Hypochlorous Acid (HOCl) Water (HAW)
A new disinfectant

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<Hotta’s Research Targets>
Antibiotic-producing actinomycetes
Antibiotic-resistant bacteria (MRSA)
Anti-MRSA antibiotics
Acidic electrolyzed water (Hypochlorous Acid Water)
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

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**Production system for HOCl water, NaOCl water and NaOCl solution**

- **Tap water**
  - 0.1% NaCl

- **Electrolysis chamber**

- **Alkaline water**
  - NaOH
  - pH11-11.5

- **Hypochlorous acid water**
  - HOCl
  - pH 2.2-2.7
  - Av.Cl2: 20-60ppm

- **Hypochlorite water**
  - NaOCl ≈ HOCl
  - pH 9-9.4
  - Av.Cl2: 30-200ppm

- **Hypochlorite solution**
  - NaOCl
  - pH 14
  - Av.Cl2: >40,000ppm

- **Saturated NaCl soln**

- **Electrolysis**
Electrolytic Reactions in the three types of electrolyzers that produce HAW

Properties
- Slightly acidic HAW
  pH 5.0-6.5
  10-80 ppm
- Weakly acidic HAW
  pH 3.0-5.0
  10-60 ppm
- Strongly acidic HAW
  pH 2.2-2.7
  20-60 ppm

Bacteria-killing activity of HAW

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

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Acidic water (pH 2.5-2.7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (MSSA)</td>
<td>&lt; 10 sec.</td>
</tr>
<tr>
<td>S. aureus (MRSA)</td>
<td>&quot;</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>&quot;</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>&quot;</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>&quot;</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>&quot;</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&quot;</td>
</tr>
<tr>
<td>Serratia marcessens</td>
<td>&quot;</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>&quot;</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>&lt; 30 sec.</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>&lt; 1 min.</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>&lt; 1 min.</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>&lt; 2.5 min.</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>&lt; 5 min.</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>&quot;</td>
</tr>
<tr>
<td>Virus including norovirus etc.</td>
<td>&lt; 10 sec.</td>
</tr>
</tbody>
</table>

Acidic water (pH 2.5-2.7)

Not treated | Treated (10 sec) | MRSA | E. coli
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.

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.  

Evidence for the formation in HAW of HOCl •OH and H₂O₂

1. Formation of hypochlorous acid at anode Cl₂ + H₂O → HOCl + HCl
2. Detection of hypochlorous acid by ion chromatography
3. Spectrophotometric spectrum identical with hypochlorous acid

•OH and H₂O₂ may cause the following. 
Degradation or damage of DNA, Protein, Amino acids
**Damages of cell surface and DNA by acid HOCl water**

AcEW treat

M C 10' 1' 5'

AcEW treat

M N C AcEW C'

519 bp

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. 10 μl + HOCl water 40 μl → 10’, rt → TLC → Ninhydrin reaction
Oxidation of Met by HAW

Similarity between neutrophile (left) and HOCI water (right) in terms of elements for bactericidal mechanism.
Difference between HAW and NaOCl solution

<table>
<thead>
<tr>
<th></th>
<th>Available chlorine concentration (mg/kg)</th>
<th>Available chlorine concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAW</strong></td>
<td>pH 2.2 ~ 7.5</td>
<td>pH &gt; 7.5</td>
</tr>
<tr>
<td><strong>NaOCl solution</strong></td>
<td>100 ~ 1000 mg/kg</td>
<td>1000 ppm</td>
</tr>
</tbody>
</table>

- **NaOCl solution**
  - pH > 7.5
  - 100 ~ 1000 mg/kg

- **HAW**
  - pH 2.2 ~ 7.5
  - 10 ~ 100 mg/kg

Anti-microbial activities of HAW and NaClO solution

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

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

Activity: \( \text{HOCl} > \text{OCl}^- \)

\[
\text{HOCl} \rightarrow \cdot \text{OH} + \cdot \text{Cl} \\
(\text{OCl})^- + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{Cl}^- + \text{H}_2\text{O}
\]

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.

