MS ISO/IEC 17025
TESTING
SAMM NO. 823

Test Report No.: VX-TR-21-0075
Copy No.: 1

DETERMINATION OF THE FUNGICIDAL ACTIVITY (EN 1650) OF BERRYC

Lab No.:	VX-63-20-0003
Sample Name:	**BerryC**
Method:	EN 1650:2019 (E) 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)
Client:	TEVO Creations Sdn Bhd Plot 155, Jln Perindustrian Bukit Minyak 7 Bukit Minyak Industrial Estate, MK 13, S.P.T. 14100 Bukit Mertajam, Penang Malaysia
Sample Receipt Date:	4 December 2020
Report Date:	2 February 2021

Kuala Lumpur, 2 February 2021

Dr Peter Cheong
Head of Microbiological Testing

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Materials and Method

Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the food, industrial, domestic, and institutional areas according to EN 1650:2019 (E)

1. Testing laboratory identification

Viroxy Sdn. Bhd.
6th Floor, Menara RKT
50300 Kuala Lumpur
Malaysia

2. Sample identification

2.1	Sample name:	BerryC
2.2	Batch no.:	Not specified
2.3	Product appearance:	Clear, colourless solution
2.4	Manufacturer:	TEVO Creations Sdn Bhd Plot 155, Jln Perindustrian Bukit Minyak 7 Bukit Minyak Industrial Estate, MK 13, S.P.T. 14100 Bukit Mertajam, Penang Malaysia
2.5	Active substances:	Not specified
2.6	Sample receipt date:	4 December 2020
2.7	Storage conditions:	Room temperature
2.8	Product diluent:	Distilled water

3. Experimental conditions

3.1	Testing period:	21 December 2020
3.2	Test organism(s):	*Candida albicans* ATCC 10231
3.3	Concentration / contact time:	100.00 %* / 5, 15, and 30 minutes
3.4	Loading:	0.30 g/L bovine albumin solution
3.5	Test temperature:	20 °C ± 1 °C
3.6	Counting method:	Pour plate
3.7	Incubation period:	48 hours, 30 °C ± 1 °C

4. Test method and its validation

4.1	Testing method:	Dilution-neutralization
4.2	Inactivation combination:	60.00 g/L Tween 80 60.00 g/L Saponin 6.00 g/L Lecithin 2.00 g/L L-Histidine 2.00 g/L L-Cysteine in tryptone soya broth

The results of validation tests A, B, and C proved the viability of the method in all cases.

5. Test results

The results are stated in Tables A and B.

6. Conclusion

BerryC showed the required microbial reduction of ≥4.0 $\log_{10}$ against test strain *Candida albicans* ATCC 10231 in accordance with EN 1650:2019 (E) at 100.00 %* concentration after 5, 15, and 30 minutes under the stated conditions. According to the simple acceptance decision rule†, there is a minimal risk of false acceptance.

Kuala Lumpur, 2 February 2021

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7. Note

Yeasticidal activity – the capability of a product to produce a reduction in the number of viable yeasts belonging to reference strains under defined conditions by at least 4 orders (10^4).

$R = N_0/N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

* The product can only be tested at 80.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

† The decision rule applied is simple acceptance rule with no guard band and up to 50 % risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

Table A: Evaluation of the yeasticidal activity of BerryC on test strains according to EN 1650

Product: BerryC
Loading: 0.30 g/L bovine albumin solution

Test strain: *Candida albicans* ATCC 10231

N	V_{C1}	V_{C2}	Test suspension, N
10^{-5}	233	223	N: 2.27×10^7 N_0: 2.27×10^6 lg N_0: **6.36**
10^{-6}	20	24	

Test concentration (%) / contact time (min)	Dilution	V_{C1}	V_{C2}	Test procedure, N_a $N_a = \bar{x} \times 10$
100.00* / 5	10^0	<14	<14	N_a: $<1.40 \times 10^2$ lg N_a: **<2.15** lg R: **>4.21**
100.00* / 15	10^0	<14	<14	N_a: $<1.40 \times 10^2$ lg N_a: **<2.15** lg R: **>4.21**
100.00* / 30	10^0	<14	<14	N_a: $<1.40 \times 10^2$ lg N_a: **<2.15** lg R: **>4.21**

