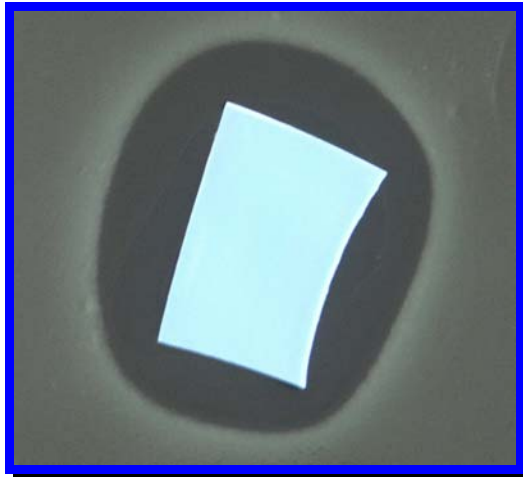


Growth inhibition test with *Legionella pneumophila* - Agar diffusion test -

Material testing of foils containing inhibitor

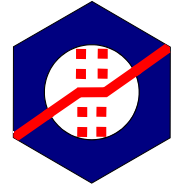


Münster, September 2003

Mikrobiologisches Labor

Dr. J. Balfanz - Dr. M. Lohmeyer - GbR
Biotechnologie, Forschung, Analytik

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Auftrags-Nr.: Au0821-07-30-03	Prüfende: 1.9.2003	Berichts-Nr.: Be0821-09-15-03

Results

(The following data refer to the examined samples exclusively)

Aim:

- Demonstration of the growth-inhibiting property of foils

Test samples:

- Blue foil segments (about 2 cm²) with inhibitor
- Black foil segments (about 2 cm²) without inhibitor addition

Test organism:

- *Legionella pneumophila* subsp. *pneumophila* ATCC 33152

Methods:

Nutritional agar: GVPC Legionella selective agar

Soft agar: bacteriological agar, 1.5 %, 45°C

Dilution tubes: physiological NaCl-solution

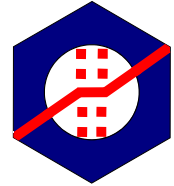
1. Opening of the vial and preparation of the freeze-dried culture due to the recommendations of DSMZ
2. Cultivation of Legionella on GVPC-agar
3. Preparation of a dense Legionella suspension with NaCl solution
4. Microscopic enumeration of the original solution and dilution (about 10⁶ Legionellen/ml = test solution 1)
5. Test solution 1
6. Test solution 2 dilution of test solution 1 (1 : 100)
7. Test solution 3 dilution of test solution 2 (1 : 100)
8. **Spread plates:**
Spreading of 1 ml dilution each on 2 parallel plates, drying of the spread plates and placing of the test samples onto the agar surface
9. **Layered plates:**
Addition of 1 ml dilution each into 2 ml liquid agar (1.5%), mixing and pouring of the soft agar onto the GVPC-plate. After congelation, placing of the test samples onto the agar surface.
10. Determination of the total viable counts of the test solution number 1: Preparation of decimal dilutions, spreading of 0.1 ml each onto the surface of GVPC-agar
11. Incubation of all tests at 36° C for 72 h
12. Evaluation