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**Anti-microbial Activity of Disinfectants**

<table>
<thead>
<tr>
<th>Endospore &gt;</th>
<th>Tubercul. Bac. Virus &gt;</th>
<th>Fungi &gt;</th>
<th>Bacteria Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Level</td>
<td>Glutaraldehyde(GA)</td>
<td>Peracetic Acid(PAA)</td>
<td>Phthalic acid(PA)</td>
</tr>
<tr>
<td>Intermediate Level</td>
<td>NaOCl soln</td>
<td>HOCI water</td>
<td>O₃ water</td>
</tr>
<tr>
<td>Low Level</td>
<td>Ethanol</td>
<td>Surfactants</td>
<td>Quartery Ammonium Chlorhexidin</td>
</tr>
</tbody>
</table>
### Features of disinfectants

<table>
<thead>
<tr>
<th></th>
<th>HOCl w.</th>
<th>NaOCl soln.</th>
<th>EtOH</th>
<th>PVI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Available concn.</strong></td>
<td>10-80ppm</td>
<td>40,000ppm</td>
<td>75-80%</td>
<td>7-10%</td>
</tr>
<tr>
<td><strong>Concn. for use</strong></td>
<td>10-80ppm</td>
<td>100~10,000ppm</td>
<td>同上</td>
<td>250-500ppm</td>
</tr>
<tr>
<td><strong>How to use</strong></td>
<td>washing</td>
<td>immersion</td>
<td>spray/rubbing</td>
<td>smearing</td>
</tr>
<tr>
<td><strong>Target</strong>&lt;sup&gt;1)&lt;/sup&gt;: Food m.</td>
<td>○</td>
<td>○</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>: Metal</td>
<td>Δ</td>
<td>Δ</td>
<td>○</td>
<td>X</td>
</tr>
<tr>
<td>: non-metal</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>X</td>
</tr>
<tr>
<td>: environ.</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>X</td>
</tr>
<tr>
<td>: skin</td>
<td>○</td>
<td>X</td>
<td>○</td>
<td>O</td>
</tr>
<tr>
<td>: mucosa</td>
<td>○</td>
<td>X</td>
<td>X</td>
<td>O</td>
</tr>
<tr>
<td><strong>Anti-microbial</strong></td>
<td>broad</td>
<td>broad</td>
<td>broad</td>
<td>semi-broad&lt;sup&gt;2)&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Anti-viral&lt;sup&gt;3)&lt;/sup&gt;</strong></td>
<td>broad</td>
<td>broad</td>
<td>semi-broad&lt;sup&gt;4)&lt;/sup&gt;</td>
<td>broad</td>
</tr>
<tr>
<td>influenza</td>
<td>◎</td>
<td>◎</td>
<td>◎</td>
<td>◎</td>
</tr>
<tr>
<td>noro</td>
<td>○</td>
<td>○</td>
<td>▽</td>
<td>◎</td>
</tr>
</tbody>
</table>

<sup>1)</sup>〇no problem, △some problem(rust), Xproblematic.  
<sup>2)</sup>not effective to bacteria with endospores  
<sup>3)</sup>virus-inactivating activity: ◎markedly effective, ◎effective, ▽weakly effective  
<sup>4)</sup>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

**Trihalomethane (THM: CHCl₃ etc.) formation:** Little

**Emergence of resistant bacteria:** No

**Influence on environment:** Very weak if any

**Results indicated good bio-safety**

**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, PVI, HAW (rubbing) (smear) (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, O₃, H₂O₂, CH₃COOOH

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

<table>
<thead>
<tr>
<th>Electrolyzed water (Denkaisui)</th>
<th>Strongly acidic</th>
<th>Weakly acidic</th>
<th>Slightly acidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH:</td>
<td>2.2-2.7</td>
<td>2.7-5.0</td>
<td>5.0-6.5</td>
</tr>
<tr>
<td>Av.Cl₂:</td>
<td>20-60ppm</td>
<td>10-60ppm</td>
<td>10-80ppm</td>
</tr>
</tbody>
</table>

