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# Expert Report

## Testing of the fungistatic and bacteriostatic/bactericidal action of plasma cluster ions, generated by the plasma cluster generator 1 (from Sharp), in a test chamber under static test conditions

## 1. Objective

The growth inhibiting action of selected test microorganisms (fungi and bacteria) is to be tested under the influence of plasma cluster ions. The reference value must be determined in a test chamber of the same construction without a plasma cluster generator.

## 2. Material and methods

### 2.1. Fungi:

The fungistatic action of the plasma cluster ions on the germination capacity of fungal spores was tested by means of a modified ASTM C665 test (ASTM = American Society of Testing and Materials). Roughened beechwood spatulas (2 x 15 cm spatulas for medical use) were stored for 7 days in a desiccator above distilled water (approx. 95% relative humidity). The gravimetric determination of the set material moisture content of the beechwood spatula indicated a value of approx. 20% (w/w). 500 µl of a penicillium expansum spore suspension (7.8 x $10^9$ CFU's/ml) or Aspergillus fumigatus spore suspension (1.7 x $10^6$ CFU's/ml) or Cladosporium herbarum spore suspension (4.4 x $10^6$ CFU'S/ml) were then applied to the surface of the spatula. The spatulas were treated with plasma cluster ions for 9 and 14 days respectively in a closed test chamber at 22° - 23° C and a predetermined relative humidity of 83 – 93%. At the same time a reference sample without plasma cluster action was tested. For a quantitative microscopic evaluation of the inhibition of spore germination the fungal material was sampled by means of cellophane adhesive film at predetermined intervals. The material was then mounted on object carriers (colouring agent: 0.5% lactophenol blue). The following evaluation scoring was applied:

\+ = spores only
++ = germination (isolated hyphae)
+++ = hypha formation
++++ = formation of fructifying organs ("new spore chains").

### 2.2 Bacteria

The testing of the bacteriostatic and bactericidal action of plasma ions on the reproducibility of selected bacteria was carried out by the quantitative cultural method. The following bacteria were tested:
Pseudomonas aeruginosa (ATCC 15442) initial concentration = 9.68 x $10^9$ CFU's/ml )
Enterococcus faecium (ATCC 6057): initial concentration = 9.67 x $10^9$ CFU's/ml)
Staphylococcus epidermidis (ATCC 12228): initial concentration = 3.87 x $10^9$ CFU'S/ml)
10 µl of a 1:$10^6$ dilution of the above-mentioned initial bacteria concentrations, poured out on to the solid nutrient CASO agar (ø 85 mm) with a spatula, was used on each occasion. This was followed by plasma cluster ion treatment for 12 hours. In parallel with this the reference value was determined in the same test structure without plasma cluster ion action. The bacteria cultures were then incubated for 12 hours for 12 and 5 hours respectively for Pseudomonas aeruginosa, and at 37°C, and the colony counts then determined.

## 3. Results

### Fungi:

The beechwood spatulas coated with fungal spores were treated with and without plasma cluster ion field for 9 days at 22° - 23° C under a relative humidity of 83 – 93% in a closed test chamber.
Exception: penicillium expansum was treated for 14 days, the relative humidity being here set to 83 – 93% by means of distilled water after the 6 $^{th}$ day.

As can be seen from Tables 1 to 3, the fungi Penicillium expansum, Aspergillus fumigatus and Cladosporium herbarum are inhibited in their germinating capacity to

varying degrees by the plasma customer ion field In this case Cladosporium herbarum appears to be somewhat less inhibited than the other two fungus species in their germinating capacity.

