CLIENT:	EKOKIM TEMIZLIK URUNLERI SAN. VE TIC. LTD. STI.
ADDRESS:	Sanayi Sk. No: 30 Posta Kodu: 203 Pelitlikoy GEBZE / KOCAELI – TURKEY
SAMPLES:	Bucket Of Sanitizer Wipes
SAMPLE RECEIVED ON:	01.06.2020
REPORT NUMBER:	2020156990
BRANDS OF THE PRODUCT:	CLARK

Tested by: Michael Jones

Checked by: Jennifer Richardson

Signed by: Walter Lee

Rabi-Laboratories

Test Standard: EN 14476+A1:2015

Test Purpose

Quantitative suspension test for the evaluation of virucidal activity of disinfectants intended for use in the medical area.

EN 14476 is a phase 2 step 1 suspension test to evaluate the virucidal activity of chemical disinfectants intended for use in the medical area.

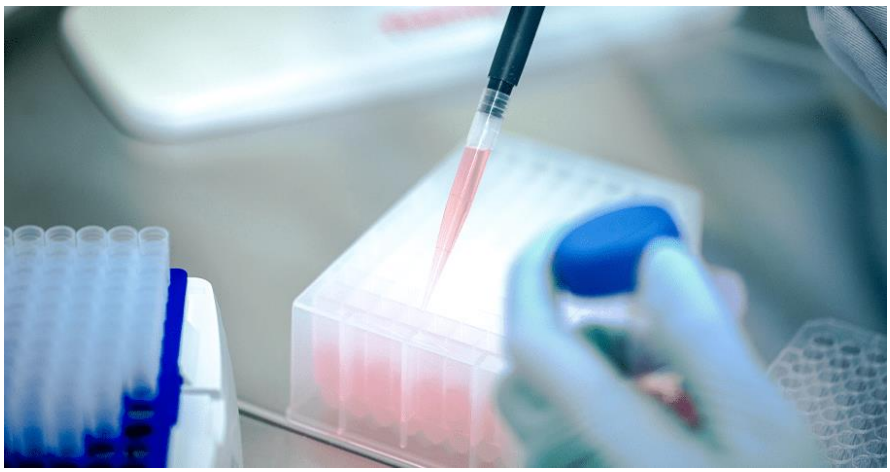
Refer to the table below for the minimum requirements for EN 14476+A1 test:

Table 1: EN 14476 mandatory test viruses

	Hygienic handrub and handwash	Instrument disinfection	Surface disinfection	Textile disinfection
Mandatory test microorganisms	Fully virucidal Adenovirus Norovirus Poliovirus Limited spectrum virucidal activity Adenovirus Norovirus Virucidal activity against enveloped viruses Vaccinia virus	Adenovirus Norovirus When temperature is 40°C or higher, only parvovirus	Adenovirus Norovirus Poliovirus	Parvovirus
Test temperature	according to the manufacturer's recommendation but at / between			
	20°C	20°C and 70°C	4°C and 30°C	30°C and 70°C
Contact time	according to the manufacturer's recommendation			
	but between	but no longer than	but no longer than	but no longer than
	30 sec and 2 min	60 min	5 min or 60 min	20 min
Interfering substance – clean condition	0,3 g/l bovine albumin solution (hygienic handrub)	0,3 g/l bovine albumin solution and/or	0,3 g/l bovine albumin solution and/or	
Interfering substance – dirty condition	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes (hygienic handwash)	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes
Reduction (lg)	≥ 4	≥ 4	≥ 4	≥ 4

Test Conditions

All the viruses listed are non-enveloped viruses (except vaccinia virus) and as such are more resistant to chemical disinfectants compared to enveloped viruses. Poliovirus is the most resistant among non-enveloped viruses and manufacturers often struggle to attain efficacy against this microorganism. If EN 14476 is performed against all three non-enveloped viruses above for a hand product and it passes the test for adenovirus or norovirus but fails against poliovirus, the product is deemed as limited spectrum virucidal or limited virucidal. For a hand product to be fully virucidal or acknowledged as capable of inactivating all enveloped and non-enveloped viruses, it must be effective against adenovirus, norovirus and poliovirus. Instrument and surface disinfectants intended for the medical area however, must pass the test against all three non-enveloped viruses.



In preparing for the suspension test, the test virus is added to an interfering substance in a suspension. The choice of interfering substance used in the test depends on the product claim. The test product or disinfectant is then added to the virus suspension for the duration of the exposure time at the temperature specified by the manufacturer.

At the end of the exposure time, samples are retrieved and the activity of the test product is neutralised by dilution in ice-cold test medium. Serial dilutions are performed, and the dilutions are examined for viral infectivity.

Unlike bacteria or fungus, most viruses are too small (ranging from 25nm to 400nm) for observation under a light microscope. The presence of viruses in a suspension before and after product exposure is therefore determined by inoculating live host cells with suspension samples. These cells are then observed after 7 days (depending on the cell type) for structural changes. If the test product had not been successful in inactivating test viruses before neutralization, they invade and damage the live cells to display cytopathic effect (CPE). These are the effects virologists look for when observing the cells under a light microscope.

Control Tests

In addition to the efficacy test, 5 control tests are run concurrently to eliminate other possible explanations for the test results. The 5 control tests are:

1. Virus control

Determines the infectivity of the test virus suspension. To pass the test, the concentration of virus in the control test must be sufficiently high to enable a 4-log reduction or reduction of viruses by 10 000-fold.

2. Cytotoxicity control

Reveals the possible alteration in cell structure caused by the test product or disinfectant. To pass the test, live cells must not display toxic reaction or damage to a level where achieving 4-log reduction is not possible.

