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SAMM 1016

Test Report No.: TR-22-0080

Determination of the Bactericidal Activity of **BLEACHING LIQUID (5.20%) – REGULAR** according to EN 1276:2019

Test Method
EN 1276:2019
Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas – Test method and requirements (Phase 2, step 1)

Testing Laboratory
TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
No. 115, Jalan Kepayang, Off Jalan Kuching
51200 Kuala Lumpur
Malaysia

Kuala Lumpur, 2 March 2022

Dr Marven Lee Cheng Shoou
Managing Director

IDENTIFICATION OF TESTING LABORATORY

TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
No. 115, Jalan Kepayang, Off Jalan Kuching
51200 Kuala Lumpur
Malaysia

IDENTIFICATION OF TEST ITEM

Test item name:	Bleaching Liquid (5.20%) – Regular
Lab ID:	D001-21-001
Batch no.:	Not specified
Receipt date:	24 November 2021
Storage conditions:	Room temperature away from sunlight
Product diluent recommended by manufacturer:	Not specified
Active substances:	Sodium hypochlorite (3.9%)
Product appearance:	Clear, pale yellow solution

TEST METHOD & VALIDATION

Test method:	EN 1276:2019 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas – Test method and requirements (Phase 2, step 1)
Inactivation method:	Dilution-neutralization method
Inactivator:	30 g/L Tween 80 3 g/L Sodium thiosulphate 3 g/L Lecithin

EXPERIMENTAL CONDITIONS

Date of test:	16 February – 17 February 2022
Product diluent:	Hard water
Concentration / contact time:	0.6%* / 20 minute ± 10 seconds
Test temperature:	(20 ± 1) °C
Interfering substance:	Clean condition (0.3 g/L bovine serum albumin)
Test organism:	*Enterococcus hirae* ATCC 10541 *Escherichia coli* ATCC 10536 *Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 15442
Incubation temperature:	(37 ± 1) °C
Incubation period:	24 hours
Appearance of the product dilutions:	Clear, colourless solution
Stability and appearance of product dilutions during test:	Homogenous without any precipitate

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

CONTROLS AND VALIDATION

Test Organism	Validation Suspension	Experimental Conditions Control	Neutralizer or Filtration Control	Method Validation
E. hirae ATCC 10541	$N_V/10$: 91.0	A: 94.5	B: 93.5	C: 89.5
E. coli ATCC 10536	$N_V/10$: 159.0	A: 100.0	B: 105.0	C: 109.0
P. aeruginosa ATCC 15442	$N_V/10$: 121.5	A: 123.5	B: 132.5	C: 65.0
S. aureus ATCC 6538	$N_V/10$: 90.0	A: 113.5	B: 90.5	C: 94.5

The control and validation tests A, B, and C were within the basic limits:

- The number of cells per mL in the validation suspension, $N_V/10$, must be between 30 and 160,
- A must be equal to or greater than 0.5 x $N_V/10$ to verify the absence of any lethal effect in the experimental conditions,
- B must be equal to or greater than 0.5 x $N_V/10$ to verify the absence of neutralizer toxicity or to validate the filtration procedure, and
- C must be equal to or greater than 0.5 x $N_V/10$ to validate the dilution-neutralization method or membrane filtration method.

TEST RESULTS

For each product concentration and contact time, the log reduction (lg R) is calculated using the formula lg R = lg N_0 – lg Na, in which:

- N_0 is the number of cells per mL in the test mixture at the beginning of the contact time, and
- Na is the number of cells per mL in the test mixture at the end of the contact time and before neutralization or filtration.

Test organism: *Enterococcus hirae* ATCC 10541

Test suspension, N	N: 4.90×10^8 lg N_0: 7.69

Concentration / Contact Time	Test, Na	Reduction, lg R = lg N_0 – lg Na
0.6%* / 20 minutes	Na: $<1.40 \times 10^2$ lg Na: <2.15	lg R: >5.54 ± 0.10 %R: >99.9997%

Test organism: *Escherichia coli* ATCC 10536

Test suspension, N	N: 4.80×10^8 lg N_0: 7.68

Concentration / Contact Time	Test, Na	Reduction, lg R = lg N_0 – lg Na
0.6%* / 20 minutes	Na: $<1.40 \times 10^2$ lg Na: <2.15	lg R: >5.54 ± 0.10 %R: >99.9997%

Test organism: *Pseudomonas aeruginosa* ATCC 15442

Test suspension, N	N: 4.77×10^8 lg N_0: 7.68

Concentration / Contact Time	Test, Na	Reduction, lg R = lg N_0 – lg Na
0.6%* / 20 minutes	Na: $<1.40 \times 10^2$ lg Na: <2.15	lg R: >5.53 ± 0.10 %R: >99.9997%

Test organism: *Staphylococcus aureus* ATCC 6538

Test suspension, N	N: 4.35×10^8 lg N_0: 7.64

Concentration / Contact Time	Test, Na	Reduction, lg R = lg N_0 – lg Na
0.6%* / 20 minutes	Na: $<1.40 \times 10^2$ lg Na: <2.15	lg R: >5.49 ± 0.10 %R: >99.9997%

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

CONCLUSION

The test item achieved a reduction of ≥5.00 log against the test organisms *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, and *Staphylococcus aureus* ATCC 6538 under tested conditions.

Therefore, **Bleaching Liquid (5.20%) – Regular** has demonstrated a bactericidal activity according to EN 1276:2019 under the following conditions:

Concentration	Contact Time	Test Temperature	Soiling
0.6%*	20 minutes	20 °C	Clean condition

Kuala Lumpur, 2 March 2022

Norazzira Zulkharnain
Microbiologist

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

EXPERT OPINION

This expert opinion is based on the test report TR-22-0080 dated 2 March 2022. Opinions and interpretations expressed herein are outside the scope of the Laboratory Accreditation Scheme of Malaysia (SAMM).

The product **Bleaching Liquid (5.20%) – Regular** was tested according to EN 1276:2019 against *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, and *Staphylococcus aureus* ATCC 6538. These organisms are the minimum test organisms and they have been chosen as representative species taking into account their relative resistance, relevance to practical use, handling properties, and microbiological safety.

Bactericidal activity is defined as a capability of a product or active substance to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions. According to EN 1276, a general-purpose disinfectant is considered to possess a bactericidal activity if it demonstrates a reduction of ≥5.00 log against the minimum spectrum of test organisms within 60 minutes when tested at 4 to 60 °C under clean (0.3 g/L bovine serum albumin) or dirty (3.0 g/L bovine serum albumin) condition.

When tested under the following conditions, **Bleaching Liquid (5.20%) – Regular** achieved a reduction of ≥5.00 log against *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, and *Staphylococcus aureus* ATCC 6538:

Concentration	Contact Time	Test Temperature	Soiling
0.6%*	20 minutes	20 °C	Clean condition

Therefore, **Bleaching Liquid (5.20%) – Regular** has demonstrated a bactericidal activity against conforming to EN 1276:2019 under the aforementioned conditions.

Kuala Lumpur, 2 March 2022

Dr Marven Lee Cheng Shoou
Managing Director

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

INFORMATION ON MEASUREMENT UNCERTAINTY & DECISION RULE

The statement of conformity given by EN 1276:2019 states that the test item shall be considered to have passed EN 1276 if it demonstrates ≥5.00 log reduction under the defined conditions.

The laboratory employs the simple acceptance decision rule to account for the measurement uncertainty when stating the statement of conformity. The measurement uncertainty and conformance probability are shown in the raw data and are summarized as follows:

Test Organism	Concentration / Contact Time	Log Reduction	Conformance	Conformance Probability†
E. hirae ATCC 10541	0.6%* / 20 minutes	>5.54 ± 0.10	Yes	<0.001% chance of false acceptance
E. coli ATCC 10536	0.6%* / 20 minutes	>5.54 ± 0.10	Yes	<0.001% chance of false acceptance
P. aeruginosa ATCC 15442	0.6%* / 20 minutes	>5.53 ± 0.10	Yes	<0.001% chance of false acceptance
S. aureus ATCC 6538	0.6%* / 20 minutes	>5.49 ± 0.10	Yes	<0.001% chance of false acceptance

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

† The conformance probability follows a normal distribution. Therefore, the percentage of conformance can never be zero or 100% due to the asymptotic tails.

RAW DATA

Test Method:	EN 1276:2019				
Product Name:	Bleaching Liquid (5.20%) - Regular			Batch No.:	-
Product Diluent:	Hard water			Lab ID:	D001-21-001
Appearance of Product Dilutions:	Clear , colourless solution				
Inactivation:	Dilution-neutralization	Dilution Method:	Standard (80%)	Test Temperature (°C):	20
Neutralizer:	30 g/L Tween 80, 3 g/L Sodium thiosulphate, 3 g/L Lecithin				
Interfering Substance:	0.3 g/L bovine serum albumin				
Test Organism:	Enterococcus hirae ATCC 10541			Plating Method:	Pour plate
Incubation Temperature (°C):	37	Passing Criteria (lg):	5.00	Measurement Uncertainty (±):	0.10
Testing Period:	16/2/2022		Tested By: AZZ	Verified By:	CSE

Validation & Controls

Validation Suspension (N_V)	V_{C1}	V_{C2}	N_{V0} = 91.0 $N_{V0} = N_V/10$
	92	90	Limit: $30 \le N_{V0} \le 160$
Validation Suspension (N_{VB})	V_{C1}	V_{C2}	N_{V0} = $N_{V0} = N_{VB}/1000$
	-	-	Limit: $30 \le N_{V0} \le 160$

Experimental Conditions Control (A)	V_{C1}	V_{C2}	A = 94.5
	96	93	Limit: $A \ge 0.5 \times N_V/10$
Neutralizer Control (B)	V_{C1}	V_{C2}	B = 93.5
	96	91	Limit: $B \ge 0.5 \times N_V/10$ or $N_{VB}/1000$
Method Validation (C)	V_{C1}	V_{C2}	C = 89.5
Conc.: 0.6%	89	90	Limit: $C \ge 0.5 \times N_V/10$

Test Suspension & Procedure

Test Suspension (N)	N	V_{C1}	V_{C2}	$\overline{x}_{wm}$ = N = 4.90E+08
	10^{-6}	>330	>330	N_0 = N/10 lg N_0 = 7.69
	10^{-7}	41	57	Limit: 7.17 ≤ lg N_0 ≤ 7.70

