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Preliminary Study of Treatment of Spent Test Tubes Used for Blood Tests by Acidic Electrolyzed Water

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1. Introduction

Test tubes are widely used in medical facilities, for example, for collecting blood specimens of patients undergoing health checkups. Plastic-made and disposable tubes are increasingly replacing glass-made tubes, owing to the fact that they are convenient and hygienic. Because of the increase in the population of senior citizens in Japan and the increase in people's interest in their health, the amount of used test tubes will be much higher in the future. In Japan, recycling of medical waste is not a common practice, but there has been some research on medical waste management (Kagawa et al., 2006; Tamiya, 2004, Yamaguchi et al., 2002). Recycling of medical waste is gaining increasing popularity abroad, and it continues to attract the attention of researchers (Kushida, 2000; Bohlmann et al., 2005; Lee et al., 2002; Bartholomew et al., 2002). Test tubes used for blood tests are mostly made from polyethylene terephthalate (PET). In 2005, the total domestic demand for PET resin was 544,500 tons (Editorial Office of Monthly the Waste, 2006). Materials made of PET can be sold at a high price in the market; consequently, recycling industries in Japan are finding it increasingly difficult to source used PET materials. China in particular has a high demand and pays a good price for PET materials: Japan exported 338,000 tons of PET to China in 2009 (The Council for PET Bottle Recycling, 2010).
Incineration has been the main treatment method for PET tubes; however, social consensus against dioxins discourages incineration. Heating treatment followed by direct disposal is another option for treating the tubes, but this option is not reliable since complete inactivation of pathogens in the tubes by heating treatment is not guaranteed. Besides, the heating treatment has another problem. Unlike the incineration treatment, heating leaves blood in the tubes after the treatment. The blood that remains in the tubes drips from the tubes during direct disposal process, which has ethical non-acceptance and implications even though pathogens in the blood would be completely killed.

Acidic electrolyzed water has been used in various fields, such as agriculture, dentistry, food industry, livestock industry, and medicine, for the purpose of disinfection. Used blood testing tubes could be safe if they are treated with acidic electrolyzed water properly, which could introduce new ways of recycling. Tubes treated with acidic electrolyzed can be recycled. For example, the treated tubes can be used as feed stock for alternative energy source and waste heat recovery technologies; they can also used for recycling cloth. However, the main purpose of the complete disinfection of blood testing tubes is the reduction of hospital management cost. In Japan, since the disposal cost of infectious waste by a third party waste management company is approximately five times higher than that of non-infectious or general waste (Tanaka, 2007), hospitals could save significant management cost if they could achieve complete disinfection of blood testing tubes before disposal.
The purpose of this study is to investigate the total annual generation of the used test tubes used for blood tests and the possibility of treating the tubes by acidic electrolyzed water to reduce hospital management cost and to promote material recycling. The effective and proper treatment of the spent tubes by acidic electrolyzed water was also studied. This is the first report on the application of acidic electrolyzed water to the treatment of test tubes used for blood tests and on the recycling of the disinfected tubes.

2. Proposal of a treatment process for used test tubes used for blood tests

Fig. 1 shows the treatment process for used test tubes used for blood tests. The process consists two steps: the pretreatment and the disinfection processes.

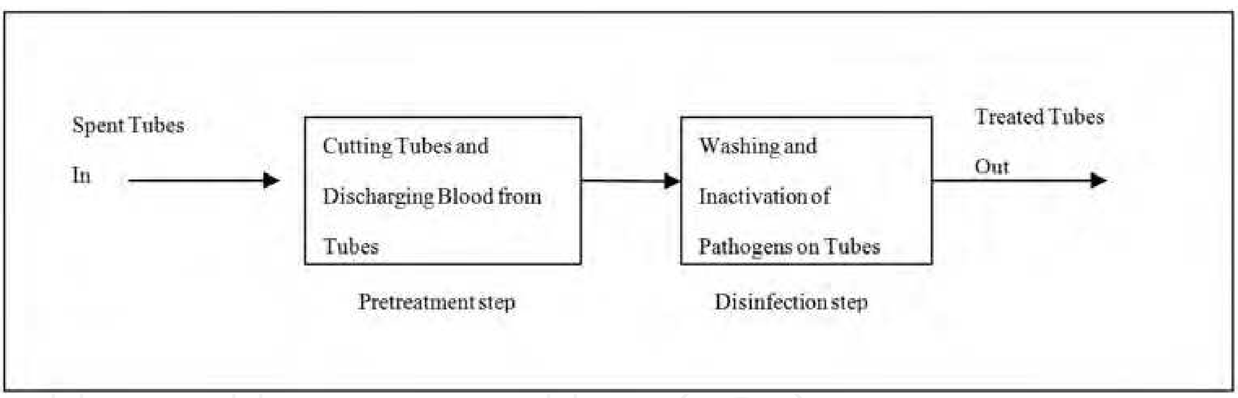


Fig. 1. Proposed treatment system for spent test tubes used for blood tests

The tubes are cut into the most appropriate shape, and the blood in the tubes is discharged during the pretreatment step. The cut tubes are sent to the disinfection step and are washed by acidic electrolyzed water. The ultimate goal is to complete the process in one box and to let the tubes fed to the process come out automatically after complete disinfection.

3. Materials and methods

3.1 Questionnaire survey for the annual generation of test tubes used for blood tests in Japan

The annual production of disposed test tubes used for blood tests was 800 million tubes in 2003, and all of these were consumed domestically (Muranaka, 2005). Then, when the relationship of “production = generation” was valid, the annual generation can be easily estimated. To confirm the relationship, flows of test tubes used for blood tests in hospitals were investigated by sending questionnaires to 80 hospitals nationwide through the postal service; these hospitals had large bed numbers and were randomly selected. Questions and information needed in the questionnaire were as follows. 1. Is the following relation on test tubes for blood tests "purchase numbers = disposal numbers" valid in your hospital? (Does your hospital store or keep test tubes for blood tests for a long period of time for the purpose of such as sample storage?) 2. What are the reasons if the answer in question 1 is "no"? 3. What is the annual number of purchased test tubes used for blood tests in your hospital? 4. Name of your hospital. 5. Number of beds. 6. Address of your hospital., 7. Name.

3.2 Test tubes for blood tests

Ten ml Venoject II vacuum test tube for blood tests for blood coagulation promotion (15.6 × 100 mm, TERUMO Corporation) was used for the experiments. The tube was made from PET. A coagulation promotion sheet in a tube was removed before the experiments.

3.3 Acidic electrolyzed water (AEWater)

AEWater was produced by the Hoshizaki electrolyzed water generator (ROX-10WA, Hoshizaki Electric Company, Ltd., Japan). The electronic current and voltage for the generator were set at 1.5 A and 100 V (single-current phase), respectively.

