# Memorandum for using microspin columns testing disinfectants for virus-inactivating properties

(dating 18.05.2013)

For testing the virus-inactivating properties of chemical disinfectants it is necessary to demonstrate a reduction of virus titre by 4 $\log_{10}$ steps within the recommended exposure time. Since some disinfectants show a strong cytotoxicity, this reduction of virus titre cannot always be achieved. In the EN 14476 und the Guideline of DVV/RKI a detoxification of test mixtures by molecular sieving e.g. with Sephadex™ LH 20 is recommended. With this method cytotoxicity in dependence of the active ingredients can be reduced in general by a one $\log_{10}$ step. In some laboratories the molecular sieving is even used to stop the virus-inactivating properties testing non-cytotoxic disinfectants.

Many tests in different laboratories have shown that by molecular sieving infectious viruses can be restrained in an unpredictable manner leading to false positive results. A virus-inactivating property is pretended which is not given. This effect was shown with different viruses and disinfectants based on various active ingredients. Table 1 shows different examples with and without the usage of columns for molecular sieving: with QAC based disinfectants 1, 3 and 4 a high restraining of infectious virus in the columns exists, whereas with the QAC based disinfectant 2 no influence of the columns was shown.

In the direct comparison of test mixtures with and without filtration differences of 3 $\log_{10}$ steps were measured. In contrast, examining the virus controls in parallel such effect of restraining were not observed.

From these data it is assumed that the composition of the test mixture based upon disinfectant, test virus suspension and soil loading is the critical factor.

In some test reports and publications testing of disinfectants are found that were exclusively performed by molecular sieving. An estimation of the reliability of these data is difficult as parallel examinations without columns allowing detecting the restraint are missing.

Therefore, we want to give the strong advise that testing disinfectants with high cytotoxicity and molecular sieving a parallel examination without columns at all exposure times is necessary. This would allow the detection of restraint viruses. In this case other measures (e.g. higher virus titres) should be used. For stopping the virus-inactivation properties of test mixtures columns are not suited because by doing so the exact time point is not well defined in contrast to immediate dilutions.

Table 1: Examples of virus titres in test mixtures with and without the usage of columns for molecular sieving

| active ingredient | conc. | test virus | soil load | cytotox. | columns | virus titre control | virus titre ($log_{10}$ TCID50/ml) after | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | **5 min** | **15 min** | **30 min** | **60 min** |
| QAC 1 | 1% | rota | Aqua bidest. | 3.50 | no | 7.13 ± 0.37 | 6.00 ± 0.38 | 5.63 ± 0.41 | 5.63 ± 0.41 | n.d. |
| | | | | 2.50 | yes | 7.00 ± 0.38 | ≤ 2.50 ± 0.0 | ≤ 2.50 ± 0.0 | ≤ 2.50 ± 0.0 | n.d. |
| QAC 2 | 0.25% | rota | Aqua bidest. | 3.50 | no | 7.13 ± 0.37 | 6.75 ± 0.33 | 6.75 ± 0.44 | 5.88 ± 0.37 | n.d. |
| | | | | 2.50 | yes | 7.00 ± 0.38 | 6.63 ± 0.25 | 6.50 ± 0.35 | 6.75 ± 0.44 | n.d. |
| QAC 3 | 1% | adeno | FCS | 3.50 | no | 7.64 ± 0.29 | n.d. | n.d. | n.d. | 5.79 ± 0.37 |
| | | | | 3.50 | yes | 7.50 ± 0.40 | n.d. | n.d. | n.d. | ≤ 3.50 ± 0.0 |
| | | SV40 | | 3.50 | no | 7.79 ± 0.37 | n.d. | n.d. | n.d. | 5.21 ± 0.55 |
| | | | | 3.50 | yes | 7.79 ± 0.37 | n.d. | n.d. | n.d. | ≤ 3.50 ± 0.0 |
| QAC 4 | 1% | adeno | FCS | 3.50 | no | 7.64 ± 0.29 | n.d. | n.d. | n.d. | 6.07 ± 0.49 |
| | | | | 2.50 | yes | 7.50 ± 0.40 | n.d. | n.d. | n.d. | ≤ 2.50 ± 0.0 |
| | 0.5% | SV40 | | 4.50 | no | 7.79 ± 0.37 | n.d. | n.d. | n.d. | 6.36 ± 0.29 |
| | | | | 3.50 | yes | 7.79 ± 0.37 | n.d. | n.d. | n.d. | ≤ 3.50 ± 0.0 |
| | | | | | | | **1 min** | **2 min** | **3 min** | **5 min** |
| Alcohol + CHG | 80% | adeno | Aqua bidest. | 4.50 | no | 8.00 ± 0.46 | n.d. | 6.75 ± 0.33 | n.d. | n.d. |
| | | | | 2.50 | yes | 7.25 ± 0.44 | n.d. | ≤ 2.63 ± 0.25 | n.d. | n.d. |