Product Concentration	Contact Time	Dilution	V_{C1}	V_{C2}	Na = $\overline{x}$ or $\overline{x}_{wm}$ x 10	lg Na	lg R = lg N_0 - lg Na	Conformance Probability
0.6%	20 minutes	10^{0}	<14	<14	<1.40E+02	<2.15	>5.54 ± 0.10	>99.999%
		10^{-1}	<14	<14				
		10^{-2}	<14	<14			>99.9997%	
		10^{-3}	<14	<14				
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						

Raw Data of Colony Count

	N_V	N_{VB}	A	B	C	N^{-6}	N^{-7}
V_{C1}	92	-	96	96	89	>330	41
V_{C2}	90	-	93	91	90	>330	57

Product Concentration	Contact Time	Na^{0}		Na^{-1}		Na^{-2}		Na^{-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
0.6%	20 minutes	0	0	0	0	0	0	0	0

RAW DATA

Test Method:	EN 1276:2019				
Product Name:	Bleaching Liquid (5.20%) - Regular			Batch No.:	-
Product Diluent:	Hard water			Lab ID:	D001-21-001
Appearance of Product Dilutions:	Clear , colourless solution				
Inactivation:	Dilution-neutralization	Dilution Method:	Standard (80%)	Test Temperature (°C):	20
Neutralizer:	30 g/L Tween 80, 3 g/L Sodium thiosulphate, 3 g/L Lecithin				
Interfering Substance:	0.3 g/L bovine serum albumin				
Test Organism:	Escherichia coli ATCC 10536			Plating Method:	Pour plate
Incubation Temperature (°C):	37	Passing Criteria (lg):	5.00	Measurement Uncertainty (±):	0.10
Testing Period:	17/2/2022		Tested By: AZZ	Verified By:	CSE

Validation & Controls

Validation Suspension (N_V)	V_{C1}	V_{C2}	N_{V0} = 159.0 $N_{V0} = N_V/10$
	157	161	Limit: $30 \le N_{V0} \le 160$
Validation Suspension (N_{VB})	V_{C1}	V_{C2}	N_{V0} = $N_{V0} = N_{VB}/1000$
	-	-	Limit: $30 \le N_{V0} \le 160$

Experimental Conditions Control (A)	V_{C1}	V_{C2}	A = 100.0
	101	99	Limit: $A \ge 0.5 \times N_V/10$
Neutralizer Control (B)	V_{C1}	V_{C2}	B = 105.0
	98	112	Limit: $B \ge 0.5 \times N_V/10$ or $N_{VB}/1000$
Method Validation (C)	V_{C1}	V_{C2}	C = 109.0
Conc.: 0.6%	99	119	Limit: $C \ge 0.5 \times N_V/10$

Test Suspension & Procedure

Test Suspension (N)	N	V_{C1}	V_{C2}	$\overline{x}_{wm}$ = N = 4.80E+08
	10^{-6}	>330	>330	N_0 = N/10 lg N_0 = 7.68
	10^{-7}	40	56	Limit: 7.17 ≤ lg N_0 ≤ 7.70

Product Concentration	Contact Time	Dilution	V_{C1}	V_{C2}	Na = $\overline{x}$ or $\overline{x}_{wm}$ x 10	lg Na	lg R = lg N_0 - lg Na	Conformance Probability
0.6%	20 minutes	10^{0}	<14	<14	<1.40E+02	<2.15	>5.54 ± 0.10	>99.999%
		10^{-1}	<14	<14				
		10^{-2}	<14	<14			>99.9997%	
		10^{-3}	<14	<14				
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						

Raw Data of Colony Count

	N_V	N_{VB}	A	B	C	N^{-6}	N^{-7}
V_{C1}	157	-	101	98	99	>330	40
V_{C2}	161	-	99	112	119	>330	56

Product Concentration	Contact Time	Na^{0}		Na^{-1}		Na^{-2}		Na^{-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
0.6%	20 minutes	0	0	0	0	0	0	0	0

RAW DATA

Test Method:	EN 1276:2019				
Product Name:	Bleaching Liquid (5.20%) - Regular			Batch No.:	-
Product Diluent:	Hard water			Lab ID:	D001-21-001
Appearance of Product Dilutions:	Clear , colourless solution				
Inactivation:	Dilution-neutralization	Dilution Method:	Standard (80%)	Test Temperature (°C):	20
Neutralizer:	30 g/L Tween 80, 3 g/L Sodium thiosulphate, 3 g/L Lecithin				
Interfering Substance:	0.3 g/L bovine serum albumin				
Test Organism:	Pseudomonas aeruginosa ATCC 15442			Plating Method:	Spread plate
Incubation Temperature (°C):	37	Passing Criteria (lg):	5.00	Measurement Uncertainty (±):	0.10
Testing Period:	17/02/2022	Tested By:	NII	Verified By:	CSE

Validation & Controls

Validation Suspension (N_V)	V_{C1}	V_{C2}	N_{V0} = 121.5 $N_{V0} = N_V/10$
	126	117	Limit: $30 \le N_{V0} \le 160$
Validation Suspension (N_{VB})	V_{C1}	V_{C2}	N_{V0} = $N_{V0} = N_{VB}/1000$
	-	-	Limit: $30 \le N_{V0} \le 160$

Experimental Conditions Control (A)	V_{C1}	V_{C2}	A = 123.5
	127	120	Limit: $A \ge 0.5 \times N_V/10$
Neutralizer Control (B)	V_{C1}	V_{C2}	B = 132.5
	127	138	Limit: $B \ge 0.5 \times N_V/10$ or $N_{VB}/1000$
Method Validation (C)	V_{C1}	V_{C2}	C = 65.0
Conc.: 100%	65	65	Limit: $C \ge 0.5 \times N_V/10$

Test Suspension & Procedure

Test Suspension (N)	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = N = 4.77E+08
	10^{-6}	490	466	N_0 = N/10 lg N_0 = 7.68
	10^{-7}	50	44	Limit: 7.17 ≤ lg N_0 ≤ 7.70

Product Concentration	Contact Time	Dilution	V_{C1}	V_{C2}	Na = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg Na	lg R = lg N_0 - lg Na	Conformance Probability
0.6%	20 minutes	10^{0}	<14	<14	<1.40E+02	<2.15	>5.53 ± 0.10	>99.999%
		10^{-1}	<14	<14				
		10^{-2}	<14	<14			>99.9997%	
		10^{-3}	<14	<14				
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						

Raw Data of Colony Count

	N_V		N_{VB}		A		B		C		N^{-6}		N^{-7}	
V_{C1}	60	66	-	-	58	69	58	69	40	25	244	246	25	25
V_{C2}	62	55	-	-	60	60	60	78	30	35	215	251	25	19

Product Concentration	Contact Time	Na^{0}		Na^{-1}		Na^{-2}		Na^{-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
0.6%	20 minutes	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0

RAW DATA

Test Method:	EN 1276:2019				
Product Name:	Bleaching Liquid (5.20%) - Regular			Batch No.:	-
Product Diluent:	Hard water			Lab ID:	D001-21-001
Appearance of Product Dilutions:	Clear , colourless solution				
Inactivation:	Dilution-neutralization	Dilution Method:	Standard (80%)	Test Temperature (°C):	20
Neutralizer:	30 g/L Tween 80, 3 g/L Sodium thiosulphate, 3 g/L Lecithin				
Interfering Substance:	0.3 g/L bovine serum albumin				
Test Organism:	Staphylococcus aureus ATCC 6538			Plating Method:	Pour plate
Incubation Temperature (°C):	37	Passing Criteria (lg):	5.00	Measurement Uncertainty (±):	0.10
Testing Period:	16/2/2022		Tested By: NII	Verified By:	CSE

Validation & Controls

	V_{C1}	V_{C2}	
Validation Suspension (N_V)	92	88	N_{V0} = 90.0 $N_{V0} = N_V/10$ Limit: $30 \le N_{V0} \le 160$
Validation Suspension (N_{VB})	-	-	N_{V0} = $N_{V0} = N_{VB}/1000$ Limit: $30 \le N_{V0} \le 160$

	V_{C1}	V_{C2}	
Experimental Conditions Control (A)	120	107	A = 113.5 Limit: $A \ge 0.5 \times N_V/10$
Neutralizer Control (B)	91	90	B = 90.5 Limit: $B \ge 0.5 \times N_V/10$ or $N_{VB}/1000$
Method Validation (C) Conc.: 0.6%	99	90	C = 94.5 Limit: $C \ge 0.5 \times N_V/10$

Test Suspension & Procedure

	N	V_{C1}	V_{C2}	
Test Suspension (N)				$\bar{x}_{wm}$ = N = 4.35E+08
	10^{-6}	>330	>330	N_0 = N/10 lg N_0 = 7.64
	10^{-7}	50	37	Limit: 7.17 ≤ lg N_0 ≤ 7.70

Product Concentration	Contact Time	Dilution	V_{C1}	V_{C2}	Na = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg Na	lg R = lg N_0 - lg Na	Conformance Probability
0.6%	20 minutes	10^{0}	<14	<14	<1.40E+02	<2.15	>5.49 ± 0.10	>99.999%
		10^{-1}	<14	<14				
		10^{-2}	<14	<14			>99.9997%	
		10^{-3}	<14	<14				
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						

Raw Data of Colony Count

	N_V	N_{VB}	A	B	C	N^{-6}	N^{-7}
V_{C1}	92	-	120	91	99	>330	50
V_{C2}	88	-	107	90	90	>330	37

Product Concentration	Contact Time	Na^{0}		Na^{-1}		Na^{-2}		Na^{-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
0.6%	20 minutes	0	0	0	0	0	0	0	0

TEST PROCEDURE

1. Test *Na*: Determination of Bactericidal Concentrations

   1.1 1.0 mL of the interfering substance was pipetted into a tube. 1.0 mL of the test suspension *N* (1.5 – 5.0 x 10^8 cfu/mL) was added to the tube.

   1.2 The stopwatch was started immediately and the tube was mixed and placed in a water bath controlled at test temperature (θ ± 1) °C for 2 minutes ± 10 seconds.

   1.3 At the end of the 2 minutes, 8.0 mL of the product test solution was added to the tube. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at θ for the contact time *t*. Just before the end of *t*, the tube was mixed again.

   1.4 At the end of *t*, the test mixture was inactivated by one of the following methods:

      1.4.1 For dilution neutralization method:

         1.4.1.1 1.0 mL sample of the test mixture *Na* was transferred into a tube containing 8.0 mL of neutralizer and 1.0 mL of distilled water. The neutralizer tube was mixed and placed in a water bath controlled at (20 ± 1) °C.

