Seminar at OIST; 2017.10.12

Hypochlorous Acid (HOCl) Water (HAW)
A new disinfectant
次亜塩素酸水：電解製殺菌料

Kunimoto HOTTA, Ph.D.　堀田国元

Functional Water Foundation & National Inst. of Infectious Diseases
一般財団法人機能水研究振興財団理事長・元国立感染症研究所生物活性物質部室長

<Hotta's Research Targets>

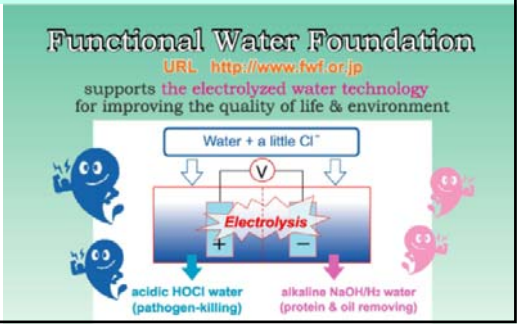
Antibiotic-producing actinomycetes

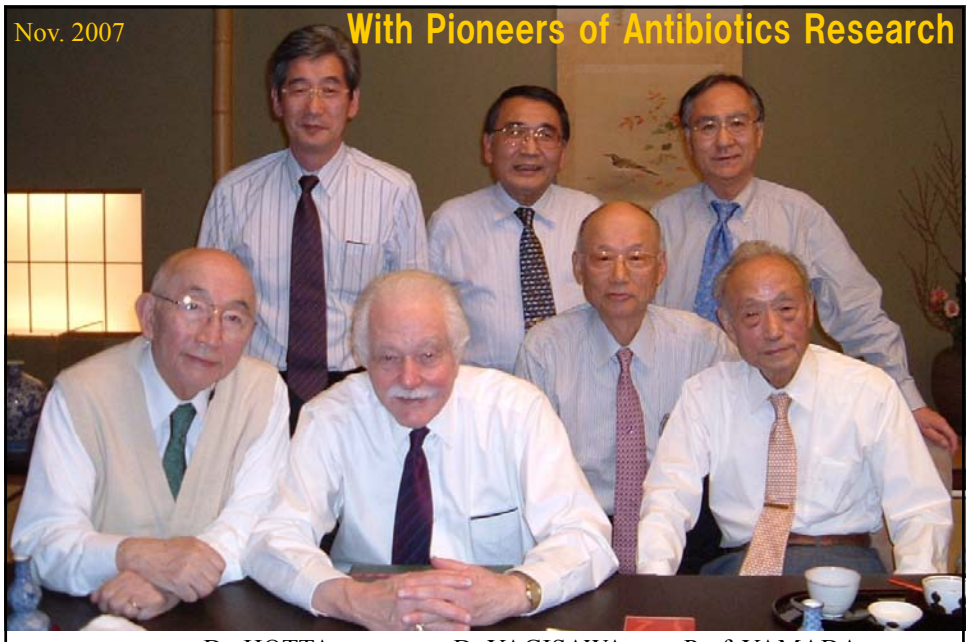
⇩

Antibiotic-resistant bacteria (MRSA)
Anti-MRSA antibiotics

⇩

Acidic electrolyzed water
(Hypochlorous Acid Water)





Dr. HOTTA　Dr.YAGISAWA　Prof. YAMADA

Dr.OKAMI　Prof. A.L. DEMAIN　Prof. OMURA　Dr. KINOSHITA

CONTENTS of MY TALK

- The HAW is an acid aqueous solution which is generated by electrolyzing water containing chloride ion (Cl^-) such as NaCl or HCl.
- It shows strong and broad microbicidal activity with remarkably low toxicity that should be valuable for research activities, especially for biological, medical and life science.

Requirements for disinfectants

What is functional water? and how to make HAW?

Anti-microbial activity of HAW

Mechanism (factors) of anti-microbial activity of HAW

Difference between HAW and NaOCl solution

Biosafety of HAW

Reliability of HAW: Governmental approval, JIS, guidelines

Application of HAW: Hand-washing, Endoscope-reprocessing

Requirements for Disinfectants in Hygienic Management

Disinfection, Sanitation etc. at Hospitals, Food Services, BSL etc.

Good and Broad Activity against

pathogenic bacteria/virus
no emergence of resistance

Good Bio-Safety (friendly to)

not only to human (regardless healthy & sick),
but also to animals, plants & environment

Easy to handle

Acidic Electrolyzed Water
(Hypochlorous acid water =HAW)

What is HAW?

A kind of functional water which is defined as follows.

Aqueous solutions that acquired useful & reproducible functions by science-based artificial treatment.

Electrolyzed water or 'Denkaisui'