**Bacteria:**
The vital bacteria Pseudomonas aeruginosa, Enterococcus faecium and Staphylococcus epidermidis applied to CASO agar on a spatula (germ counts: 989 CFU's/plate, 997 CFU's/plate and 387 CFU's/plate respectively) were inactivated or inhibited to varying degrees in their growth by the plasma cluster ion field (see Table 4 and Figures 1 – 3). After 12 hours of plasma cluster action only 1 CFU/plate could be demonstrated in the case of Pseudomonas aeruginosa and Enterococcus faecium. The inactivated bacteria Enterococcus faecium and Staphylococcus epidermidis could not be reproduced either on CASO agar at 37°C/12 hours incubation time after being stored in the closed condition for 5 days at room temperature (Ø 22°C), then cluster ion free for 3 days in the refrigerator at approx. 4°C. Compared to the control, Pseudomonas aeruginosa showed varied growth after 12 months of plasma cluster action , since the colony count was reduced from 968 to 794 CFU 's/plate and there was variable macroscopic morphology of the colonies. Microcolonies were observed which showed no variation in macroscopic morphology or total gem count, even after 8 days of storage (5 days at 22°C, then 3 days at 4°C), followed by incubation at 37°C for 12 hours.

## 4. Conclusions

The three different species of fungi (Penicillium expansum, Aspergillus fumigatus, Cladosporium herbarum were observed after 14 and 9 days of plasma cluster ion treatment respectively in a closed test chamber on the carrier material at a relative humidity of 83 – 93% and an air temperature of 22 to 23°C, a species-dependent growth inhibition was observed.
The bacteria Enterococcus faecium (ATCC 6057) and Staphylococcus epidermis (ATCC 1228) displayed a bactericidal action after 12 hours of plasma cluster ion action on the solid nutrient CASO agar (83 – 93% relative humidity and 22°/23°C). On the other hand a varied macroscopic morphology (microcolony formation) was observed in the case of Pseudomonas aeruginosa after 12 hours of plasma cluster action on CASO agar under the above-mentioned conditions, a morphology which remained irreversible even after storage for 8 days without plasma cluster action (5 days at 22°C/23°C and 3 days at 4°C) (Enclosed: Tables 1 – 4, Figs. 1 – 3).

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Enclosures

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**Table 1: Testing the action of the plasma cluster on the germinating capacity of Penicillium expansum spores according to the modified ASTM C 665 test**

**Cluster ion generator 1: Plasma cluster unit (from Sharp)**
**Initial concentration of the test germ: 7.8 x $10^9$ CFU's/ml Penicillium expansum spores in physiological common salt solution + 0.01% (w/w) Tween 80**
**Test carrier material: Beechwood spatula (roughened, 20% (w/w) material moisture content); 2 x 15 cm spatulas for the medical requirement**
**Application volume: 500 µl**
**Test chamber: Plastic box (22 x 20 x 15 cm)**
**Water reservoir: 350 ml distilled water in plastic box (13 x 11 x 4 cm) with perforated screen cover**
**Method of demonstration: Surface contact sampling with Tesafilm, coloration with lactophenol blue, microscopic determination**

| Serial no. | Date | With plasma cluster action Microscopic determination of the growth phases | | | | | | Without plasma cluster action Microscopic determination of the growth phases | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | 5th | 6th spatula | 1st | 2nd | 3rd | 4th | 5th | 6th spatula |
| 1 | 25.03.02 | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | 27.03.02 | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | 29.03.02 | + | + | + | + | + | + | + | + | + | + | + | + |
| 4 | 31.03.02*) | + | + | + | + | + | + | + | + | + | + | + | + |
| 5 | 02.04.02 | + | + | + | + | + | + | ++++ | +++ | ++ | ++++ | ++ | ++ |
| 6 | 06.04.02 | + | + | + | + | ++++ | ++++ | ++++ | ++++ | ++ | ++++ | +++ | ++++ |
| 7 | 08.04.02 | + | + | + | + | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |

**Evaluation:**
+ Spores only
++ Germination (isolated hyphae)
+++ Hypha formation
++++ Formation of fructifying organs ("new spore chains")

*) Plastic boxes each filled with 350 ml of distilled water
ASTM = American Society for Testing and Materials

