

BioCote®

Support Pack







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Q. What is BioCote®?

A. BioCote® is silver ion technology. It has been used in the manufacture of AquaVENT® breathing circuits to give the product antimicrobial properties. Ionic silver particles are homogeneously dispersed through the polymers used to produce AquaVENT®.

Q. Why Silver?

A. Silver (Ag) antimicrobial technology is safe, natural and sustainable. Silver has been used for centuries for its abilities to aid preservation, for example, the ancient Greeks used silver vessels to keep their water fresh and Chinese Emperors ate with silver chopsticks. Ag comes from the Latin Argentum, meaning 'grey' or 'shining'.

Prior to the introduction of antibiotics, silver was used widely in hospitals to combat bacteria and today it is used in a wide range of medical devices, such as wound dressings and catheters. Its use has been shown to reduce rates of hospital acquired infections.

Recent advances in antimicrobial technology allow silver to be added to everyday products at the manufacturing stage. Silver is an ideal antimicrobial agent due to its effectiveness against a range of microorganisms. It is non-toxic to non-target cells.

Q. How does the silver work?

A. BioCote® antimicrobial technology, in the form of silver ions in a carrier polymer, is added to the product during moulding or extrusion of the components. Silver ions are present on the surface of the components and are available to act against contaminating bacteria. Silver ions bind with the bacteria and damage their cells in a number of ways; disrupting their normal function, stopping them from replicating and rendering them harmless. (For more information see page 6).

Q. Why is BioCote® different to other antimicrobial technologies?

A. BioCote® is the only antimicrobial company to have conducted environmental studies on a wide range of treated products to demonstrate real-life efficacy. BioCote® offers microbiological and regulatory support. BioCote® in AquaVENT® has a rating for antimicrobial efficacy of >99.3%. (For more information see page 7).

Q. Are bacteria resistant to silver?

A. There is no evidence that BioCote® functions in the same way as antibiotics and therefore, to date, no bacteria have become resistant to BioCote® as they have to some antibiotics.

Q. Is BioCote® effective against antibiotic resistant bacteria?

A. Yes, BioCote® is currently effective against antibiotic-resistant bacteria, including MRSA and CDifficile.

Q. Is BioCote® effective against H1N1 (swine influenza A)?

A. BioCote® has been shown to significantly reduce infectious viruses, including H1N1 on the surface of polymers to which it has been added. (For more information see page 10).

Q. Is silver safe?

A. Yes, silver is a safe, natural antimicrobial.

Q. What is the expected life cycle of BioCote® protection?

A. Products containing BioCote® have been subjected to 25-years accelerated life cycle testing.

Results show over 99.3% reduction in targeted cells; however the maximum period of use of AquaVENT® breathing systems is 7-days, from date of initial use, in accordance with manufacturer's guidelines and local hospital policy.

Q. Will silver ions separate from the polymer to which it is added?

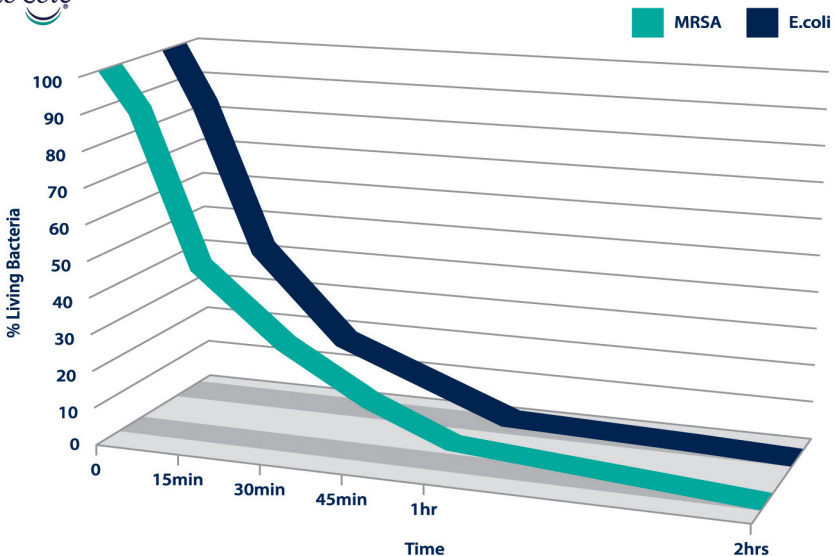
A. BioCote® is present at the surface until absorbed by bacteria; it will not leach from the surface.

Q. How quickly can BioCote® act against microbes?

A. See graph below

Q. Do I still need to use a breathing filter or filter HME?

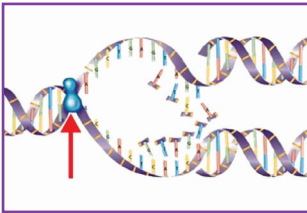
A. Yes. Although BioCote® protects both the internal and external surfaces of the breathing system a suitably validated filter should be used to protect the patient and the anaesthetic machine or ventilator from gas-borne pathogens.



How does BioCote® work?



1. Silver ions combine with microbial proteins located in the cell wall and cytoplasm, which interferes with their normal functioning.

