

Clinical Test Report

Applicator	G-PLAN Co., Ltd
Testing Device	Magnetic vitalized Neojikun (Product) (Early Version of the MIMURA)
Testing Clause	“Antimicrobial Effectiveness”

With regard to the above requirement, we herewith report the result of Clinical tests.

23rd October 1997

**Itabashi Head Clinical Test Institution Co., Ltd
Environmental Sanitary Div.**

**With an official company authorized Stamp
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Ministry of Japanese Law**

(Translation from Original Document in Japanese)

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Anti-Microbial Effectiveness on Clinical Tests

1. Testing bacillus

Separate stump for clinical test

- . Escherichia coli
- . Diseased colon bacillus O157:H7
- . Salmonella typhi

2. Testing bacillus culture medium : Tryptic Soy Agar (TSA)

- . Normal a.gar-a.agar culture (Eiken Chemical Co.)
- . Agar-a agar culture including enzyme X-GAL (Nissui Pharmaceutical Co.)

3. The method used for sterilizing power test

- after installing Neojikun products
- to the testing setup (see diagram 1)

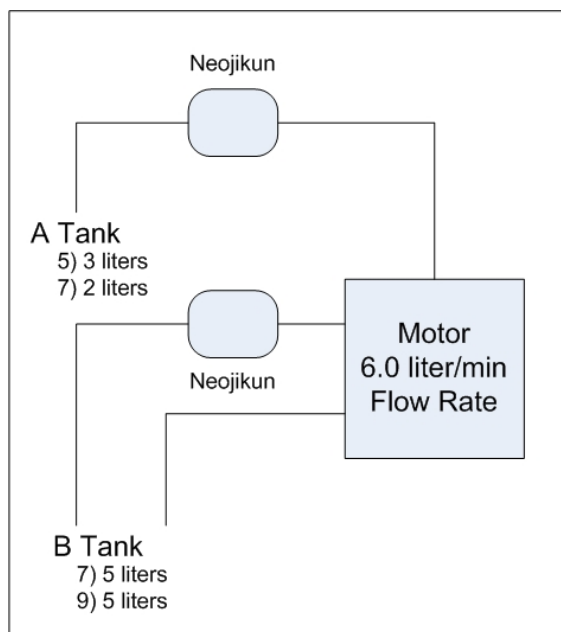


Diagram 1

- we created an experimental test system with the following procedures -
 - a. Pipe water which had chlorine in it was channeled into B tank (10 litres)
 - b. The water was pumped from Tank B to Tank A with Pump at the rate of 6.0 litter/min for total sterilization
 - c. After completing the sterilization, the pump was stopped
 - d. Water in Tank A was disposed off by draining the tank.
 - e. Fresh pipe water was channeled into Tank B with 10 litres.
 - f. The chemical Chio sulfuric acid sodium was passed into Tank B to eliminate the chlorine in Tank B and confirming the ratio of remaining chlorine (Less than 0.1 ppm), 3 litres was pumped into Tank A and the pump was stopped. Then all the water in the Tank A was disposed.

- g. Contaminated water with comprising of E.Coli, O157H7, and Salmonella samples were introduced into Tank B in separate sessions and was stirred. Samples of each strain were collected in individual sessions.
(Note: The test apparatus was reset with the above procedures (1-5) before each strains of the abovementioned bacteria colonies were introduced)**
- h. By controlling the pump sequence, the pickup of samples was done when tank B reached 2 liters in volume of the contaminated. The water was then channeled into Tank A and the pump stopped.
This sample was labeled as the 1st pass sample.**
- i. Tank B was connected to allow the water from Tank A to recirculate within the system.**
- j. The water circulation rate was for 5 liters at the flow rate of 6 liters per minute.**
- k. The pump was operated at the following time intervals - at 3 min, 5 min, 15 min, 30 min, 60 min, 120 min**

The samples were then tested for bacterial count.

