Summary of antibacterial effect test results of natural calcined calcium aqueous solution

Product Name： **A B A N T**

Natural calcined calcium aqueous solution 【Product name : ABANT】, developed from Apr. 2010, is a safe product that uses 100% natural ingredients and has a strong antibacterial deodorizing effect with a wide range of applications.

300ml

30ml

ARROWFIELD CO., LTD.
5-16 Honjo, Koyoen, Nishinomiya, Hyogo 662-0015, Japan
TEL +81-798-75-1100
✉ abant@arrowfield.co.jp

With excellent antibacterial properties and effective against most of food poisoning bacteria

It is not only effective against general food poisoning bacteria such as Vibrio parahaemolyticus, Salmonella spp., O-157, etc., but also have a great effective against viral food poisoning.

Bacteria	Sterilization time	Viable count		Testing laboratory
		At the start	After sterilization	
E. coli: serotype O157：H7	10 mins	2.8×10^5	＜10	Tokyo Metropolitan Food Technology Research Center
Staphylococcus aureus	10 mins	2.7×10^5	40	
Salmonella	10 mins	2.3×10^5	≤ 30	
Pseudomonas aeruginosa	10 mins	3.2×10^5	≤ 30	
Listeria monocytogenes	60 mins	6.0×10^5	≤ 30	
Bacillus subtilis	after 3 mins	1.3×10^5	≤ 300	
Histamine-producing bacteria	5 mins	3.0×10^5	≤ 300	
Vibrio parahaemolyticus	5 mins	1.2×10^5	＜ 10	Kyoto Microbio Laboratory
Methicillin-resistant Staphylococcus aureus	after 3 hours	1.6×10^5	＜ 10	
Legionella	10 mins	0.82×10^5	＜ 10	Laboratory of food environment and hygiene
Campylobacter	10 mins	10-fold serial dilution of bacterial solution	No occurrence	Japan Meat Science & Technology Institute

Add the bacterial suspension to the natural calcined calcium saturated aqueous solution (0.11%), stir it , and measure the bacterial counts according to the method for measuring general bacterial counts.

Virus	Virus removal time	Virus infectious titer		Testing laboratory
		At the start	After sterilization	
Norovirus（Feline calicivirus）	10 mins	7.0	＜ 1.5	Japan Food Research Laboratories

Although there is no effective drug or vaccine against Norovirus currently, natural calcined calcium saturated aqueous solution can prevent the onset of disease with virus removal effect.

Sterilize 99.99% of coronavirus

Virus inactivation effect test by natural calcined calcium

【 Virus inactivation test】

・Mixed the test solution (0.9 ml) with the virus solution (0.1 ml) and allowed them to act for a predetermined time.

・After the action, sampled 0.1 ml from the mixed solution and diluted it about 10 times with 0.1 M HEPES buffer (7.0) to stop the action of the test product against the virus.

・Measured the infectious titer by the TCID50 method using the solution as a stock solution for the infectious titer measurement sample.

Sample virus：
Feline enteric coronavirus（WSU 79-1683）

Specimen	Infectious titer(TCID50/ml)			LRV	
	0(at the start)	after 5 mins	after 10 mins	after 5 mins	after 10 mins
Contrast(PBS)	2.4E+05		5.0E+05		-0.3
Natural calcined calcium		1.1E+02	＜1.3E+01	3.3	＞4.2

As a control, phosphate buffered saline (PBS) was used.
Detection limit: 1.3E+01 TCID50/ml
LRV (log reduction of infectious titer): log10 (initial infectious titer ÷ infectious titer after each action time)

Testing organization: Kitasato Research Center for Environmental Science

Based on the above results, natural calcined calcium is proved to be sterilizable as following
5 minutes later ➡ 99.95%
10 minutes later ➡ 99.99%.

Mold Reproduction Deterrence

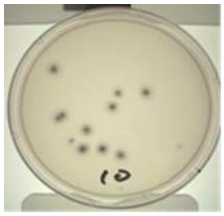
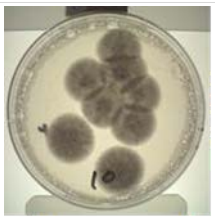
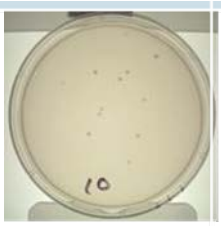
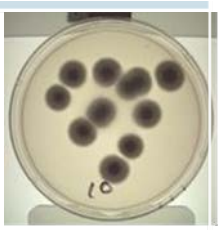
<Test method>

(1) Add a predetermined amount of natural calcined calcium powder to potato dextrose medium*.

