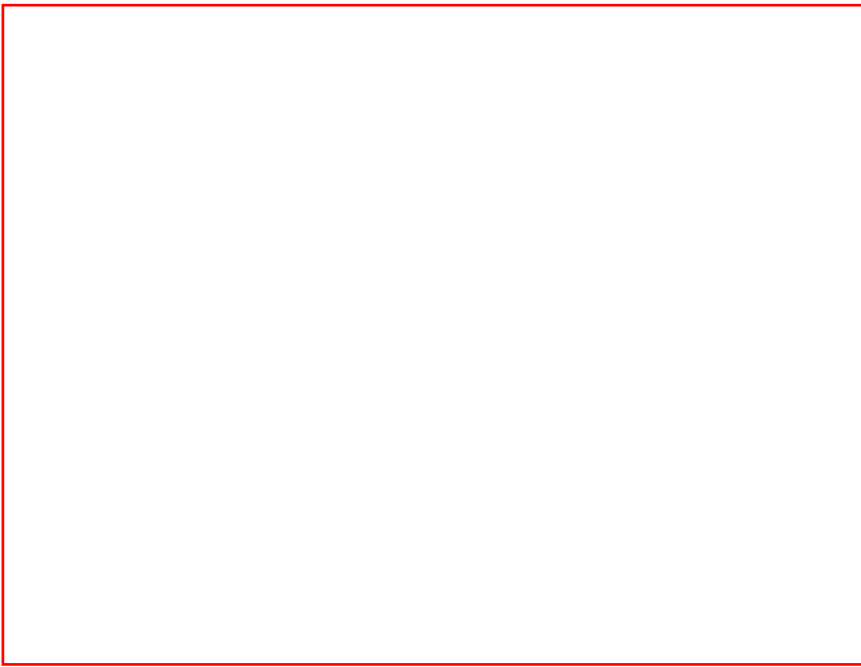
**Outer layer of the Safetyhood filter.**



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**Active charcoal layer of Safetyhood filter. In fig 3 some micro fibres of the outer layer are sticking to the charcoal layer**



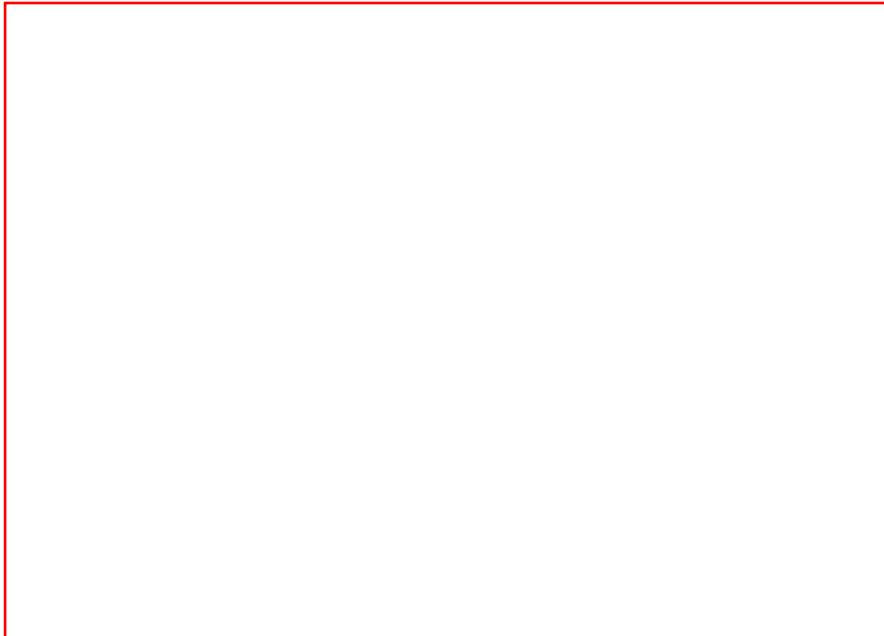
**Active charcoal layer of Safetyhood filter. In fig 3 some micro fibres of the outer layer are sticking to the charcoal layer**