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**Table 2: Testing the action of the plasma cluster on the germinating capacity of Aspergillus fumigatus spores according to the modified ASTM C 665 test**
**Cluster ion generator 1: Plasma cluster unit (from Sharp)**
**Initial concentration of the test germ: 1.7 x $10^6$ CFU's/ml Aspergillus fumigatus spores in physiological common salt solution + 0.01% (w/w) Tween 80**
**Test carrier material: Beechwood spatula (roughened, 20% (w/w) material moisture content); 2 x 15 cm spatulas for the medical requirement**
**Application volume: 500 µl**
**Test chamber: Plastic box (22 x 20 x 15 cm)**
**Water reservoir: 350 ml distilled water in plastic box (13 x 11 x 4 cm) with perforated screen cover**
**Method of demonstration: Surface contact sampling with Tesafilm, coloration with lactophenol blue, microscopic determination**

| Serial no. | Date | With plasma cluster action Microscopic determination of the growth phases | | | Without plasma cluster action Microscopic determination of the growth phases | | |
|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd spatula | 1st | 2nd | 3rd spatula |
| 1 | 10.04.02 | + | + | + | + | + | + |
| 2 | 12.04.02 | + | + | + | ++ | ++ | ++ |
| 3 | 15.04.02 | + | + | + | +++ | ++++ | +++ |
| 4 | 17.04.02 | + | + | + | ++++ | ++++ | ++++ |
| 5 | 19.04.02 | + | ++ | ++ | ++++ | ++++ | ++++ |

**Evaluation:**
+ Spores only
++ Germination (isolated hyphae)
+++ Hypha formation
++++ Formation of fructifying organs ("new spore chains")

*) Plastic boxes each filled with 350 ml of distilled water

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**Table 3: Testing the action of the plasma cluster on the germinating capacity of Cladosporium herbarum spores according to the modified ASTM C 665 test**
**Cluster ion generator 1: Plasma cluster unit (from Sharp)**
**Initial concentration of the test germ: 4.4 x $10^6$ CFU's/ml Cladosporium herbarum spores in physiological common salt solution + 0.01% (w/w) Tween 80**
**Test carrier material: Beechwood spatula (roughened, 20% (w/w) material moisture content); 2 x 15 cm spatulas for the medical requirement**
**Application volume: 500 µl**
**Test chamber: Plastic box (22 x 20 x 15 cm)**
**Water reservoir: 350 ml distilled water in plastic box (13 x 11 x 4 cm) with perforated screen cover**
**Method of demonstration: Surface contact sampling with Tesafilm, coloration with lactophenol blue, microscopic determination**

| Serial no. | Date | With plasma cluster action Microscopic determination of the growth phases | | | Without plasma cluster action Microscopic determination of the growth phases | | |
|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd spatula | 1st | 2nd | 3rd spatula |
| 1 | 10.04.02 | + | + | + | + | + | + |
| 2 | 12.04.02 | + | + | + | ++ | ++ | ++ |
| 3 | 15.04.02 | + | + | + | +++ | ++++ | ++++ |
| 4 | 17.04.02 | +++ | ++ | ++++ | ++++ | ++++ | ++++ |
| 5 | 19.04.02 | +++ | ++ | ++++ | ++++ | ++++ | ++++ |

**Evaluation:**
+ Spores only
++ Germination (isolated hyphae)
+++ Hypha formation
++++ Formation of fructifying organs ("new spore chains")

*) Plastic boxes each filled with 350 ml of distilled water
ASTM = American Society for Testing and Materials

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**Table 4: Testing of the bacteriostatic and bactericidal action of the plasma cluster on the reproducibility of selected bacteria**