HOCl water & Ozonated water

killing activity against microbes including viruses

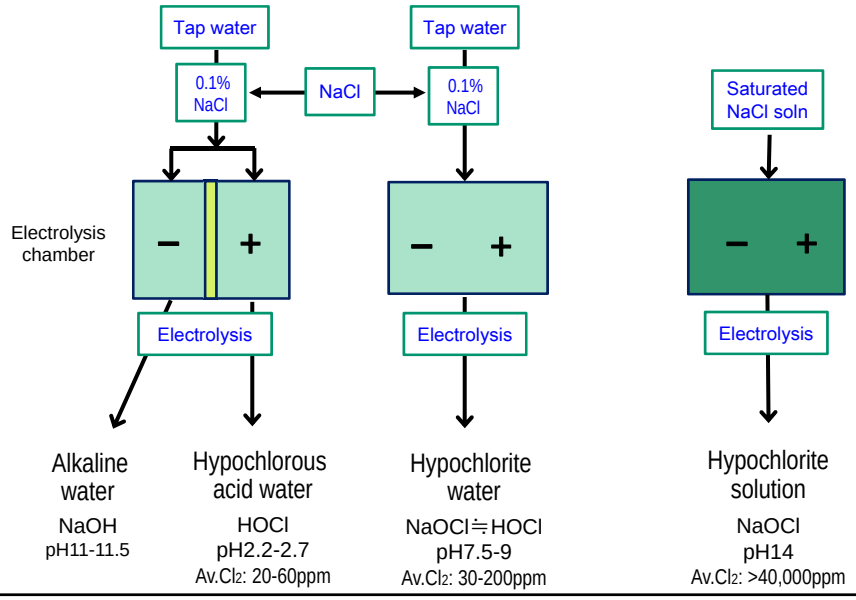
Strongly alkaline electrolyzed water: pH>10.5

capability of removing oily & organic substances

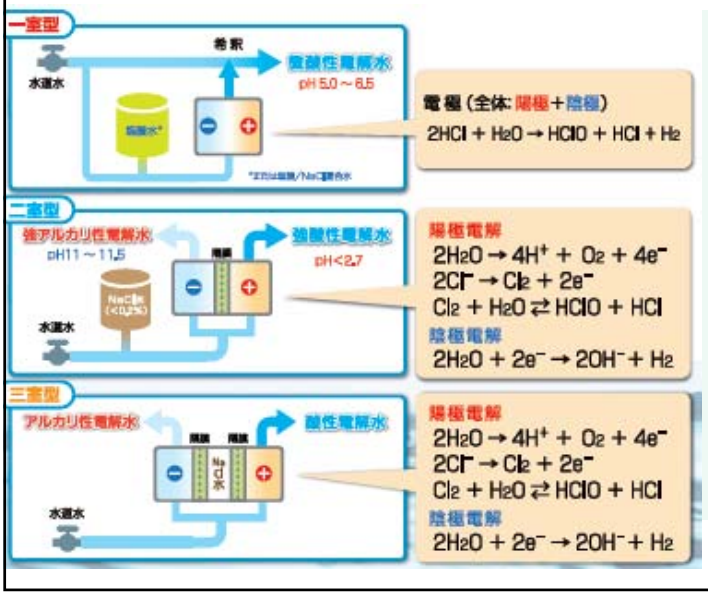
Potable alkaline ionized water: pH9-10

capability of improving gastro-intestine conditions

Production system for HOCl water, NaOCl water and NaOCl solution



Electrolytic Reactions in the three types of electrolyzers that produce HAW



Properties

Slightly acidic HAW
pH5.0-6.5
10-80ppm

Weakly acidic HAW
pH3.0-5.0
10-60ppm

Strongly acidic HAW
pH2.2-2.7
20-60 ppm

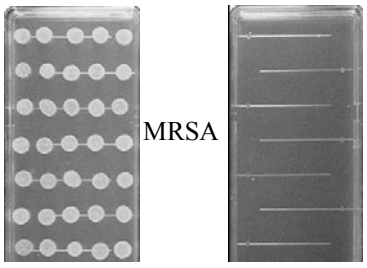
Bacteria-killing activity of HAW

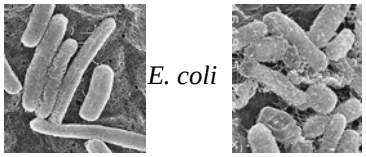
10^6cfu cells in 0.02 ml were mixed with 0.1ml of HOCl water

Staphylococcus aureus (MSSA)	<10 sec.
S. aureus (MRSA)	〃
S. epidermidis	〃
Enterococcus faecalis	〃
Escherichia coli	〃
Klebsiella pneumoniae	〃
Pseudomonas aeruginosa	〃
Serratia marcescens	〃
Vibrio parahaemolyticus	〃
Candida alibicans	<30 sec.
Cryptococcus neoformans	< 1 min.
Mycobacterium avium	< 1 min.
M. tuberculosus	<2.5 min.
Bacillus cereus	< 5 min.
B. subtilis	〃
Virus including norovirus etc.	< 10 sec.

Acidic water (pH2.5-2.7)

Not treated **Treated(10sec)**





Inactivation of Prion

- Prof. N. Nishida and his colleagues at Nagasaki University School of Medicine demonstrated the inactivation of prion adhered to metal surface by washing with strongly alkaline electrolyzed water under sonication followed by washing with acidic electrolyzed water as reported in the following journals.

T. Mori et al.: A direct assessment of human prion adhered to steel wire using realtime quaking-induced conversion.

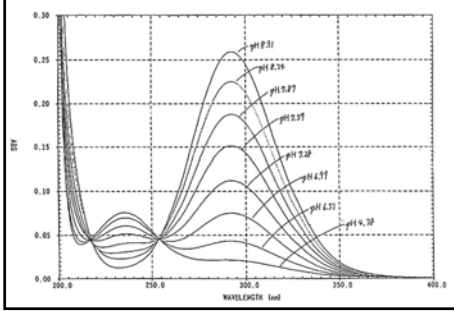
SCIENTIFIC REPORTS/6:24993/DOI:10.1038/srep24993. Apr.26,2016 www.nature.com/scientificreports

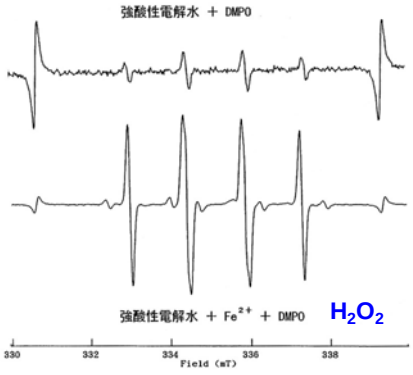
Y. Nakano et al.: Sequential washing with electrolyzed alkaline and acidic water effectively removes pathogens from metal surface.

