

**Bactericidal tests of Neutral Anolyte produced in
Eurostel EE-90 units on Legionella**

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UNIT SPECIFICATION

EUROSTEL® EE-90 unit (serial no: 0003) and *all* other EE-90 or EE-160 Eurostel units designed to produce Neutral Analyte supplied by the Aquastel Group incorporating the following patented diaphragmatic Electrolysers.

DISINFECTANT TESTED

Superoxidised water (Neutral Analyte) produced in hereafter-mentioned diaphragmatic electrolysers accordingly the following methods

Electrolysers:

- Electrochemical Plant
(Patent *Western Pacific Company Inc.*, Tallinn; No. 2100285; RU96122382; WO98/23793; EP0882815A1)
- Electrochemical Installation
(Patent *Harrisson Investments Ltd.*, Tallinn; No. 2104961; RU97103204; WO98/40536; EP0922788A1)

Methods:

- A Method for Sterilising water and device for realising the same
(Patent *Western Pacific Company Inc.*, Tallinn; No. 2100286; RU96123069; WO98/25855, EP0885849A1)
- A Method for electrochemical treatment of aqueous solutions and device for realising the same
(Patent *Western Pacific Company Inc.*, Tallinn; No. 2100287; RU96123170; WO98/27012; EP0885848A1)

1. Introduction

Eurostel EE-90 units produce Neutral Anolyte. The bactericidal properties of this Neutral Anolyte were tested against *Legionella pneumophila*.

Drinking water

The microbiological properties of drinking water in Germany are determined by the German Drinking water Standards (TVO: Trinkwasserverordnung, last edition:12.12.1990).

The Drinking Water Standard explains in chapter 1., § 1. (properties of drinking water) the following:

(1) Drinking water must be free of pathogens. This requirement is not fulfilled if in 100ml drinking water *Escherichia coli* is measured (Limit). Germs of *E. coli* are not allowed in 100ml (limit).

Swimming pool water

The microbiological properties of swimming pool water in Germany are determined by the German Industry Standard (DIN: 19643-1: "Aufbereitung von Schwimm –und Badebeckenwasser").

The DIN explains in Table 2 the following requirements:

(1) Swimming pool and drinking water must be free of pathogens. This requirement is not fulfilled if in 100ml Swimming pool and Drinking water *Escherichia coli* and *Pseudomonas aeruginosa* is measured (Limit). Germs of *E. Coli* and *Ps. Aeruginosa* are not allowed in 100ml (limit).

(2) Moreover, swimming pool and drinking water must be free of *Legionella pneumophila*. Germs of *L. Pneumophila* are not allowed in 100ml drinking water (limit). Germs of *L. Pneumophila* are not allowed in 1ml swimmingpool water (limit).

2. Execution of the tests and analyses

Bacteria tested:

To execute the tests Aquastel Europe BV supplied us with a Eurostel EE-90 unit. Several Neutral Anolyte solutions were produced by this Eurostel unit with an amount of free chlorine between 300 and 500 mg/l.

With a view to simulate a practical application, the following pathogen

- *Legionella pneumophila*

was used and isolated in water-samples and in caso-bouillon cultivated. By means of dilution of the pathogens with sterile physiological saline solutions were the needed concentrations produced. For all test samples tap water (drinking water) of the city of Lollar was used.

Production of Neutral Anolyte

A Eurostel EE-90 unit was used to produce Neutral Anolyte samples. During the production the current was set for ~30A. after one-hour production the current was still ~30A and Neutral Anolyte samples were taken for analyses.

Free Chlorine:	300-500mg/l
PH- Value:	8.2
Conductivity:	11.700µS/cm
ORP:	790mV

3. Preparation of *Legionella* –samples and Neutral Anolyte-samples

Preparation of *Legionella*-samples

A *Legionella* – agar was prepared. The agar consisted of 2 colonies in 5ml *Legionella*-agar. All dilutions were made with sterile tap water of the city of Lollar. The tests were conducted with 100ml tap water of the city of Lollar to which *Legionella Pneumophila* bacteria was added in order to get a concentration of *L. pneumophila* of 300 CFU/ml. (100ml tap water of the city of Lollar and 0.1ml of the *Legionella*-agar.)