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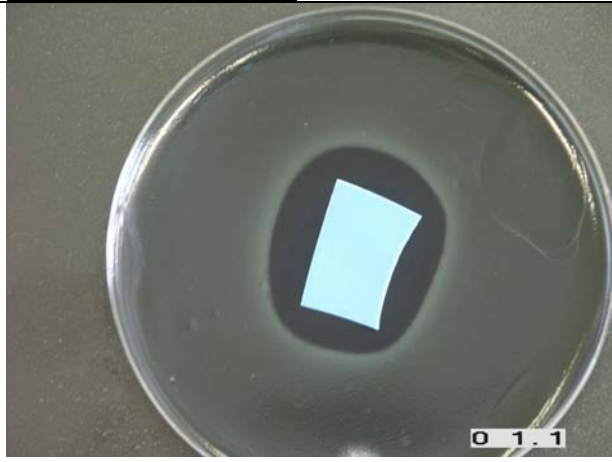
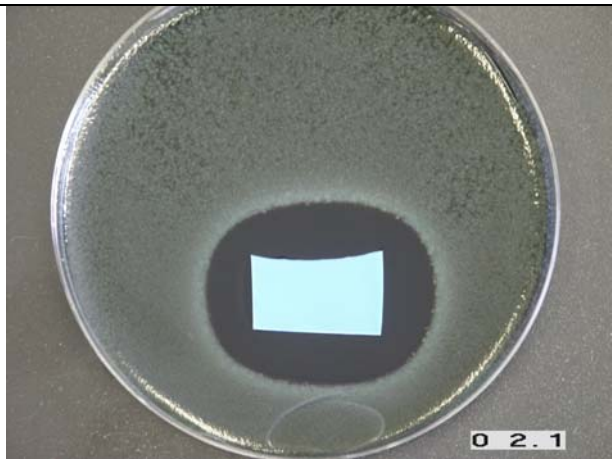
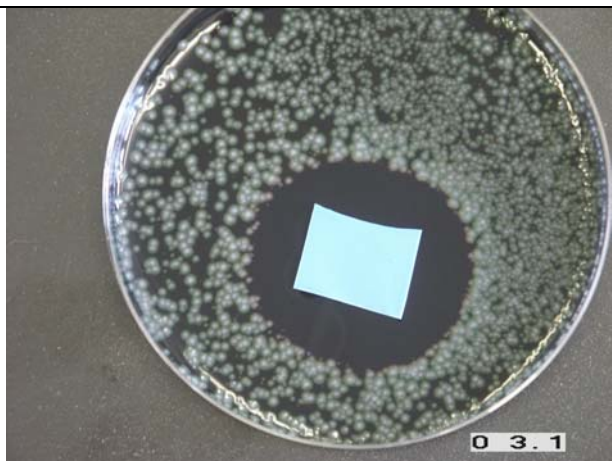
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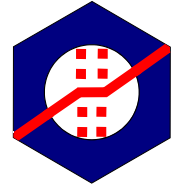
Picture documentation

	<p>Sample 1 with inhibitor</p> <p>Figure 1: Spread plate with Test solution 1: $7,9 \times 10^6$ Legionella / ml</p>
	<p>Sample 1 with inhibitor</p> <p>Figure 2: Spread plate with Test solution 2: $7,9 \times 10^4$ Legionella / ml</p>
	<p>Sample 1 with inhibitor</p> <p>Figure 3: Spread plate with Test solution 3: $7,9 \times 10^2$ Legionella / ml</p>

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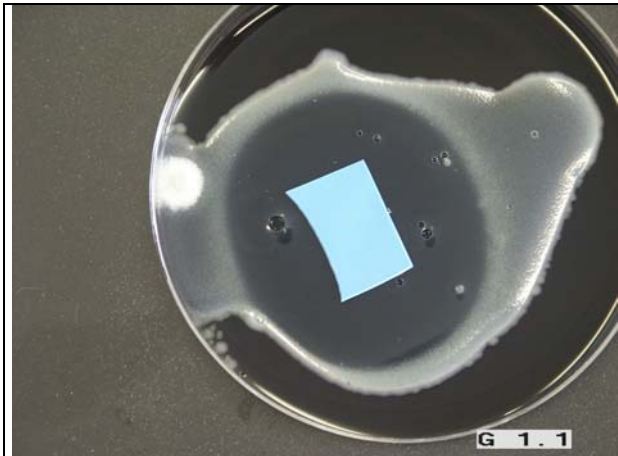
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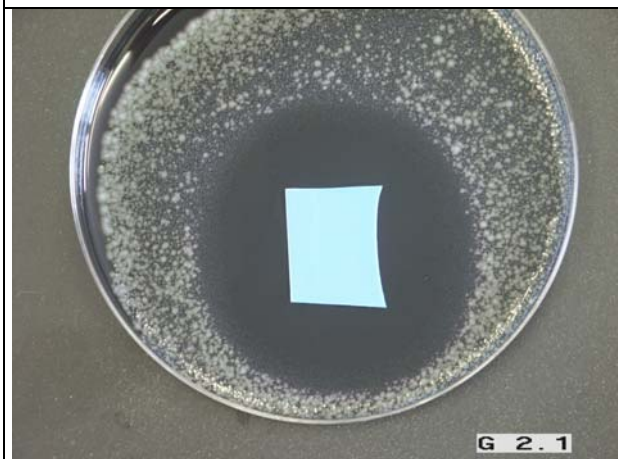
Sample 1 with inhibitor

