Final test report C05ML260
submitted to

AS Chemi-Pharm
Põllu 132
Tallinn 10917
Estonia

Evaluation of the effectiveness of

Chemisept G/Chemisept G Color

Against

Bovine Viral Diarrhea Virus (BVDV) (surrogate of HCV)

in
a quantitative suspension test

Test method according to the guideline of BGA and DVV

Dr. Jochen Steinmann
MikroLab GmbH
Norderoog 2
D-28259 Bremen

Phone: +49 (0) 421-27819102
Fax: +49 (0) 421-2760283
E-mail: Mikrolab.GmbH@t-online.de
http://www.mikrolab-gmbh.de
2005-12-30

5.6 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

6. Results

In parallel with the inactivation tests, cytotoxicity of Chemisept G/G COLOR (80,0 %) and 0.7 % formaldehyde solution was measured. The formaldehyde solution was toxic for the *KOP-R cells* in the 1:1.000-dilutions. This corresponded to a $log_{10}CD_{50}$/mL of 4.50. (Table 1)

Examinations also showed that without treatment Chemisept G/G Color (80,0 %) had a $log_{10}CD_{50}$/mL of 2.50 (cytotoxicity in the 1:100 and 1:10-dilutions),where-as no cytotoxitc effect after treatment with the colomns was measured resulting in a $log_{10}CD_{50}$/mL of ≤ 1,50 (Table 1).

These tests to measure cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated BVDV could be determined.

Virus titres without treatment with MicroSpin™ S-400 HR columns were 5,50 (assay without protein load), 5,88 (assay with BSA) and 5,75 $log_{10}CD_{50}$/mL (assay with FCS) (data not shown in table)

Results of inactivation tests are found in table 2. Formaldehyde (0.7 %) reduced the BVDV titre after 5 minutes by ≥ 0,25 log_{10}-steps. After 15, 30 and 60 minutes identical reduction factors werw ≥ 1,25 (Table 2).

Chemisept G/G Color was examined undeluted .Due the addition of virus suspension and interfering substance a test concsntration of 80,0 % resulted.Exposure time were 30, 60,120 seconds.

Testing Chemisept G/G Color undiluted, an effisncy was measured after exposure time 30 s (Table 2). At this time , no BVDV was detecable any longer. The reduction factors were ≥ 4.25 (assays without soil load) and ≥ 4.25 (assays with BSA) and ≥ 4,38 (assays with FCS). This

corresponds in all cases to an inactivation of ≥99,99%. According to the guidline of BGA/DVV, a disinfectant or a disinfectant solution at a particular concentrations is having virus-inactivating efficacy if within the recommended exposure period the titre is reduced at least by four log_{10}-steps.

Due to the lack of virological guidelines simulating practical conditions in Europe (phase 2, step 2 tests) the data of this quantitative suspension test lead to the recommendation to use the disinfectant Chemisept G/G Color for inactivation of BVDV (surrogate of hepatitis C virus) as follows:

undiluted **30 s**

Dr. J. Steinmann