3. Suppression control

Verifies the efficiency of the neutralising method in suppressing the virucidal activity of the test product after the required exposure time.

4. Interference control

Verifies the susceptibility of infection in cells is not influenced negatively by the test product (passed cytotoxicity test).

Ensures the test virus can be inactivated and non-resistant to antimicrobial agents, enabling it to achieve 4-log reduction.

ANTIMICROBIAL TEST RESULTS		
MICROORGANISMS NAME	CLARK (product) BIOLOGICAL ACTIVITY	ANTIBACTERIAL EFFECT (% REDUCE)
Escherichia coli ATCC 25922	+	%99,9
Staphylococcus aureus ATCC 25923	+	%92
Enterococcus faecalis ATCC 29212	+	%99,9
Pseudomonas aeruginosa ATCC 27853	+	%98
MRSA (Methicillin-resistant Staphylacoccus aureus)	+	%98
Salmonella typhimurium ATCC 14028	+	%80,8
Listeria monocytogenes ATCC 15313	+	%99,9

Influenza A (H1N1)

	Refrance of virus	% 0.1 Product Effect			
		30 Seconds		60 Seconds	
Virus Titre*	5.5	**Clean Environment**	**Dirty Environment**	**Clean Environment**	**Dirty Environment**
Virus Titre With Product**		1.5	1.5	1.3	1.5
The Rate Of Decrease In The Titre Of The Virus***		4.0	4.0	4.2	4.0

* Logarithmic TCID50 value of virus in M1.
**Logarithmic TCID50 value of the virus treated with the product in different time periods and environments.
***Logarithmic TCID50 ratio between virus titer and product virus titer.

It tested the effectiveness of a liquid wet cloth solution of Ekokim Temizlik Urunleri San. Ve Tic. Ltd. Sti. against the influenza A (H1N1) virus in accordance with EN 14476 standards. The lowest non-toxic rate of the disinfectant solution in question of the product tested in this experiment was used in this study because the 100% ratio showed toxic effects to cells in the test environment. As a result of the Test, the product at 0.1% pda temperature (20 C), in clean and dirty conditions, 30 and 60 seconds of application time, in all experimental conditions virus titer (see result table) was found to cause at least 4 log reductions. According to Antimicrobial Division US EPA standards, disinfectants are required to reduce virus titers by 4 logs or more for their virusidal activity.

As a result; the results of this experiment show that the product tested was 99.99% effective against the influenza A (H1N1) virus at the 0.1% suspension of 30 and 60 seconds of application time.

This product can maintain its effectiveness if used with one of the methods of washing, wiping, impregnation (wetting/dipping) and spraying, provided that it is used at least in the above-mentioned resolutions and times.

Coronavirus (COVID-19)

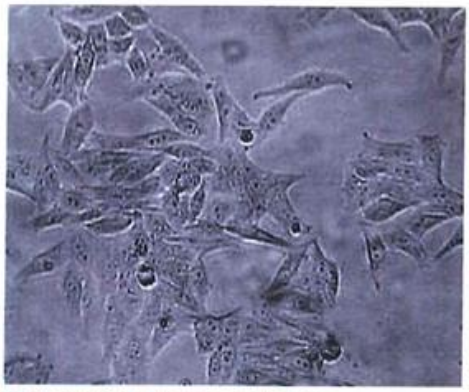
It tested the effectiveness of a liquid wet cloth solution of Ekokim Temizlik Urunleri San. Ve Tic. Ltd. Sti. against the coronavirus (COVID-19) in accordance with EN 14476 standards. The lowest non-toxic rate of the disinfectant solution in question of the product tested in this experiment was used in this study because the 100% ratio showed toxic effects to cells in the test environment. As a result of the Test, the product at 0.1% pda temperature (20 C), in clean and dirty conditions, 5 and 30 seconds of application time, in all experimental conditions virus titer (see result table) was found to cause at least 4 log reductions. According to Antimicrobial Division US EPA standards, disinfectants are required to reduce virus titers by 4 logs or more for their virusidal activity.

As a result; the results of this experiment show that the product tested was 99.99% effective against the coronavirus (COVID-19) at the 0.1% suspension of 30 seconds of application time.

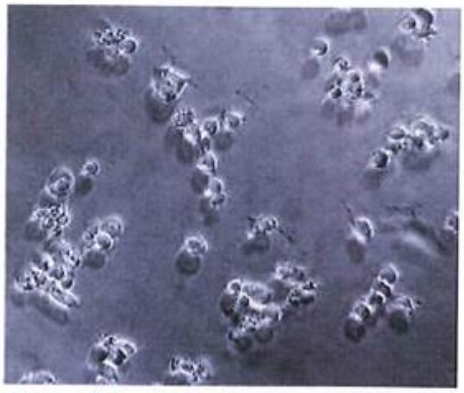
This product may retain its effectiveness when used in one of the methods of washing, wiping, impregnation (wetting/dipping) and spraying, provided it is used at specified resolutions and times.

Determination of Results

CPEs if present, can be viewed through a light microscope and the reduction in virus infectivity is calculated through the difference in virus concentration before and after treatment with the test product. A 4-log reduction or reduction of viruses by 10 000-fold demonstrates the ability of the test product in inactivating viruses to a level acceptable to the European standards. Log reductions are calculated by determining 50% Tissue Culture Infective Dose (TCID50) or the viral dose required to display CPE in 50% of the cell culture.



Cell lines before exposure to Poliovirus



Cell lines displaying CPE after exposure to Poliovirus

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