Figure 4:

Layered plate with

Test solution 1: $7,9 \times 10^6$ Legionella / ml

(the agar congelated very quickly)

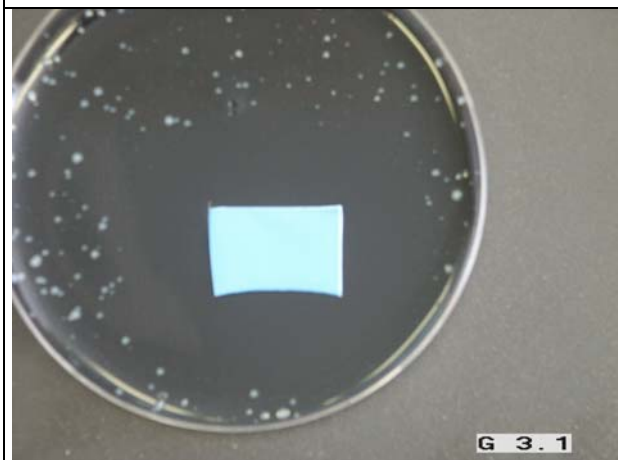


Sample 1 with inhibitor

Figure 5:

Layered plate with

Test solution 2: $7,9 \times 10^4$ Legionella / ml



Sample 1 with inhibitor

Figure 6:

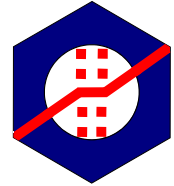
Layered plate with

Test solution 3: $7,9 \times 10^2$ Legionella / ml

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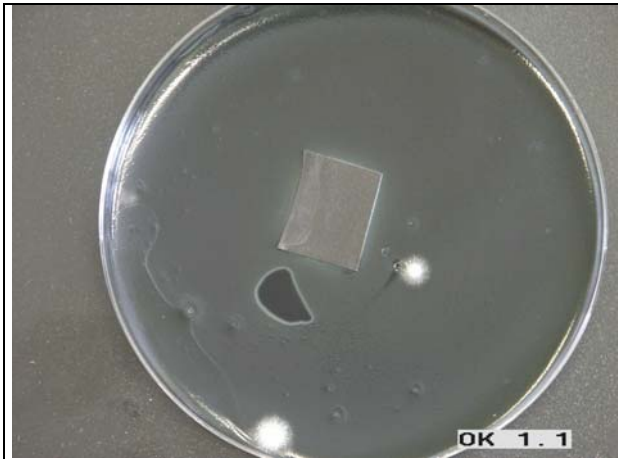
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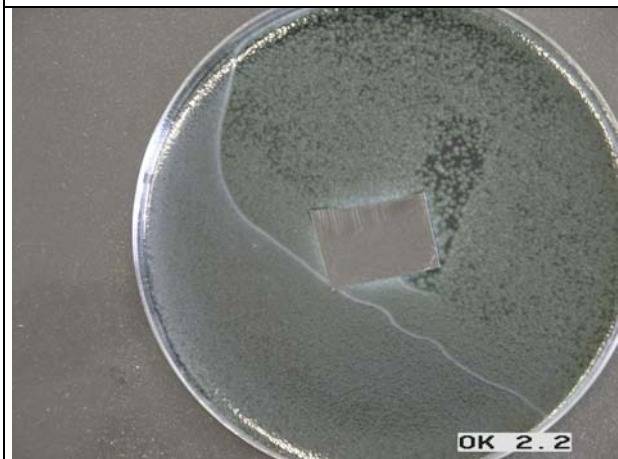