* The product can only be tested at 80.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

Table B: Control tests and method validation for Table A

Test strain	Validation suspension	Validation of experimental conditions	Neutralizer toxicity control	Method validation control
C. albicans ATCC 10231	N_{V0}: 9.60 x 10^1	A: 8.50 x 10^1	B: 7.80 x 10^1	C: 8.40 x 10^1

Note

cfu: Colony forming units

V_C: Number of cfu counted per 1.0 ml sample

$\overline{x}$: Average V_{C1} and V_{C2} values

N: Number of cfu per ml in the test suspension

N_0: Number of cfu per ml at the beginning of the contact time

N_{V0}: Number of cfu per ml in the mixtures A and C at the beginning of the contact time

N_{VB}: Number of cfu per ml in the mixture B at the beginning of the contact time

N_a: Number of survivors per ml in the test mixture at the end of the contact time and before neutralization

A: Number of cfu per ml in the experimental conditions control

B: Number of cfu per ml in the neutralizer toxicity control

C: Number of cfu per ml in the dilution-neutralization method validation

Table C: Summary of the log reductions of the quantitative suspension test according to EN 1650

Test strain	Test concentration (%) / contact time min)	Log reduction	Associated risk†
C. albicans ATCC 10231	100.00* / 5	**>4.21 ± 0.06**	Minimal risk of false acceptance
	100.00* / 15	**>4.21 ± 0.06**	Minimal risk of false acceptance
	100.00* / 30	**>4.21 ± 0.06**	Minimal risk of false acceptance

* The product can only be tested at 80.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

† The decision rule applied is simple acceptance rule with no guard band and up to 50 % risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

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Plot 155, Jln Perindustrian Bukit Minyak 7
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Efficacy of BerryC against *Candida albicans* ATCC 10231 in a quantitative suspension test at 20 ˚C according to EN 1650:2019 (E) under clean condition

EXPERT OPINION*

This expert opinion is based on the test report VX-TR-21-0075 dated 2 February 2021.

The yeasticidal activity of the disinfectant BerryC of TEVO Creations Sdn Bhd against *Candida albicans* ATCC 10231 was investigated by a quantitative suspension test according to EN 1650:2019 (E) under clean condition (0.30 g/L bovine albumin solution).

According to this suspension test, a disinfectant or a disinfectant solution at a particular concentration is considered as having fungicidal or yeasticidal activity if the number of viable fungi or yeasts is reduced by ≥4 $\log_{10}$ (inactivation ≥99.99 %) within the recommended exposure period.

BerryC was examined at 20 ˚C at the concentration of 100.00 %** for the exposure time of 5, 15, and 30 minutes. After the exposure time, the yeasticidal reduction exceeded 4 $\log_{10}$-steps in all assays. According to the simple acceptance decision rule†, there is a minimal risk of false acceptance. Therefore, a yeasticidal activity against *Candida albicans* ATCC 10231 was measured as follows:

Clean condition 100.00 %** 5, 15, and 30 minutes

Kuala Lumpur, 2 February 2021

Dr Peter Cheong
Head of Microbiological Testing

Maizatul Ismail
Microbiologist

* Opinions and interpretations expressed here are outside the scope of SAMM (Laboratory Accreditation Scheme of Malaysia) accreditation.

** The product can only be tested at 80.00 % concentration or less, as some dilution always occurs when test organisms and interfering substance are added.

† The decision rule applied is simple acceptance rule with no guard band and up to 50 % risk of false acceptance or rejection. This rule has been determined by the laboratory and agreed with the client prior to testing.

Appendix 1

QAU CERTIFICATE*

The results stated in test report VX-TR-21-0075 dated 2 February 2021 were compared to the raw data of the tests and checked for correct transfer. No deviations were detected.