Preparation of Neutral Anolyte samples

In order to determine at what concentration of free chlorine the *L. pneumophila* bacteria completely were inactivated, several Neutral Anolyte samples were prepared. Neutral Anolyte with a free chlorine concentration of 300-500mg/l was diluted with tap water of the city of Lollar to get the following concentration of free chlorine.

- 20mg/L
- 40 mg/L
- 60mg/L
- 80mg/L

Then after

- 1 minute
- 5 minutes
- 10 minutes
- 30 minutes
- 60 minutes

Sodium thiosulphate, a reagent that free chlorine destroys were added to 10ml samples to avoid that the free chlorine after sample taken continues to react.

Finally 0.05ml of the sample was poured with the Legionella contaminated agar, and at 36°C cultivated for a week.

4. Analysing the effect of Neutral Anolyte samples on Legionella–agar.

Table 1: Test sample with 0.1 Legionella-agar in 100ml tap water at various concentrations of free chlorine.

Initial concentration of Legionella in test sample: 300CFU/ml (= 30.000 CFU/100ml)
0.7 ml Neutral Anolyte: 20 mg/L free chlorine

Contact time, min	Concentration, CFU/ml	Concentration, CFU/ 100 ml
1	>20.000	> 2.000.000
5	>20.000	> 2.000.000
10	>20.000	> 2.000.000
30	>20.000	> 2.000.000
60	>20.000	> 2.000.000

Table 2: Test sample with 0.1 Legionella-agar in 100ml tap water at various concentrations of free chlorine.

Initial concentration of Legionella in test sample: 300CFU/ml (= 30.000 CFU/100ml)
1.3 ml Neutral Analyte: 40 mg/L free chlorine

Contact time, min	Concentration, CFU/ml	Concentration, CFU/ 100 ml
1	>20.000	> 2.000.000
5	>20.000	> 2.000.000
10	>20.000	> 2.000.000
30	80	8.000
60	0	0

Table 3: Test sample with 0.1 Legionella-agar in 100ml tap water at various concentrations of free chlorine.

Initial concentration of Legionella in test sample: 300CFU/ml (= 30.000 CFU/100ml)
2.0 ml Neutral Analyte: 400 mg/L free chlorine

Contact time, min	Concentration, CFU/ml	Concentration, CFU/ 100 ml
1	N/A	N/A
5	280	28.000
10	100	10.000
30	60	6.000
60	0	0

Table 4: Test sample with 0.1 Legionella-agar in 100ml tap water at various concentrations of free chlorine.

Initial concentration of Legionella in test sample: 300CFU/ml (= 30.000 CFU/100ml)
2.6 ml Neutral Analyte: 80 mg/L free chlorine

Contact time, min	Concentration, CFU/ml	Concentration, CFU/ 100 ml
1	0	0
5	0	0
10	0	0
30	0	0
60	0	0

5. Conclusion

In this tests Neutral Anolyte produced by Eurostel EE-90 units was tested for its sporicidal activity against *Legionella Pneumophila*. Neutral Anolyte with different concentrations of free chlorine were prepared and added to a Legionella-agar with 300 CFU/ml contamination. During one hour contact time samples were taken and the effect of Neutral Anolyte observed.

The concentration of *Legionella Pneumophila* was relative low ($3 \times 10^2 = 30.000$ CFU/100ml). However a sporicidal effect could only be observed when the Neutral Anolyte samples contained more than 40mg/l free chlorine and only after 30 minutes contact time.

When the concentration of free chlorine in Neutral Anolyte samples is 80mg/l of more, then *Legionella Pneumophila* spores are inactivated within a minute. No spores' could be observed during the whole test period.

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