PLOS ONE/DOI: 10.1371/journal.pone.0156058 May25,2016.

Evidence for the formation in HAW of HOCl ・OH and H_2O_2

1. Formation of hypochlorous acid at anode $Cl_2 + H_2O \rightleftharpoons HOCl + HCl$
2. Detection of hypochlorous acid by ion chromatography
3. Spectrophotometric spectrum identical with hypochlorous acid

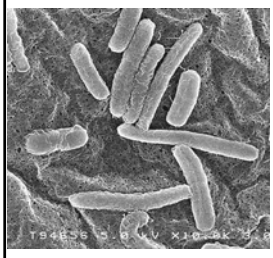




・OH and H_2O_2 may cause the following.

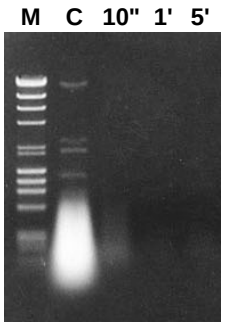
Degradation or damage of DNA, Protein, Amino acids

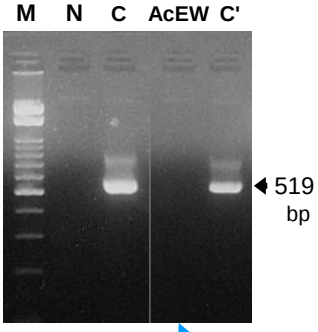
Damages of cell surface and DNA by acid HOCl water