Kuala Lumpur, 2 February 2021

Maizatul Ismail
Microbiologist

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Appendix 2 Raw data

Test Method	EN 1650:2019				
Product	BerryC		**Batch No.**	N/A	
Product Diluent	Distilled water		**Lab No.**	VX-63-20-0003	
Test Organism	*Candida albicans ATCC 10231*				
Interfering Substance	0.30 g/L bovine albumin solution				
Test Temperature (°C)	20	**Incubation Temperature (°C)**		36	
Neutralizer or Rinsing Liquid	60.00 g/L Tween 80, 60.00 g/L Saponin, 6.00 g/L Lecithin, 2.00 g/L Histidine, 2.00 g/L Cysteine in Tryptone Soya Broth				
Inactivation Method	Dilution-neutralization		**Plating Method**	Spread plate	
Test Date	21/12/2020	**Analyzed By**	MIS	**Verified By**	ANE

Test and Validation Suspension

Test Suspension (N)	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = N = 2.27E+07	N_0 = N/10
	10^{-5}	233	223	lg N_0 = 6.36	6.17 ≤ lg N_0 ≤ 6.70
	10^{-6}	20	24	Pass? **Yes**	

Validation Suspension (N_V)	V_{C1}	V_{C2}	N_{V0} = 96.0	N_{V0} = N_V/10
	90	102	30 ≤ N_{V0} ≤ 160	Pass? **Yes**
Validation Suspension (N_{VB})	V_{C1}	V_{C2}	N_{V0} =	N_{V0} = N_{VB}/1000
	-	-	30 ≤ N_{V0} ≤ 160	Pass? **N/A**

Validation and Control Procedures

Experimental Conditions Control (A)	V_{C1}	V_{C2}	A = 85.0	Pass? **Yes**
	86	84	A ≥ 0.5 x N₩10	
Neutralizer Toxicity or Filtration Control (B)	V_{C1}	V_{C2}	B = 78.0	Pass? **Yes**
	85	71	B ≥ 0.5 x N_{VB}/1000 or N₩10	
Method Validation (C)	V_{C1}	V_{C2}	C = 84.0	Pass? **Yes**
Concentration: 100 %	88	80	C ≥ 0.5 x N₩10	

Test Procedure

Product Concentration	Contact Time (minutes)	Dilution	V_{C1}	V_{C2}	Na = $\bar{x}$ or $\bar{x}_{wm}$ x 10	lg Na	lg R = lg N_0 - lg Na
100 %	5	10^0	<14	<14	<1.40E+02	<2.15	>4.21
		10^{-1}	-	-			
		10^{-2}	-	-			
		10^{-3}	-	-			
100 %	15	10^0	<14	<14	<1.40E+02	<2.15	>4.21
		10^{-1}	-	-			
		10^{-2}	-	-			
		10^{-3}	-	-			
100 %	30	10^0	<14	<14	<1.40E+02	<2.15	>4.21
		10^{-1}	-	-			
		10^{-2}	-	-			
		10^{-3}	-	-			

Raw Data of Colony Count

	N^{-5}	N^{-6}	N_V	N_{VB}	A	B	C
V_{C1}	233	20	90	-	86	85	88
V_{C2}	223	24	102	-	84	71	80

Product Concentration	Contact Time (minutes)	Na^0		Na^{-1}		Na^{-2}		Na^{-3}	
		V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}	V_{C1}	V_{C2}
100 %	5	0	0	-	-	-	-	-	-
100 %	15	0	0	-	-	-	-	-	-
100 %	30	0	0	-	-	-	-	-	-

Note

cfu: Colony forming units

V_C: Number of cfu counted per 1.0 ml sample

$\bar{x}$: Average V_{C1} and V_{C2} values

N: Number of cfu per ml in the test suspension

N_0: Number of cfu per ml at the beginning of the contact time

N_{V0}: Number of cfu per ml in the mixtures A and C at the beginning of the contact time

N_{VB}: Number of cfu per ml in the mixture B at the beginning of the contact time

N_a: Number of survivors per ml in the test mixture at the end of the contact time and before neutralization