3.3 Washing apparatus

Toshiba AW-422V5 (TOSHIBA Corporation, Japan), a commercially and widely available home washing machine, was used to wash the tubes. The electric current and voltage were 3.3 A and 100 V, respectively; the maximum volume of the washing machine was 45 liters. Since the washing machine started with laminar flow mixing when the operation started with the ON/OFF switch button, the washing machine started at stand-by mode in order to obtain turbulent flow mixing at the beginning of the wash. The water level chosen for the experiments was 24 liters, or half of the volume of the washing drum.

3.4 Indicator microorganism

Strain *Escherichia coli* ATCC10798 K-12 was used as an indicator microbe for disinfection. *E. coli* K-12 was cultured in 100 ml LB broth at 30°C with an agitation of 120 rpm. After two rounds of 24-hour precultivation, a culture of *E. coli* K-12 was used for the experiments. Plating count of *E. coli* K-12 was done using deoxycholate agar (Oxoid, United Kingdom).

3.5 Marker

Tomato ketchup (KAGOME, Japan, hereafter called "artificial marker") was used as a marker to evaluate the efficacy of washing. The ketchup (1,000–10,000 cP) was selected on the basis of the following criteria: color, economical value, high accessibility, constant quality, and high viscosity than blood (approximately 4.6 cP). The evaluation of washing efficacy was done through visual observation for HACEP Mate (wiping type simple culture medium kit) assay.

3.6 *E. Coli* assay

HACEP Mate for detecting *E. coli* and total coliform bacteria (F&S Research Center, Japan) was used for the disinfection assay. This kit is widely used for checking hygienic safety of food and in the kitchen. Knives or cutting boards were wiped carefully and thoroughly with cotton swab, and the swab was submerged in prepared agar for incubation. After 24 hours of incubation at 35°C, the survival of *E. coli* K-12 was evaluated, and the color of the agar turned to yellow from red when it reacted with *E. coli* or the coliform. The color stayed red if *E. coli* or the coliform was inactivated. The sensitivity of HACEP Mate was as low as 1 CFU/ml.

For a submerged assay, deoxycholate agar (Oxoid, United Kingdom) was used. After the test tubes were treated with AEWater, they were placed in a Petri dish, and then deoxycholate agar was poured on the tubes until the tubes were submerged. The Petri dish was incubated at 37°C and observed after 24 and 48 hours.

3.7 Experiment on investigation disinfection capacity of AEWater

The disinfection capacity of AEWater against *E. coli* K-12 was studied. Five, 10, 15, and 20 ml of *E. coli* K-12 (5.6×10^7 CFU/ml) were separately transferred into 200 ml of AEWater, and they were mixed on a magnetic stirrer with mild stirring level for 15 and 30 seconds. After mixing for the a particular period of time, HACEP Mate was used for detecting the survival of *E. coli* K-12. The effective chlorine concentration was measured before and after the experiments with chlorine test paper, 10–50 ppm (Advantec, Japan).

3.8 Experiments for finding the best cutting type and most effective washing condition

A 1.2 g of the artificial marker was placed into each test tube and was uniformly spread on the inside wall of the tubes by a touch mixer (MT-31, Yamato Japan). Then, the tubes were left for 1 hour under room temperature. Afterward, the tubes were cut by a fret saw BANDSAW K-100 (HOZAN, Japan) into the following three types: half pipe cut, half length cut, and bottom edge cut. The cut types were shown in Fig. 2. The tubes were washed with tap water (24 liters and 15°C), and the best cutting type was decided based on the least amount of the marker left on the tubes, which was done by visual observation.

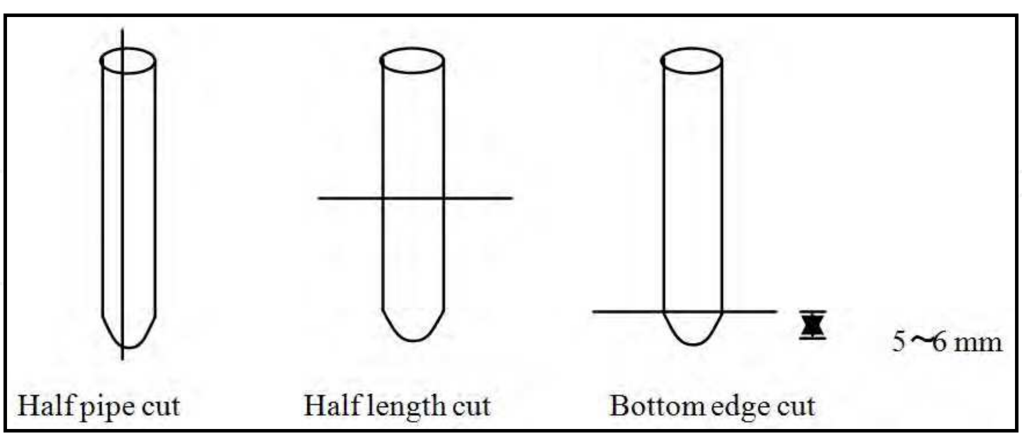


Fig. 2. Three cutting types

After the best cutting type was known, the optimal washing condition was studied. The same experimental procedure as the previous one for deciding the best cutting type was applied for finding the optimal condition. Under the optimal conditions found in the previous experiment, the disinfection test of *E. coli* K-12 was carried out. A 100 ml of *E. coli* K-12 was put in 10 liters of LB broth, and the test tubes used for blood tests, which were already cut according to the best cutting type, were placed in the broth. The broth was heated at 35°C by a ribbon heater Flexible Heater FHU-8 (ADVANTEC, Japan) controlled by a portable temperature controller TC-1N (ADVANTEC, Japan) and stirred at 120 rpm on Hyper Starter HPS-200 (AS ONE, Japan) for 24 hours. After 24 hours, the parts of the tubes were transferred into 24 liters of AEWater for washing. After washing under the optimal condition, the *E. coli* assay was carried out at parts of the tubes using HACEP Mate, as described in the previous *E. coli* Assay section.

3.9 Experiment for investigating dead spots on tubes against disinfection by AEWater

A 100 ml of *E. coli* K-12 was put into 10 liters of LB broth, and then the test tubes used for blood tests, which were already cut in several parts (upper part and bottom part) were put into the broth. The broth was heated at 35°C by a ribbon heater Flexible Heater FHU-8 (ADVANTEC, Japan) controlled by a portable temperature controller TC-1N (ADVANTEC, Japan) and stirred at 120 rpm on Hyper Starter HPS-200 (AS ONE, Japan) for 24 hours.

