



VIRUCIDAL ACTIVITY PHASE2 / STEP 1 QST TEST ACCORDING TO NORM EN14476:2007

Application field

This European normative is applicable to products for which disinfectant activity against viruses is claimed.

It is applicable to products to be used for surface, skin or instrumental disinfection. It is applicable to chemical disinfectants and antiseptics to be used in areas and situations where disinfection or antisepsis are indicated. Such indications occur in patient care:

- in hospitals, in community medical facilities and dental institutions,
 - in clinics of schools, of kindergartens and of nursing homes
- and may also occur in the workplace, in public areas and in the home.

It may also include services such as in laundries and kitchens supplying products.

Interests

The aim of the test below schematically reported is to evaluate the capability of a chemical disinfectant formulation to produce a reduction in the number of infecting viral particles on suitable human derived host cells.

Principle of the test

The virucidal activity is verified as follows:

two different viral suspensions, *Poliovirus type 1* and *Adenovirus type 5*, are incubated with the test item in the following conditions test:

- Concentrations: three different dilutions of the test item are tested
- contact time: 60 minutes or lower according to Sponsor requirements
- temperature test 20°C ±1°C.

The test may be performed by using as interfering substance either a solution of bovine albumin and sheep erythrocytes with a final concentration of 0.3% (simulating dirty conditions) or a solution of bovine albumin with a final concentration of 0.03% (simulating clean conditions).

After the prescribed time/s, both viral suspensions are inoculated in a cellular monolayer of HeLa (cells of human uterine carcinoma).

After 7 days, cellular cultures are observed with the inverted microscope for the detection of cytopathic effects (CPE) produced by viral multiplication.

Before checking virucidal activity, a test to validate the method based on 5 steps is performed:

1. ASSAY OF VIRAL ACTIVITY (virus titration)
2. CHECK OF CYTOTOXICITY OF THE TEST SUBSTANCE
3. CHECK OF CELLULAR SENSITIVITY TO VIRUS
4. CHECK OF SUPPRESSION OF DISINFECTANT ACTIVITY
5. CHECK OF VIRAL INACTIVATION (*Poliovirus type 1*)
6. CHECK OF VIRUS CONTROL VITALITY (Virus control)



The infecting activity is determined by means of Spaerman – Karber method that uses the following formula to calculate the value of TCID₅₀ (tissue culture infecting dose which kills 50% of the host cells):

$$-\text{Log}_{10} \text{TCID}_{50} = -(-x_0) - \{[R/100] - 0.5\} \times \log_{10} \text{dilution factor}$$

where:

x_0 = log₁₀ of the lowest dilution with 100% of positive reaction (CPE)

R = sum (%) of positive cultures

Log TCID₅₀ values are rounded to two significant ciphers as shown in Annex D table D.1 of EN 14476. Rounding is automatically performed by excel datasheet.

Normative references

EN14476:2007 - Chemical Disinfectants and Antiseptics – Virucidal Quantitative Suspension Test.

Restrictions

It is not applicable to formulations not soluble in water.

Expression and interpretation of the results

The virucidal activity of the product test solution is evaluated for each exposure time. The log-reduction is calculated by subtracting the logarithmic titre TCID₅₀ at any test point from the logarithmic titre TCID₅₀ of the virus control.

The test substance is considered virucidal when, after 60 minutes of contact, it causes a reduction of viral assay of at least 4log₁₀ compared to control virus.

Other test conditions (contact time, temperature, viruses) may be considered upon Sponsor's request.

Amount of samples necessary to the analysis/TAT from sample arrival

2x100 ml per test.

TAT: 28 days

Information to be provided with the sample

- name of product or formula code (compulsory);
- batch number (compulsory for studies to be performed under GLP accreditation);
- manufacture date;
- expiry date;
- storage and stability conditions (compulsory for studies to be performed under GLP accreditation);
- qualitative/quantitative composition (at least % of active ingredient)