**Cluster ion generator 1: Plasma cluster unit (from Sharp)**
**Initial concentration of the test germs:** **$9.68 \times 10^9$ CFU's/ml Pseudomonas aeruginosa in physiological common salt solution**
**(24 h cultures in broth)** **$9.97 \times 10^9$ CFU's/ml Enterococcus faecium in physiological common salt solution**
**$3.87 \times 10^9$ CFU's/ml Staphylococcus epidermidis in physiological common salt solution**
**Solid nutrient: CASO agar**
**Application volume: 100 µl of the $1:10^6$ dilutions**
**Test chamber: plastic box (22 x 20 x 15 cm)**
**Water reservoir: 350 ml of distilled water in plastic box (13 x 11 x 4 cm) with perforated screen cover**
**Method of demonstration: Quantitative cultural methods**

| Serial No. | Method of treatment | Treatment time | Microorganisms | Incubation time | Incubation temperature | Germ count in CFU'S/plate |
|---|---|---|---|---|---|---|
| 1 | With plasma cluster | 12 h | Pseudomonas aeruginosa | 5 h | 37°C | 794 |
| | Without plasma cluster | 0 h | ATCC 15442 | 5 h | 37°C | 968 |
| 2 | With plasma cluster | 12 h | Enterococcus faecium | 12 h | 37°C | 1 |
| | Without plasma cluster | 0 h | ATCC 6057 | 12 h | 37°C | 997 |
| 3 | With plasma cluster | 12 h | Staphylococcus epidermidis | 12 h | 37°C | 1 |
| | Without plasma cluster | 0 h | ATCC 12228 | 12 h | 37°C | 387 |

*) Plastic boxes each filled with 350 ml of distilled water; CFU 's = Colony forming units;
ATCC = American Type Culture Collection

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**Fig. 1: Macroscopic photographs of the bacteriostatic action of the plasma cluster (fro Sharp) on the reproducibility of Pseudomonas aeruginosa (ATCC 15442)**
**l: With plasma cluster action (12 h): 794 CFU's/plate**
**r: Without plasma cluster action: 968 CFU's/plate**
**Incubation tie/temperature: 5 h/37°C**

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**Fig. 2: Macroscopic photographs of the bacteriostatic action of the plasma cluster (fro Sharp) on the reproducibility of Enterococcus faecium (ATCC 6057))**
**l: With plasma cluster action (12 h): 1 CFU/plate**
**r: Without plasma cluster action: 997 CFU's/plate**
**Incubation tie/temperature: 12 h/37°C**

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**Fig. 3: Macroscopic photographs of the bacteriostatic action of the plasma cluster (fro Sharp) on the reproducibility of Staphylococcus epidermides (ATCC 12228)**
**l: With plasma cluster action (12 h): 1 CFU/plate**
**r: Without plasma cluster action: 387 CFU's/plate**
**Incubation tie/temperature: 12 h/37°C**

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**Abb.1: Makroskopische Aufnahmen der bakteriostatischen Wirksamkeit des Plasma clusters (Fa. Sharp) auf die Wiederanzüchtbarkeit von Pseudomonas aeruginosa (ATCC 15442)**
**l: Mit Plasma cluster Einwirkung (12 h): 794 KBE/Platte**
**r: Ohne Plasma cluster Einwirkung: 968 KBE/Platte**
**Bebrütungszeit/-temperatur: 5 h/37°C**