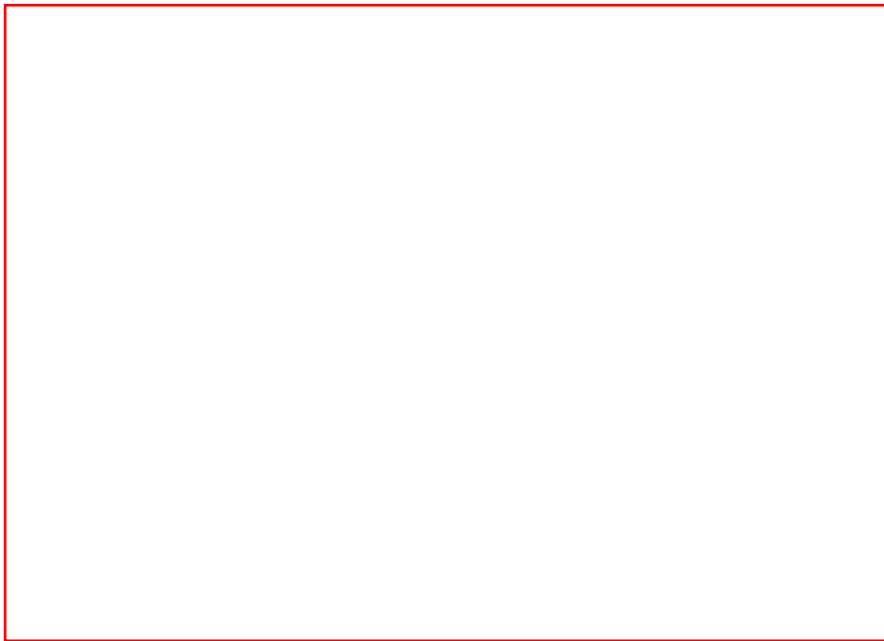
**Intermediate layer of Safetyhood filter**



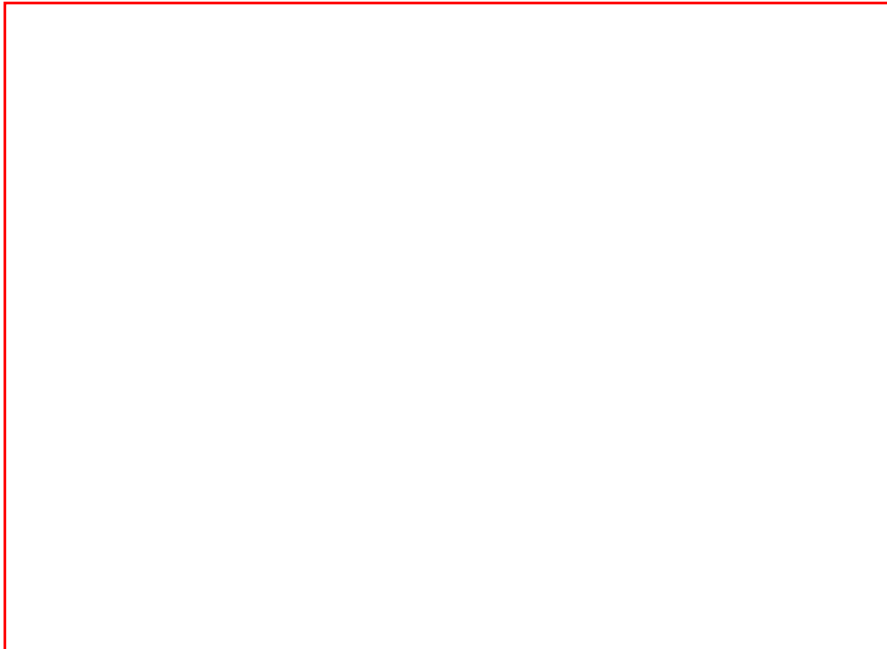
**Intermediate layer of Safetyhood filter**



**Inner layer of Safetyhood filter**



**Inner layer of Safetyhood filter**



Scanning electron micrograph of *Streptococcus pyogenes*



Scanning electron micrograph of *Serratia marcescens*



Scanning electron micrograph of *Pseudomonas fluorescens*



Scanning electron micrograph of *Pseudomonas fluorescens*



Scanning electron micrograph of *Bacillus stearothermophilus* spores  
Scanning



Scanning electron micrograph of *Bacillus clausii* spores



Scanning electron micrograph of *Bacillus subtilis* spores