Test number	Test number of tubes	Cutting type	Cut condition	Treatment time (min)
1	5	Top edge cut	Cut litter remained With aluminum cap	5
2	100			
3	24		Cut litter removed With aluminum cap	
4	5		Cut litter removed Without aluminum cap	
5	4	Bottom edge cut		

Table 1. Test conditions

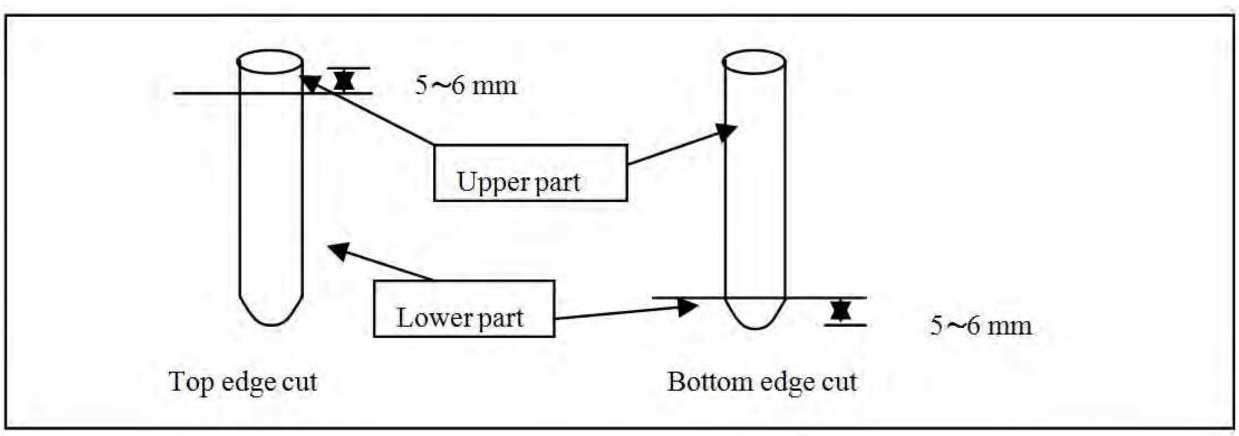


Fig. 3. Tube cutting and cutting parts

After 24 hours, the parts of the tubes were transferred into 24 liters of AEWater for washing. After washing, those parts were placed into Petri dishes for the assay to be submerged, which is described in the previous *E. coli* Assay section. The test conditions are shown in Table 1. The cutting types of a tube and the cut parts for this experiment are described in Fig. 3 and Photos 1 and 2. The conditions of cut litter that remained and was removed are shown in Photo 3.

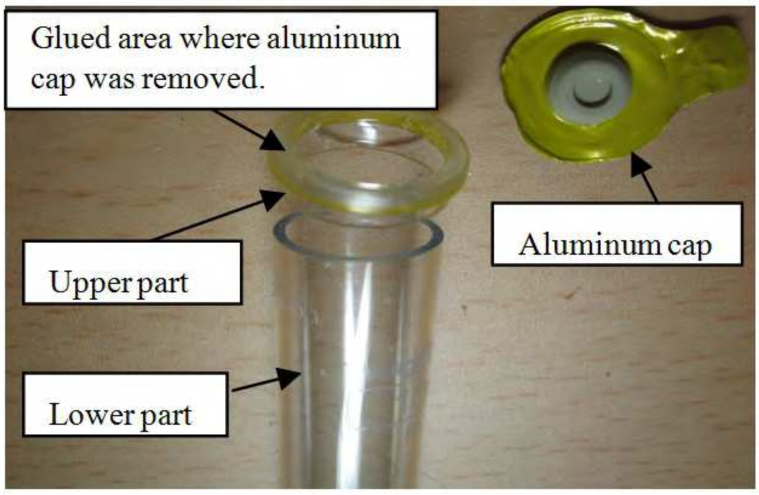


Photo 1. Top edge cut

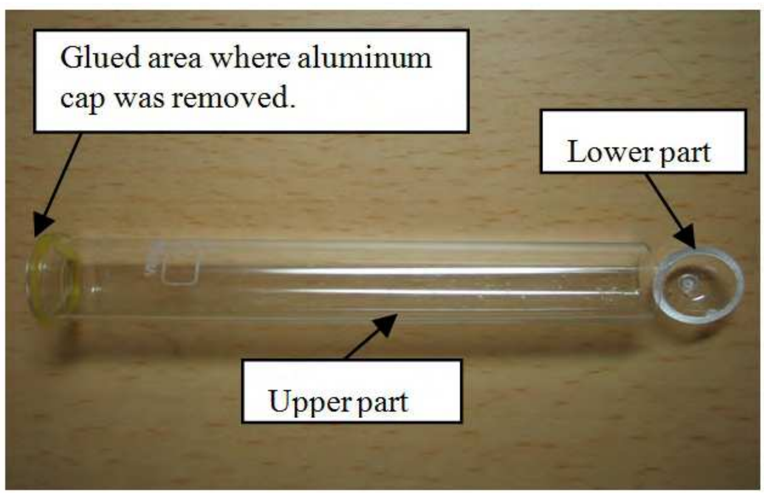


Photo 2. Bottom edge cut

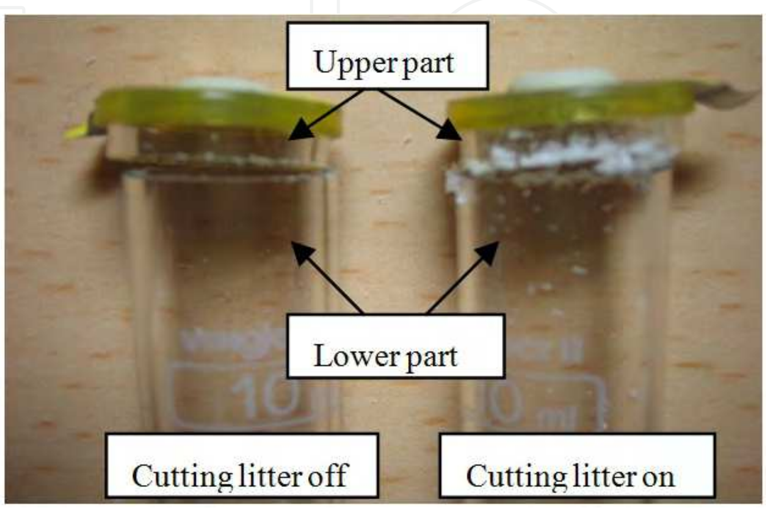


Photo 3. Tubes with cutting litter off and on

4. Results and discussions

4.1 Results of questionnaire survey for the annual generation of test tubes used for blood tests in Japan

Twenty-eight hospitals out of 80 responded to the survey questionnaires (collection percentage of about 35%). The results were summarized in Table 2. To avoid the specification of hospital names, the locations of the hospitals were stated through the prefecture level and bed numbers were expressed as more than or less than 700 beds. Most of the hospitals gave exact numbers for their test tube purchase; however, the numbers were expressed by only the third digit. According to the results, 24 of 28 hospitals answered that the relationship "purchase = disposal" on test tubes used for blood tests was valid (86%). Three hospitals answered in the negative with regard to the relationship "purchase = disposal," and the answer of "unknown" was obtained from one hospital. As Table 2 shows, the flow of disposal test tubes used for blood tests was very smooth from purchase to disposal in hospitals, and the tubes were disposed within a period of one month including sample storage. Hospital ID Nos. 18, 19, and 20 answered "not valid" to the relationship "purchase = disposal." At Hospital ID No.18, blood tests were not conducted in the hospital but in other organizations; that is why the relation was not valid. At Hospital ID No.19, the relationship was not valid because it found a large number of storage in wards, and a large number of test tubes used for blood tests purchased for tests became unnecessary due to cancellation of the tests for some reason. This hospital showed the relationship "purchase ≄ sample number," and the sample number tallied 95% of the purchase number, which was approximately 850,000 sample tubes. Hospital ID No. 20 had always some stock of the tubes in case of emergency, and that is the reason why "purchase = disposal" was not balanced. At Hospital ID No.16, which answered "unknown" to the relationship "purchase = disposal," spent test tubes were disposed mixed and along with other infectious medical wastes; therefore, the disposed number of spent test tubes was unknown. Observing the purchase number of test tubes used for blood tests, a wide range of 17,000–880,000 on purchase number can be noticed.