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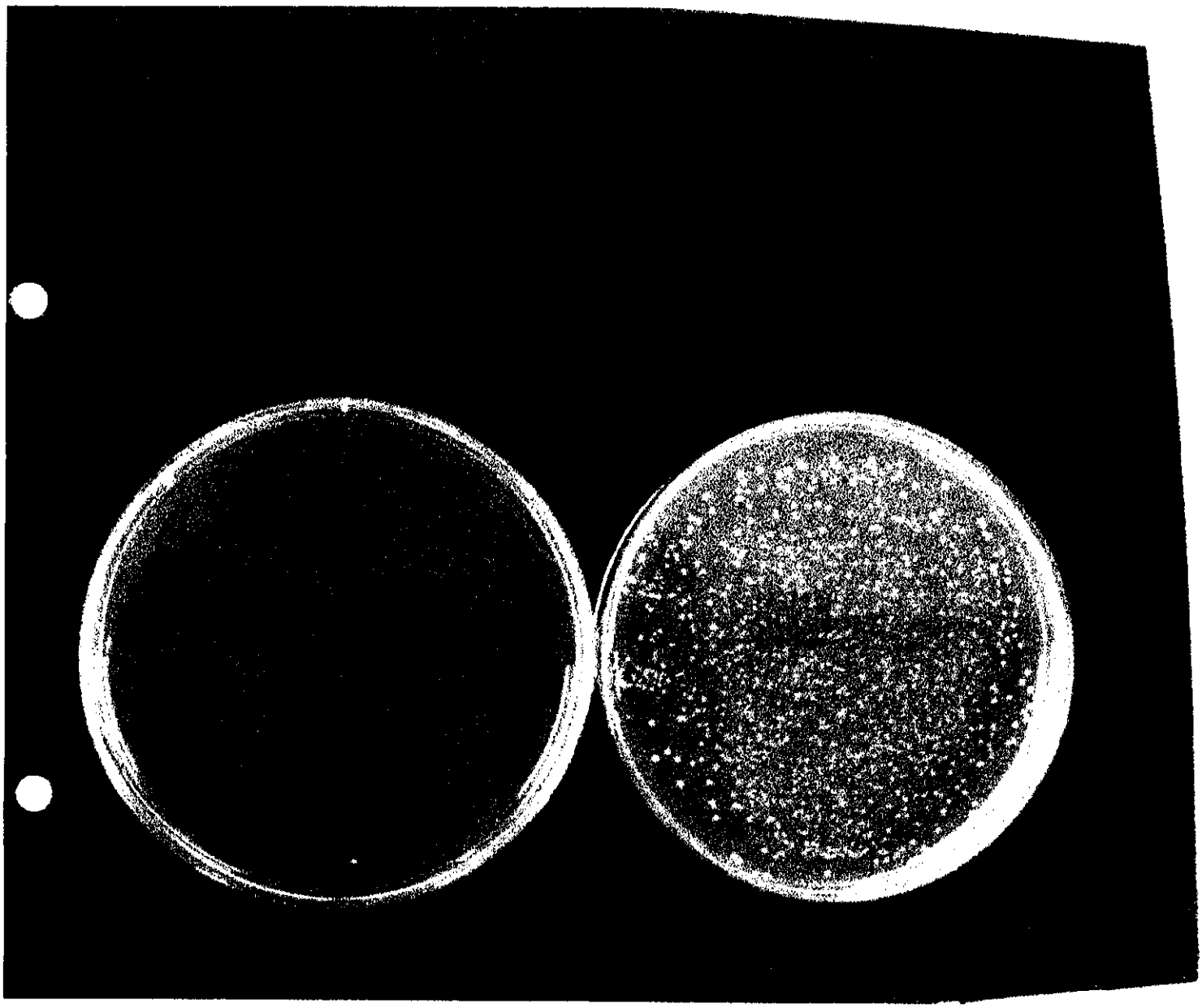

**Abb.2: Makroskopische Aufnahmen der bakteriziden Wirksamkeit des Plasma clusters (Fa. Sharp) auf die Wiederanzüchtbarkeit von Enterococcus faecium (ATCC 6057)**
**l: Mit Plasma cluster Einwirkung (12 h): 1 KBE/Platte**
**r: Ohne Plasma cluster Einwirkung: 997 KBE/Platte**
**Bebrütungszeit/-temperatur: 12 h/37°C**

