

Executive Summary: Determination of the MIC and MBC in the Presence of Organic Matter for 0.05% CHG Solution and 0.35% PVP-I



Background

Irrisept® Antimicrobial Wound Lavage is intended for mechanical cleansing and removal of debris, dirt, and foreign materials, including microorganisms from wounds. The Irrisept single-use, manual, self-contained irrigation device is comprised of 0.05% Chlorhexidine Gluconate (CHG) in 99.95% sterile water for irrigation. The CHG acts as a preservative to inhibit microbial growth in the solution. The test results summarized here assess CHG and Povidone-Iodine (PVP-I), a commonly used wound irrigant, as preservatives in solutions to inhibit microbial growth upon exposure of the solution to organic matter.

Objective

An *in vitro* test was carried out to assess the ability of CHG and PVP-I to inhibit the growth of microorganisms in the presence of organic matter as would be the case in wound debridement. The test used Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays. Human serum was used to simulate the organic matter.

Methodology

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration evaluation of the test articles versus five microorganisms was performed based upon the Microdilution Broth Method outlined in CLSI Document M07-A10, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Tenth Edition, as well as NCCLS (currently known as CLSI) Document M26-A, Methods for Determining Bactericidal Activity of Antimicrobial Agents (September 1999).

Organisms Tested

Clinically relevant microorganisms were selected, including Gram-positive bacteria and Gram-negative bacteria:

- *Escherichia coli* (ATCC® 25922™)
- *Pseudomonas aeruginosa* (ATCC® 15442™)
- *Staphylococcus aureus* (ATCC® 29213™)
- *Staphylococcus epidermidis* (ATCC® 35984™)
- *Streptococcus agalactiae* (ATCC® 13813™)

Test Articles - Antimicrobials

- 0.05% Chlorhexidine Gluconate (CHG) in 99.95% sterile water for irrigation
- 0.05% Chlorhexidine Gluconate (CHG) in 99.95% sterile water for irrigation with 10% human serum
- 0.35% Povidone-Iodine† (PVP-I)
- 0.35% Povidone-Iodine with 10% human serum

0.35% PVP-I compounded at test laboratory using Sterile 10% PVP-I and 0.9% normal sterile saline. This formula is routinely used and can be commonly referred to as dilute Betadine®.

Results

MIC assay

- Without organic matter (human serum):
 - No visible microbial growth was observed with 0.05% CHG for all organisms tested, whereas microbial growth was observed with 0.35% PVP-I for *Escherichia coli*. See Table 1.
 - 0.05% CHG showed no visible microbial growth at several-fold dilution, i.e., lower than 0.05% CHG. See Table 2. The highest dilution where there was no visible microbial growth observed was 1:256 for 0.05% CHG, whereas it was only 1:4 for 0.35% PVP-I.
- With organic matter (human serum):
 - No visible microbial growth was observed with 0.05% CHG + 10% serum for all organisms tested, whereas microbial growth was observed with 0.35% PVP-I + 10% serum for *all organisms* tested. See Table 1.
 - 0.05% CHG + 10% serum showed no visible microbial growth at several-fold product dilution. Microbial growth was observed with PVP-I + 10% serum at concentrations lower than 0.35%. See Table 2. In all cases excluding *Streptococcus agalactiae* where results were inconclusive, 0.35% PVP-I + 10% serum showed visible microbial growth. The highest dilution where there was no visible microbial growth observed was 1:512 for 0.05% CHG + 10% serum.

Results

MBC assay

- Without organic matter (human serum):
 - The microbial count reduction was greater than $4.24 \log_{10}$ with 0.05% CHG for all organisms tested. The microbial count reduction was less than $2 \log_{10}$ with 0.35% PVP-I for *Escherichia Coli* and *Pseudomonas aeruginosa*. See Table 3.
- With organic matter (human serum):
 - The microbial count reduction was greater than $4.24 \log_{10}$ with 0.05% CHG + 10% serum for all organisms tested. The microbial count reduction was less than $2.15 \log_{10}$ with 0.35% PVP-I + 10% serum for all organisms. See Table 3.

Table 1: MIC Assay Results

Test Article	Visible Microbial Growth?				
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Streptococcus agalactiae</i>
0.05% CHG	No	No	No	No	Inconclusive*
0.05% CHG + 10% serum	No	No	No	No	Inconclusive
0.35 PVP-I	Yes	No	No	No	Inconclusive
0.35 PVP-I + 10% serum	Yes	Yes	Yes	Yes	Inconclusive

*Unable to assess due to product turbidity

Table 2: MIC Assay Results at Lower Concentrations**

Test Article	Highest Dilution (v/v) at No Visible Microbial Growth Observed				
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Streptococcus agalactiae</i>
0.05% CHG	1:256	1:64	1:256	1:256	Inconclusive*
0.05% CHG + 10% serum	1:512	1:64	1:512	1:512	Inconclusive
0.35 PVP-I	<1:2	1:2	1:4	1:4	Inconclusive
0.35 PVP-I + 10% serum	<1:2	<1:2	<1:2	<1:2	Inconclusive

*Unable to assess due to product turbidity

** Higher dilution means lower concentration and vice versa

Table 3: MBC Assay Results

Test Article	Microbial Reduction									
	<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>		<i>Staphylococcus epidermidis</i>		<i>Streptococcus agalactiae</i>	
	%	\log_{10}	%	\log_{10}	%	\log_{10}	%	\log_{10}	%	\log_{10}
0.05% CHG	99.99	4.24	99.99	4.31	99.99	4.55	99.99	4.49	99.99	4.45
0.05% CHG + 10% serum	99.99	4.24	99.99	4.31	99.99	4.55	99.99	4.49	99.99	4.45
0.35 PVP-I	≤98.55	1.84	≤98.77	1.91	99.99	4.55	99.99	4.49	99.99	4.45
0.35 PVP-I + 10% serum	≤98.55	1.84	≤98.77	1.91	≤99.30	2.15	≤99.20	2.09	≤99.11	2.05

Conclusions

Under the test conditions:

- With 0.05% CHG, there was no microbial growth for tested organisms irrespective of presence of organic matter.
- With 0.35% PVP-I, there was microbial growth for *Escherichia coli* without organic matter present. With 0.35% PVP-I in the presence of organic matter, there was microbial growth for all tested organisms.
- 0.05% CHG was effective in inhibiting microbial growth even when diluted resulting in a concentration lower than 0.05%. For example, at 1:64 dilution, i.e., 0.0008% concentration, CHG effectively inhibited microbial growth for *Pseudomonas aeruginosa*, irrespective of organic matter presence. Conversely, the 0.35% PVP-I was less effective in inhibiting microbial growth when diluted to lower than 0.35% concentration in the presence of organic matter. Dilution of 0.35% PVP-I resulted in reduced inhibition of microbial growth.
- In the MBC assay, 0.05% CHG demonstrated a greater than $4.24 \log_{10}$ reduction in inhibiting microbial growth for all tested organisms irrespective of organic matter presence. Conversely, 0.35% PVP-I demonstrated less than $2.15 \log_{10}$ reduction inhibiting microbial growth for all tested microorganisms in the presence of organic matter.