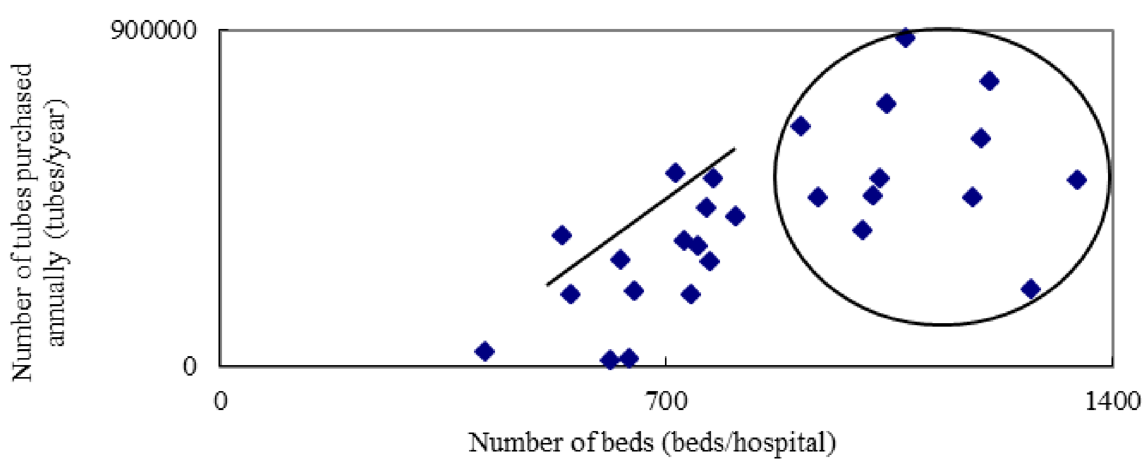


Fig. 4. Number of disposable test tubes used for blood tests purchased annually as a function of number of beds in the hospital

Fig. 4 shows the relationship between bed number and annual purchase number of test tubes used for blood tests. Avoiding the exact number of beds, the scale in Fig. 4 was made very roughly on purpose.

ID No.	Location	Quantity of beds	Quantity of Tubes Purchased per Year	"Quantity Purchased = Quantity Disposed" What can be said?	Remarks
1	Hokkaido	< 700	190,000	Yes	Stays for 1 month in Biochemistry and Immune serum Division. Stays for 2 days in Blood Test Division.
2	Iwate	> 700	335,000	Yes	
3		> 700	700,000	Yes	There are some stocks, but consumption and disposal are smoothly taken place in a short period.
4	Miyagi	< 700	40,000	Yes	No long stay in the hospital. There is a time lag from purchase to consumption.
5		> 700	200,000	Yes	
6	Saitama	< 700	22,000	Yes	
7	Kanagawa	> 700	765,000	Yes	
8	Shizuoka	< 700	350,000	Yes	In case of a long storage, transfer the samples to special storage tubes.
9		-	244,000	Yes	
10	Niigata	> 700	280,000	Yes	Consumption and disposal are smoothly taken place within 20 days.
11		> 700	323,000	Yes	
12	Toyama	> 700	400,000	Yes	
13	Ishikawa	> 700	453,000	Yes	Consumption and disposal are smoothly taken place within 1 week. Dispose the tubes as infectious waste.
14		< 700	200,000	Yes	
15	Fukui	< 700	290,000	Yes	No long stay in the hospital. Dispose the tubes as industrial waste after autoclave treatment.
16	Aichi	> 700	500,000	No answer	Since the tubes are disposed with other infectious waste; the quantity of the tubes disposed is unknown.
17		> 700	190,000	Yes	
18	Shiga	< 700	17,000	No	Since a part of blood analysis is ordered from outside affiliations, a number of the tubes disposed are different from those purchased. Some samples are stored for 1 year.
19	Osaka	> 700	880,000	No	"Quantity purchased = Quantity disposed" is not correct but "Quantity sampled = Quantity

					disposed" is, because some tubes purchased are forgotten and left in a ward and blood sampling was sometimes suddenly canceled due to unexpected events. Quantity of sample was 840,000.
20		> 700	364,000	No	The tubes were stocked for emergency use. Stays in freezers for 2 weeks.
21		> 700	456,000	Yes	
22	Hyogo	> 700	640,000	Yes	Some are stored but do not stay for long in the hospital.
23		> 700	610,000	Yes	
24	Okayama	> 700	450,000	Yes	
25		> 700	520,000	Yes	Stays for 1 week in the hospital
26	Hiroshima	> 700	500,000	Yes	Serum and plasma are separated and stored in special tubes. "Quantity purchased = Quantity disposed" is not correct for small hospitals that do not have basic analyzing equipments because they ask blood testing from outside testing affiliations.
27		> 700	425,000	Yes	
28	Fukuoka	> 700	500,000	Yes	

Table 2. Summary of questionnaires on management of blood sampling tubes

The purchase number of the tubes increased as the number of beds increased until some level. With regard to the data in the circle, there was no relationship between the purchase number and bed number. According to the results, it cannot say that the hospital with large number of beds always purchased a large number of disposal test tubes used for blood tests, and the purchase number of the tubes totally depended on the hospital condition.

Hospital ID No.17 used extra number of test tubes used for blood tests so that the extra number of the tubes should be also included in the calculation of the balance of "purchase = disposal." Moreover, Hospital ID No.19 proposed that the sample number, not purchase number, should be counted in order to know the disposal number of the tubes. Taking those comments into account, the trend seen from 28 hospital results implied that it would be acceptable even if the relationship "purchase = disposal" on test tubes used for blood tests was concluded as valid for the estimation of annual disposal tubes. Hence, the annual generation number of spent disposal test tubes used for blood tests was 800 million tubes in 2003.