         1.4.1.2 After a neutralization time of 5 minutes ± 10 seconds (for products with *t* of 10 minutes or shorter, neutralization time is (10 ± 1) seconds), the neutralizer tube was mixed and 1.0 mL of the neutralized test mixture *Na* (containing neutralizer, product test solution, interfering substance, and test suspension) was taken in duplicate and inoculated using the pour or spread plate technique.

         1.4.1.3 For hygienic handwash products, two additional decimal dilutions were plated.

      1.4.2 For membrane filtration method:

         1.4.2.1 0.1 mL sample of the test mixture *Na* was taken in duplicate and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

         1.4.2.2 For hygienic handwash products, a 0.1 mL sample of the test mixture *Na* was diluted 10-fold and 100-fold in 0.9 ml and 9.9 mL of rinsing liquid to obtain 10^{-1} and 10^{-2} dilutions, respectively. The mixture was mixed and 0.1 mL of each dilution was transferred in duplicate into two separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

   1.5 The procedure was performed using other product test solutions at the same time.

2. Experimental Conditions Control *A*: Verification of the Absence of Any Lethal Effect in the Experimental Conditions

   2.1 1.0 mL of the interfering substance used in the test *Na* was pipetted into a tube. 1.0 mL of the validation suspension N_V (0.3 – 1.6 x 10^3 cfu/mL) was added to the tube.

   2.2 The stopwatch was started immediately and the tube was mixed and placed in a water bath controlled at test temperature θ for 2 minutes ± 10 seconds.

2.3 At the end of the 2 minutes, 8.0 mL of hard water (distilled water for ready-to-use or handwash products) was added to the tube. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at θ for the contact time t. Just before the end of t, the tube was mixed again.

2.4 For dilution-neutralization method, 1.0 mL sample of the test mixture *A* was taken in duplicate at the end of t and inoculated using the pour or spread plate technique for dilution-neutralization method.

2.5 For membrane filtration method, 1.0 mL sample of the test mixture *A* was taken in duplicate at the end of t and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

3. Neutralizer or Filtration Control *B*: Verification of the Absence of Toxicity of the Neutralizer or the Filtration Procedure

3.1 For dilution neutralization method:

3.1.1 8.0 mL of the neutralizer used in the test *Na* and 1.0 mL of distilled water were pipetted into a tube. 1.0 mL of the validation suspension N_V was added to the tube.

3.1.2 The stopwatch was started at the beginning of the addition and the tube was mixed and placed in a water bath controlled at the test temperature θ for 5 minutes ± 10 seconds ((10 ± 1) seconds for products with t of 10 minutes or shorter). Just before the end of this time, the tube was mixed.

3.1.3 At the end of the time, 1.0 mL sample of the test mixture *B* was taken in duplicate and inoculated using the pour or spread plate technique.

3.2 For membrane filtration method:

3.2.1 0.1 mL of the validation suspension *Nv* was taken in duplicate and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

4. Method Validation *C*: Validation of the Dilution-Neutralization or Membrane Filtration Method

4.1 1.0 mL of the interfering substance used in the test *Na* was pipetted into a tube. 1.0 mL of diluent was added and then, starting a stopwatch, 8.0 mL of the product test solution of the highest concentration used in the test *Na* was added to the tube. The tube was mixed and placed in a water bath controlled at test temperature θ for contact time t. Just before the end of t, the tube was mixed again.

4.2 For dilution neutralization method:

4.2.1 At the end of t, 1.0 mL of the mixture was transferred into a tube containing 8.0 mL of neutralizer used in the test *Na*. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at the test temperature θ for 5 minutes ± 10 seconds ((10 ± 1) seconds for products with t of 10 minutes or shorter).

4.2.2 1.0 mL of the validation suspension N_V was added. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at (20 ± 1) °C for (30 ± 1) minutes. Just before the end of this time, the tube was mixed again.

4.2.3 At the end of this time, 1.0 mL sample of the test mixture *C* was taken in duplicate and inoculated using the pour or spread plate technique.

4.3 For membrane filtration method:

4.3.1 At the end of *t*, 0.1 mL of the test mixture *C* was taken in duplicate and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed as before and the membranes were covered with 50 mL of rinsing liquid again.

4.3.2 0.1 mL of the validation suspension *Nv* was added to each filtration apparatus. The mixture was filtered and rinsed again and each membrane was transferred onto the surface of separate agar plates.

5. Incubation and Counting

5.1 The plates were incubated for 20 to 24 hours. The plates were counted to determine the number of cfu. Any plates which were not countable for any reason were discarded.

5.2 The plates were incubated for a further 20 to 24 hours. The plates were recounted and if the number of colonies had increased, only the higher number was used for further evaluation. Plates that no longer showed well-separated colonies were not recounted.

5.3 For each plate, the exact number of colonies were noted but any counts higher than 330 colonies were recorded as '>330'. For membrane filtration method, record '>165' for any counts higher than 165 colonies.

5.4 All experimental data were reported as V_C values, in which a V_C value is the number of cfu counted per 1.0 mL sample inoculated. For membrane filtration method, a V_C value is the number of cfu counted per 0.1 mL sample of *Na*, *B*, and *C*, and per 1.0 mL sample of *A*.

5.5 Only V_C values within the counting limits, i.e., 14 to 330/165 colonies, were taken into account for further calculation, except in the case of *Na*.

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Test Report No.: TR-22-0081

Determination of the Fungicidal and Yeasticidal Activities of **BLEACHING LIQUID (5.20%) – REGULAR** according to EN 1650:2019

Test Method
EN 1650:2019
Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas – Test method and requirements (Phase 2, step 1)

Testing Laboratory
TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
No. 115, Jalan Kepayang, Off Jalan Kuching
51200 Kuala Lumpur
Malaysia

Kuala Lumpur, 2 March 2022

Dr Marven Lee Cheng Shoou
Managing Director

IDENTIFICATION OF TESTING LABORATORY

TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
No. 115, Jalan Kepayang, Off Jalan Kuching
51200 Kuala Lumpur
Malaysia

IDENTIFICATION OF TEST ITEM

Test item name:	Bleaching Liquid (5.20%) – Regular
Lab ID:	D001-21-001
Batch no.:	Not specified
Receipt date:	24 November 2021
Storage conditions:	Room temperature away from sunlight
Product diluent recommended by manufacturer:	Not specified
Active substances:	Sodium hypochlorite (3.9%)
Product appearance:	Clear, pale yellow solution

TEST METHOD & VALIDATION

Test method:	EN 1650:2019 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas – Test method and requirements (Phase 2, step 1)
Inactivation method:	Dilution-neutralization method
Inactivator:	30 g/L Tween 80 3 g/L Sodium thiosulphate 3 g/L Lecithin

Inactivator: 30 g/L Tween 80
3 g/L Sodium thiosulphate
3 g/L Lecithin

Test method accredited according to MS ISO/IEC 17025. This test report may not be reproduced, in whole or in part, without the prior permission of the laboratory. The test results relate only to the test item provided by the client.
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EXPERIMENTAL CONDITIONS

Date of test:	21 February 2022
Product diluent:	Hard water
Concentration / contact time:	0.6%* / 20 minute ± 10 seconds
Test temperature:	(20 ± 1) °C
Interfering substance:	Clean condition (0.3 g/L bovine serum albumin)
Test organism:	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404
Incubation temperature:	(30 ± 1) °C
Incubation period:	48 hours for yeast 72 hours for mould
Appearance of the product dilutions:	Clear, colourless solution
Stability and appearance of product dilutions during test:	Homogenous without any precipitate

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

CONTROLS AND VALIDATION

Test Organism	Validation Suspension	Experimental Conditions Control	Neutralizer or Filtration Control	Method Validation
C. albicans ATCC 10231	$N_V/10$: 139.0	A: 139.0	B: 146.5	C: 108.5
A. brasiliensis ATCC 16404	$N_V/10$: 37.0	A: 54.0	B: 47.0	C: 42.0

The control and validation tests A, B, and C were within the basic limits:

- The number of cells per mL in the validation suspension, $N_V/10$, must be between 30 and 160,
- A must be equal to or greater than 0.5 x $N_V/10$ to verify the absence of any lethal effect in the experimental conditions,
- B must be equal to or greater than 0.5 x $N_V/10$ to verify the absence of neutralizer toxicity or to validate the filtration procedure, and
- C must be equal to or greater than 0.5 x $N_V/10$ to validate the dilution-neutralization method or membrane filtration method.

TEST RESULTS

For each product concentration and contact time, the log reduction (lg R) is calculated using the formula lg R = lg N_0 – lg Na, in which:

- N_0 is the number of cells per mL in the test mixture at the beginning of the contact time, and
- Na is the number of cells per mL in the test mixture at the end of the contact time and before neutralization or filtration.

Test organism: *Candida albicans* ATCC 10231

Test suspension, N	N: 4.90×10^7 lg N_0: 6.69

Concentration / Contact Time	Test, Na	Reduction, lg R = lg N_0 – lg Na
0.6%* / 20 minutes	Na: $<1.40 \times 10^2$ lg Na: <2.15	lg R: >4.54 ± 0.20 %R: >99.997%

Test organism: *Aspergillus brasiliensis* ATCC 16404

Test suspension, N	N: 1.74×10^7 lg N_0: 6.24

Concentration / Contact Time	Test, Na	Reduction, lg R = lg N_0 – lg Na
0.6%* / 20 minutes	Na: $<1.40 \times 10^2$ lg Na: <2.15	lg R: >4.09 ± 0.20 %R: >99.992%

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

CONCLUSION

The test item achieved a reduction of ≥4.00 log against the test organisms *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 under the tested conditions.

Therefore, **Bleaching Liquid (5.20%) – Regular** has demonstrated a fungicidal activity according to EN 1650:2019 under the following conditions:

Concentration	Contact Time	Test Temperature	Soiling
0.6%*	20 minutes	20 °C	Clean condition

Kuala Lumpur, 2 March 2022

Norazzira Zulkharnain
Microbiologist

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

EXPERT OPINION

This expert opinion is based on the test report TR-22-0081 dated 2 March 2022. Opinions and interpretations expressed herein are outside the scope of the Laboratory Accreditation Scheme of Malaysia (SAMM).

The product **Bleaching Liquid (5.20%) – Regular** was tested according to EN 1650:2019 against *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404. These organisms are the minimum test organisms and they have been chosen as representative species taking into account their relative resistance, relevance to practical use, handling properties, and microbiological safety.