A: Number of cfu per ml in the experimental conditions control

B: Number of cfu per ml in the neutralizer toxicity control

C: Number of cfu per ml in the dilution-neutralization method validation

Appendix 3 Summary of test description

1. Materials and reagents

1.1 Malt Extract Agar (MEA, Oxoid, catalogue no. CM0059)
1.2 Tryptone, pancreatic digest of casein (Oxoid, catalogue no. LP0042)
1.3 Sodium chloride (Merck, catalogue no. 1.06404.0500)
1.4 Magnesium chloride ($MgCl_2$, Acros Organics, catalogue no. AC223211000)
1.5 Calcium chloride ($CaCl_2$, R&M Chemicals, catalogue no. 9924-00)
1.6 Sodium bicarbonate ($NaHCO_3$, Fisher Chemical, catalogue no. 10152780)
1.7 Bovine albumin fraction V (Merck, catalogue no. 1.12018.0100)
1.8 Neutralizer
  1.8.1 Tween 80 (Fisher Chemical, catalogue no. 10498800)
  1.8.2 Saponin (Nacalai Tesque, catalogue no. 30502-55)
  1.8.3 Lecithin (Nacalai Tesque, catalogue no. 20335-65)
  1.8.4 L-Histidine (Fisher Scientific, catalogue no. BP382-100)
  1.8.5 L-Cysteine (Merck, catalogue no. 1.02838.0100)
  1.8.6 Tryptone Soya Broth (TSB, Oxoid, catalogue no. CM0129)

2. Apparatus and glassware

2.1 Autoclave (TOMY, model SX500)
2.2 Water baths (Memmert, model WNB 29)
2.3 Incubator (Binder, model BD 260)
2.4 pH-meter (Ohaus, model 3100 Meter with ST310)
2.5 Vortex® mixer (Biosan model Biosan V-1 Plus)
2.6 Petri dishes (Wanpow Plastic)

3. Test procedure

3.1 Test Na – Determination of fungicidal or yeasticidal concentrations

3.1.1 Pipette 1.0 ml of interfering substance into a tube.

3.1.2 Add 1.0 ml of the test suspension.

3.1.3 Start the stopwatch immediately, mix and place the tube in a water bath controlled at the chosen test temperature θ for 2 minutes ± 10 seconds.

3.1.4 At the end of this time, add 8.0 ml of the product test solution.

3.1.5 Restart the stopwatch at the beginning of the addition.

3.1.6 Mix and place the tube in a water bath controlled at θ for the chosen contact time t.

3.1.7 Just before the end of t, mix again.

3.1.8 At the end of t, take 1.0 ml sample of the test mixture Na and transfer into a tube containing 8.0 ml neutralizer and 1.0 ml water.

3.1.9 Mix and place in a water bath controlled at 20 °C ± 1 °C.

3.1.10 After a neutralization time of 5 minutes ± 10 seconds, mix and immediately take a sample of 1.0 ml of the neutralized test mixture Na (containing neutralizer, product test solution, interfering substance, and test suspension) in duplicate and inoculate using the pour plate or spread plate technique.

3.1.11 When using the pour plate technique, pipette each 1.0 ml sample into separate Petri dishes and add 15 ml to 20 ml of melted MEA, cooled to 45 °C ± 1 °C.

3.1.12 When using the spread plate technique, spread each 1.0 ml sample – divided into portions of approximately equal size – on an appropriate number (at least two) of surface dried plates containing MEA.

3.1.13 Incubate the plates for 42 to 48 hours at 30 °C ± 1 °C.

3.1.14 Discard any plates which is not countable (for any reason). Count the plates and determine the number of cfu. Only for *Aspergillus brasiliensis*: incubate the plates for a further 20 to 24 hours and if the number of colonies has increased, incubate for a third additional period of 20 to 24 hours.

3.1.15 Do not recount plates which no longer show well separated colonies. Recount the remaining plates. If the number has increased, use only the higher number for further evaluation.

3.1.16 Note the exact number of colonies for each plate but record >165 (for moulds) or >330 (for yeasts) for any counts higher than 165 and 330, respectively and determine the V_C-values.

3.1.17 Perform the procedure using the other product test solutions at the same time.

3.1.18 Perform the procedure applying the other obligatory and – if appropriate – other additional experimental conditions.

