Final test report
submitted to

Amazon Bio-Guard Ltd
Unit 3 Waterfall Mill
Queen Victoria Street
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BB2 2QG

Evaluation of the effectiveness of

eradic8 A2Z

against
Feline Calicivirus
(Surrogate of human norovirus)

Test method according to guideline of BGA and DVV

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1. Introduction

It is not possible to study virus-inactivating properties of chemical disinfectants against noroviruses (synonyms: Norwalk-like viruses or small round structured viruses, SRSVs) because no suitable replication systems are available for these viruses. Therefore, feline calicivirus (FCV) is often used as a surrogate for noroviruses in inactivation tests. As requested, the surface disinfectant eradic8 A2Z of Amazon Bio-Guard Ltd was tested for its virus-inactivating properties against feline calicivirus. Investigations were carried out in accordance with the guideline on testing chemical disinfectants for effectiveness against viruses published by the Federal Health Office (Bundesgesundheitsamt, BGA, now Robert Koch-Institute, Berlin) and the German Association for the Control of Virus Diseases (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e. V., DVV) (1,2).

2. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of sample

<table>
<thead>
<tr>
<th>name of product</th>
<th>eradic8 A2Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>manufacturer</td>
<td>Amazon Bio-Guard Ltd</td>
</tr>
<tr>
<td>lot no.</td>
<td>18022005</td>
</tr>
<tr>
<td>project number</td>
<td>ML 167/05</td>
</tr>
<tr>
<td>appearance and smell of the product</td>
<td>clear, colourless liquid, product specific</td>
</tr>
<tr>
<td>pH-value(s)</td>
<td>undiluted: 13.03 (20°C)</td>
</tr>
<tr>
<td></td>
<td>4.0 %: 11.45 (20°C)</td>
</tr>
<tr>
<td>date of receipt at laboratory</td>
<td>2005-02-23</td>
</tr>
<tr>
<td>conditions of storage</td>
<td>room temperature in the dark (area with restricted access)</td>
</tr>
<tr>
<td>active substance(s) and concentration(s)</td>
<td>9.9 % Bardac 22.70</td>
</tr>
</tbody>
</table>
4. Experimental conditions

| date of examinations          | 2005-03-14 – 2005-05-22 |
| test temperature             | 20°C ± 1°C              |
| diluent of product           | 4.0 %                   |
| contact times                | 30, 60 and 120 minutes  |
| interfering substances       | 2.0 % solution of bovine serum albumin (BSA) fetal calf serum (FCS) |
| diluent                      | Aqua bidest.            |
| procedure to stop action of disinfectant | immediate dilution |
| test virus                   | FCV strain F9           |

5. Material and methods

5.1. Preparation of test virus suspension

The feline calicivirus strain F9 was obtained from Prof. Dr. H. Schirrmeier, Institute for Diagnostic Virology of the German Federal Research Centre for Viral Diseases of Animals (BFAV) on Riems Island. Prior to inactivation, FCV was passaged three times in KE-R cells (full embryo, feline, Catalog No. 138). The KE-R cells were obtained from Dr. R. Riebe, cell bank for cell lines in veterinary medicine at the BFAV.

To prepare the test virus suspension, KE-R cells which had been cultured with Eagle’s minimum essential medium (EMEM) and 10 % or 2 % fetal calf serum (FCS, Biochrom AG, Berlin, Germany) were infected with FCV in 175 cm² cell culture flasks (Nunc GmbH & Co. KG, Wiesbaden, Germany). Once a cytopathic effect had been induced (approx. 16-20 hours), freezing and thawing was carried out three times. The cell debris was removed by centrifugation at 770 x g for ten minutes and the supernatant was recovered as viral suspension.

5.2. Inactivation tests

Tests were carried out following BGA and DVV guideline. Eight parts by volume of the disinfectant in a 1.25 x of the desired concentration were mixed with one part by volume of virus suspension and one part by volume of interfering substances. In tests with interfering substances, instead of double distilled water, one part by volume of fetal calf serum or of a
2 % serum albumin solution (bovine serum albumin, BSA, Cohn fraction V, Sigma-Aldrich Chemie GmbH, D-82018 Taufkirchen, Germany) was added. A control was one part by volume of virus suspension, four parts by volume of PBS and five parts by volume of 1.4 % formaldehyde. Concentration of formaldehyde was determined by the hydroxyl-ammoniumchloride method.