Fungicidal and/or yeasticidal activity is defined as a capability of a product or active substance to produce a reduction in the number of viable fungal and/or yeast cells of relevant test organisms under defined conditions. According to EN 1650, a general-purpose disinfectant is considered to possess a fungicidal activity if it demonstrates a reduction of ≥4.00 log against the minimum spectrum of test organisms within 60 minutes when tested at 4 to 60 °C under clean (0.3 g/L bovine serum albumin) or dirty (3.0 g/L bovine serum albumin) condition. A yeasticidal activity is demonstrated if the required log reduction is achieved against the minimum spectrum of yeast only.

When tested under the following conditions, **Bleaching Liquid (5.20%) – Regular** achieved a reduction of ≥4.00 log against *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404:

Concentration	Contact Time	Test Temperature	Soiling
0.6%*	20 minutes	20 °C	Clean condition

Therefore, **Bleaching Liquid (5.20%) – Regular** has demonstrated a fungicidal activity conforming to EN 1650:2019 under the aforementioned conditions.

Kuala Lumpur, 2 March 2022

Dr Marven Lee Cheng Shoou
Managing Director

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

INFORMATION ON MEASUREMENT UNCERTAINTY & DECISION RULE

The statement of conformity given by EN 1650:2019 states that the test item shall be considered to have passed EN 1650 if it demonstrates ≥4.00 log reduction under the defined conditions.

The laboratory employs the simple acceptance decision rule to account for the measurement uncertainty when stating the statement of conformity. The measurement uncertainty and conformance probability are shown in the raw data and are summarized as follows:

Test Organism	Concentration / Contact Time	Log Reduction	Conformance	Conformance Probability†
C. albicans ATCC 10231	0.6%* / 20 minutes	>4.54 ± 0.20	Yes	<0.326% chance of false acceptance
A. brasiliensis ATCC 16404	0.6%* / 20 minutes	>4.09 ± 0.20	Yes	<32.005% chance of false acceptance

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

† The conformance probability follows a normal distribution. Therefore, the percentage of conformance can never be zero or 100% due to the asymptotic tails.

RAW DATA

Test Method:	EN 1650:2019				
Product Name:	Bleaching Liquid (5.20%) - Regular			Batch No.:	-
Product Diluent:	Hard water			Lab ID:	D001-21-001
Appearance of Product Dilutions:	Clear , colourless solution				
Inactivation:	Dilution-neutralization	Dilution Method:	Standard (80%)	Test Temperature (°C):	20
Neutralizer:	30 g/L Tween 80, 3 g/L Sodium thiosulphate, 3 g/L Lecithin				
Interfering Substance:	0.3 g/L bovine serum albumin				
Test Organism:	Candida albicans ATCC 10231			Plating Method:	Pour plate
Incubation Temperature (°C):	30	Passing Criteria (lg):	4.00	Measurement Uncertainty (±):	0.20
Testing Period:	21/2/2022	Tested By:	NII	Verified By:	CSE

Validation & Controls

Validation Suspension (N_V)	V_{C1}	V_{C2}	N_{V0} = 139.0 $N_{V0} = N_V/10$
	136	142	Limit: $30 \le N_{V0} \le 160$
Validation Suspension (N_{VB})	V_{C1}	V_{C2}	N_{V0} = $N_{V0} = N_{VB}/1000$
	-	-	Limit: $30 \le N_{V0} \le 160$
Experimental Conditions Control (A)	V_{C1}	V_{C2}	A = 139.0
	148	130	Limit: $A \ge 0.5 \times N_V/10$
Neutralizer Control (B)	V_{C1}	V_{C2}	B = 146.5
	144	149	Limit: $B \ge 0.5 \times N_V/10$ or $N_{VB}/1000$
Method Validation (C)	V_{C1}	V_{C2}	C = 108.5
Conc.: 0.6%	105	112	Limit: $C \ge 0.5 \times N_V/10$

Test Suspension & Procedure

Test Suspension (N)	N	V_{C1}	V_{C2}	$\overline{x}_{wm}$ = N = 4.90E+07
	10^{-5}	>330	>330	N_0 = N/10 lg N_0 = 6.69
	10^{-6}	48	50	Limit: 6.17 ≤ lg N_0 ≤ 6.70

Product Concentration	Contact Time	Dilution	V_{C1}	V_{C2}	Na = $\overline{x}$ or $\overline{x}_{wm}$ x 10	lg Na	lg R = lg N_0 - lg Na	Conformance Probability
0.6%	20 minutes	10^{0}	<14	<14	<1.40E+02	<2.15	>4.54 ± 0.20	>99.674%
		10^{-1}	<14	<14				
		10^{-2}	<14	<14			>99.997%	
		10^{-3}	<14	<14				
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						

Raw Data of Colony Count

	N_V	N_{VB}	A	B	C	N^{-5}	N^{-6}
V_{C1}	136	-	148	144	105	>330	48
V_{C2}	142	-	130	149	112	>330	50

Product Concentration	Contact Time	Na^{0}		Na^{-1}		Na^{-2}		Na^{-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
0.6%	20 minutes	0	0	0	0	0	0	0	0

RAW DATA

Test Method:	EN 1650:2019				
Product Name:	Bleaching Liquid (5.20%) - Regular			Batch No.:	-
Product Diluent:	Hard water			Lab ID:	D001-21-001
Appearance of Product Dilutions:	Clear , colourless solution				
Inactivation:	Dilution-neutralization	Dilution Method:	Standard (80%)	Test Temperature (°C):	20
Neutralizer:	30 g/L Tween 80, 3 g/L Sodium thiosulphate, 3 g/L Lecithin				
Interfering Substance:	0.3 g/L bovine serum albumin				
Test Organism:	Aspergillus brasiliensis ATCC 16404			Plating Method:	Pour plate
Incubation Temperature (°C):	30	Passing Criteria (lg):	4.00	Measurement Uncertainty (±):	0.20
Testing Period:	21/2/2022		Tested By: AZZ	Verified By:	CSE

Validation & Controls

Validation Suspension (N_V)	V_{C1}	V_{C2}	N_{V0} = 37.0 $N_{V0} = N_V/10$
	41	33	Limit: $30 \le N_{V0} \le 160$
Validation Suspension (N_{VB})	V_{C1}	V_{C2}	N_{V0} = $N_{V0} = N_{VB}/1000$
	-	-	Limit: $30 \le N_{V0} \le 160$

Experimental Conditions Control (A)	V_{C1}	V_{C2}	A = 54.0
	52	56	Limit: $A \ge 0.5 \times N_V/10$
Neutralizer Control (B)	V_{C1}	V_{C2}	B = 47.0
	44	50	Limit: $B \ge 0.5 \times N_V/10$ or $N_{VB}/1000$
Method Validation (C)	V_{C1}	V_{C2}	C = 42.0
Conc.: 0.6%	39	45	Limit: $C \ge 0.5 \times N_V/10$

Test Suspension & Procedure

Test Suspension (N)	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = N$ = 1.74E+07
	10^{-5}	181	160	$N_0 = N/10$ $\lg N_0$ = 6.24
	10^{-6}	19	22	Limit: $6.17 \le \lg N_0 \le 6.70$

Product Concentration	Contact Time	Dilution	V_{C1}	V_{C2}	$Na = \bar{x}$ or $\bar{x}_{wm} \times 10$	lg Na	lg R = lg N_0 - lg Na	Conformance Probability
0.6%	20 minutes	10^{0}	<14	<14	<1.40E+02	<2.15	>4.09 ± 0.20	>67.995%
		10^{-1}	<14	<14				
		10^{-2}	<14	<14			>99.992%	
		10^{-3}	<14	<14				
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						
		10^{0}						
		10^{-1}						
		10^{-2}						
		10^{-3}						

Raw Data of Colony Count

	N_V		N_{VB}		A	B	C	N^{-5}		N^{-6}	
V_{C1}	20	21	-	-	52	44	39	100	81	10	9
V_{C2}	11	22	-	-	56	50	45	100	60	12	10

Product Concentration	Contact Time	Na^{0}		Na^{-1}		Na^{-2}		Na^{-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
0.6%	20 minutes	8	9	2	0	0	0	0	0

TEST PROCEDURE

1. Test *Na*: Determination of Fungicidal or Yeasticidal Concentrations

   1.1 1.0 mL of the interfering substance was pipetted into a tube. 1.0 mL of the test suspension *N* (1.5 – 5.0 x 10^7 cfu/mL) was added to the tube.

   1.2 The stopwatch was started immediately and the tube was mixed and placed in a water bath controlled at test temperature (θ ± 1) °C for 2 minutes ± 10 seconds.

   1.3 At the end of the 2 minutes, 8.0 mL of the product test solution was added to the tube. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at θ for the contact time *t*. Just before the end of *t*, the tube was mixed again.

   1.4 At the end of *t*, the test mixture was inactivated by one of the following methods:

      1.4.1 For dilution neutralization method:

         1.4.1.1 1.0 mL sample of the test mixture *Na* was transferred into a tube containing 8.0 mL of neutralizer and 1.0 mL of distilled water. The neutralizer tube was mixed and placed in a water bath controlled at (20 ± 1) °C.

         1.4.1.2 After a neutralization time of 5 minutes ± 10 seconds (for products with *t* of 10 minutes or shorter, neutralization time is (10 ± 1) seconds), the neutralizer tube was mixed and 1.0 mL of the neutralized test mixture *Na* (containing neutralizer, product test solution, interfering substance, and test suspension) was taken in duplicate and inoculated using the pour or spread plate technique.

         1.4.1.3 For hygienic handwash products, two additional decimal dilutions were plated.

      1.4.2 For membrane filtration method:

         1.4.2.1 0.1 mL sample of the test mixture *Na* was taken in duplicate and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

         1.4.2.2 For hygienic handwash products, a 0.1 mL sample of the test mixture *Na* was diluted 10-fold and 100-fold in 0.9 ml and 9.9 mL of rinsing liquid to obtain 10^{-1} and 10^{-2} dilutions, respectively. The mixture was mixed and 0.1 mL of each dilution was transferred in duplicate into two separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

   1.5 The procedure was performed using other product test solutions at the same time.

2. Experimental Conditions Control *A*: Verification of the Absence of Any Lethal Effect in the Experimental Conditions

   2.1 1.0 mL of the interfering substance used in the test *Na* was pipetted into a tube. 1.0 mL of the validation suspension N_V (0.3 – 1.6 x 10^3 cfu/mL) was added to the tube.

   2.2 The stopwatch was started immediately and the tube was mixed and placed in a water bath controlled at test temperature θ for 2 minutes ± 10 seconds.