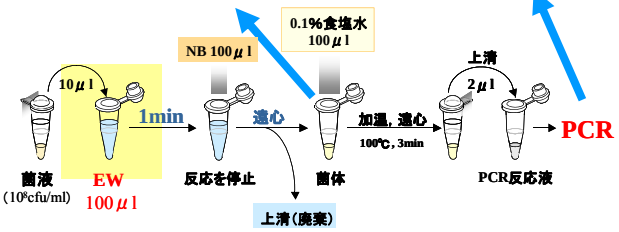


AcEW treat

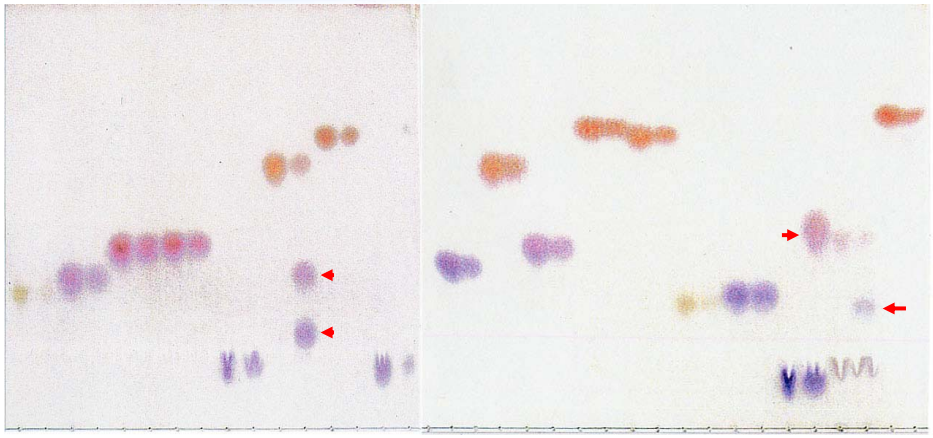
AcEW treat







Action of acidic HOCl water on amino acids



Pro Gly Ala Glu Arg Met Phe His Pro Val Thr Leu Ile Asn Gln Lys Cys Trp

2mM A.A. 10μl + HOCl water 40μl → 10', rt → TLC → Ninhydrin reaction

1 Met
2 AcEW
3 Met Sulfoxide
4 Met Sulfone
5 Mixture

Methionine

Met Sulfone

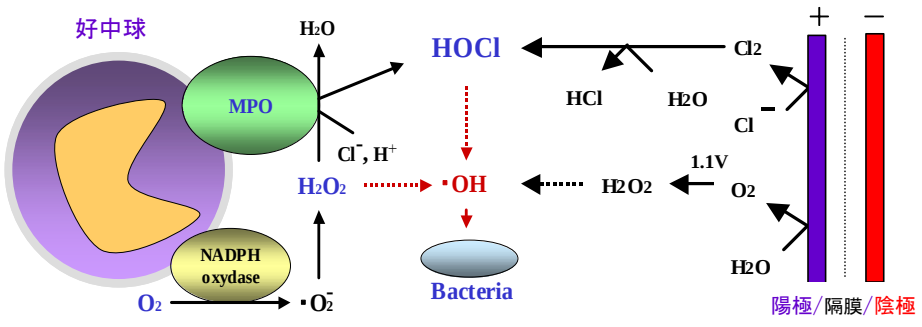
Met Sulfoxide

$CH_3S(CH_2)_3CHNH_2COOH$
Methionine

$CH_3S(CH_2)_3CHNH_2COOH$
O
Met Sulfoxide

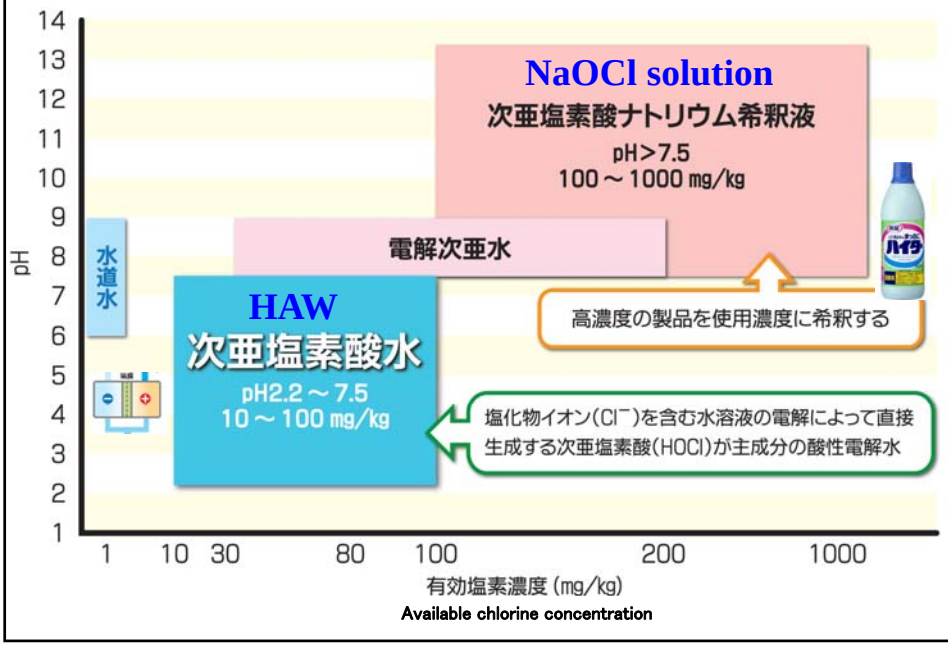
$CH_3S(CH_2)_3CHNH_2COOH$
O
O
Met Sulfone

Oxidation of Met by HAW



Similarity between neutrophile (left) and HOCl water (right) in terms of elements for bactericidal mechanism

Difference between HAW and NaOCl solution

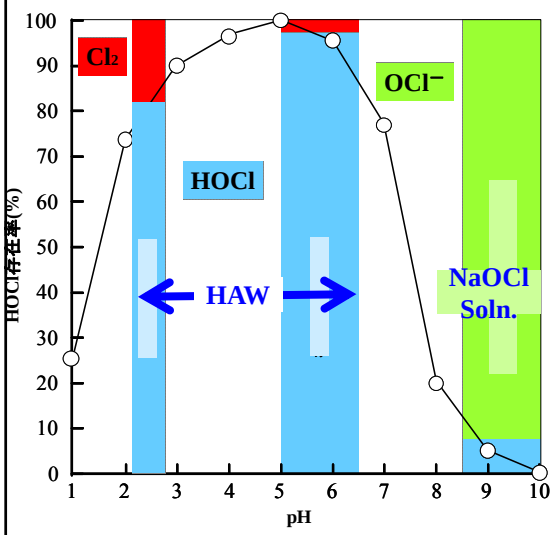


Anti-microbial activities of HAW and NaClO solution

10^6cfu cells in 0.02 ml were mixed with 0.1ml of HAW or NaClO soln.

Pathogenic bacteria & virus	HOCl water (40ppm)	NaClO solution. (1,000ppm)
Staphylococcus aureus 黄色ブドウ球菌	◎(＜10 秒)	◎(＜10 秒)
MRSA (メチシリン耐性黄色ブドウ球菌; 多剤耐性)	◎	◎
Escherichia coli 0-157 H7 (腸管出血性大腸菌)	◎	◎
Pseudomonas aeruginosa (緑膿菌)	◎	◎
Salmonella Enteritidis (サルモネラ菌)	◎	◎
Vibrio paraphaemolyticus (腸炎ビブリオ菌)	◎	◎
Other Gram-negative bacteria (他のグラム陰性菌)	◎	◎
Bacillus cereus (セレウス菌)	△(3～5min.)	△(3～5min.)
Mycobacterium tuberculosis (結核菌)	△(～2.5min.)	▲(～30min.)
Norovirus ノロウイルス(ネコカリシウイルス)	◎	○
Herpes virus ヘルペスウイルス	◎	◎
Influenza virus インフルエンザウイルス	◎	◎