3.2 Experimental conditions control A – Validation of the selected experimental conditions and/or verification of the absence of any lethal effect in the test conditions

3.2.1 Pipette 1.0 ml of interfering substance into a tube.

3.2.2 Add 1.0 ml of the validation suspension.

3.2.3 Start the stopwatch immediately, mix, and place the tube in a water bath controlled at θ for 2 minutes ± 10 seconds.

3.2.4 At the end of this time, add 8.0 ml of hard water. In the case of ready-to-use products: water instead of hard water.

3.2.5 Restart the stopwatch at the beginning of the addition.

3.2.6 Mix and place the tube in a water bath controlled at θ for *t*.

3.2.7 Just before the end of *t*, mix again.

3.2.8 At the end of *t*, take a sample of 1.0 ml of this mixture A in duplicate and inoculate using the pour plate or the spread plate technique.

3.2.9 Calculate the numbers of cfu/ml in the validation mixture A.

3.2.10 Verify according to Section 3.5.

3.3 Neutralizer control B – Verification of the absence of toxicity of the neutralizer

3.3.1 Pipette 8.0 ml of the neutralizer used in the test and 1.0 ml of water into a tube.

3.3.2 Add 1.0 ml of the validation suspension.

3.3.3 Start the stopwatch at the beginning of the addition and mix.

3.3.4 Place the tube in a water bath controlled at 20 °C ± 1 °C for 5 minutes ± 10 seconds.

3.3.5 Just before the end of this time, mix.

3.3.6 At the end of this time, take a sample of 1.0 ml of this mixture B in duplicate and inoculate using the pour plate or the spread plate technique.

3.3.7 Calculate the numbers of cfu/ml in the validation mixture B.

3.3.8 Verify according to Section 3.5.

3.4 Method validation C – Dilution-neutralization validation

3.4.1 Pipette 1.0 ml of interfering substance into a tube.

3.4.2 Add 1.0 ml of the diluent.

3.4.3 Start the stopwatch, add 8.0 ml of the product test solution only of the highest concentration used in the test.

3.4.4 Mix and place the tube in a water bath controlled at θ for *t*.

3.4.5 Just before the end of *t*, mix again.

3.4.6 At the end of *t*, transfer 1.0 ml of the mixture into a tube containing 8.0 ml of neutralizer.

3.4.7 Restart the stopwatch at the beginning of the addition.

3.4.8 Mix and place the tube in a water bath controlled at 20 °C ± 1 °C for 5 minutes ± 10 seconds.

3.4.9 Add 1.0 ml of the validation suspension.

3.4.10 Start the stopwatch at the beginning of the addition and mix.

3.4.11 Place the tube in a water bath controlled at 20 °C ± 1 °C for 30 minutes ± 1 minute.

3.4.12 Just before the end of this time, mix again.

3.4.13 At the end of this time, take a sample of 1.0 ml of the mixture C in duplicate and inoculate using the pour plate or the spread plate technique.

3.4.14 Calculate the numbers of cfu/ml in the validation mixture C.

3.4.15 Verify according to Section 3.5.

3.5 Basic limits

3.5.1 N is between 1.5 x 10^7 and 5.0 x 10^7 (7.17 ≤ lg N ≤ 7.70)

3.5.2 N_0 is between 1.5 x 10^6 and 5.0 x 10^6 (6.17 ≤ lg N_0 ≤ 6.70)

3.5.3 N_{V0} is between 30 and 160 (3.0 x 10^1 and 1.6 x 10^2)

3.5.4 N_V is between 3.0 x 10^2 and 1.6 x 10^3

3.5.5 A, B, C are equal to or greater than 0.5 x N_{V0}

3.5.6 Control of weighted mean counts: quotient is not lower than 5 and not higher than 15

4. Literature

4.1 EN 1650:2019 (E): 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)

4.2 EN 14885:2018 (E): Chemical disinfectants and antiseptics – Application of European Standards for chemical disinfectants and antiseptics

4.3 EN 12353:2013 (E): Chemical disinfectants and antiseptics – Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity

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