4. The method of measurement on the number of bacteria, virus

a. Method for dilution of bacterial sample

Each sample was diluted by the sterilized physiological salt water by 10 times to 100 times to 1,000 times

Sample	T S B(Tryptic Soy Broth)		magnification
	dilution liquid	diluted sample	
1 m L	9 m L	10 m L	No.1 X 10
1 m L	9 m L	10 m L	No.2 X 100
1 m L	9 m L	10 m L	No.3 X 1,000

- b. After collecting samples in a pipette 23 u L of No.3 diluted sample into a Petri dish, where 20.0 ml of moltened TSA(Tryptic Soy Agar) of E.coli and O157H7 in X-GAL / Salmonella typhi in normal a agar medium are poured. When the medium Soddy is incubated at 35 c for 20 hours, an analysis to determine the colony count was calculated in 1 ml measurements.**

5. The following were the result of the anti-microbial effectiveness performed on the above clinical tests.

Sample	E.coli / ml	O157H7 / ml	Salmonella/ ml
Original	140,000	1,640,000	2,460,000
1 Pass	71,200	1,870,000	2,280,000
Later 3 min	4,640	1,170,000	1,750,000
5 minutes	1,010	1,320,000	1,890,000
15 minutes	40	880,000	1,120,000
30 minutes	0	670,000	100,000
60 minutes	0	10,000	0
120 minutes	0	0	0

6. Conclusion

With regards to E. coli, diseased colon bacillus O157, Salmonella, Anti-microbial effectiveness of the magnetic vitalized device Neojikun (early version of the MIMURA) are certified exponentially as a time lapse sequence of magnetic treatment method by water flow circulation system.

Comments by Satoru Mimura – January 2007

The Itabashi Institution belongs to the Itabashi Private General Hospital Group which is well known in Tokyo and the rest of Japan. This clinical test procedure is modified from “Antimicrobial Preservative Effectiveness “ in USP-NF reference.

The present MIMURA (Neojikun) when compared with the 1997 version, sterilized numbers of 4,680,000 E.coli/ ml in one pass.

A test was conducted in 2006 Feb whereby 0.027 minute in a single pass

Clinical Tests by MIMURA & MAY Co., Ltd., Conducted at its accredited Test Facilities in Thailand in February 2006.

Extract: Anti-Microbial Effectiveness of Bio Magnet

Procedure:

Sample Collection Method:

Sterilised distilled water was passed through a simulated “bio-compound” sarcophagus for many cycles until none of the micro organisms were found. A certain amount of bacterial inocula (A) were transferred into the cycled water of the simulated circulation system. A sample of 20.0 ml of the bacterial contaminated water samples were collected at the interval times (0.3, 5, 15, 30, 60 and 120 mins.) respectively. The sample collected at 0.027 minutes was counted for 1 pass (cycle).

THE ANTIMICROBIAL EFFECTIVENESS METHOD (MODIFIED):

Each 1.0 ml of the sample collection was diluted by the serial dilution method with 9.0 ml TSB. Then pipette 1.0 ml of each dilution into a Petri dish. 20.0 ml of moltened TSA (Tryptic Soy Agar) were poured into the Petri dish. When the medium solidified and incubated at 37°C for 24 hours.

Calculation: -

Percentage of bacterial reduction = $(A-B)/A \times 100$

A = initial amount of the micro organisms

B = amount of micro organism at the interval of times

Results: -

The microbial contamination pre-test of the sterile distilled water when cycled into the simulated “Bio Crystal Compound Resin bonded” system 2 times were shown in Table 1. Anti microbial Effectiveness of the “Bio Crystal Compound Resin bonded” against *E. coli* ATCC 25922 and the temperature of the bacterial contaminated water monitored at 3 sites (T₁, T₂, T₃) by interval time (mins.) were tabulated and shown here below in Table 1

Table 1

The Microbial Amount detected in sterile distilled water (CFU/ml)	
1st Pass	2nd Pass
0	0