

Test Report: EN 14476:2005 Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine - Test method and requirements (phase 2/step 1)

Test Laboratory

BluTest Laboratories Ltd

Robertson Incubator (4th Floor)
Robertson Building
University of Glasgow
57 Dumbarton Road
Glasgow
G11 6NU

Identification of sample

Name of the product
Project Code
Manufacturer
Date of Delivery
Storage Conditions
Active substances

DUOMAX DR 1

BT-DUO-04
DUO TECH LTD, 11-12 QUEENS SQUARE, BRISTOL, BS1 4NT
7 January 2010
Room temperature
Unknown

Test Method and its validation

Method

1 part interfering substance + 1 part virus suspension + 8 parts biocide were mixed and incubated at the indicated contact temperature for the indicated contact times. Assays were validated by a cytotoxicity control and a neutralization control.

Dilution-neutralization

Neutralizer

Dulbecco's modified Eagles medium + 5% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis
Product diluent used
Product test concentrations
Contact times
Test temperature
Interfering substances
Stability of mixture
Temperature of incubation
Identification of virus

January 2010
Sterile Hard Water
4.0%V/V, 2.5 %V/V, 1.0 %V/V
5 minutes $\pm$ 10s & 60 minutes $\pm$ 10s
20°C $\pm$ 1°C
0.6 g/l foetal bovine serum + 0.03% V/V BSA
Precipitate absent throughout the test
37°C $\pm$ 1°C + 5% CO₂
Poliovirus 1 (ATCC VR-1562)/HeLa cells
Adenovirus 5 (ATCC VR-5)/HeLa cells

PROTOCOL SUMMARY

Exposure times as standard are 60 minutes (obligatory), 30, 15 and 5 minutes (optional), with test conditions as clean (0.3g/l BSA) and/or dirty (3.0g/l BSA + 3% V/V sheep erythrocytes). Three dilutions of disinfectant were used, with at least one out with the expected efficacy range. Disinfectant was diluted in sterile synthetic hard water (shH₂O).

The basic Virucidal Efficacy Test is set up with one test per concentration of disinfectant (3 concentrations as specified by client) /exposure time. Virus is exposed to disinfectant in 24-well plates. The initial dilution and neutralisation step for each test is performed in a 24-well plate at 4°C, with the subsequent dilution series from 10⁻²–10⁻⁸ made in 96-well deep well blocks. Virus was titred in 96-well tissue culture plates (TCID₅₀).

All assay plates are set up in advance. For each virus:-

Neutralisation: In a 24-well plate, to each well was added 0.9ml EMEM (+ 2% serum, 1% penicillin, Streptomycin, L-Glutamate, PSG), and the plates maintained at 4°C.

Dilution series: To each well in a 96-well deep well block is pipetted 0.9ml of EMEM (+2% serum, 1%PSG), and the plates maintained at 4°C. In a second 96 well block (which can be shared by both test viruses), 2 columns of 6 wells are prepared as follows: into rows 1 and two pipette 0.9ml EMEM + 2% FBS, and into rows 3-6 pipette 0.9ml PBS +2% FBS. In the next 2 columns, to each of 6 wells, pipette 0.9ml PBS + 2% FBS.

TCID₅₀ assay: For an exposure time of 60 minutes only, a total of 8 plates per virus/cell line are prepared in advance, with an additional 2 plates required for each extra exposure time. Into every well of rows A-F was seeded 1x10⁴ cells in 100µl EMEM + 2% FBS. The plates are incubated until required for no longer than 24 hours.

NB: The cytotoxicity control described below should be performed at least 1 day prior to the test

Exposure to disinfectant was carried out in a 24-well plate, in a single replicate/test.