   2.3 At the end of the 2 minutes, 8.0 mL of hard water (distilled water for ready-to-use or handwash products) was added to the tube. The stopwatch was restarted at the beginning of the addition.

The tube was mixed and placed in a water bath controlled at θ for the contact time t. Just before the end of t, the tube was mixed again.

2.4 For dilution-neutralization method, 1.0 mL sample of the test mixture *A* was taken in duplicate at the end of t and inoculated using the pour or spread plate technique for dilution-neutralization method.

2.5 For membrane filtration method, 1.0 mL sample of the test mixture *A* was taken in duplicate at the end of t and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

3. Neutralizer or Filtration Control *B*: Verification of the Absence of Toxicity of the Neutralizer or the Filtration Procedure

3.1 For dilution neutralization method:

3.1.1 8.0 mL of the neutralizer used in the test *Na* and 1.0 mL of distilled water were pipetted into a tube. 1.0 mL of the validation suspension N_V was added to the tube.

3.1.2 The stopwatch was started at the beginning of the addition and the tube was mixed and placed in a water bath controlled at the test temperature θ for 5 minutes ± 10 seconds ((10 ± 1) seconds for products with t of 10 minutes or shorter). Just before the end of this time, the tube was mixed.

3.1.3 At the end of the time, 1.0 mL sample of the test mixture *B* was taken in duplicate and inoculated using the pour or spread plate technique.

3.2 For membrane filtration method:

3.2.1 0.1 mL of the validation suspension *Nv* was taken in duplicate and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed and each membrane was transferred onto the surface of separate agar plates.

4. Method Validation *C*: Validation of the Dilution-Neutralization or Membrane Filtration Method

4.1 1.0 mL of the interfering substance used in the test *Na* was pipetted into a tube. 1.0 mL of diluent was added and then, starting a stopwatch, 8.0 mL of the product test solution of the highest concentration used in the test *Na* was added to the tube. The tube was mixed and placed in a water bath controlled at test temperature θ for contact time t. Just before the end of t, the tube was mixed again.

4.2 For dilution neutralization method:

4.2.1 At the end of t, 1.0 mL of the mixture was transferred into a tube containing 8.0 mL of neutralizer used in the test *Na*. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at the test temperature θ for 5 minutes ± 10 seconds ((10 ± 1) seconds for products with t of 10 minutes or shorter).

4.2.2 1.0 mL of the validation suspension N_V was added. The stopwatch was restarted at the beginning of the addition. The tube was mixed and placed in a water bath controlled at (20 ± 1) °C for (30 ± 1) minutes. Just before the end of this time, the tube was mixed again.

4.2.3 At the end of this time, 1.0 mL sample of the test mixture *C* was taken in duplicate and inoculated using the pour or spread plate technique.

4.3 For membrane filtration method:

4.3.1 At the end of *t*, 0.1 mL of the test mixture *C* was taken in duplicate and transferred into separate membrane filtration apparatus containing 50 mL of rinsing liquid. The mixture was filtered and rinsed as before and the membranes were covered with 50 mL of rinsing liquid again.

4.3.2 0.1 mL of the validation suspension *Nv* was added to each filtration apparatus. The mixture was filtered and rinsed again and each membrane was transferred onto the surface of separate agar plates.

5. Incubation and Counting

5.1 The plates were incubated for 42 to 48 hours. The plates were counted to determine the number of cfu. Any plates which were not countable for any reason were discarded.

5.2 For moulds, the plates were incubated for a further 20 to 24 hours. The plates were recounted and if the number of colonies had increased, the plates were incubated for an additional third period of 20 to 24 hours. Only the higher number was used for further evaluation. Plates that no longer showed well-separated colonies were not recounted.

5.3 For each plate, the exact number of colonies were noted but any counts higher than 330 colonies or 165 colonies (for mould) were recorded as '>330' and '>165', respectively. For membrane filtration method, record '>165' or '>55' for any counts higher than 165 or 55 colonies (for mould), respectively.

5.4 All experimental data were reported as V_C values, in which a V_C value is the number of cfu counted per 1.0 mL sample inoculated. For membrane filtration method, a V_C value is the number of cfu counted per 0.1 mL sample of *Na*, *B*, and *C*, and per 1.0 mL sample of *A*.

5.5 Only V_C values within the counting limits, i.e., 14 to 330/165/55 colonies, were taken into account for further calculation, except in the case of *Na*.

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SAMM 1016

Test Report No.: TR-22-0094

Determination of the Virucidal Activity of **BLEACHING LIQUID (5.20%) – REGULAR** according to EN 14476:2013+A2:2019

Test Method
EN 14476:2013+A2:2019
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)

Testing Laboratory
TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
No. 115, Jalan Kepayang, Off Jalan Kuching
51200 Kuala Lumpur
Malaysia

Kuala Lumpur, 17 March 2022

Dr Marven Lee Cheng Shoou
Managing Director

IDENTIFICATION OF TESTING LABORATORY

TECOLAB Sdn. Bhd.
J-2-6, Pusat Komersial Jalan Kuching
No. 115, Jalan Kepayang, Off Jalan Kuching
51200 Kuala Lumpur
Malaysia

IDENTIFICATION OF TEST ITEM

Test item name:	Bleaching Liquid (5.20%) – Regular
Lab ID:	D001-21-001
Batch no.:	Not specified
Receipt date:	24 November 2021
Storage conditions:	Room temperature away from sunlight
Product diluent recommended by manufacturer:	Not specified
Active substances:	Sodium hypochlorite (3.9%)
Product appearance:	Clear, pale yellow liquid

TEST METHOD & VALIDATION

Test method:	EN 14476:2013+A2:2019 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)
Titration method:	Quantal tests
Inactivation method:	Molecular sieving (microspin)

EXPERIMENTAL CONDITIONS

Date of test:	16 February - 4 March 2021
Product diluent:	Hard water
Concentration / contact time:	0.6%* / 20 minutes ± 10 seconds
Test temperature:	(20 ± 1) °C
Interfering substance:	Clean condition (0.3 g/L bovine serum albumin)
Test organism / passage no.:	Human coronavirus (HCoV-229E), strain 229E, ATCC VR-740 / P16 Human enterovirus 71 (EV-71), strain H, ATCC VR-1432 / P3 Influenza A virus (H3N2), strain A/Hong Kong/8/68, ATCC VR-1679 / P6
Cell line / passage no.:	MRC-5 ATCC CCL-171 / P15&16 LLC-MK2 ATCC CCL-7.1 / P14&15 MDCK ATCC CCL-34 / P13&14
Growth medium:	DMEM supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin
Incubation temperature:	(37 ± 1) °C, 5% CO_2
Incubation period:	2 to 5 days (for HCoV-229E & EV-71) 3 to 4 days (for H3N2)
Appearance of the product dilutions:	Clear, colourless liquid
Stability and appearance of product dilutions during test:	Homogenous without any precipitate

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

CONTROLS AND VALIDATION

Test Organism	Cytotoxicity Effect	Interference Control	Suppression Control	Reference Test
HCoV-229E ATCC VR-740	N: 6.50 ± 0.00 CE: 1.50 ± 0.00	A_{PBS}: 6.50 ± 0.00 A_T: 6.00 ± 0.38	B_N: 6.50 ± 0.00 B_T: 6.38 ± 0.25	C_5: ≥2.00 ± 0.00 C_{15}: ≥2.00 ± 0.00
EV-71 ATCC VR-1432	N: 5.75 ± 0.33 CE: 1.50 ± 0.00	A_{PBS}: 6.50 ± 0.00 A_T: 6.38 ± 0.25	B_N: 5.75 ± 0.33 B_T: 5.75 ± 0.33	C_{30}: 2.25 ± 0.50 C_{60}: 3.50 ± 0.38
A(H3N2) ATCC VR-1679	N: 6.25 ± 0.33 CE: 1.50 ± 0.00	A_{PBS}: 6.13 ± 0.37 A_T: 5.75 ± 0.33	B_N: 6.25 ± 0.33 B_T: 5.75 ± 0.33	C_5: ≥1.38 ± 0.37 C_{15}: ≥1.38 ± 0.37

The control and validation tests A, B, and C were within the basic limits:

- The difference between N and CE must be ≥4.00 to verify that the cytotoxicity of the product does not affect cell morphology and growth or susceptibility for the test organism which are necessary to demonstrate a 4-log reduction of the virus,
- The difference A_{PBS} and A_T must be <1.00 to verify that the susceptibility of the cells for the virus infection is not influenced negatively by the treatment with the product test solution,
- The difference between B_N and B_T must be ≤0.50 to validate the inactivation method,
- The reduction of the virus in the reference test after 5 and 15 minutes, C_5 and C_{15}. No passing criteria were given for coronavirus and influenza virus, and
- The reduction of the virus in the reference test after 30 and 60 minutes, C_{30} and C_{60}. No passing criteria were given for enterovirus 71.

TEST RESULTS

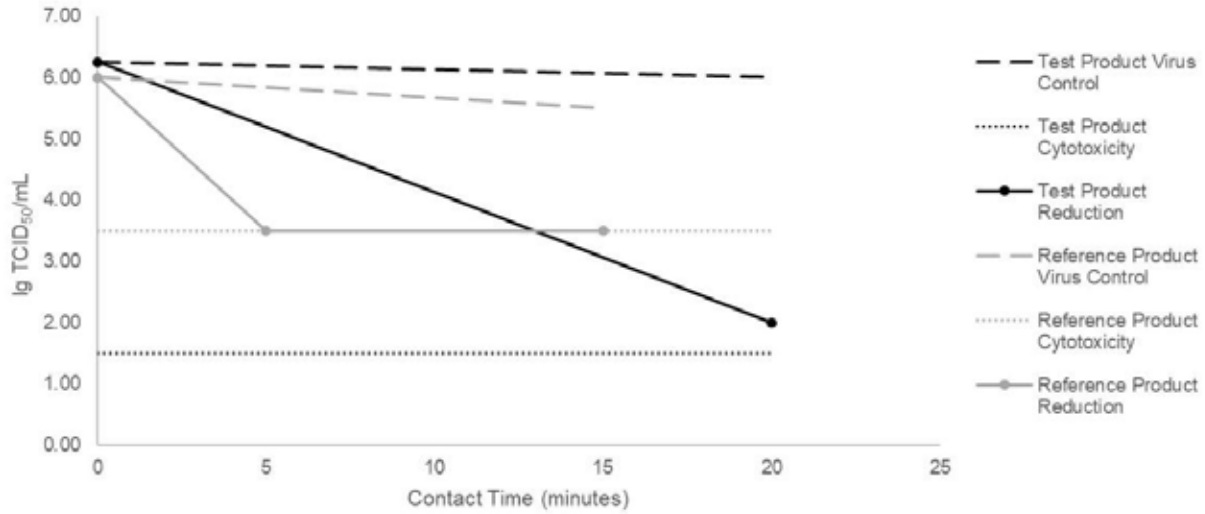
For each product concentration and contact time, the log reduction (lg R) is calculated using the formula lg R = N – Na, in which:

- N is the lg $TCID_{50}$ per mL of the virus control at the end of the contact time, and
- Na is the lg $TCID_{50}$ per mL of the test mixture at the end of the contact time.