Inactivation tests were carried out in sealed test tubes (Sarstedt AG & Co., D-51588 Nümbrecht, Germany) in a water bath at 20°C ± 1°C. Aliquots were removed after appropriate times, and residual infectivity was determined. In addition, in accordance with the guideline, virus controls were carried out. Activity of the disinfectant was stopped by immediate dilution.

5.3. Determination of infectivity

Infectivity was determined by means of end point dilution method using the microtitre process. For this, 100 µl aliquots of the samples, which had been serially diluted with ice-cold EMEM, were transferred to eight cups of a sterile polystyrol 96-well microtitre plate with a flat bottom (Nunc GmbH & Co. KG, Wiesbaden, Germany). Already on the previous day 100 µl aliquots of a freshly trypsinized CRFK cells (approx. 2.7 x 10⁴ cells) had been placed in each cup (preformed monolayer). Incubation took place at 37°C in a CO₂ incubator (5 % CO₂ content) for five to seven days. Finally, cultures were observed for cytopathic effects with a reversed microscope. Virus titres (TCID₅₀/ml) were calculated with the method of Kärber (3) and Spearman (4).

5.4. Determination of cytotoxicity

For the determination of the cytotoxicity, two volume parts PBS were mixed with eight volume parts of the disinfectant and, following serial dilution as outlined in 5.3, transferred to the 96-well microtitre plate with the preformed monolayer. The cytotoxic dose was calculated as log₁₀ CD₅₀/ml (analogous to log₁₀ TCID₅₀ value).

5.5. Calculation of virus-inactivating properties

Virus-inactivating properties of the test disinfectant were determined by calculating the titre reduction compared with the respective control titrations containing no disinfectant. Data are given as reduction factor (RF).

6. Results

In parallel with inactivation tests, cytotoxicity of eradic8 A2Z (4.0 %) and 0.7 % formaldehyde was measured. The formaldehyde solution was toxic for the CRFK cells in the 1:10,000 dilution. This corresponded to a log₁₀ CD₅₀/mL of 5.50.
Examinations showed that the tested surface disinfectant produced as a 4.0 % solution a cytotoxic effect at the dilution of 1: 100. This means a log\textsubscript{10}CD\textsubscript{50}/mL value (analogous to the TCID\textsubscript{50} value) of 3.50 (data not shown in table). These tests to measure the cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated FCV is determined.

Formaldehyde (0.7 %) reduced the FCV titre after 5 and 15 minutes by 1.25 and $\geq 1.87$ log\textsubscript{10} steps. After 30 and 60 minutes RF was $\geq 2.25$ (table 1).

Results of inactivation tests are found in table 1. eradic8 A2Z was tested as 4.0 % solution. The exposure times were 30, 60 and 120 minutes.

Testing eradic8 A2Z as 4.0 % solution, after an exposure time of 30 minutes a sufficient reduction of the virus titre was measured (table 1). The reduction factors were $\geq 4.25$ (assay without soil load), $\geq 4.38$ (assay with BSA) and $\geq 4.25$ (assay with FCS). This corresponded to an inactivation of $\geq 99.99$ % meaning virus-inactivating properties. According to the guideline of BGA/DVV, a disinfectant or a disinfectant solution at a particular concentration is having virucidal efficacy if within the recommended exposure period the titre is reduced at least by $\geq$ four logs\textsubscript{10}.

A prolongation of exposure time to 120 minutes led to identical results.

Summarizing the results of the quantitative suspension test it can be recommended to use the surface disinfectant eradic8 A2Z for the inactivation of FCV (surrogate of human norovirus) as follows:

4.0 % 30 min

Bremen, 2005-05-25

- Dr. J. Steinmann -
Literature