* A medium that makes the molds grow actively on a trial basis by adding potato extract, glucose, and agar into purified water.

(2) Approximately 10 mold spores are planted in each small schale (approximately 20 ml).

(3) Cultivate at room temperature and visually observe the growth of mold over time.

Mold type	Additive amount of natural calcined calcium	The No. of the elapsed days	
		2 days	5 days
黒コウジカビ Aspergillus b rasiliensis	0g		
	0.3g /200ml (約0.15%) (Approx.0.15%)		
コウジカビ Aspergillus oryzae	0g		
	0.3g /200ml (約0.15%) (Approx.0.15%)		
アオカビ Penicillium camemberti	0g		
	0.3g /200ml (Approx.0.15%)		

Control the growth of many types of molds.

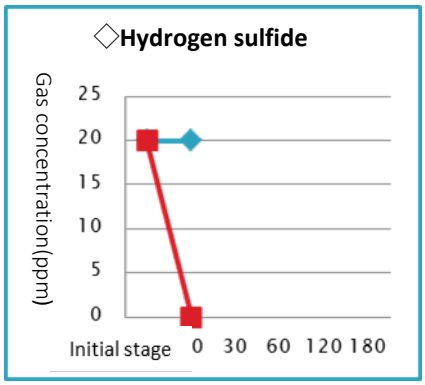
Natural calcined calcium solution is an odorless deodorant.

Blank test

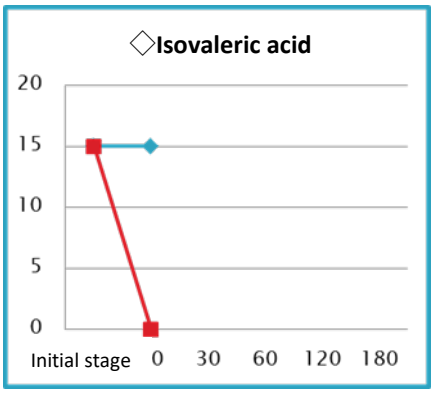
Gas concentration when 5 ml of calcined calcium saturated solution was injected

Gas concentration when 15 ml of calcined calcium saturated solution was injected

Rotten smell of eggs



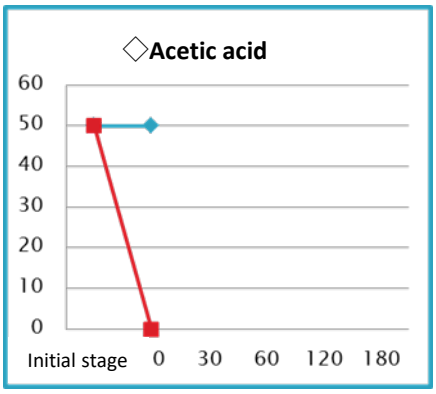
The smell of soggy socks



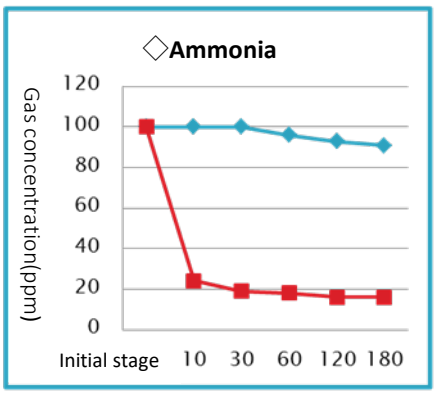
Test method:
Gas injection into the bag and detection tube method.
Each sample was put in an odor bag, and after applying a heat sheet, 3L of air was enclosed, and the test gas which concentration was obtained was added. Such sample was settled and the gas concentration in the bag was measured at each elapsed time using a gas detector tube. A blank test was conducted by performing the same operation without inserting the sample.