Test organism: Human coronavirus (HCoV-229E) ATCC VR-740

Virus control, N	N_1: 6.50 ± 0.00 N_2: 5.50 ± 0.00
Cytotoxicity effect, CE	CE_1: 1.50 ± 0.00 CE_2: 1.50 ± 0.00

Concentration / Contact Time	Test, Na	Reduction, lg R = N – Na	Average Reduction, lg R
0.6%* / 20 minutes	Na_1: 2.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: 4.00 ± 0.00 lg R_2: ≥4.00 ± 0.00	lg R: ≥4.00 ± 0.00 %R: ≥99.990%

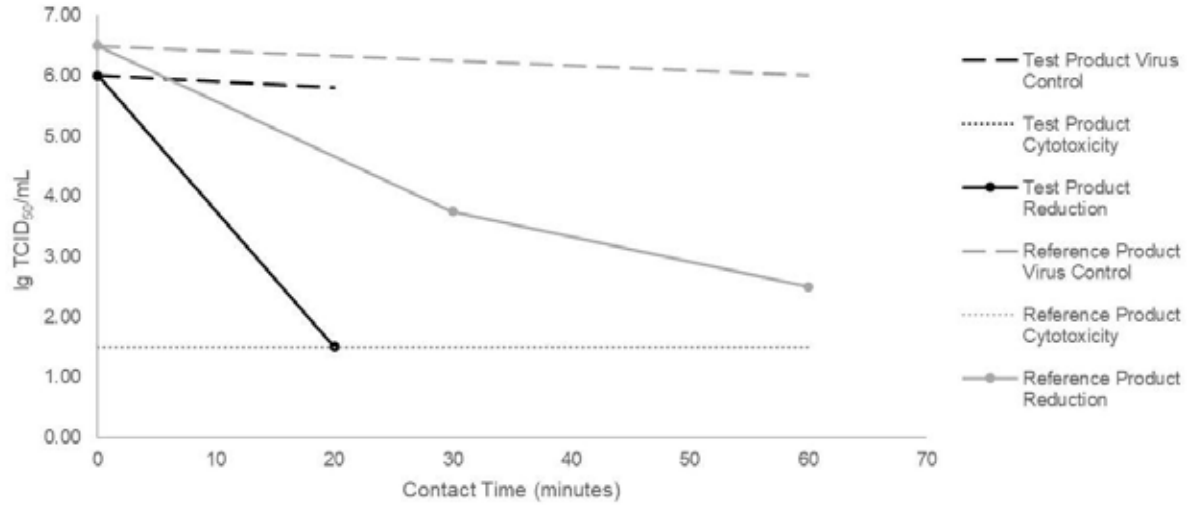


* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

Test organism: Human enterovirus 71 (EV-71) ATCC VR-1432

Virus control, N	N_1: 5.75 ± 0.33 N_2: 5.88 ± 0.45
Cytotoxicity effect, CE	CE_1: 1.50 ± 0.00 CE_2: 1.50 ± 0.00

Concentration / Contact Time	Test, Na	Reduction, lg R = N – Na	Average Reduction, lg R
0.6%* / 20 minutes	Na_1: ≤1.50 ± 0.00 Na_2: ≤1.50 ± 0.00	lg R_1: ≥4.25 ± 0.33 lg R_2: ≥4.38 ± 0.45	lg R: ≥4.31 ± 0.40 %R: ≥99.995%

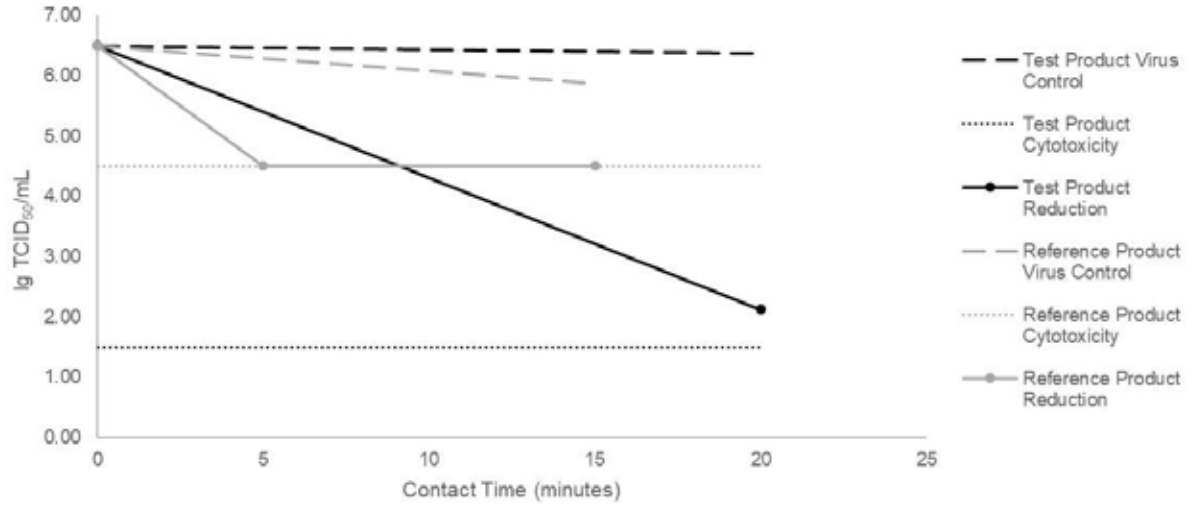


* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

Test organism: Influenza A virus (H3N2) ATCC VR-1679

Virus control, N	N_1: 6.25 ± 0.33 N_2: 6.50 ± 0.00
Cytotoxicity effect, CE	CE_1: 1.50 ± 0.00 CE_2: 1.50 ± 0.00

Concentration / Contact Time	Test, Na	Reduction, lg R = N – Na	Average Reduction, lg R
0.6%* / 20 minutes	Na_1: 1.75 ± 0.33 Na_2: 2.50 ± 0.00	lg R_1: 4.50 ± 0.46 lg R_2: 4.00 ± 0.00	lg R: 4.25 ± 0.33 %R: 99.994%



* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

CONCLUSION

The test item achieved a reduction of ≥4.00 log against the test organisms Human coronavirus 229E (HCoV-229E) ATCC VR-740, Human enterovirus 71 (EV-71) ATCC VR-1432, and Influenza A virus (H3N2) ATCC VR-1679 under the tested conditions.

Therefore, **Bleaching Liquid (5.20%) – Regular** has demonstrated a virucidal activity against human coronavirus, enterovirus 71, and influenza A virus according to EN 14476:2013+A2:2019 under the following conditions:

Concentration	Contact Time	Test Temperature	Soiling
0.6%*	20 minutes	20 °C	Clean condition

Kuala Lumpur, 17 March 2022

Chan Yanqi
Microbiologist

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

EXPERT OPINION

This expert opinion is based on the test report TR-22-0094 dated 17 March 2022. Opinions and interpretations expressed herein are outside the scope of the Laboratory Accreditation Scheme of Malaysia (SAMM).

The product **Bleaching Liquid (5.20%) - Regular** was tested according to EN 14476:2013+A2:2019 against Human coronavirus (HCoV-229E), strain 229E, ATCC VR-740, Human enterovirus 71 (EV-71), strain H, ATCC VR-1432, and Influenza A virus (H3N2), strain A/Hong Kong/8/68, ATCC VR-1679. These organisms are chosen as a surrogate species for the clinical strains of human coronavirus such as SARS-CoV-2, enterovirus 71, and influenza A virus, taking into account their relative resistance, relevance to practical use, handling properties, and microbiological safety.

Virucidal activity is defined as a capability of a product or active substance to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions. According to EN 14476, a surface disinfectant is considered to possess a virucidal activity if it demonstrates a reduction of ≥4.00 log against the representative virus within 60 minutes (or 5 minutes for surfaces in contact with patient or medical staff) when tested at 4 to 30 °C under clean (0.3 g/L bovine serum albumin) or dirty (3.0 g/L bovine serum albumin and 3.0 mL/L sheep erythrocytes) condition.

When tested under the following conditions, **Bleaching Liquid (5.20%) - Regular** achieved a reduction of ≥4.00 log against Human coronavirus (HCoV-229E) ATCC VR-740, Human enterovirus 71 (EV-71) ATCC VR-1432, and Influenza A virus (H3N2) ATCC VR-1679:

Concentration	Contact Time	Test Temperature	Soiling
0.6%*	20 minutes	20 °C	Clean condition

Therefore, **Bleaching Liquid (5.20%) – Regular** has demonstrated a virucidal activity against human coronavirus, enterovirus 71, and influenza A virus according to EN 14476:2013+A2:2019 under the following conditions:

Kuala Lumpur, 17 March 2022

Dr Marven Lee Cheng Shoou
Managing Director

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

INFORMATION ON MEASUREMENT UNCERTAINTY & DECISION RULE

The statement of conformity given by EN 14476:2013+A2:2019 states that the test product shall be considered to have passed EN 14476 if it demonstrates ≥4.00 log reduction under the defined conditions.

The laboratory employs the simple acceptance decision rule to account for the measurement uncertainty when stating the statement of conformity. The measurement uncertainty and conformance probability are shown in the raw data and are summarized as follows:

Test Organism	Concentration / Contact Time	Log Reduction	Conformance	Conformance Probability†
HCoV-229E ATCC VR-740	0.6%* / 20 minutes	≥4.00 ± 0.00	Yes	50.000% chance of false acceptance
EV-71 ATCC VR-1432	0.6%* / 20 minutes	≥4.31 ± 0.40	Yes	≤21.460% chance of false acceptance
A(H3N2) ATCC VR-1679	0.6%* / 20 minutes	4.25 ± 0.33	Yes	22.250% chance of false acceptance

* Expressed as a percentage of sodium hypochlorite, calculated from the neat product which has 3.9% of sodium hypochlorite.