A 10 ml Venoject II vacuum test tube for blood tests for blood coagulation promotion (15.6 × 100 mm, TERUMO Corporation) is 6.8 g. Suppose 800 million tubes estimated above were all 10 ml Venoject II vacuum test tube for blood tests for blood coagulation promotion (15.6 × 100 mm, TERUMO Corporation), 5,440 tons of PET resin was disposed annually. Since the annual generation of infectious medical wastes was estimated as 290,000 tons (Tanaka, 2007), the annual generation number of spent disposal test tubes used for blood tests amounted to 2% (probably more than 3%, including specimens). Regarding treatment cost, suppose the weight of a test tube used for blood test with blood is approximately 12 g (blood density of 1.0), then the total weight of the tubes becomes 9,600 tons, resulting from the multiplication of 5,440 by 12/6.8. The 9,600 tons was multiplied with 160,000 yen/ton (Tanaka, 2007) of treatment cost by third party waste management companies for infectious medical wastes, and the total treatment cost of the tubes that hospitals have to pay to is 1,540 million yen. In case that the disposal was made after the complete disinfection treatment, changing the condition from infectious medical waste to general medical waste, the total cost treatment cost of the tubes becomes 290–670 million yen, a half to one-fifth reduction of the cost, since it is 30,000–70,000 yen/ton (Tanaka, 2007) of treatment cost by third party waste management companies for general medical wastes. The treatment cost estimation of used test tubes used for blood tests at each hospital is shown in Table 3. The estimation was done by assuming that the weight of a used test tube used for blood tests with blood was 12 g, and the treatment cost by third party waste management companies for infectious medical wastes was 161 yen/kg (Tanaka, 2007). According to the table, the minimum treatment cost was 32,844 yen and the maximum was 1.7 million yen at Hospital ID Nos.18 and 19, respectively.

At Hospital ID No.19, it can be said that the treatment capacity of a treatment system should be 2,500 tubes/day at least if the system for treating spent test tubes used for blood tests was developed according to Table 3. In Fig. 5, the relationship between daily treatment capacity of used test tubes used for blood tests and the production cost for making a used tube disinfection treatment system. The production cost was calculated by a fixed rate method (annual depreciation = (actual cost – remaining price) / duration period), the while annual treatment cost of spent tubes is equal to depreciation and the duration period of the machine's lifetime is 10 years.

For instance, in the case of Hospital ID No.19, an estimated price of a spent tube disinfection treatment system would be around 19 million yen since the current annual treatment cost for spent tubes was 1.7 million yen. In case that the treated used tubes went for material recycling under an assumption of complete disinfection of used tubes, the selling revenue would be that as shown in Table 3, assuming 140 yen/PET resin kg. From Fig. 5 and Table 3, simply excluding running and maintenance cost, the treatment of used tubes at each hospital by purchasing the machine reduces the annual treatment cost of spent tubes and produces new revenue by selling treated tubes. Kagawa et al. (2006) reported that increase in the use of disposal goods in hospitals greatly contributed to increase in infectious medical wastes at hospitals. It is also already commonly known that plastics compose most of the medical waste in hospitals (Lee et al., 2002; Yamaguchi et al., 2002). It is obvious that the disposal of plastic medical goods will increase further in the future and that the treatment cost of these goods would become a tremendous burden to hospital management. Test tubes used for blood tests, unlike other medical goods, have an advantage over those treatments because those tubes have a very low possibility to be mixed with other medical waste during disposal; these are handled through a special room called a central analysis room. It can be said that changing infectious waste to being non-infectious and selling non-infectious wastes as resources reduce the economical burden of hospital management. Hospitals with low generation of used test tubes used for blood tests should cooperate with other hospitals for the treatment in order to reduce the treatment cost of its medical wastes.

ID No.	Estimated number of tubes disposed annually (tubes/year)	Estimated number of tubes disposed monthly (tubes/m)	Estimated number of tubes disposed daily (tubes/d)	Annual disposal weight (kg)	Annual treatment cost (yen)	Annual revenue by selling (yen)
1	190,000	15,833	528	2,280	367,080	319,200
2	335,000	27,917	931	4,020	647,220	562,800
3	700,000	58,333	1,944	8,400	1,352,400	1,176,000
4	40,000	3,333	111	480	77,280	67,200
5	200,000	16,667	556	2,400	386,400	336,000
6	22,000	1,833	61	264	42,504	36,960
7	765,000	63,750	2,125	9,180	1,477,980	1,285,200
8	350,000	29,167	972	4,200	676,200	588,000
9	244,000	20,333	678	2,928	471,408	409,920
10	280,000	23,333	778	3,360	540,960	470,400
11	323,000	26,917	897	3,876	624,036	542,640
12	400,000	33,333	1,111	4,800	772,800	672,000
13	453,000	37,750	1,258	5,436	875,196	761,040
14	200,000	16,667	556	2,400	386,400	336,000
15	290,000	24,167	806	3,480	560,280	487,200
16	500,000	41,667	1,389	6,000	966,000	840,000
17	190,000	15,833	528	2,280	367,080	319,200
18	17,000	1,417	47	204	32,844	28,560
19	880,000	73,333	2,444	10,560	1,700,160	1,478,400
20	364,000	30,333	1,011	4,368	703,248	611,520
21	456,000	38,000	1,267	5,472	880,992	766,080
22	640,000	53,333	1,778	7,680	1,236,480	1,075,200
23	610,000	50,833	1,694	7,320	1,178,520	1,024,800
24	450,000	37,500	1,250	5,400	869,400	756,000
25	520,000	43,333	1,444	6,240	1,004,640	873,600
26	500,000	41,667	1,389	6,000	966,000	840,000
27	425,000	35,417	1,181	5,100	821,100	714,000
28	500,000	41,667	1,389	6,000	966,000	840,000

Table 3. Estimation of annual treatment cost and revenue on spent test tubes used for blood tests

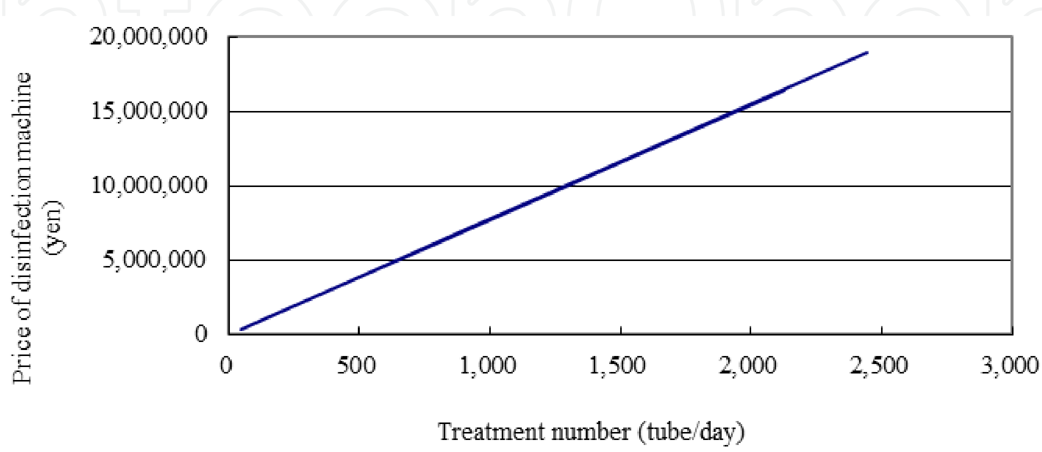


Fig. 5. Price of a used tube disinfection system as a function of treatment capacity of tubes

4.2 Results of experiment on investigating the disinfection capacity of AEWater

Results of disinfection capacity of AEWater are shown in Table 4. Despite the difference in mixing time, the results showed the same trend. A 5 ml or 2.8 × 10^8 CFU of *E. coli* K-12 was inactivated in 200 ml of AEWater in 15 and 30 second mixing times. Any cases that were more than 10 ml or 5.6 × 10^8 CFU of *E. coli* K-12 did not show disinfection capacity of AEWater. According to the results, it can be said that it requires more than 35 ppm of effective chlorine concentration to reach complete disinfection of *E. coli* K-12.