Testing laboratory:
Japan Food Research Laboratories

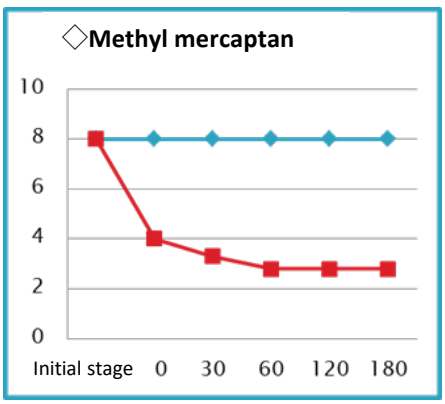
Vinegar smell



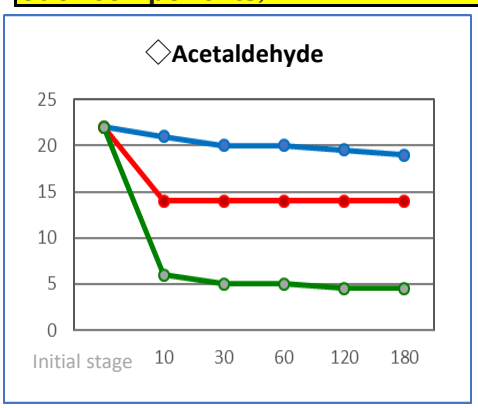
Excreta smell



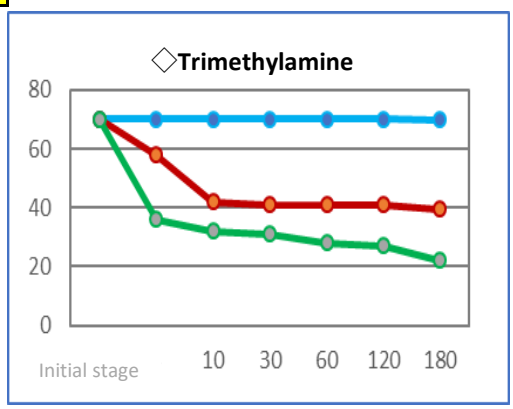
Rotten smell of onions



Pungent smell of grass（tobacco odor components）



Rotten smell of fish



Safety tests of natural calcined calcium

Natural calcined calcium in the form of powder has passed the following four safety tests.

1. Acute oral toxicity test Test No.N17137-1

2.Primary skin irritation test Test No.N17137-2

3.Skin sensitization test Test No.N17137-3

4.Reverse mutation test Test No.AN170026

Acute oral toxicity test

Safety was evaluated by acute oral toxicity test using mice and primary skin irritation examined by using rabbits.

An acute oral toxicity test was carried out in mice using a calcium-saturated aqueous solution of shellfish as a sample.

A 2000 mg/kg dose of the test substance was orally administered to the test group, and water for injection as a solvent control was orally administered to the male and female mice control group once, and observation was performed for 14 days.

（Testing organization: Japan Food Research Laboratories）

Body Weight Change(Male)

Administration group	Before administration	After administration（day）	
		7	14
Test group	33.5±2.5（5 animals）	37.1±2.7	40.1±3.7
Control group	33.3±1.7（5 animals）	37.9±2.1	40.9±2.5

No abnormalities or deaths were observed during the observation period in either male or female administration group.

No difference was found in the body weight between the test group and the control group in both males and females 7 and 14 days after the administration.

Body Weight Change (Female)

Administration group	Before administration	After administration（day）	
		7	14
Test group	27.7±1.6（5 animals）	30.1±2.1	32.7±3.1
Control group	27.8±1.4（5 animals）	30.0±1.4	32.7±1.5

At the end of the observation period, necropsy revealed no abnormalities in all test animals of both males and females.

Based on the above data, in the acute oral toxicity test using mice, the LD50 value of the sample was evaluated to exceed 2000 mg/kg for both males and females.

Skin irritation evaluation category 「non-irritant」

Primary skin irritation test

Three Japanese white rabbits were used as test animals, and the administration part was the dorsal part of the dehaired animal, and healthy and damaged skin parts were provided.
The administration method was such that 0.5 g of the administration sample was placed on a 2.5 × 2.5 cm lint cloth, and a non-permeable adhesive plaster, an adhesive sponge adhesive plaster, and an adhesive elastic bandage were used to occlude and apply the adhesive for 24 hours.
The irritative reaction was observed at 3, 24 and 48 hours after removal of the patch.

(Testing institution: Yoshimi Research Center, Drug Safety Testing Center Co., Ltd.)