pH dependent transition of HOCl ratio



Activity: HOCl > OCl⁻
80 : 1

HOCl ⇒ ·OH + ·Cl
$(OCl)^- + 2H^+ + 2e^- \Rightarrow Cl^- + H_2O$

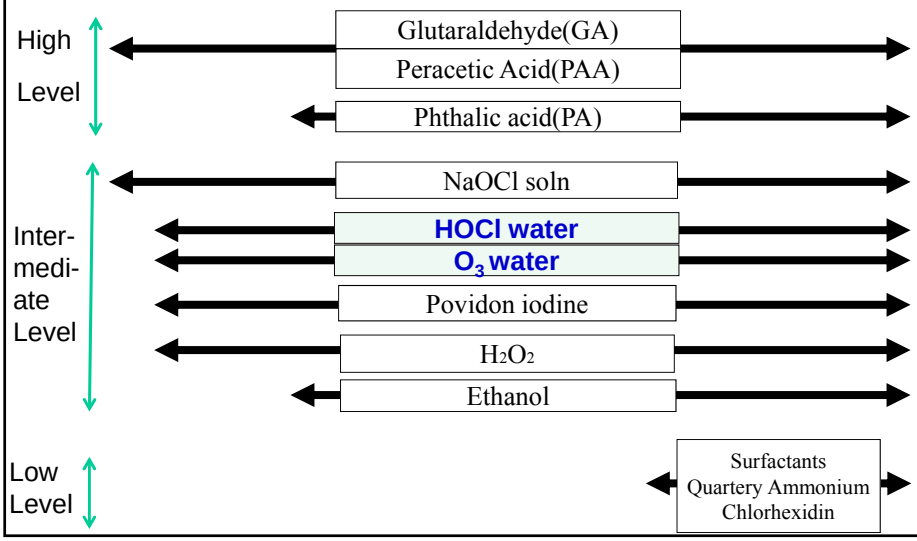
Due to radical formation, HOCl shows high bactericidal activity

Because of high levels of HOCl content compared to NaOCl soln., HAW shows markedly high bactericidal activity.

However, if organic material is contaminated, serious reduction of activity occurs, since HOCl (available chlorine) concentration of HAW is very low

Anti-microbial Activity of Disinfectants

Endospore > Tubercul . Bac. Virus > Fungi > Bacteria Yeast



Features of disinfectants

	HOCl w.	NaOCl soln.	EtOH	PVI
Available concn.	10-80ppm	≧40,000ppm	75-80%	7-10%
Concn. for use	10-80ppm	100～10,000ppm	同上	250-500ppm
How to use	washing	immersion	spray/rubbing	smearing
Target*1) : Food m.	○	○	×	×
: Metal	△	△	○	×
: non-metal	○	○	○	×
: environ.	○	○	○	×
: skin	○	×	○	○
: mucosa	○	×	×	○
Anti-microbial	broad	broad	broad	semi-broad*2)
Anti-viral*3)	broad	broad	semi-broad*4)	broad
influenza	◎	◎	◎	◎
noro	○	◎	▽	◎

*1)○no problem, △some problem（rust）、×problematic. *2)not effective to bacteria with endospores
*3)virus-inactivating activity：◎markedly effective, ○effective,▽weakly effective
*4) viruses without envelope like noro-virus shows resistance.

Safety of Acidic HOCl water

General toxicity tests at GLP level:
- **Acute & sub-acute toxicities**
- **Irritation of skin & mucous membrane (eye, oral cavity, gullet & stomach)**

Mutagenicity
Damage to hand skin

⇨ **Results indicated good bio-safety**

Trihalomethane (THM: $CHCl_3$ etc.) formation: Little
Emergence of resistant bacteria: No
Influence on environment: Very weak if any

Safe or friendly to human, animal and environment
Little harmful to human skin : Body washable
Accidental drinking results in no harmful effect

Use of HAW

1. Use for skin:

   Low level disinfection: Chlorhexidine

   Intermediate level disinfection: EtOH, (rubbing) PVI, (smear) **HAW** (washing)

2. Use for instruments & environment:

   Intermediate disinfection: NaOCl solution, **HAW** etc.

   High level disinfection: Glutaraldehyde

3. Facility space

   Regular working space: Plasma, Air-purifier, UV, **HAW** etc.

   Clean room: HCHO, O_3, H_2O_2, CH_3COOOH

Requirements for Reliability

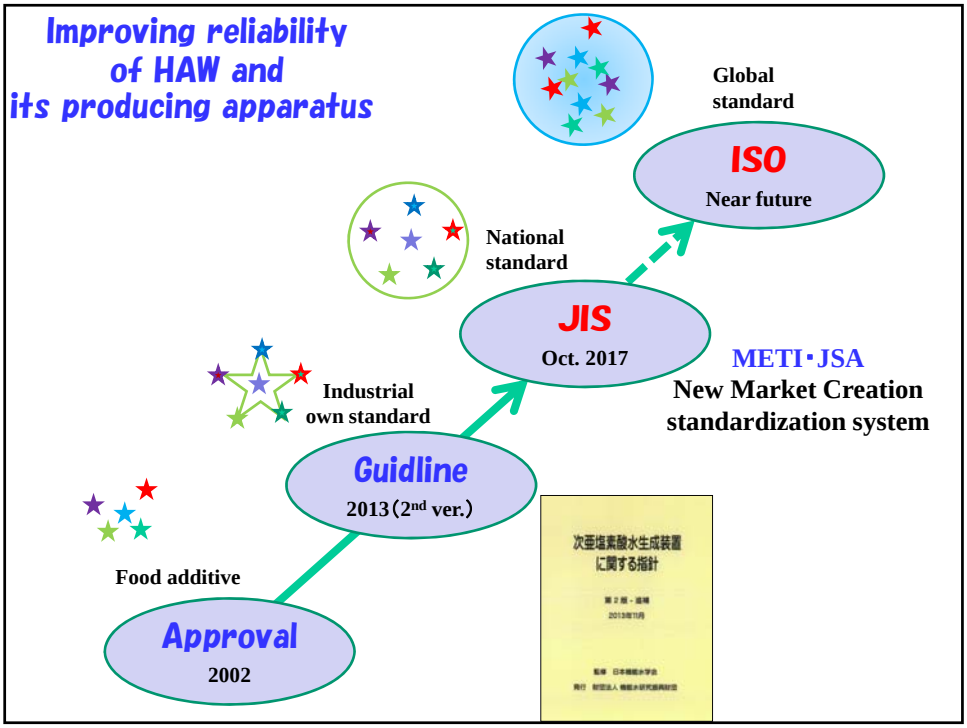
- **Apparatus**
  - Scientific principle for production of functional water
  - Specified standard of apparatus
  - Specified standard of functional water to produce
  - Evaluation by the third public organization
- **Functional water**
  - Reproducible scientific data on function
  - Scientific basis for function
  - Monitoring method for function
  - Evaluation by the third public organization

