

Clinical Trial Endoscope disinfection test with Cleantop® WM-1

Reference data

Endoscope disinfection test (clinical trial Cleantop WM-1)

We examined cleaning and bactericidal effect for endoscope by CLEANTOP WM-1 in the clinical field by performing general bacteria test (aerobic and anaerobic culture) and identification of Helicobacter Pylori (nested-PCR and cultivation method). We also performed HCV test (RT-PCR method) as necessary.

Test organization:

Upper gastrointestinal tract:

Osaka University Medical Department, Internal medicine section1, 2

Tokyo University Medical Department, Internal medicine section 2

Lower gastrointestinal tract

Foundation of Keio Cancer Center

Specimen number:

(upper) 30 x 2, total 60 cases for each organization

(lower) 30 cases

Test method:

Cleaning and disinfection method for endoscope

Performed cleaning and bactericidal activity by using endoscope cleaner / sterilizer WM-1 for seven minutes. We used 5.5 l of acid purified water produced from the electrolysis performed by WM-1 using 10 l of tap water added and mixed with 5 g of NaCl.

Sampling method:

After wiping off outside of endoscope (inserting area) with sterile absorbent gauze immediately after endoscope checkup without hand manual cleaning & brushing and after sterilization. Put the gauze in the sterile container. Injected 40 ml of sterile saline solution by injector in order to cleanse adhesive bacteria in the forceps channel. Add this liquid to the above sterile container and collected bacteria adhered to the forceps channel and outside of inserting area.

1. For general bacteria check, we injected 10 ml of collected solution separately in sterile polypsits as sterile as possible and stored them at low temperature.
2. For Helicobacter Pylori test (nested-PCR method), we injected 10 ml of collected solution separately in sterile polypsits aside from general bacteria test as sterile as possible and stored them at -202 or less.
3. For Helicobacter Pylori test (culture method), we applied collected solution on DENT culture medium using inoculating loop and put it in the Canpy-pouch to seal. After that, transported the specimens to SRL, Inc.

Test items and method:

General bacteria test and identification of Helicobacter Pylori (nested-PCR and cultivation method) were performed. HCV test (RT-PCR method) was also performed as necessary.

1. General bacteria test

Performed 10 times dilution on the collected solution in the method of 2. using sterile saline solution. With regard to the collected solution before sterilizing, we used stock and 10^{-10} times diluent. And for the collected solution after sterilizing, we used stock and 10 to 10^2 times diluent. For upper digestive tract, 0.1 ml of solution was seeded on blood agar to cultivate at 37°C for 48 hours. For lower digestive tract, 0.1 ml of solution was seeded on blood agar for aerobic culture to perform cultivation at 37°C for 48 hours and for anaerobic culture, seeded the solution on Brucella HK blood agar to perform cultivation at 37°C for 72 hours. We measured colony number to compute the total number of cell adhered to endoscope.

We also identified the types of colony concerning separated bacteria before and after sterilization.

2. Helicobacter Pylori

We performed the identification of Helicobacter Pylory on the collected solution with the method shown in 2. by the nested -PCR method. And performed aerobic cultivation (CO₂ 10%, O₂ 5%, NO₂ 85%) on the medium (DENT medium) obtained by the method of 2. at 37°C for 3 to 7 days in order to observe the breeding of bacteria.

3. Measurement of pH and ORP of electrolytic acid water

Generally, bactericidal and virucidal effect is recognized at least under the condition pH 2.7 or less, ORP 1000 mV or more. When blood or body fluid is contacted to this electrolytic acid water, pH and ORP change to the level in which the water shows no effect.

So, WM-1 keep on supplying maintenance electric current in order to maintain defined value of pH and ORP. We examined and measured whether pH and ORP maintain the defined value in the clinical field. We also measured the concentration of residual free chloride produced by the electrolysis.

Test results 1:

Upper digestive tract

Osaka University, Medical Department, Internal medical section 1, 2

Types and number of bacteria (before sterilization)	Before sterilization	After sterilisation	Remarks
Neisseriae spp (20~4 x 10 ³ /ml)	12/30	0/30	
Sreptococcus spp (90~1.5 x 10 ⁴ /ml)	16/30	0/30	
Staphylococcus spp (20~2.3 x 10 ² /ml)	2/30	0/30	
Staphylococcus aureus (40/ml)	1/30	0/30	
Staphylococcus epidermidis (10/ml)	1/30	0/30	
Corynebacterium sp (70~2 x 10 ³ /ml)	3/30	0/30	
Micrococcus spp (1.2 x 10 ² /ml)	1/30	0/30	
Helicobacter pylori	16/30	0/30	nested PCR method