Mixing Time (sec.)	*E. coli* (ml)	Effective Chlorine Conc. before (ppm)	Effective Chlorine Conc. after (ppm)	HACEP Mate Color
15	5	more than 50	35	RED
	10	more than 50	20	YELLOW
	15	more than 50	10	-
	20	more than 50	less than 10	YELLOW
30	5	more than 50	35	RED
	10	more than 50	30	YELLOW
	15	more than 50	20	YELLOW
	20	more than 50	10	YELLOW

Table 4. Change in population of *E. coli* K-12 and disinfection capacity. Note: RED means no detection of *E. coli* K-12, YELLOW means detection of *E. coli* K-12, " -" means experimental error.

Suppose that the thickness of *E. coli* K-12 attached to the inner surface of the blood testing tubes was 0.1 mm. Since the inner surface area of a tube was approximately 41 cm^2, then the volume of *E .coli* K-12 on a tube was 0.41 ml or 2.3 × 10^7 CFU. Considering a 24 liter of AEWater in the washing apparatus, 3.4 × 10^{10} CFU or 600 ml of *E. coli* K-12 could be treated. Hence, it could be estimated that 1460 tubes could be theoretically treated with a 24 liter of AEWater.

4.3 Results of experiments of finding the best cutting type and most effective washing condition

Results of finding the best cutting are shown in Table 5 and Fig. 6. Control (no cut) tubes were completely washed for 300 seconds, but the efficacy became just 2% when washing time was shortened to 120 seconds. All tubes in half pipe cut type was almost washed as the tubes in the bottom edge cut type showed a very good efficacy. Among the cut types, the tubes in half length cut type showed poor efficacy. Photo 4 showed the washing performance on each cut type. For the washing of control tubes, the marker remained mainly at the bottom of the tubes and drew a line from the bottom to the upper sites of the tubes (Photo 4 (a)). The washing performance in Photo 4 (a) indicated that water current did not reach sufficiently the bottom sites of the tubes in 30 seconds and resulted in the marker being left at the bottom sites of the tubes. In Photo 4 (b), the washing performance on half pipe cut type was shown. As seen in the figure, the tubes were completely washed, which indicated that water current reached the entire parts of tubes and removed the marker thoroughly in 30 seconds. The washing performance of the half length cut type showed differences in upper and lower parts (Photo 4 (c)). Almost a complete washing was shown in upper parts of the tubes. It could be said that water flowed sufficiently through the pipes and washed out the marker. In the case of lower parts, like control tubes, the marker was not cleaned and a lot of it was left in the lower parts. The washing performance of the bottom edge cut type was very good and showed almost complete removal of the marker at the upper and bottom parts, like the performance on half pipe cut (Photo 4 (d)). Unlike the performance of the half length cut type, the upper and lower parts in bottom edge cut type were thoroughly cleaned. The lower parts could receive sufficient water flow to remove the marker. According to the results, the washing performance of both of half pipe cut and bottom edge cut types was very good, and none is apparently inferior than the other. Considering the ease of cutting and least time consumption, it can be said that the bottom edge cut was the best cutting type for washing the tubes.

Cut type	Number of tubes	Washing time (sec.)	Washed	Not Washed
Control (no cut)	50	300	50 tubes	0 tube
	50	120	1 tube	49 tubes
Half pipe cut	50	30	98 parts	2 parts
Half length cut	50	30	(upper) 22 parts	28 parts
			(lower) 0 parts	50 parts
			(sum) 22 parts	78 parts
Bottom edge cut	50	30	(upper) 50 parts	0 part
			(lower) 47 parts	3 parts
			(sum) 97 parts	3 parts

Table 5. Efficacy of washing on different cut types

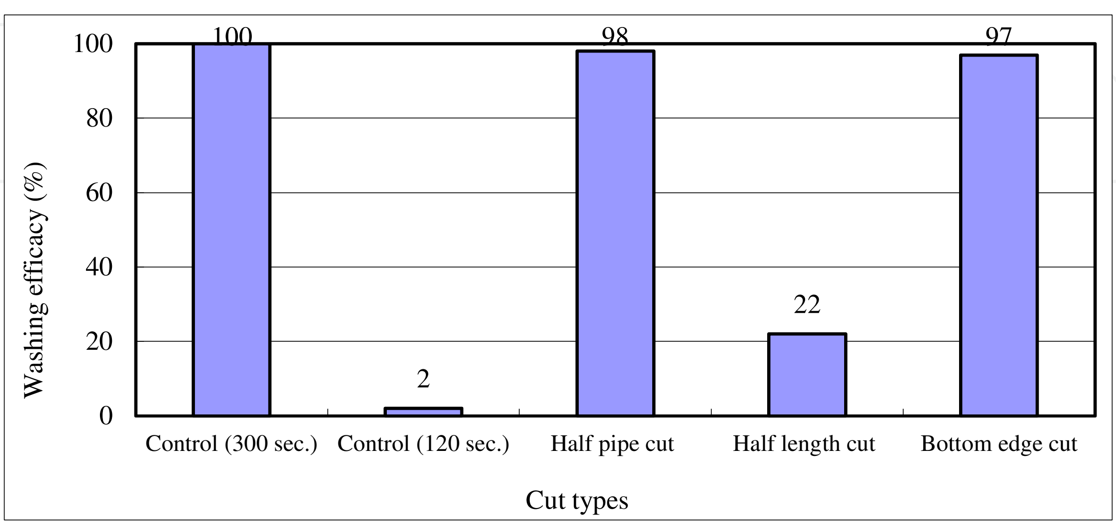


Fig. 6. Washing efficacy of the different cut types

(a) Control (no cut) tubes after 120 second washing

(b) Half pipe cut tubes after 30 second washing

(c) Half length cut tubes after 30 second washing

(d) Bottom edge cut tubes after 30 second washing

Photo 4. Washing performance of each tube cut type. Note:Tube(s) with a full of marker showed tubes before washing.

