KLINIK FÜR VÖGEL, REPTILIEN,
AMPHIBIEN UND FISCHE
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# Certificate

on the virucidal activity of the disinfectant

Anolyte

of the company **Envirolyte-Euregio,** Am Rosengarten 5, 52080 Aachen, Germany, represented by Mr. Oliver Röder. The named preparation was provided to me to test its virucidal activity against the causative organisms of highly pathogenic avian influenza (classical fowl plague) under laboratory conditions.

The complete testing (estimation of cytotoxicity and suspension tests) was done on the basis of the regulations that were generated by the German Veterinary Society e. V. Giessen. Toxicity tests and suspension tests were performed during the time of August 08 to September 06, 2006 in the institution named above.



## 1 Materials and Methods

### 1.1 Material

**Disinfectant: Anolyte (neutral)**

Container: plastic container with a content of approx. 10 liters.

Packaging: no further details

Composition of the Anolyte: see savety data sheet

Lot No: none provided

Best for use: no date declared on container; according to oral communication of Mr. Oliver Röder approximately six month.

Danger labeling: Acute not toxic

**Test virus:**

The highly pathogenic avian influenza A virus (A/carduelis/Germany/72, H7N1) was used in form of infectious allantoic fluid that was derived from infected chicken SPF embryos following allantoic cavity inoculation

**Cell cultures for virus assays**
Primary chicken embryo fibroblast cultures (CEF cultures) were used for toxcicity tests and and for testing of residual influenza A virus in suspension tests.

## 1.2 Methods

Details on the methods used for toxicity and suspension tests are laid down in the regulations of the German Veterinary Society e. V. Giessen. All testing was done without a protein load.
All toxicity and suspension tests were repeated once.

**Test temperatures**
All suspension test were performed according to an aggreement with Mr. Röder at 10 °C, 20 °C and 30 °C.

**Test times**
The suspension tests were performed according to an aggreement with Mr. O. Röder at exposure times of one minute, 10 minutes and 30 minutes.

**Mixing and testing of the reaction components**
Efficacy tests consists of one ml virus plus 9 ml anolyte

Control tests consists of one ml of virus plus 9 ml of phosphate buffered saline solution

Sampling of mixtures (0.1 ml each) was done after the end of the given time periods and temperatures. Each sample was further diluted and inoclated into five wells. Recording of results was done seven days after start of the cultures.



# 2 Results

## 2.1 Toxicity tests

The titration of the disinfectant Anolyte in primary CEF cultures yielded toxic effect of undiluted and one in ten diluted preparations. (see Tble 1). On repetition, the same result was obtained.

**Table1**: Toxicity tests of Anolyte for chicken embryofibroblast cultures

| Cell culture | Concentration of the disinfectant | Toxicity at dilution step | | |
|---|---|---|---|---|
| | | Undiluted | 1 : 10 diluted | 1 : 100 diluted |
| CEF | Pure, undiluted | Toxic | Toxic | Not toxic |

Toxic = the cell sheat of the CEF cultures displays structural alterations that are entirely due to the action of the preparation Anolyte
Not toxic = Alterations on the cells are microscopically not visible.

## 2.2 Results of the suspension tests

The results of the suspension tests using three exposure times and three temperature intervalls are provided in Table 2.

All numbers given in Table 2 represent log 10 values of culture infective dose 50 %.

The virus content of the test virus is in all cases $\log_{10}$ = 7.0 $CID_{50}$.

**Table 2: Results of Suspension tests**

| Test Temperature | Test-set | Virus content in CID50 post exposure times (minutes) | | | |
|---|---|---|---|---|---|
| | | Control | 1 | 10 | 30 |
| 10 °C | 1 | 7,0 | ≤ 2.5 | ≤ 2.5 | ≤ 2.5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 1 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 1 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| 20 °C | 1 | 7,0 | ≤ 2.5 | ≤ 2.5 | ≤ 2.5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 1 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 1 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| 30 °C | 1 | 7,0 | ≤ 2.5 | ≤ 2.5 | ≤ 2.5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 1 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 1 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |
| | 2 | 7,0 | ≤ 2,5 | ≤ 2,5 | ≤ 2,5 |



The efficacy of the undiluted preparation Anolyte against the highly pathogenic avian influenza A virus is completely independed on the chosen test temperature of ten, 20 and 30 °C. The efficacy is also independend on the duration of the exposure times of one, ten or 30 minutes.

The difference of the virus content between the untreated virus control test and the virus plus Anolyte preparations is more than 4.5 $\log_{10}$ indicating a reduction of more that 99.995 %.

## 3 Evaluation of the results

The toxicity tests prove a low level of toxicity of the preparation Anolyte.

The results of the suspension test without the addition of a protein load prove for all temperatures and exposure times good efficay of Anolyte against the tested influenza A virus.

Prof. Dr. Erhard F. Kaleta