Sample 2 without additives

Figure 7:

Spread plate with

Test solution 1: $7,9 \times 10^6$ Legionella / ml

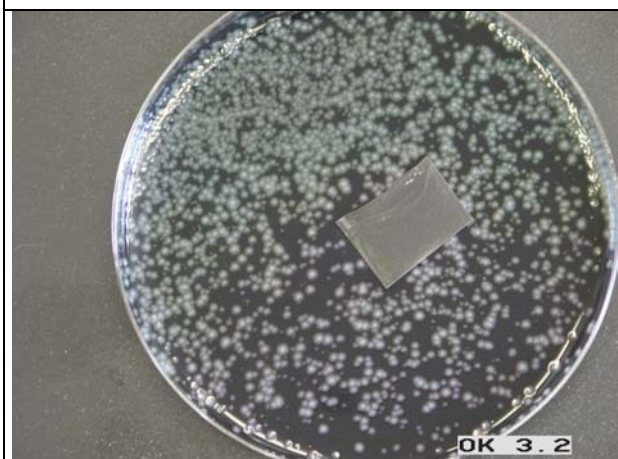


Sample 2 without additives

Figure 8:

Spread plate with

Test solution 2: $7,9 \times 10^4$ Legionella / ml



Sample 2 without additives

Figure 9:

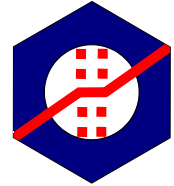
Spread plate with

Test solution 3: $7,9 \times 10^2$ Legionella / ml

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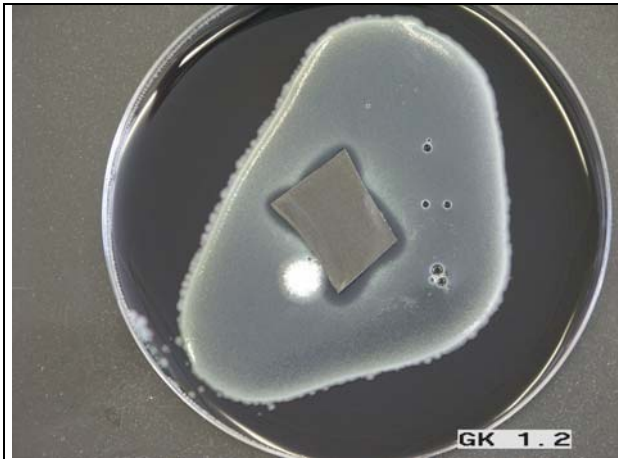
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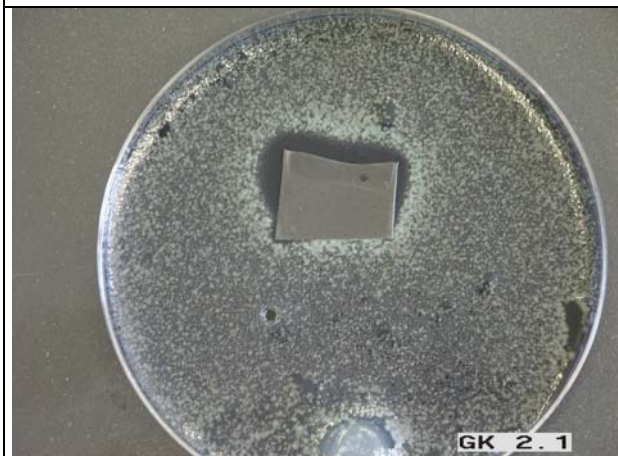
Sample 2 without additives