Current governmental approval of electrolyzed water in Japan

Current approval		Electrolyzed water (Denkaisui) Strongly acidic	Weakly acidic	Slightly acidic
	pH:	2.2-2.7	2.7-5.0	5.0-6.5
	Av.Cl2:	20-60ppm	10-60ppm	10-80ppm
Medical instrument*				
Hand-washing (pre-operation)		Yes(1996)	No	No
Endoscope reprocessing (washing & disinfection)		Yes(1997-)	No	No
Food additives** (HOCl water)		Yes(2002)	Yes(2012)	Yes(2002)

* Only strongly acidic electrolyzed water-producing apparatus was approved and no water itself has been approved.
Drug, Cosmetics & Medical Instrument Act（薬事法）

** Water itself was approved in the name of hypochlorous acid (HOCl) water. Therefore various types of apparatus can be used.
Food Sanitation Law （食品衛生法）



Merits of HAW

1. High and broad bactericidal activity at low concentration
   ⇒ active against viruses such as norovirus tolerant to EtOH
2. High biosafety: approved as a food additive
   ⇒ can be used like tap water
3. Reliability of apparatus: JIS
4. Easy to produce
   ①Safe and easy production by persons w/o professional knowledge
   ②Capable of continuous production like a tap water flow
5. Economical
   ①Low running cost: ＜1yen/L
   ②Water cut: Remarkable reduction of water for rinsing
   ③Easy for wasting

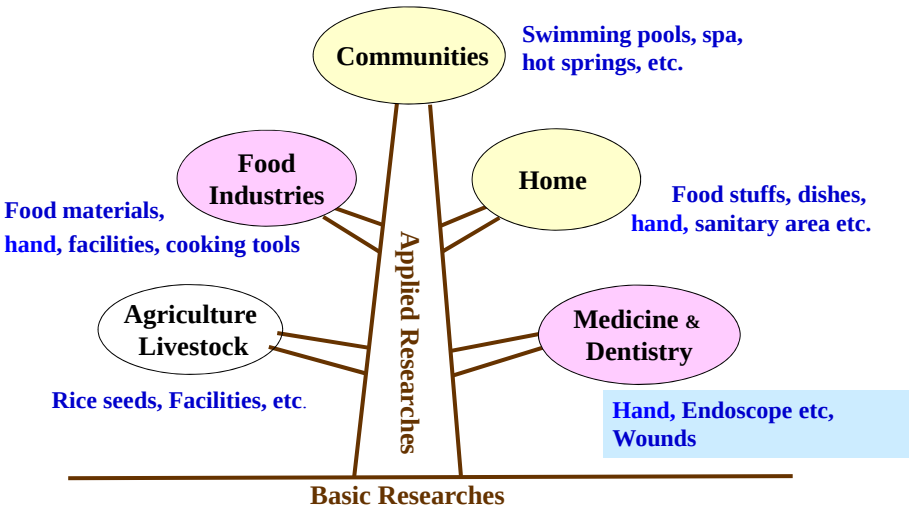
Risk of HAW

1. Chlorine gas formation
   1）Use in a small and closed room without ventilation
   2）Metal corrosion
   ①Metal quality: No problem with SUS304 or higher stainless steel
   ②Weak point: welding part

2. Easy loss of activity:
   1) Continual check of available chlorine concentration is needed
   → Use after confirmed the appropriate range of available chlorine

Apparatus and Technologies of Acidic Denkaisui were born and developed in Japan and contribute to various fields

Disinfection & Sanitation



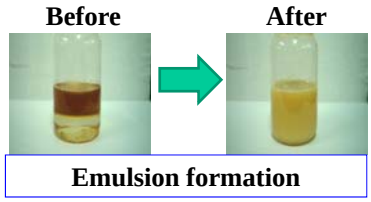
Effective use of hypochlorous acid water

In the presence of organic materials such as proteinous and oily materials, HOCl reacts quickly with them, resulting in the critical loss of bactericidal activity.

Removal of contaminated organic materials before using HOCl water is necessary for the effective disinfection.

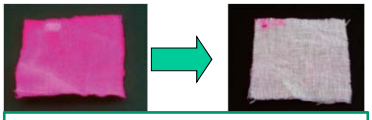
The use of strongly alkaline Denkaisui prior to the use of HOCl water turned out to be good for disinfection.