† The conformance probability follows a normal distribution. Therefore, the percentage of conformance can never be zero or 100% due to the asymptotic tails.

RAW DATA

Test Method:	EN 14476:2013+A2:2019			Dilution Method:	Standard (80%)	Passing Criteria (lg):	4.00
Product Name:	Bleaching Liquid (5.20%) - Regular			Batch No.:	-	Lab ID:	D001-21-001
Product Diluent:	Hard water	Appearance of Product Dilutions:	Clear, colourless solution			Titration Method:	Quantal test
Inactivation:	Molecular sieving (microspin)	Interfering Substance:	0.3 g/L bovine albumin solution				
Test Organism:	Human coronavirus, strain 229E, ATCC VR-740			Passage No.:	16	Test Temperature (°C):	20
Cell Line:	MRC-5 cells, ATCC CCL-171			Passage No.:	15 & 16	Incubation Temperature (°C):	36
First Assay Testing Period:	16/02/2022	Second Assay Testing Period:	23/02/2022	Tested By:	CYQ	Verified By:	CSE

Validation & Controls

	Product Concentration	Dilution	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	$\lvert A_{PBS} - A_T \rvert$ = 0.50
Interference Control (A)	PBS	N/A	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 3 4 2 4 4 2	1 2 1 1 1 1 1 1	0 0 0 0 0 0 0 0					6.50 ± 0.00	
	0.6%	10^-1	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 2 1 4 4 2 1	0 0 0 0 1 1 1 1	0 0 0 0 0 0 0 0					6.00 ± 0.38	Limit: $\lvert A_{PBS} - A_T \rvert$ < 1

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	$\lvert B_N - B_T \rvert$ = 0.13
Suppression Control (B)	Virus Control (N)	5	4 4 4 4 4 4 4 4	4 4 4 4 3 4 3 4	1 1 4 3 2 1 3 3	1 3 1 1 1 1 1 1	1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0					6.50 ± 0.00	
	0.6%	5	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 4 4 4 4 3 4 4	1 3 1 1 3 1 1 1	1 1 0 1 1 1 1 1	0 0 0 0 0 0 0 0					6.38 ± 0.25	Limit: $\lvert B_N - B_T \rvert \leq 0.5$

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_C = C_N - C_T$
Reference Test (C)	0.7% Formaldehyde	5	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					≤3.50 ± 0.00	≥2.00 ± 0.00
		15	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					≤3.50 ± 0.00	≥2.00 ± 0.00
	Virus Control (N)	0	2 4 2 2 3 2 2 4	2 2 2 2 2 2 2 2	2 1 2 1 1 2 2 2	2 1 1 2 2 1 1 1	1 1 1 1 0 0 0 0	0 0 0 0 0 0 0 0					6.00 ± 0.38	
		15	2 2 2 2 3 3 2 2	2 2 2 2 1 1 2 1	2 2 1 1 1 1 1 1	2 2 2 2 1 1 1 1	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					5.50 ± 0.00	
	Cytotoxicity Effect (CE)	N/A	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					3.50 ± 0.00	

RAW DATA

Test Method:	EN 14476:2013+A2:2019	Dilution Method:	Standard (80%)	Passing Criteria (lg):	4.00
Product Name:	Bleaching Liquid (5.20%) - Regular	Batch No.:	-	Lab ID:	D001-21-001
Product Diluent:	Hard water	Appearance of Product Dilutions:	Clear, colourless solution	Titration Method:	Quantal test
Inactivation:	Molecular sieving (microspin)	Interfering Substance:	0.3 g/L bovine albumin solution		
Test Organism:	Human enterovirus 71 (EV-71), Strain H, ATCC VR-1432	Passage No.:	3	Test Temperature (°C):	20
Cell Line:	LLC-MK2 cells, ATCC CCL-7.1	Passage No.:	14 & 15	Incubation Temperature (°C):	36
First Assay Testing Period:	24/02/2022	Second Assay Testing Period:	01/03/2022	Tested By:	CYQ
Verified By:	CSE				

Validation & Controls

	Product Concentration	Dilution	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Interference Control (A)	PBS	N/A	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2	0 0 0 0 0 0 0 0					6.50 ± 0.00	$\lvert A_{PBS} - A_T \rvert$ = 0.13
	0.6%	10^-2	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 1 2 2 2 2 0	0 0 0 0 0 0 0 0					6.38 ± 0.25	Limit: $\lvert A_{PBS} - A_T \rvert$ < 1

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Suppression Control (B)	Virus Control (N)	20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2	0 1 0 1 0 0 0 0	0 0 0 0 0 0 0 0					5.75 ± 0.33	$\lvert B_N - B_T \rvert$ = 0.00
	0.6%	20	4 4 4 4 4 4 4 4	4 4 3 4 4 4 4 3	2 2 2 2 2 2 2 2	2 1 1 1 1 1 2 1	0 0 2 1 0 0 0 0	0 0 0 0 0 0 0 0					5.75 ± 0.33	Limit: $\lvert B_N - B_T \rvert \leq 0.5$

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_C = C_N - C_T$
Reference Test (C)	0.7% Formaldehyde	30	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	0 1 0 0 2 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					3.75 ± 0.33	2.25 ± 0.50
		60	4 4 3 4 4 4 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					2.50 ± 0.00	3.50 ± 0.38
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 3 2 4 2 2 1 2	1 1 1 2 1 1 1 1	0 0 0 0 0 0 0 0					6.50 ± 0.00	
		60	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 3 2 4 1 2 1 1	0 0 2 2 0 0 2 2	0 0 0 0 0 0 0 0					6.00 ± 0.38	
	Cytotoxicity Effect (CE)	N/A	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					1.50 ± 0.00	

RAW DATA

Test Method:	EN 14476:2013+A2:2019	Dilution Method:	Standard (80%)	Passing Criteria (lg):	4.00
Product Name:	Bleaching Liquid (5.20%) - Regular	Batch No.:	-	Lab ID:	D001-21-001
Product Diluent:	Hard water	Appearance of Product Dilutions:	Clear, colourless solution	Titration Method:	Quantal test
Inactivation:	Molecular sieving (microspin)	Interfering Substance:	0.3 g/L bovine albumin solution		
Test Organism:	Human enterovirus 71 (EV-71), Strain H, ATCC VR-1432	Passage No.:	3	Test Temperature (°C):	20
Cell Line:	LLC-MK2 cells, ATCC CCL-7.1	Passage No.:	14 & 15	Incubation Temperature (°C):	36
First Assay Testing Period:	24/02/2022	Second Assay Testing Period:	01/03/2022	Tested By:	CYQ	Verified By:	CSE

Validation & Controls

	Product Concentration	Dilution	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Interference Control (A)	PBS	N/A	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2	0 0 0 0 0 0 0 0					6.50 ± 0.00	$\lvert A_{PBS} - A_T \rvert$ = 0.13
	0.6%	10^-2	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 1 2 2 2 2 0	0 0 0 0 0 0 0 0					6.38 ± 0.25	Limit: $\lvert A_{PBS} - A_T \rvert$ < 1

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Suppression Control (B)	Virus Control (N)	20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2	0 1 0 1 0 0 0 0	0 0 0 0 0 0 0 0					5.75 ± 0.33	$\lvert B_N - B_T \rvert$ = 0.00
	0.6%	20	4 4 4 4 4 4 4 4	4 4 3 4 4 4 4 3	2 2 2 2 2 2 2 2	2 1 1 1 1 1 2 1	0 0 2 1 0 0 0 0	0 0 0 0 0 0 0 0					5.75 ± 0.33	Limit: $\lvert B_N - B_T \rvert \leq 0.5$

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_C = C_N - C_T$
Reference Test (C)	0.7% Formaldehyde	30	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	0 1 0 0 2 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					3.75 ± 0.33	2.25 ± 0.50
		60	4 4 3 4 4 4 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					2.50 ± 0.00	3.50 ± 0.38
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 3 2 4 2 2 1 2	1 1 1 2 1 1 1 1	0 0 0 0 0 0 0 0					6.50 ± 0.00	
		60	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 3 2 4 1 2 1 1	0 0 2 2 0 0 2 2	0 0 0 0 0 0 0 0					6.00 ± 0.38	
	Cytotoxicity Effect (CE)	N/A	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					1.50 ± 0.00	

RAW DATA

Test Procedure

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_1 = N_1 - Na_1$
First Assay (Na_1)	0.6%	20	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					≤1.50 ± 0.00	≥4.25 ± 0.33
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 1 2 2 2 2 2	0 1 0 1 1 1 0 0	0 0 0 0 0 0 0 0					6.00 ± 0.38	$N_1 - CE_1 = 4.25$ Limit: $N_1 - CE_1 \geq 4$
		20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2	0 1 0 1 0 0 0 0	0 0 0 0 0 0 0 0					5.75 ± 0.33	
	Cytotoxicity Effect (CE)	N/A	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					1.50 ± 0.00	

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_2 = N_2 - Na_2$
Second Assay (Na_2)	0.6%	20	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					≤1.50 ± 0.00	≥4.38 ± 0.45
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 3 2 2 3 2 2 2	1 1 1 1 0 0 0 0	0 0 0 0 0 0 0 0					6.00 ± 0.38	$N_2 - CE_2 = 4.38$ Limit: $N_2 - CE_2 \geq 4$
		20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 3 4 1 4 3 0 2	2 0 0 0 0 1 1 1	0 0 0 0 0 0 0 0					5.88 ± 0.45	
	Cytotoxicity Effect (CE)	N/A	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					1.50 ± 0.00	

Product Concentration	Contact Time (minutes)	First Assay (Na_1) lg $TCID_{50}$/mL	First Assay (Na_1) lg $R_1 = N_1 - Na_1$	Second Assay (Na_2) lg $TCID_{50}$/mL	Second Assay (Na_2) lg $R_2 = N_2 - Na_2$	Average Reduction (lg R)	Conformance Probability
0.6%	20	≤1.50 ± 0.00	≥4.25 ± 0.33	≤1.50 ± 0.00	≥4.38 ± 0.45	≥4.31 ± 0.40 ≥99.995%	≥78.540%