**Medical instrument***

<table>
<thead>
<tr>
<th>Activity</th>
<th>Strongly acidic</th>
<th>Weakly acidic</th>
<th>Slightly acidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand-washing (pre-operation)</td>
<td>Yes(1996)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Endoscope reprocessing</td>
<td>Yes(1997-)</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

**Food additives***

<table>
<thead>
<tr>
<th>Type</th>
<th>Strongly acidic</th>
<th>Weakly acidic</th>
<th>Slightly acidic</th>
</tr>
</thead>
</table>

* Only strongly acidic electrolyzed water-producing apparatus was approved and no water itself has been approved.

** Water itself was approved in the name of hypochlorous acid (HOCl) water. Therefore various types of apparatus can be used.

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**Improving reliability of HAW and its producing apparatus**

- **Approval**: 2002
- **Guideline**: 2013 (2nd ver.)
- **JIS**: Oct. 2017
- **ISO**: Near future
- **METI-JSA**: New Market Creation standardization system
- **Global standard**: Near future
- **National standard**: Industrial own standard

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*Drug, Cosmetics & Medical Instrument Act (薬事法)*

*Food Sanitation Law (食品衛生法)*

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### 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
   ① Safe and easy production by persons w/o professional knowledge
   ② Capable of continuous production like a tap water flow
5. Economical
   ① Low running cost: <$1 yen/L
   ② Water cut: Remarkable reduction of water for rinsing
   ③ Easy for wasting

### Risk of HAW

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

- **Communities**
  - Swimming pools, spa, hot springs, etc.
- **Food Industries**
  - Food materials, hand, facilities, cooking tools
- **Home**
  - Food stuffs, dishes, hand, sanitary area etc.
- **Agriculture Livestock**
  - Rice seeds, facilities, etc.
- **Medicine & Dentistry**
  - Hand, Endoscope etc, Wounds

**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.
Non-touch automatic hand-washing system using Denakisui

IC sensor for recording hand-washing (Traceability of hand-washing)

Infrared sensor

Sink

Drain

Denkaisui outlet

Hygienic hand-washing
1st: Alk. Denkaisui 15 sec. automatically
2nd: Acid. Denkaisui 15 sec.

Electrolyzer

1st: Alk. Denkaisui 15 sec. automatically
2nd: Acid. Denkaisui 15 sec.
**Hygienic handwashing with Denkaisui**

1. Washing with strongly alkaline EW for 15 seconds
2. Washing with strongly acidic EW for 15 seconds

Reduction of organic dirt

Reduction of general bacteria

**Surgical Handwashing with Denkaisui**

*Removal of organic materials*

*ATP level*

N=40
Baseline: 3185 ± 2378

P<0.01

Group 1: Soap → Hibiscrub
Group 2: Soap → HOCl w.
Group 3: alk. EW → Hibiscrub
Group 4: alk. EW → HOCl w.
Group W: Med. Soap → Weluplotion
**Surgical Handwashing with Denkaisui**

*Removal of general bacteria*

**Palm Stamp**

N=40
Baseline:127±108

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soap → Hibiscrub</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>Soap → Strongly Acid. EW → Hibiscrub</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>al. EW → Hibiscrub</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>al. EW → Strongly Acid. EW</td>
<td>1.3</td>
</tr>
<tr>
<td>W</td>
<td>Med. Soap → Weluplotion</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Full-automatic endoscope reprocessing apparatus**

*Total Processing Time: GIF Mode 6min. & CF Mode 9min.*
Disinfection results at 3 different facilities

Logarithmic decrease (mean score) of bacteria

n=147

CFU/ml

before | after  
--- | ---
Channel A | 2.4±1.0 | 2.9±1.5
Channel B | before | after
Reprocessor-side | Patient-side

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

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


監修 日本機能水学会

Japanese Society for Functional water

Functional Water Foundation

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

次世代衛生管理ツール
「菌未来」を創造する
Thank you for your attention!