Alkaline water (pH11-11.5)

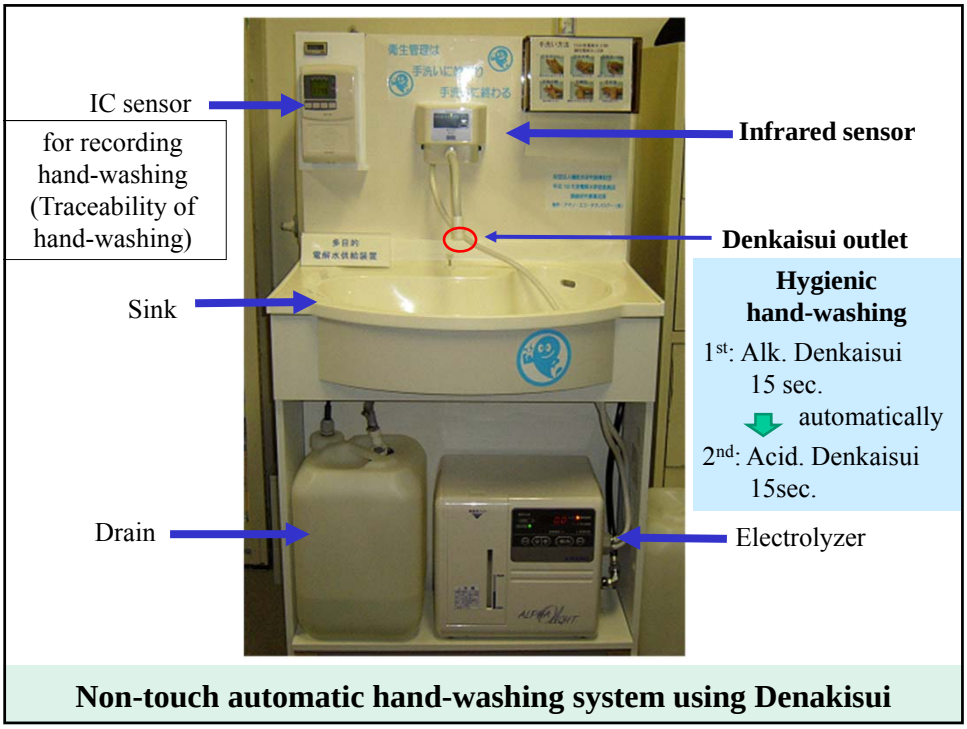


Emulsion formation

Removal of mayonnaise

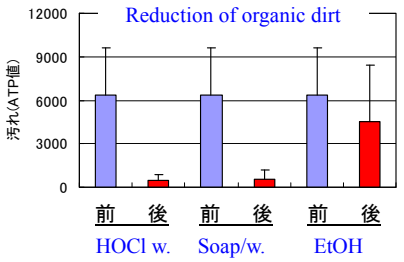


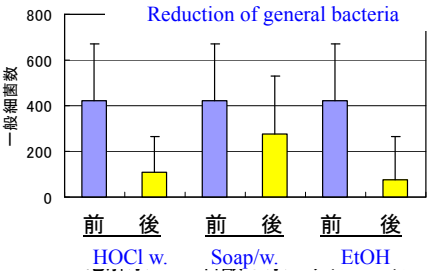
Removal of blood-like protein



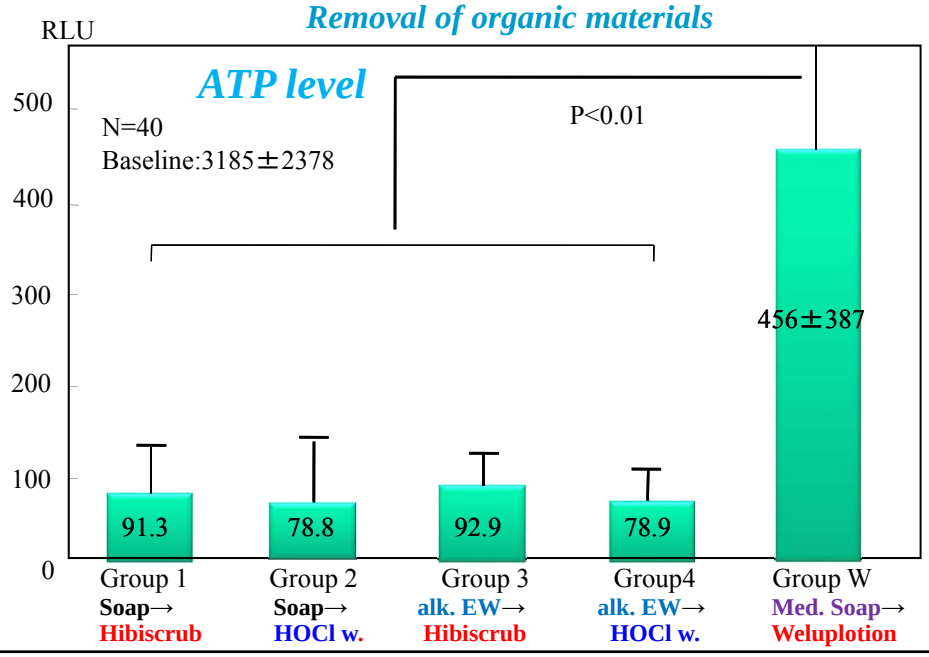
Non-touch automatic hand-washing system using Denakisui