Number of tubes (tube)	Water Temp. (°C)	Washing time (sec.)	Upper part (part)	Lower part (part)	Total (part)
60	30	15	55/60	57/60	112/120
70	45		70/70	70/70	140/140
80			77/80	80/80	157/160
50	15	30	50/50	47/50	97/100
60			49/60	56/60	105/120
70			14/70	47/70	61/140
70	30		70/70	70/70	140/140
80			40/80	73/80	113/160
80	45		80/80	80/80	160/160
90			90/90	90/90	180/180
100			100/100	100/100	200/200
120			120/120	120/120	240/240
150			150/150	150/150	300/300
170			169/170	170/170	339/340
200			197/200	200/200	397/400

Table 6. Washing conditions and washing results. Note: washed numbers/total numbers.

With the best cutting type, the best condition for washing the tubes was investigated. Although it could be estimated in the previous experiment that 1,460 tubes could be theoretically treated with a 24 liter of water, disinfection efficacy may be significantly different from the estimation when *E. coli* K-12 on the tubes was tried to be disinfected because the tubes became obstacles against the flow of AEWater. Table 6 and Fig. 7 showed the washing conditions and their results. According to the results, water temperature influenced washing efficacy more than washing time did. At 15°C of water temperature and 30 seconds of washing time, washing efficacy on 60 and 70 tubes was 87.5% and 43.6%, respectively, whereas the efficacy was 93.3% on 60 tubes and 100% on 70 tubes for 30 and 45°C of water temperature, respectively, for 15 second washing time. At 70 tubes and 30 second washing time, washing efficacy changed drastically from 43.6% to 100% when water temperature increased from 15 to 30°C. The same trend could be seen when 80 tubes were washed at a 30 second washing time. The efficacy increased from 70.6% to 100% when water temperature increased from 30 to 45°C. The advantage of a longer washing time could be seen on 80 tube washing at 45°C water temperature. Washing efficacy was 98.1% for 15 seconds and that was 100% for 30 seconds. A difference of 15 seconds contributed an increase in efficacy from 98.1% to 100%. At 45°C of water temperature and 30 seconds of washing time, 100% efficacy was shown on up to 150 tubes. In the case of 170 and 200 tubes, the efficacy did not reach 100% and was 99.7% and 99.3%, respectively.

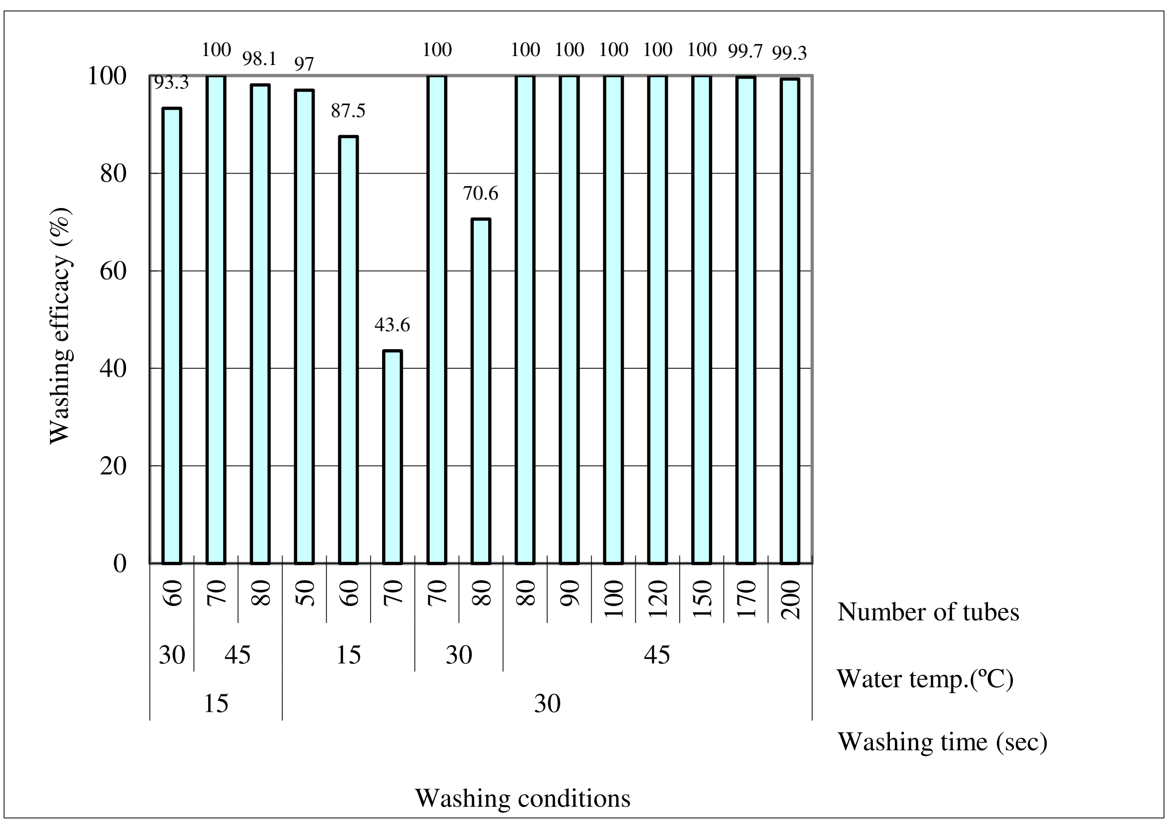


Fig. 7. Washing efficacy of different washing conditions

Number of tubes (tubes)	AEWater temp. (°C)	Washing time (sec.)	Positive part numbers (parts)	Negative part numbers (parts)	Disinfection percentage (%)	*E. coli* positive/negative in AEWater
50	10	30	0	100	100	negative
200	45	30	1	399	99.8	negative

Table 7. Disinfection test on the optimal condition

The disinfection test was performed under optimal conditions, i.e., a water temperature of 45º**C** and a washing time of 30 seconds. Two hundred tubes were used for 24 liters of water in this experiment as it was the maximum number of tubes used in the previous experiment. This experiment was also performed for a water temperature of 10º**C**. This temperature was considered, as 10º**C** is the temperature of tap water; hence, minimum operating cost can be expected using AEWater obtained from tap water without heating, thus conserving the energy supply that would otherwise be unnecessarily used for increasing water temperature. The results are shown in Table 7. Fifty tubes (9.36 $\log_{10}$ CFU) were completely disinfected in 24 liters of AEWater at 10°C. In the case of 200 tubes (9.96 $\log_{10}$ CFU), 1 part of a tube remained positive; therefore, it can be said that 150 tubes could be the safe amount for complete disinfection under the optimal condition. In both cases, the *E. coli* reaction in AEWater was negative.

Venkitanarayanan et al. (1999) reported that *E. coli* 157:H7 on the 100 m^2 area of a plastic cutting board was disinfected from 8.14 $\log_{10}$ CFU to 2.43 $\log_{10}$ CFU and from 8.01 $\log_{10}$ CFU to 0 $\log_{10}$ CFU for 5 and 10 minutes of washing time, respectively, at 45°C of AEWater temperature. The disinfection time in their study was much longer than that of this study: 30 seconds in this study and 5 or 10 minutes in their study. The reason for that could be attributed to the disinfection conditions with agitation or without agitation. Venkitanarayanan et al. (1999) used no agitation during disinfection, whereas this study used agitation during disinfection since a home cloth washing machine was used as a washing apparatus. According to this comparison, the efficacy of disinfection with AEWater dramatically improved when agitation was performed, which agreed with the result of the study conducted by Park et al. (2002).

