

Test about Sterilizing effect of AQUASSIMO on baby products(bottle of milk)
-Test report-

株式会社 食環境衛生研究所
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Institution Corporation for food environment and hygiene
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1. The title of test

Test about Sterilizing effect of AQUASSIMO on baby products(bottle of milk)

2. The objective

This is conducted to verify the sterilizing effect by attaching the officially-announced microorganisms into a product which is for sale and measuring the number of viruses after several hours.

3. Committer of this test

Name : Michwa Chemical corporation

Sunny date operation department

Location : Gunma ken takasaka city hanatakazo 43-13

person in charge of committing : Snaga Tomonori

4. Conducting Institute

Name : Food Environment Sanitation laboratory\

Location : Gunma ken Maebasi city araguchizo 561-21

Operation manager : Kubo Gazhiro

5. Conductor of the test

A person responsible for the test : Machmotto syohei

A person in charge of the test : Kamiya Tomohide

6. Schedule

1) Launching date : April 26 2010

2) Finishing date : May 10 2010

7. Writer of report

Kamiya Tomohide

8. the officially-announced microorganisms

Black Fungus Cladsporinum sp.(external separative)

Staphyococcus aureus (ATCC6538p)

E. coli o and 157 Esherichia coli(ATCC700728)

9. A sample of a subject for experiment

AQUASSIMO(Concentration of available chlorine : 10ppm)

10. Agar medium used as respective species of microorganism

Microorganism	Medium	Cultivation Time
Cladsporinum sp.	PDA Medium	25°C, for 7 hours
staphylococcus aureus	Mannitol Salt Agar with Egg Yolk	35°C, for 48 hours
Esherichia coli	CT-SMAC agar culture medium	35°C, for 24 hours

11. Set up and a method of test

Researchers sprayed the officially-announced microorganism into respective test groups of bottle of milk, Dried it(The goal about the number of attached microorganisms is about $10^7 \sim 10^8$) and then use it in the test.

Test 1 : Spray test

Nursery Plot	subject water	moment of inspection
Nursery Plot 1	ACQASSIMO	at 15, 30 second
Nursery Plot 2(Comparison)	Sterilization normal saline solution	at 0, 15, 30 second

Researchers sprayed subject water(about 6 ml) 5 times with an adjunctive nozzle into a bottle of milk inside which public announced microorganism is attached, inoculated mixture for several hours, cultivated inoculated mixture subject water in an agar medium and the measured the number of microorganisms.

Test 2: Inoculated mixture test

Nursery Plot	subject water	moment of inspection
Nursery Plot 3	ACQASSIMO	at 15, 30 second
Nursery Plot 4(Comparison)	Sterilization normal saline solution	at 0, 15, 30 second

Researchers sprayed subject water 25ml, 1/10 of size of the mottle inside which public announced microorganism is attached, inoculated mixture for several hours, cultivated inoculated mixture subject water in an agar medium and the measured the number of microorganisms.

Test 3: Warming inoculated mixture Test

Nursery Plot	subject water	moment of inspection
Nursery Plot 5	ACQASSIMO	at 15, 30 second
Nursery Plot 6(Comparison)	Sterilization normal saline solution	at 0, 15, 30 second

Researchers sprayed warmed subject water(41°C) 25ml, 1/10 of size of the bottle inside which the officially-announced microorganism is attached, inoculated mixture for several hours, cultivated inoculated mixture subject water in an agar medium and measured the number of microorganisms

12. Results

Respective results of the test

Test 1 : Spray Test

Staphyococcus aureus and Esherichia coli were not found at all moments. In Nursery Plot 2, which is comparison, Staphyococcus aureus were found and the number of those is $2.6 \times 10^8 \sim 3.0 \times 10^8$ cfu/ml, The number of Esherichia colis was $7.8 \times 10^7 \sim 8.0 \times 10^7$ cfu/ml, Cladsporinum's alive spores were found in more than half part of Nursery Plot 2, which is comparison. The number of alive spores in Nursery Plot 2 is $1.9 \times 10^4 \sim 2.1 \times 10^4$ cfu/ml at all moments.