Tokyo University, Medical Department, Internal medical section 2

Types and number of bacteria (before sterilization)	Before sterilization	After sterilisation	Disinfect condition after sterilization
Neisseriae spp (50~2 x 10 ⁵ /ml)	21/30	0/30	
Sreptococcus spp (30~1 x 10 ⁶ /ml)	26/30	2/30	*A 1.3 x 10 ⁴ □20
γ-Sreptococcus (10~5.9 x 10 ³ /ml)	3/30	0/30	*B 2 x 10 ³ □20
Staphylococcus spp (1.1 x 10 ⁴ /ml)	1/30	0/30	
Staphylococcus aureus (20~7 x 10 ³ /ml)	2/30	0/30	
Bacteriodes sp (6 x 10 ² /ml)	1/30	0/30	
Candida spp (1.6 x 10 ² ~1 x 10 ³ /ml)	2/30	0/30	
Enterobacter agglomerans (9 x 10 ³ /ml)	1/30	0/30	
Enterococcus spp (2.4 x 10 ² ~9 x 10 ³ /ml)	2/30	0/30	
Eescherichia coli (2 x 10 ³ /ml)	2/30	0/30	
Klebsiella oxytoca (1.4 x 10 ³ /ml)	1/30	0/30	
Klebsiella pneumoniae (20~9 x 10 ³ /ml)	2/30	1/30	*B 4 x 10 ³ □20
Veillonella sp (2 x 10 ³ /ml)	1/30	0/30	
Helicobacter pylori	16/30	1/30	nested PCR method

Test results 2:

Lower digestive tract

Types and number of bacteria (before sterilization)	Before sterilization	After sterilisation	Disinfect condition after sterilization
Escherichia coli ($20 \sim 4.9 \times 10^6$)	26/30	2/30	^{*B} $5.6 \times 10^5 \rightarrow 20$
Streptococcus agaladiae ($1 \times 10^2 \sim 4 \times 10^4$)	5/30	0/30	^{*C} $1.0 \times 10^4 \rightarrow 10$
Streptococcus spp ($20 \sim 6 \times 10^5$)	20/30	1/30	^{*B} $2.1 \times 10^4 \rightarrow 10$
Enterococcus spp ($3 \times 10^2 \sim 1 \times 10^5$)	10/30	1/30	^{*B} $3.0 \times 10^2 \rightarrow 10$
Enterobacter aurogenes ($80 \sim 1 \times 10^3$)	3/30	0/30	
Enterobacter cloacae ($2 \times 10^2 \sim 3 \times 10^4$)	6/30	0/30	
Enterobacter spp ($2.2 \times 10^2 \sim 2.3 \times 10^4$)	8/30	0/30	
Citrobacter freundii ($10 \sim 5 \times 10^4$)	8/30	0/30	
Citrobacter diversus (3×10^2)	1/30	0/30	
Pseudomonas aeruginosa ($10 \sim 1.1 \times 10^5$)	6/10	0/30	
Pseudomonas sp ($10 \sim 6 \times 10^5$)	6/30	0/30	
Klebsiella oxytoca ($1 \times 10^3 \sim 5 \times 10^5$)	5/30	1/30	^{*C} 5×10^5
Klebsiella pneumoniae ($1 \times 10^2 \sim 8.1 \times 10^4$)	6/30	0/30	$\rightarrow 1.9 \times 10^2$
Klebsiella spp (1×10^3)	1/30	0/30	
Coagulase negative Staphylococcus ($20 \sim 9 \times 10^5$)	5/30	0/30	
Staphylococcus aureus (3×10^2)	1/30	0/30	
Bacilyl subtilis ($70 \sim 1.7 \times 10^4$)	2/30	1/30	^{*A} $1.7 \times 10^4 \rightarrow 10$
Bacilyl spp ($20 \sim 8 \times 10^3$)	4/30	0/30	
Corynebacterium sp (1×10^4)	1/30	0/30	
Group G streptococcus (70)	1/30	0/30	
Mircococcus (50)	1/30	0/30	
Morganella morganii (1×10^3)	1/30	0/30	
Proteusvulgaris mirabilis (10)	1/30	0/30	
Proviencia rettgeri (1×10^2)	1/30	0/30	
Serratia marcescems (1×10^3)	1/30	0/30	
Xanthomonas maltophilia (5×10^3)	1/30	0/30	^{*B} 1.0×10^4
Bacteroides fragilis group ($2.1 \times 10^2 \sim 1.7 \times 10^6$)	22/30	2/30	$\rightarrow 1.3 \times 10^2$
Bacteroides ovatus ($60 \sim 1.6 \times 10^2$)	2/30	0/30	^{*C} 5.6×10^5
Bateroides distasonis (2×10^3)	1/30	0/30	$\rightarrow 6.0 \times 10^2$
Bateroides spp ($10 \sim 1 \times 10^7$)	19/30	1/30	^{*B} $\rightarrow 60$
Eubacterium spp ($1.4 \times 10^4 \sim 1 \times 10^6$)	3/30	0/30	
Bifidobacterium spp ($7 \times 10^3 \sim 9 \times 10^3^*$)	2/30	0/30	
Propionibacterium sp ($3 \times 10^3 \sim 3 \times 10^3$)	2/30	0/30	
Peptostreptococcus asaccharolyticus (1×10^6)	1/30	0/30	
Peptostreptococcus sp (5×10^3)	1/30	0/30	