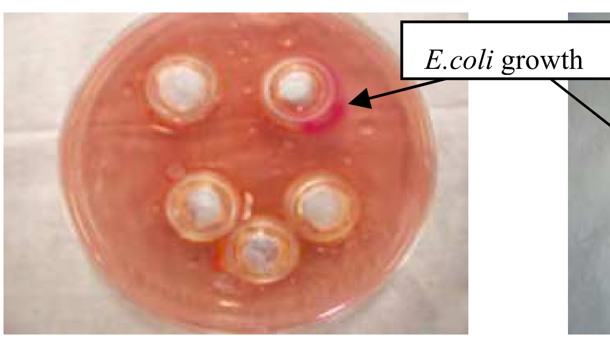
4.4 Results of experiment of investigating dead spots on tubes against disinfection by AEWater

In order to specify dead spots that the disinfectant cannot reach or is difficult to approach on the surface of the test tubes used for blood tests, a submerged tube assay in deoxycholate agar was carried out. Results were shown in Table 8. Comparing among test conditions 1 to 3, it is obvious that the existence of cut litter on tubes influenced efficacy during disinfection. The complex structure of the litter would play a role of a shelter for *E. coli* K-12 and protect *E. coli* K-12 from being exposed by AEWater. As a result, more numbers of positive tubes were seen in test numbers 1 and 2. The growth of *E. coli* K-12 was seen in Photo 5.

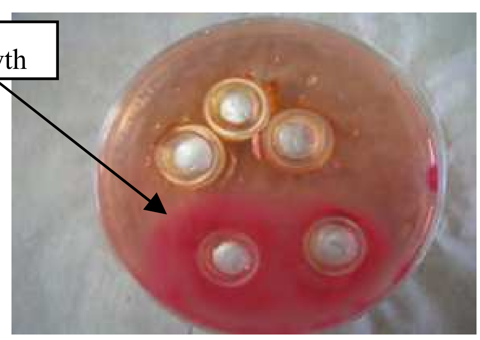
The effect of areas glued where an aluminum cap was attached on the efficacy of disinfection could be seen by comparing test numbers 3 and 4. Three parts over five showed positive for *E. coli* K-12 growth in test number 4. In test 5, a positive sample was also recognized on the area where an aluminum cap was removed after 48 h (Photo 6). As a

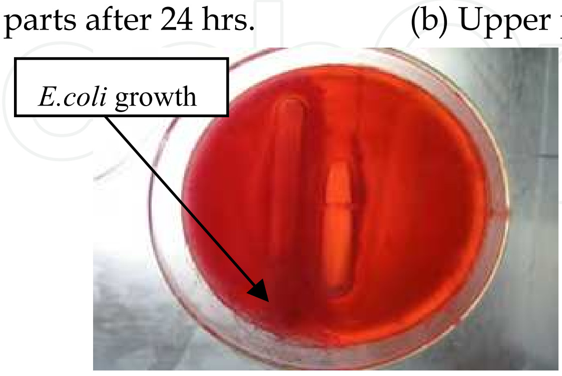
Test number	1		2		3		4			5		
Cut part of a tube (part)	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Aluminum cap removed	Lower	Upper	Aluminum cap removed	Lower
Positive tube numbers after 24 hrs	4	0	2	0	1	0	0	3	0	0	0	0
Positive tube numbers after 48 hrs	4	4	4	2	1	0	0	3	0	4	1	0

Table 8. Results of the assay submerged



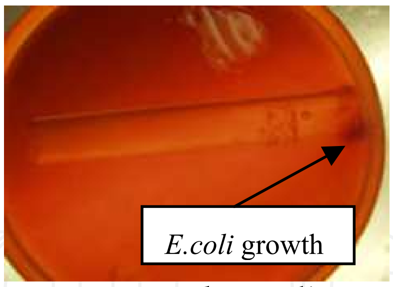
(a)Upper parts after 24 hrs.


(b) Upper parts after 48 hrs.



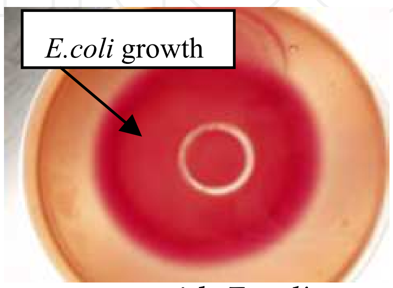
(c) Bottom part after 48 hrs.

Photo 5. Submerged assay for top edge cutting



(a) An upper part with *E. coli* growth.

(b) An upper part completely disinfected.



(c) A bottom part with *E. coli* growth.

(d) A bottom part completely disinfected.

Photo 6. Submerged assay for bottom cutting

conclusion, the existence of cut litter and the area glued where an aluminum cap was removed influenced in a negative way the efficacy of disinfection against *E. coli*. Those findings were very important aspects of developing a used tube disinfection treatment machine. According to Table 8, all lower parts in test conditions 3–5 were negative on *E. coli* K-12 growth. Disinfection washing in this experiment lasted for 5 minutes, which was far longer than the washing time in previous experiments (30 seconds). It can be said that 5 minutes is good enough as disinfection time. This corresponds to the result in Table 5 and the result in the study of Venkitanarayanan et al. (1999).

5. Conclusion

The total number of disposable test tubes used for blood tests was 800 million in 2003. Results of questionnaires reveal that the cost of disposing test tubes used for blood tests became a heavy burden to hospitals. It can also be deduced that hospitals with a large number of beds were always a large generator of used test tubes. The price of a used tube disinfection system would be 19 million yen for hospitals with a daily generation of about 2,500 tubes according to the calculations. A system that turns waste into resources will contribute to hospital health management; therefore, the development of this system is extremely important. The following conclusions are obtained from this experiment.

1. Acidic electrolyzed water can be successfully applied to the disinfection of test tubes used to collect blood samples.
2. The best cut type was the bottom edge cut type.
3. One hundred and fifty tubes were effectively disinfected by acidic electrolyzed water under these conditions: 24 liters of acidic electrolyzed water, 45°C of the water temperature, and 30 seconds of washing time.

4. The existence of cut litter and some special spots such as sticky areas reduced the efficacy of disinfection.

Further research, for example, on the disinfection efficacy for Hepatitis B and C, is absolutely needed for completing disinfection data collection; however, this preliminary study will contribute to the production of a complete system for a spent test tube used for blood tests, which will reduce drastically hospital medical waste management costs.

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This book reports mostly on institutional arrangements under policy and legal issues, composting and vermicomposting of solid waste under processing aspects, electrical and electronic waste under industrial waste category, application of GIS and LCA in waste management, and there are also several research papers relating to GHG emission from dumpsites.

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