1. A 100µl volume of shH₂O at ambient room temperature (22°C, ± 2°) is pipetted into the test well. A 100µl volume of virus suspension (minimum 1x10⁹ TCID₅₀/ml) is added and mixed by pipetting.
2. To this add 800µl of disinfectant, and the mixture incubated for the required exposure time, timed by stopwatch.
3. The reaction was neutralised by transferring 100µl to 0.9ml ice cold medium prepared as above, and incubated at 4°C for 5 minutes
4. After incubation, a ten-fold dilution series from 10⁻² – 10⁻⁷ was made by serial transfer of 100µl through each of the prepared wells of the 96-well deep block.
5. A 100µl volume of each dilution was transferred to each of 6 adjacent wells in the TCID₅₀ assay 96-well tissue culture plates.
6. All 96-well plates were incubated at 37°C with 5%CO₂ and scored for cytopathic effect (cpe) after 4-5 days.

Controls

1. Cytotoxicity; a 200µl volume of shH₂O was added to 800µl of the highest concentration of disinfectant used. The mixture was immediately diluted to 10⁻⁷ in culture medium as before, and 100µl transferred to each of six wells per dilution in a TCID₅₀ assay 96-well tissue culture plates. The lowest dilution of disinfectant which showed no cytotoxic effect was determined for use in the following control.
2. Efficiency of disinfectant suppression; the test mixture was prepared as per steps 1 and 2 above. A 1 in 10 dilution was made immediately, in ice cold culture medium, and incubated on ice for 30 minutes. After incubation a tenfold dilution series was performed in ice cold culture medium, and 100µl transferred to each of 6 adjacent wells in a 96-well assay plate.
3. Virus recovery; virus recovery was performed at t=0 and at t=60. VR was performed as in steps 1-6 above, with the exception that in step 2, 800µl shH₂O was added instead of disinfectant.

RESULTS SUMMARY

ADENOVIRUS-5 RESULT UNDER CLEAN CONDITIONS

Table of results of virucidal activity against ADENOVIRUS 5 (ADV5) under clean conditions for DUOTECH

Product	Interfering substance	Concentration	Level of cytotoxicity	lg TCID50			>4 lg reduction
				0 min	5 min	60 min	
DUOMAX	0.3g/l BSA	4.0% (v/v)	5.50	7.50	5.50	5.50	(<5)
Column*		4.0% (v/v)	2.50	7.67	7.50	2.50	<5
		2.5% (v/v)	5.50	7.50	5.50	5.50	>5
		1.0% (v/v)	5.50	7.50	4.50	4.50	>5
Formaldehyde		0.7% (w/v)	4.50	7.50	5.00	4.50	
Virus Control		n.a.	n.a.	7.50	7.50	7.50	n.a.

* Application of chromatography neutralization of the 4.0% V/V (and the virus recovery at 5 minutes) resulted in a reduction of cytotoxicity that allows a 5 log reduction to be demonstrated in 5 minutes.

POLIOVIRUS – 1 RESULT UNDER CLEAN CONDITIONS

Table of results of virucidal activity against POLIOVIRUS 1 (PV1) under clean conditions for DUOTECH

Product	Interfering substance	Concentration	Level of cytotoxicity	lg TCID50			>4 lg reduction
				0 min	5 min	60 min	
DUOMAX	0.3g/l BSA	4.0% (v/v)	5.50	7.50	5.50	5.50	(<5)
		2.5% (v/v)	5.50	7.50	5.50	5.50	(<5)
		1.0% (v/v)	5.50	7.50	4.50	4.50	<5
Formaldehyde		0.7% (w/v)	4.50	7.50	7.50	5.50	>60
Virus Control		n.a.	n.a.	7.50	8.50	8.17	n.a.