Figure 10:

Layered plate with

Test solution 1: $7,9 \times 10^6$ Legionella / ml

(the agar congelated very quickly)

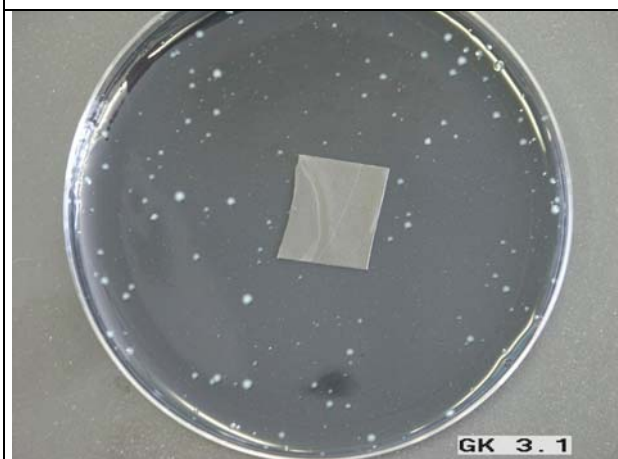


Sample 2 without additives

Figure 11:

Layered plate with

Test solution 2: $7,9 \times 10^4$ Legionella / ml



Sample 2 without additives

Figure 12:

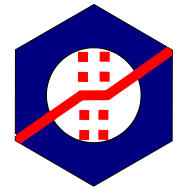
Layered plate with

Test solution 3: $7,9 \times 10^2$ Legionella / ml

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Determination of inhibition:

Due to the irregular profile of the sample segments the determination of the inhibition zone was performed according to Figure 13, considering the respective longest distance between sample and the edge of the inhibition zone.

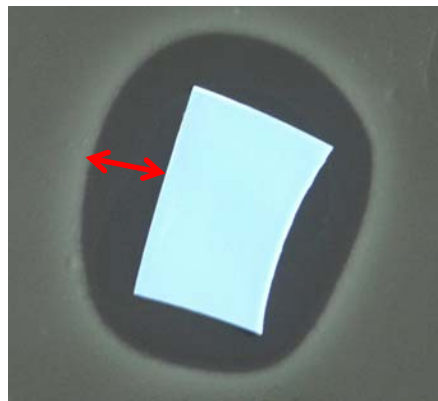


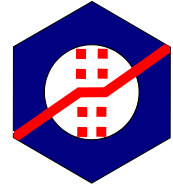
Figure 13: Determination of the inhibition zone, red arrow

Spread plates	Inhibition		Layered plates	Inhibition
Spread plate 1.1	0.7 cm		Layered plate 1.1	1.2 cm
Spread plate 1.2	0.7 cm		Layered plate 1.2	1.4 cm
Spread plate 2.1	0.8 cm		Layered plate 2.1	1.4 cm
Spread plate 2.2	0.6 cm		Layered plate 2.2	1.3 cm
Spread plate 3.1	1.0 cm		Layered plate 3.1	1.5 cm
Spread plate 3.2	1.0 cm		Layered plate 3.2	1.3 cm

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Discussion:

A growth inhibition against the used strain *Legionella pneumophila* subsp. *pneumophila* ATCC 33152 was clearly demonstrated to come from the samples containing the inhibitor (blue foil).

The inhibition is increased with decreasing cell densities, i.e., high cell densities have to be defeated by corresponding increased inhibitor concentrations. This is an usual effect, well known for many inhibition testings. This phenomenon is due to bacterial protection mechanisms, which are developed during high cell densities. The basic requirement for this effect is the absence of consumption or biological degradation of the inhibitor.

The used control samples showed a clearly reduced inhibition with layer plates, and hardly detectable inhibition effects with spread plates, respectively.

Münster, 15.9.2003

Dr. Michael Lohmeyer