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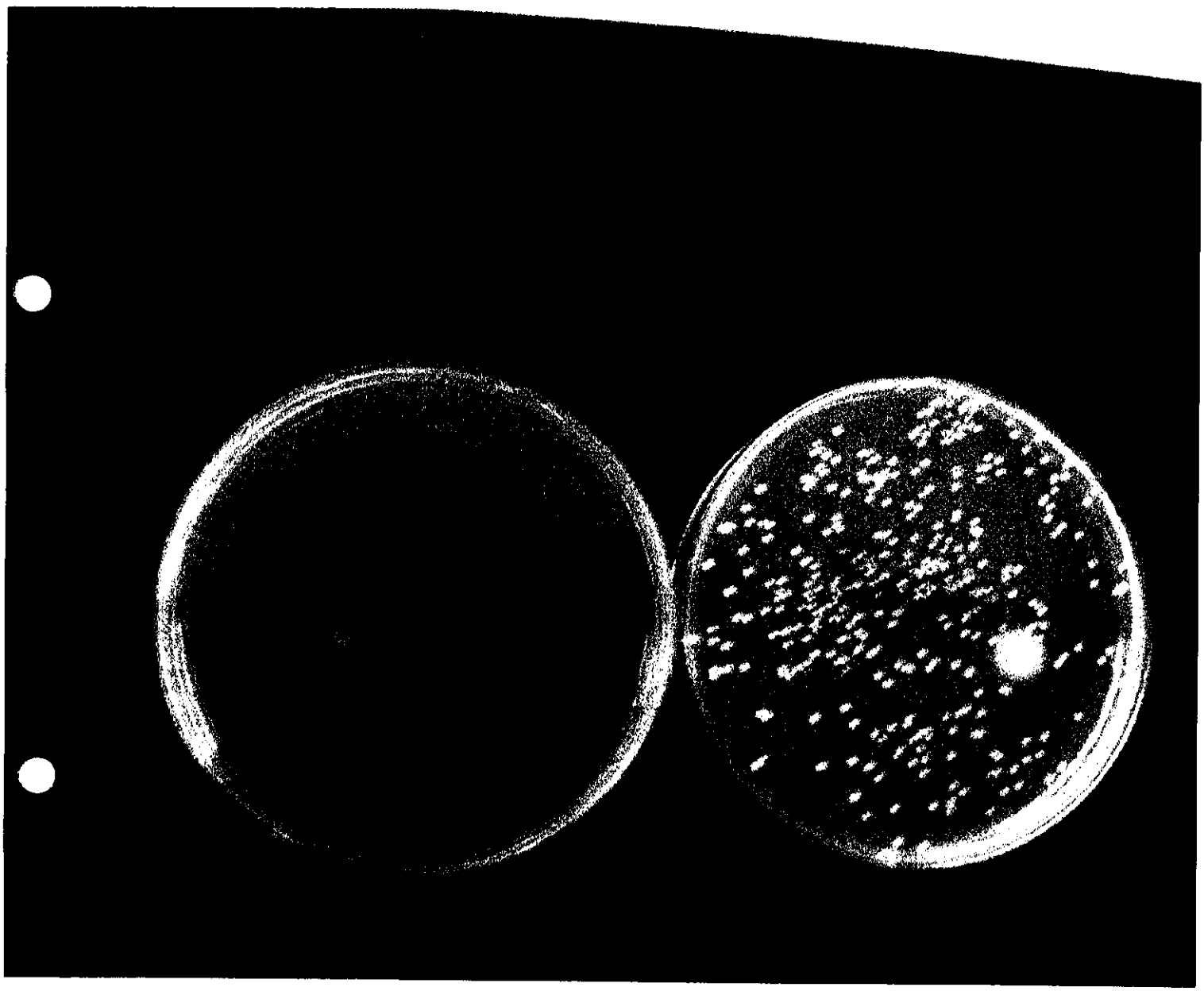

**Abb.3: Makroskopische Aufnahmen der bakteriziden Wirksamkeit des Plasma clusters (Fa. Sharp) auf die Wiederanzüchtbarkeit von Staphylococcus epidermides (ATCC 12228)**
**l: Mit Plasma cluster Einwirkung (12 h): 1 KBE/Platte**
**r: Ohne Plasma cluster Einwirkung: 38 7 KBE/Platte**
**Bebrütungszeit/-temperatur: 12 h/37°C**

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