

DIN EN 14476:2013-10 (E)

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (Phase 2/Step 1)

Contents	Page
Foreword	4
Introduction	5
1 Scope	6
2 Normative references	6
3 Terms and definitions	6
4 Requirements	7
5 Test methods	8
5.1 Principle	8
5.2 Materials and reagents, including cell cultures	9
5.2.1 Test organisms	9
5.2.2 Culture media, reagents and cell cultures	9
5.3 Apparatus and glassware	12
5.4 Preparation of test organism suspensions and product test solutions	14
5.4.1 Test organisms suspensions (test virus suspension)	14
5.4.2 Product test solutions	14
5.5 Procedure for assessing the virucidal activity of the product	15
5.5.1 General	15
5.5.2 Test procedure	16
5.5.3 Modified method for ready-to-use products	17
5.5.4 Cytotoxicity caused by product test solutions	17
5.5.5 Control of efficiency of suppression of product's activity	18
5.5.6 Reference test for virus inactivation	18
5.5.7 Titration of the virus control	19
5.5.8 Titration of test samples	19
5.6 Experimental data and calculation	19
5.6.1 Protocol of results	19
5.6.2 Calculation of infectivity titer (TCID ₅₀ or PFU)	19
5.7 Verification of the methodology	19
5.8 Expression of results	20
5.8.1 General	20
5.8.2 Calculation of the virucidal activity of products	20
5.9 Test report	20
Annex A (informative) Examples of viruses sorted according to their presence in the human body in case of virus infection	22
Annex B (informative) Detoxification of test mixtures by molecular sieving	24
B.1 Molecular sieving with Sephadex TM LH 20	24
B.1.1 Principle	24
B.1.2 Sephadex suspension	24
B.1.3 Procedure	24
B.2 Molecular sieving using MicroSpin TM S 400 HR	26

Annex C (informative) Calculation of the viral infectivity titre	27
C.1 Quantal tests Example of TCID₅₀ determination by the Spearman-Kärber method	27
C.2 Plaque test	28
C.3 Biometrical evaluation of experimental approaches and assessment of the disinfecting effect on the virus (reduction [R]):	28
C.3.1 General	28
C.3.2 Calculating the virus titre with 95 % confidence interval	29
C.3.3 Calculating the reduction and its 95 % confidence interval	29
C.3.4 Calculating the average reduction (R(mi)) and its 95 % confidence interval	30
C.3.5 Practical example	31
Annex D (informative) Presentation of test results of one active concentration	33
Annex E (informative) Quantitative determination of formaldehyde concentrations	36
Bibliography	37