Hygienic handwashing with Denkaisui



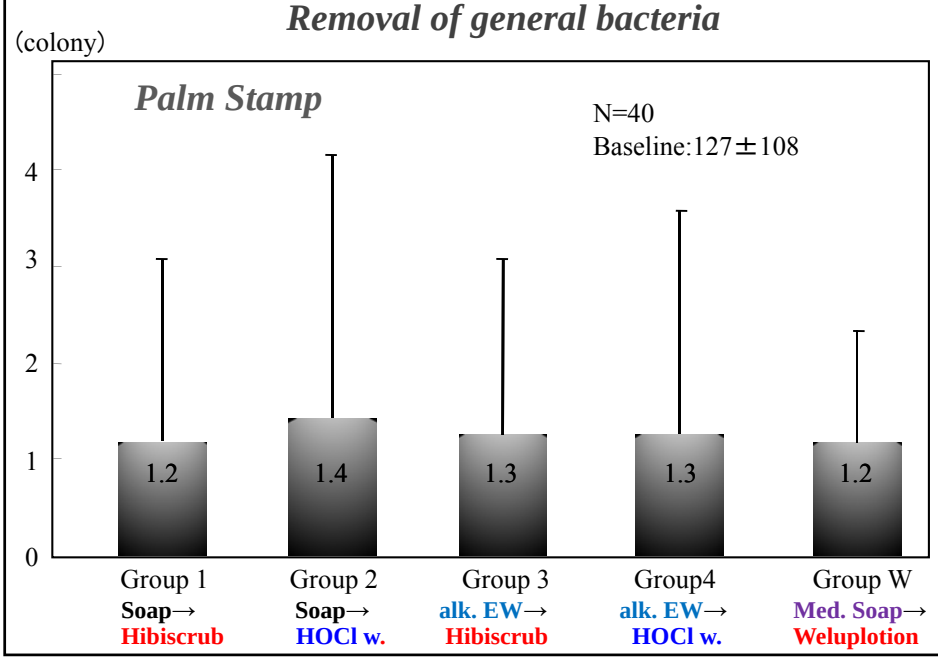


Surgical Handwashing with Denkaisui



Surgical Handwashing with Denkaisui

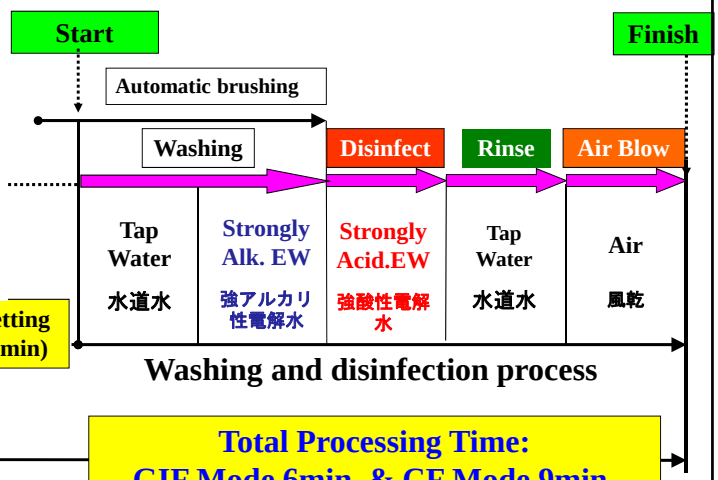
Removal of general bacteria



Full-automatic endoscope reprocessing apparatus

Kagami-Naishi
KOM-ED-01
(Koken Ltd.)

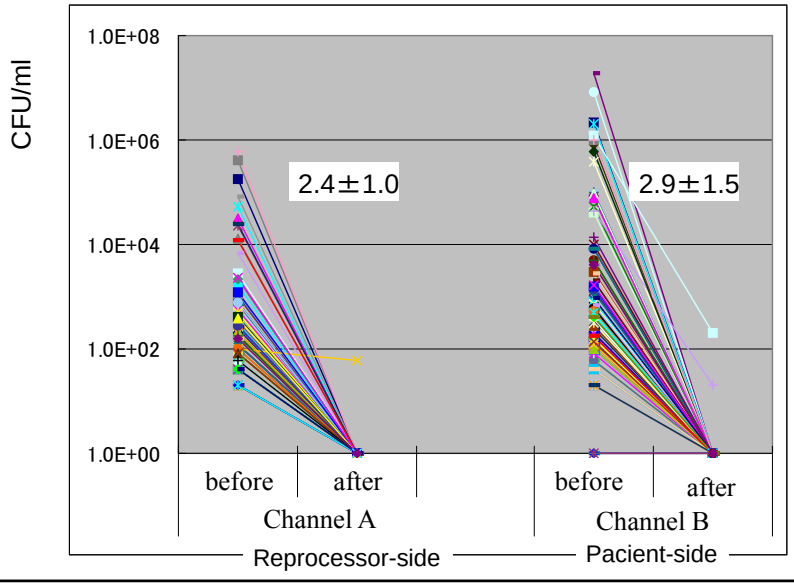
Automatic Brushing System



Total Processing Time:
GIF Mode 6min. & CF Mode 9min.

Disinfection results at 3 different facilities

Logarithmic decrease (mean score) of bacteria n=147



Handbook and Guideline

Guideline on the HOCl water-producing apparatus

次亜塩素酸水生成装置に関する指針

第２版

2012年12月

2nd Edition (Dec, 2012)

監修　日本機能水学会

発行　財団法人 機能水研究振興財団

Functional Water Foundation

How to use the endoscope reprocessors with functional water

機能水による消化器内視鏡洗浄消毒器の使用手引き

2nd Edition (May 30, 2015)

第２版

監修 日本機能水学会

Japanese Society for Functional water

一般財団法人 機能水研究振興財団

Functional Water Foundation

現場における結果の確認法

- 洗浄効果判定
  ATPふき取り法 ⇨

- 生成電解水中の有効塩素濃度
  化学的方法：　チオ硫酸ナトリウム
  DPD比色法：　有効塩素濃度計 ⇨

  新しい方法：　ダイヤモンド電極法 ⇨ **流水測定**

- 殺菌効果判定
  即時判定：　スマホ活用顕微鏡 ⇨

スマホで見るモバイル顕微鏡

いつでも　どこでも　すぐに　みて記録できる

⇨

2017年6月30日新発売
JIS化を目指している。

次世代衛生管理ツール

「菌未来」を創造する

Thank you for your attention!