Suspension test results for the efficacy of DUOMAX, Batch DR1 from DUOTECH against ADENOVIRUS 5 under clean conditions														
Exposure time	Virus recovery 0 min (TCD ₅₀ /ml)		Virus recovery t min (TCD ₅₀ /ml)		Cytotoxicity (TCD ₅₀ /ml)		Disinfectant Suppression (TCD ₅₀ /ml)		4% Disinfectant (TCD ₅₀ /ml)		2.5% Disinfectant (TCD ₅₀ /ml)		1.0% Disinfectant (TCD ₅₀ /ml)	
	raw data		raw data	TCD ₅₀	raw data		raw data	TCD ₅₀	raw data		raw data		raw data	
t=60 min	6.00	3.16E+07	6.00	3.16E+07	4.00	3.16E+05	4.00	3.16E+05	4.00	3.16E+05	4.00	3.16E+05	3.00	3.16E+04
		3.16E+07		3.16E+07		3.16E+05		3.16E+05		3.16E+05		3.16E+05		3.16E+04
log		7.50		7.50		5.50		5.50		5.50		5.50		4.50
difference								2.00		2.00		2.00		3.00
t=5 min	6.17	4.68E+07	6.00	3.16E+07	4.00	3.16E+05	4.00	3.16E+05	4.00	3.16E+05	4.00	3.16E+05	3.00	3.16E+04
		4.68E+07		3.16E+07		3.16E+05		3.16E+05		3.16E+05		3.16E+05		3.16E+04
log		7.67		7.50		5.50		5.50		5.50		5.50		4.50
difference								2.00		2.00		2.00		3.00
t=5min* (spin column)	6.17	4.68E+07	6.00	3.16E+07	1.00	3.16E+02			1.00	3.16E+02				
		4.68E+07		3.16E+07		3.16E+02				3.16E+02				
		7.67		7.50		2.50				2.50				
Stock Virus	7.33	6.76E+08	(60 minutes)											
	7.00	3.16E+08	(5 minutes)											

Suspension test results for the efficacy of DUOMAX, Batch DR1 from DUOTECH against POLIOVIRUS 1 (PV1) under clean conditions														
Exposure time	Virus recovery 0 min (TCID ₅₀ /ml)		Virus recovery t min (TCID ₅₀ /ml)		Cytotoxicity (TCID ₅₀ /ml)		Disinfectant Suppression (TCID ₅₀ /ml)		4% Disinfectant (TCID ₅₀ /ml)		2.5% Disinfectant (TCID ₅₀ /ml)		1.0% Disinfectant (TCID ₅₀ /ml)	
	raw data	TCID ₅₀	raw data	TCID ₅₀	raw data	TCID ₅₀	raw data	TCID ₅₀	raw data	TCID ₅₀	raw data	TCID ₅₀	raw data	TCID ₅₀
t=60 min	6.00	3.16E+07	6.17	4.68E+07	4.00	3.16E+05	4.83	2.14E+06	4.00	3.16E+05	4.00	3.16E+05	3.00	3.16E+04
log difference		3.16E+07		4.68E+07		3.16E+05		2.14E+06		3.16E+05		3.16E+05		3.16E+04
		7.50		8.17		5.50		6.33		5.50		5.50		4.50
								1.84		2.67		2.67		3.67
t=5 min	6.00	3.16E+07	7.00	3.16E+08	4.00	3.16E+05	4.83	2.14E+06	4.00	3.16E+05	4.00	3.16E+05	3.00	3.16E+04
log difference		3.16E+07		3.16E+08		3.16E+05		2.14E+06		3.16E+05		3.16E+05		3.16E+04
		7.50		8.50		5.50		6.33		5.50		5.50		4.50
								2.17		3.00		3.00		4.00
Stock Virus (TCID ₅₀)	7.50	1.00E+09												

CONCLUSION

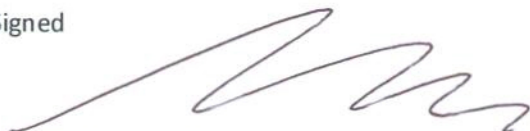
Verification of the methodology

A test is only valid if the following criteria are fulfilled:

- a) test virus suspension has TCID₅₀ 10⁸/ml, or possesses at least a concentration which allows the determination of a 4 lg reduction of the virus titre;
- b) detectable titre reduction is at least 4 lg;

According to EN 14476: 2005, DUOMAX DR 1 from Duotech Ltd, possesses virucidal activity against Poliovirus 1 (ATCC VR-1562) and Adenovirus 5 (ATCC VR-5) at 20°C following 5 MINUTES CONTACT UNDER CLEAN CONDITIONS at a concentration of 4.0% V/V. This result was demonstrated for adenovirus by the use of column chromatography to neutralize the disinfectant, that showed that a 5 log reduction at 5 minutes had been achieved.

Signed



Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
January 2010

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