Test 2 : inoculated mixture Test

Staphyococcus aureus and Esherichia colis were not found at all moments. In Nursery Plot 4 which is comparison, The number of Staphyococcus aureus was $4.1 \times 10^6 \sim 4.5 \times 10^6$ cfu/ml at all moments and The number of Esherichia colis was $2.2 \times 10^6 \sim 2.6 \times 10^6$ cfu/ml. Cladsporinum's spores were found at all moment and the number of those is 1.7×10^2 cfu.ml 15 seconds after sensitization and 4.0×10 cfu/ml 30 seconds after sensitization. The number of alive spores in Nursery Plot 4, which is a comparison, was $1.8 \times 10^3 \sim 2.1 \times 10^3$ cfu/ml

Test 3 : Warming inoculated mixture Test

Staphyococcus aureus and Esherichia colis were not found at all moments. In Nursery Plot 6 which is comparison, The number of staphyococcus aureus was $4.8 \times 10^6 \sim 5.2 \times 10^6$ cfu/ml and the number of Esherichia colis was $2.7 \times 10^6 \sim 3.1 \times 10^6$ cfu/ml. Cladsporinum's spores were found at all moments and the number of them was 2.3×10 cfu/ml 15 seconds after sensitization and 8 cfu/ml 30 seconds after sensitization. The number of alive spores was $4.8 \times 10^3 \sim 5.2 \times 10^3$ cfu/ml at all moments.

13. Consideration

Three experiments of which processes were different from one another, were conducted. In all tests, It was verified that Staphyococcus aureus and Esherichia colis could sterilize even below the limit(0cfu/ml). In all tests on Cladsporinum, Its alive spores were found. But In Test 2 and 3, It was verified that the number of its alive spores was more decreased compared to the groups of comparisons.

According to above fact, ACQUASSIMO sterilizes Staphyococcus aureus and Esherichia colis within 15 seconds. And Cladsporinum is not sterilized within 30 seconds. but, According to the group of comparison of test 2 and 3, The number of its alive spores is decreased by extending sterilizing time. It is assumed that extending the time makes more effective.

試験責任者: 松本彰平 

A person responsible for the test : Mach Moto shohei

Supplementation : Notification for the result of the test

試験 1: スプレー試験

Cladsporinum sp.

	0 秒	15 秒	30 秒
試験区 1		5.6×10^3	5.5×10^3
試験区 2	1.9×10^4	2.1×10^4	1.9×10^4

Escherichia coli

	0 秒	15 秒	30 秒
試験区 1		0	0
試験区 2	8.1×10^7	7.8×10^7	8.0×10^7

Staphylococcus aureus

	0 秒	15 秒	30 秒
試験区 1		0	0
試験区 2	2.9×10^8	3.0×10^8	2.6×10^8

単位: cfu/mL

試験 2: 攪拌試験

Cladsporinum sp.

	0 秒	15 秒	30 秒
試験区 1		1.7×10^2	4.0×10
試験区 2	2.1×10^3	1.8×10^3	2.0×10^3

Escherichia coli

	0 秒	15 秒	30 秒
試験区 1		0	0
試験区 2	2.4×10^6	2.6×10^6	2.2×10^6

Staphylococcus aureus

	0 秒	15 秒	30 秒
試験区 1		0	0
試験区 2	4.2×10^6	4.1×10^6	4.5×10^6

単位: cfu/mL

試験 3: 加温攪拌試験

Cladsporinum sp.

	0 秒	15 秒	30 秒
試験区 1		2.3×10	8
試験区 2	5.0×10^3	4.8×10^3	5.2×10^3

Escherichia coli

	0 秒	15 秒	30 秒
試験区 1		0	0
試験区 2	3.1×10^6	2.7×10^6	3.0×10^6

Staphylococcus aureus

	0 秒	15 秒	30 秒
試験区 1		0	0
試験区 2	4.8×10^6	5.2×10^6	4.9×10^6

単位: cfu/mL