RAW DATA

Test Method:	EN 14476:2013+A2:2019	Dilution Method:	Standard (80%)	Passing Criteria (lg):	4.00
Product Name:	Bleaching Liquid (5.20%) - Regular	Batch No.:	-	Lab ID:	D001-21-001
Product Diluent:	Hard water	Appearance of Product Dilutions:	Clear, colourless solution	Titration Method:	Quantal test
Inactivation:	Molecular sieving (microspin)	Interfering Substance:	0.3 g/L bovine albumin solution		
Test Organism:	Influenza A virus (H3N2), Strain A/Hong Kong/8/68, ATCC VR-1679	Passage No.:	6	Test Temperature (°C):	20
Cell Line:	MDCK cells, ATCC CCL-34	Passage No.:	13 & 14	Incubation Temperature (°C):	36
First Assay Testing Period:	24/02/2022	Second Assay Testing Period:	01/03/2022	Tested By:	CYQ
Verified By:	CSE				

Validation & Controls

	Product Concentration	Dilution	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Interference Control (A)	PBS	N/A	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	3 4 4 4 4 3 4 4	0 1 4 4 4 4 0 0	0 0 0 0 0 0 0 0					6.13 ± 0.37	$\lvert A_{PBS} - A_T \rvert$ = 0.38
	0.6%	10^-3	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 4 4 4 4 4 2 4	0 0 0 1 0 0 0 1	0 0 0 0 0 0 0 0					5.75 ± 0.33	Limit: $\lvert A_{PBS} - A_T \rvert$ < 1

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	
Suppression Control (B)	Virus Control (N)	20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 1 3 1 1 3 2 1	1 1 2 2 1 1 0 0	0 0 0 0 0 0 0 0					6.25 ± 0.33	$\lvert B_N - B_T \rvert$ = 0.50
	0.6%	20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 4 4 4 4 4 4 4	3 4 4 1 3 4 4 1	0 2 0 0 1 0 0 0	0 0 0 0 0 0 0 0					5.75 ± 0.33	Limit: $\lvert B_N - B_T \rvert \leq 0.5$

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_C = C_N - C_T$
Reference Test (C)	0.7% Formaldehyde	5	t t t t t t t t	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					≤4.50 ± 0.00	≥1.38 ± 0.37
		15	t t t t t t t t	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					≤4.50 ± 0.00	≥1.38 ± 0.37
	Virus Control (N)	0	4 4 3 3 4 4 4 3	3 3 3 3 3 3 3 3	2 2 3 2 2 1 2 2	2 2 1 2 1 1 1 2	1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0					6.50 ± 0.00	
		15	4 4 2 2 2 2 2 3	4 1 1 1 2 3 1 2	2 2 2 2 1 2 1 1	1 1 1 0 1 2 1 1	0 1 1 0 0 0 0 1	0 0 0 0 0 0 0 0					5.88 ± 0.37	
	Cytotoxicity Effect (CE)	N/A	t t t t t t t t	t t t t t t t t	t t t t t t t t	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					4.50 ± 0.00	

RAW DATA

Test Procedure

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_1 = N_1 - Na_1$
First Assay (Na_1)	0.6%	20	1 0 0 0 1 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					1.75 ± 0.33	4.50 ± 0.46
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	1 2 4 2 2 2 1 4	1 1 1 2 2 1 1 1	0 0 0 0 0 0 0 0					6.50 ± 0.00	$N_1 - CE_1 = 4.75$ Limit: $N_1 - CE_1 \geq 4$
		20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	2 1 3 1 1 3 2 1	1 1 2 2 1 1 0 0	0 0 0 0 0 0 0 0					6.25 ± 0.33	
	Cytotoxicity Effect (CE)	N/A	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					1.50 ± 0.00	

	Product Concentration	Contact Time (minutes)	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	lg $TCID_{50}$/mL	lg $R_2 = N_2 - Na_2$
Second Assay (Na_2)	0.6%	20	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					2.50 ± 0.00	4.00 ± 0.00
	Virus Control (N)	0	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0					6.50 ± 0.00	$N_2 - CE_2 = 5.00$ Limit: $N_2 - CE_2 \geq 4$
		20	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	4 4 4 4 4 4 4 4	0 0 0 0 0 0 0 0					6.50 ± 0.00	
	Cytotoxicity Effect (CE)	N/A	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0					1.50 ± 0.00	

Product Concentration	Contact Time (minutes)	First Assay (Na_1) lg $TCID_{50}$/mL	First Assay (Na_1) lg $R_1 = N_1 - Na_1$	Second Assay (Na_2) lg $TCID_{50}$/mL	Second Assay (Na_2) lg $R_2 = N_2 - Na_2$	Average Reduction (lg R)	Conformance Probability
0.6%	20	1.75 ± 0.33	4.50 ± 0.46	2.50 ± 0.00	4.00 ± 0.00	4.25 ± 0.33 99.994%	77.750%

TEST PROCEDURE

1. Test Procedure *Na*: Determination of Virucidal Concentrations

   1.1 100 µL of interfering substance was pipetted into a tube. 100 µL of virus test suspension was added to the tube and mixed.

   1.2 800 µL of the product test solution was added to tube. The tube was mixed and the stopwatch was started at once. The tube was placed in a water bath controlled at the chosen test temperature θ for the contact time t.

   1.3 Immediately at the end of t, the tube was mixed and the activity of the product test solution was inactivated or removed using one of the following methods:

      1.3.1 Dilution in ice-cold maintenance medium. 50 µL of the mixture was transferred into 450 µL ice-cold maintenance medium and put in an ice bath, or if the cytotoxicity of the product test solution is too high,

      1.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the residual virus was obtained as per manufacturer's instructions.

   1.4 Within 30 minutes of product inactivation, a series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

   1.5 The virus titre for *Na* was determined using quantal test (endpoint titration). 100 µL of each dilution was transferred into 8 wells of a microtitre plate containing a confluent (>90%) cell monolayer without any medium. 100 µL of maintenance medium was added to the last row of wells to serve as the cell control.

   1.6 After 1 hour incubation at 37 °C, 100 µL of maintenance medium was added to each well.

   1.7 The cells were incubated for the appropriate incubation period until cytopathic effect (CPE; morphological alteration of cells and/or their destruction as a consequence of virus multiplication) was observed. The results were recorded as '0' for no CPE, or '1' to '4' for approximately 25%, 50%, 75%, and 100% CPE, respectively.

   1.8 The virus titre was calculated using the Spearman-Kärber method and expressed as lg $TCID_{50}$/mL, i.e., the 50% infecting dose of a virus suspension that induces a CPE in 50% of cell culture units.

2. Virus Control *N*

   2.1 The virus control *N* was performed in parallel to the test *Na* at two contact times: at 0 minute and the longest contact time used in the test *Na*. The product test solution was substituted with hard water (distilled water for ready-to-use products).

   2.2 The inactivation method chosen must be the same as the one chosen in *Na*. A series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

   2.3 The virus titre for *N* was determined using quantal test according to Sections 1.5 to 1.8.

3. Cytotoxicity Effect *CE*: Verification for Possible Morphological Alteration of Cells by the Test Product

   3.1 100 µL of hard water (distilled water for ready-to-use products) and 100 µL of interfering substance were mixed with 800 µL of the product test solution.

   3.2 The product test solution was inactivated or removed using the same method as the one chosen in *Na*.

3.3 A series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

3.4 The cytotoxicity of the product test solution was determined using quantal test according to Sections 1.5 to 1.8.

3.5 The results were recorded as 't' for cytotoxicity, i.e., the morphological alteration of cells and/or their destruction or their reduced sensitivity to virus multiplication caused by the product.

4. Interference Control *A*: Verification that the Susceptibility of the Cells for the Virus Infection is Not Influenced Negatively by the Treatment with the Test Product

4.1 To check the reduction of the sensitivity of the cells to virus, comparative virus titrations were performed in cells that have or have not been treated with product test solution.

4.2 For the test A_T, 100 µL of the lowest apparently non-cytotoxic dilution (determined from the cytotoxicity effect test *CE*) of the product test solution and 100 µL of maintenance medium were transferred into each 8 wells of a microtitre plate containing a confluent (>90%) cell monolayer without any medium.

4.3 In parallel, the negative interference control A_{PBS} was performed using PBS instead of the product test solution.

4.4 After 1 hour incubation at 37 °C, the supernatant was discarded. A series of ten-fold dilutions of the virus test suspension was prepared in maintenance medium. 100 µL of each dilution was titrated to each well. The virus titre for A_T and A_{PBS} was determined using quantal test according to Sections 1.5 to 1.8.

5. Suppression Control *B*: Validation of the Inactivation Method

5.1 100 µL of interfering substance was pipetted into a tube. 100 µL of maintenance medium was added to the tube and mixed.

5.2 800 µL of the product test solution of the highest concentration used in the test *Na* was added to tube and mixed.

5.3 The activity of the product test solution was inactivated or removed using the same method employed for the test *Na* using one of the following methods:

5.3.1 Dilution in ice-cold maintenance medium. 50 µL of the mixture was transferred into 400 µL ice-cold maintenance medium. 50 µL of the virus test suspension was added to the mixture. The tube was mixed and put in an ice bath for 30 minutes ± 10 seconds, or if the cytotoxicity of the product test solution is too high,

5.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the eluate was obtained as per manufacturer's instructions. 50 µL of the eluate was transferred into 400 µL ice-cold maintenance medium. 50 µL of the virus test suspension was added to the mixture. The tube was mixed and put in an ice bath for 30 minutes ± 10 seconds.

5.4 At the end of the 30 minutes incubation, a series of ten-fold dilutions of the inactivated mixture B_T was prepared in ice-cold maintenance medium.

5.5 The virus titre for B_T was determined using quantal test according to Sections 1.5 to 1.8.

6. Reference Test *C*: Verification of Virus Inactivation

6.1 200 µL of the virus test suspension and 800 µL of PBS were mixed with 1 mL of 1.4% (w/v) formaldehyde.

6.2 The tube was mixed and the stopwatch was started at once. The tube was placed in a water bath controlled at the chosen test temperature θ for the contact time t.

6.3 Immediately at the end of t, the tube was mixed and the activity of the product test solution was inactivated or removed using one of the following methods:

6.3.1 Dilution in ice-cold maintenance medium. 20 µL of the mixture was transferred into 180 µL ice-cold maintenance medium and put in an ice bath, or if the cytotoxicity of the formaldehyde is too high,

6.3.2 Ultrafiltration using MicroSpin™ S-400 HR columns. 100 µL of the mixture is transferred to the column and the residual virus was obtained as per manufacturer's instructions.

6.4 Within 30 minutes of product inactivation, a series of ten-fold dilutions of the inactivated mixture was prepared in ice-cold maintenance medium.

6.5 The virus titre for C was determined using quantal test according to Sections 1.5 to 1.8.

6.6 The cytotoxicity effect for formaldehyde was determined according to Section 3, using the same inactivation method chosen for the reference test C.