Conclusion:

- General bacteria (upper digestive tract)
As shown in the table, 43 cases out of 45 (95.6%) where bacteria were detected before sterilization were converted to negative. Bacteria was detected in the other two cases after sterilization. All detected general bacteria was indigenous bacteria in oral cavity. And the level was low as 20 cfu/ml. Reduction rate of bacteria compared with that before sterilization was 1/1000 or less. It is reported that bacteria negative conversion rate of 15 minute immersion with 3% glutaraldehyde agent (Product name: Steriscope, Maruishi Pharmacy) currently used for sterilization of endoscope was 94.8% (Shigeru Okuda, et al. Clinical evaluation of 3% glutaraldehyde agent for endoscope sterilization, Clinical Adult Disease, vol. 22 No.9: 121-128, 1992). From the results mentioned above, it is supposed that sterilization effect of CLEANTOP WM-1 using electrolytic acid water is the same or better than 3% glutaraldehyde agent.
- Helicobacter pylori
With regard to Helicobacter pylori, gene amplification was not recognized in 31 cases out of 32 (96.9%) where bacteria was detected before sterilization. It was recognized in the residing case. Dead bacteria are amplified as well as viable in PCR method. So, it is difficult to decide that was the dead bacteria estimated from disinfect condition of general the possibility that it should be dead bacteria so, sterilization effect for Helicobacter Pylori is supposed to be verified.
- General bacteria (lower digestive tract)
As shown in the table, 27 cases out of 30 (90.0%) where bacteria were detected before sterilization were converted to negative. Bacteria were detected in residing 3 cases. In lower digestive tract, compared with upper digestive tract, types and quantity bacteria are larger. In addition that, bacteria amount detected was as small as 10 to 40 cfu/ml, the measurement limit (from the viewpoint of dilution times). And as the bacteria reduction rate compared with that of before sterilization was 1/1000 or less, the results were judged to be effective.
- In the case multiple sterilization was performed with the same electrolytic acid water, it was supposed that pH and ORP changed and the sterilization effect fell. However, stable sterilization effect up to 10 times of continuous use was verified. We recognized the effect caused by that maintenance electric current keeps pH and ORP in the condition where sterilization is possible.
- Residual chloride concentration decreases gradually from about 4 ppm by every sterilization. It decreases to about 1.5 ppm at tenth sterilization. As chloride gas is poisonous, the less gas exist the safer for patients. However, considering that residual chloride concentration is about 1 ppm in the tap water, it was not so large problem.
- In this time, we cleaned and sterilized the endoscope used in the checkup by CLEANTOP WM-1 without performing any procedure. There were no problem though mucin, blood, xylocaine, etc. adhered to the outside and forceps channel of endoscope. In worse condition, bacteria may be concealed in the mucin which could not be cleansed and not contacted with electrolytic acid

water. Operational procedure must include wiping of outer surface and water flow through forceps channel before sterilization.

- Sterilization between the use of endoscope for patients must be completed in short period of time. This kind of device, which uses tap water for multiple times in batch method, does not have the drudgery to flow water with direct connection to water piping or connection of water supply and can be used in comparatively small space. However, there may be complaints such as fear of water pollution and disability of sterilizing control section. With regard to water pollution, owing to the effect of the filter, we could not recognize the impurities of water after ten times of continuous use. We considered the fact control section cannot be sterilized is dissolved by the caution of operator.
- Electrolytic acid water can be obtained by performing electrolysis on tap water added with a very small quantity of sodium chloride. So, no hazardous agent is used. Though it has strong acidity such as pH 2.7 or less, different from the same level acid such as hydrochloric acid, when it comes into contact with skin or mucosa it will discharge electrons and the acidity is weakened. So, there is little danger.
The electrolytic acid water leaves some to be desired for cleaning and sterilization performed at the end of the day. However, it can be used for sterilization between patients in the ordinal clinical field.