Animal No.	Body Weight（kg）		General Condition			
	administration date	final judgement date	administration date	1 day after administration	2 days after administration	3 days after administration
1	2.29	2.36	no abnormality	no abnormality	no abnormality	no abnormality
2	2.24	2.29	no abnormality	no abnormality	no abnormality	no abnormality
3	2.21	2.3	no abnormality	no abnormality	no abnormality	no abnormality

Animal No.	Healthy Skin				Damaged Skin			
	Erythema・Scab		Edema		Erythema・Scab		Edema	
	3 hours	48 hours	3 hours	48 hours	3 hours	48 hours	3 hours	48 hours
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
Average	0		0		0		0	
Strength	0				0			
P.1.1	0							

No skin reaction was observed in both normal and damaged skin, and P.I.I was 0. No abnormalities were observed in the general conditions during the observation period, and the gain in body weight was also shown.
Based on the above results, it was concluded that this test substance had P.I.I=0, and the skin irritation evaluation category was non-irritant.

Reaction Category　P.I.I	Evaluation Category
0	non-irritant

Skin sensitization test in guinea pigs

Natural Calcined Calcium was examined by Guinea Pig Maximization Test method using guinea pig.

As test animals, 4 animals for preliminary tests, 10 animals for sensitization groups, and 5 animals for control groups, 19 animals in total were used.

For intradermal sensitization, a: Freund's complete adjuvant (FCA) and physiological saline equivalent emulsion, b: 0.05 w/w% solution of test substance (vehicle: physiological saline solution), c: 0.1 w/w% solution of test substance(vehicle: physiological saline solution) and equivalent emulsion of FCA were intradermally administered to neck skin at 0.1 ml × 2 places (however, shortening the administration interval of administration samples a and b). For contact sensitization, 0.2 g of the test substance drug substance (100%) was placed on a 2 × 4 cm lint cloth on the 7th day, and the test substance was occluded and applied on the intradermal administration site for 48 hours. The control group was treated in the same manner by impregnating 2 × 4 cm of lint cloth with 0.2 ml of water for injection. And, since the drug substance of this test substance was non-irritant, about 0.5g of the IO 3/4 SDS vaseline mixture was applied openly to the intradermal administration site where hair was removed on the sixth day.

On the 21st day after the sensitization test, 0.1g of the drug substance of this test substance was placed on a 1.5 × 1.5 cm lint cloth and occluded on the flank for 24 hours. The skin reaction was observed 24 and 48 hours after the removal of the adhesive patch.

As a result, no skin reaction was found in both the test substance sensitized group and the test substance control group. There were no abnormalities found under its general conditions and the body weight also gained."

(Testing institution: Yoshimi Research Center, Drug Safety Testing Center Co., Ltd.)

Based on the above results,

no skin sensitization was found in this test substance.

Reverse mutation test

As part of the safety assessment of natural calcined calcium using bacteria, we aimed to clarify the presence or absence of gene mutagenicity.

"To examine the presence or absence of gene mutagenesis of natural calcined calcium, Salmonella typhimurium (hereinafter abbreviated as S. typhimurium) TAIOO, TAI535, TA98, TAI 537 and Escherichia coli (hereinafter abbreviated as E. coli) WP2 uvrA were used by the preincubation method under the conditions of metabolic activation and metabolic inactivation. Water for injection was used as the solvent for the test substance.

In order to set the dose for this study, a dose setting study was conducted with 5 doses of 5000, 1250, 313, 78.1, and 19.5 μg/plate, which were serially diluted at a common ratio of 4 with 5000 μg/plate as the highest dose. According to the results, the lowest dose showing growth inhibition was taken as the highest dose. The test of this study was carried out with all strains under the conditions of metabolic inactivation, and with S. typhimurium TA strains under the conditions of metabolic activation in 6 doses of 313, 156, 78.1, 39.1, 19.5, 9.77 μg/plate, and with E. coli WP2 uwpA under the conditions of metabolic activation in 6 doses of 1250, 625, 313, 156, 78.1, 39.1 μg/plate.

Regardless of the presence or absence of metabolic activation in both the dose setting study and this study,in both the base pair substitution type and frameshift type strains, no increase in the number of revertant colonies, which is more than double the negative control value, was observed. No dose response was observed.

(Testing institution: Yoshimi Research Center, Drug Safety Testing Center Co., Ltd.)

Based on the above test results, it was determined that natural calcined calcium does not have the ability to induce gene mutation in bacteria (negative) under the conditions of this test.☒