PMS Data Cleantop WM-1

Post Marketing Surveillance Report

No.	The name of hospital	Date of test	Initial names of patient	Spec. of EAW			Disinfection	Side-effect Yes/No	Doctor's Judge
				pH	ORP	Cl			
001	Akashi City Hospital	1998.05.25	KS	-	-	-	Negative	No	Good
002	Akashi City Hospital	1998.05.25	TT	-	-	-	Negative	No	Good
003	Akashi City Hospital	1998.05.25	RN	-	-	-	Negative	No	Good
004	Akashi City Hospital	1998.05.25	HY	-	-	-	Negative	No	Good
005	Akashi City Hospital	1998.05.25	SF	-	-	-	Negative	No	Good
006	Akashi City Hospital	1998.05.25	SS	-	-	-	Negative	No	Good
007	Akashi City Hospital	1998.05.25	MF	-	-	-	Negative	No	Good
008	Akashi City Hospital	1998.05.25	ER	-	-	-	Negative	No	Good
009	Akashi City Hospital	1998.05.25	KM	-	-	-	Negative	No	Good
010	Akashi City Hospital	1998.05.25	YY	-	-	-	Negative	No	Good
011	Akashi City Hospital	1998.05.26	MH	-	-	-	Negative	No	Good
012	Akashi City Hospital	1998.05.26	MK	-	-	-	Negative	No	Good
013	Akashi City Hospital	1998.05.26	TU	-	-	-	Negative	No	Good
014	Akashi City Hospital	1998.05.26	TM	-	-	-	Negative	No	Good
015	Akashi City Hospital	1998.05.26	JT	-	-	-	Negative	No	Good
016	Akashi City Hospital	1998.05.26	HY	-	-	-	Negative	No	Good
017	Akashi City Hospital	1998.05.26	KA	-	-	-	Negative	No	Good
018	Akashi City Hospital	1998.05.26	YN	-	-	-	Negative	No	Good
019	Akashi City Hospital	1998.05.26	YT	-	-	-	Negative	No	Good
020	Akashi City Hospital	1998.05.26	YY	-	-	-	Negative	No	Good
021	Sosei Hospital	1998.06.02	TO	-	-	-	Negative	No	Good
022	Sosei Hospital	1998.06.02	MH	-	-	-	Negative	No	Good
023	Sosei Hospital	1998.06.02	SK	-	-	-	Negative	No	Good
024	Sosei Hospital	1998.06.02	JA	-	-	-	Negative	No	Good
025	Sosei Hospital	1998.06.02	IY	-	-	-	Negative	No	Good
026	Sosei Hospital	1998.06.02	YM	-	-	-	Negative	No	Good
027	Sosei Hospital	1998.06.03	TY	-	-	-	Negative	No	Good
028	Sosei Hospital	1998.06.03	OS	-	-	-	Negative	No	Good
029	Sosei Hospital	1998.06.03	KF	-	-	-	Negative	No	Good
030	Sosei Hospital	1998.06.03	DS	-	-	-	Negative	No	Good

Total number of examinations in this multicenter test were 617. Test results no. 31 - 617 are available on request. Outcome of total test please find in the following remarks:

Remarks:

The mark of "-" means that EAW specification was within specification specified and desinged.

Negative ratio was 99.18%, 5 positive against 612 negative.

Rate of good was 99.5%, 3 fair of 614 good, which doctors judged.

"Good" means very useful and "fair" means fairly useful.

Sampling Method:

After disinfecting by Cleantop, wipe the surface of endoscope with sterile gauze and put it into sterile beaker.

40 ml of sterile saline is flushed through biopsy channel into sterile beaker. Then, 10 ml of sterile saline is collected by sterile tube and the tube is sealed and kept in cool condition. Thin sample collected by tube is forwarded to Testing laboratory for culture.

Action taken:

As 4 of 5 positive cases were found at the same hospital, we searched the cause and found the possibility of insufficient hand manual pre-cleaning. Then, we advised them to care about pre-cleaning by hand manual. In 2000, we conducted again PMS at the same hospital and no positiven case was found