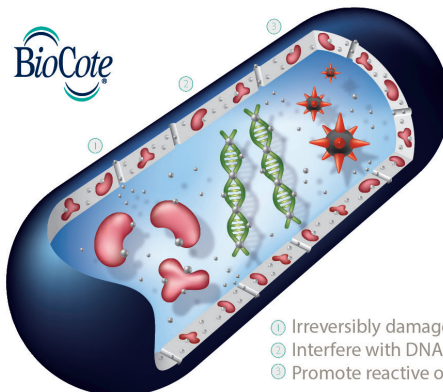


2. Silver ions stop the microbes replicating by blocking the copying of their genetic material.



3. Silver ions are known to promote the formation of harmful chemicals called reactive oxygen species (ROS) inside microbial cells.

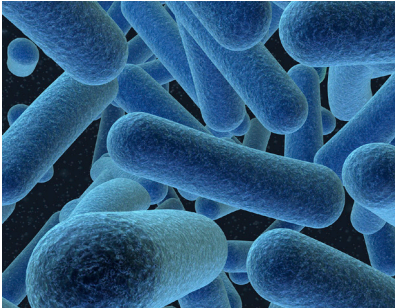
Damage caused by ROS is a major contributor to ageing that results in further inhibition of microbial growth.



- ① Irreversibly damage bacterial proteins
- ② Interfere with DNA replication
- ③ Promote reactive oxygen species

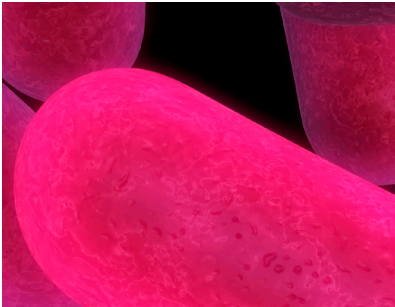
Tested Microbes

BioCote® antimicrobial additives have been tested and found to perform against a wide range of microbes including bacteria, fungi and viruses. Some of the most common are listed below. Efficacy against all of these microbes can vary and specific data are available on request.



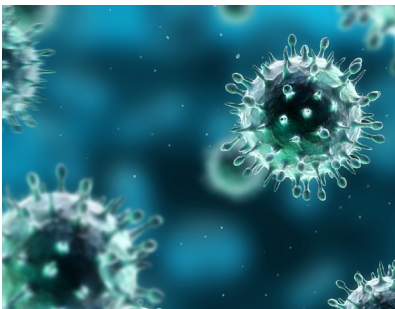
Bacteria

- Acinetobacter baumannii
- Bacillus subtilis
- Campylobacter coli
- Campylobacter jejuni
- Clostridium difficile (excluding spore form)
- E.coli
- E.coli O157
- Enterobacter aerogenes
- Enterococcus faecalis
- Escherichia coli ESBL
- Legionella pneumophila
- Listeria monocytogenes
- MRSA
- Pseudomonas aeruginosa
- Salmonella enteritidis
- Salmonella typhimurium
- Shigella sp.
- Staph aureus
- Staph epidermidis
- Streptococcus faecalis
- Vancomycin Resistant Enterococcus



Fungi

- Aspergillus niger
- Aspergillus brasiliensis
- Candida albicans
- Penicillium sp.



Virus

- Influenza A H1N1

Tested Microbes

In Armstrong Medical Ltd's efforts to reduce the risks associated with hospital acquired infections, AquaVENT® heated breathing circuits are supplied with BioCote® antimicrobial additive to limit the spread of bacterium species between patients and users, should transferable bacteria come into contact with the surfaces of the breathing circuit.

BioCote® technology in AquaVENT® heated breathing circuits uses inorganic silver ion particles dispersed homogenously throughout the polymer used to form components of the breathing circuit.

On the 9th April 2013 our manufacturing partner BioCote® issued a press release (please click [here](#) for a link to the full press release) stating they had commissioned testing of their antimicrobial technology against a carbapenem-resistant Enterobacteria (CRE). Their independently-performed ISO 22196 test demonstrated elimination of over 99.9% of the bacterium *Klebsiella pneumoniae*.

BioCote® stated that their technology has already been proven to be effective against a wide range of bacteria and fungi and this brings the total number of multi-drug resistant bacteria which BioCote® technology is known to be effective against to four:

1. MRSA (Methicillin-resistant *Staphylococcus aureus*)
2. VRE (Vancomycin-resistant *Enterococcus*)
3. ESBL (Extended Spectrum Beta Lactamase producing *E. coli*)
4. CRE (Carbapenem-resistant *Enterobacteriaceae*, *Klebsiella pneumoniae*)

Legionella

BioCote® Limited (Wolverhampton, UK) recently commissioned independent laboratory Industrial Microbiological Services Limited (Hants, UK) to test BioCote® additives used in the manufacture of Armstrong Medical's AquaVENT® heated breathing circuits. Testing aimed to establish the additive's effectiveness in reducing bacteriums.

Legionella when present on the surfaces of the breathing circuit. A reduction of >99.9% (log 10⁻³) was observed after 24-hours. These results demonstrate efficacy of the additive under the test conditions and add to the efficacy previously established in respect of other bacterium s. including MRSA, Escherichia Coli, Acinetobacter baumannii and Influenza A (H1N1).

Bacterium s. Legionella

is a gram-negative pathogen carried in water vapour and known to cause L. Pneumophila in vulnerable subjects by inhalation of respirable water particles carrying the bacterium. Elderly subjects and those with compromised immune system are most susceptible. Person-to person transfer is not considered possible.

Recent outbreaks in England and Scotland have resulted in in-patient deaths at University of North Staffordshire NHS Trust and at NHS Lothian. Whilst the source of these outbreaks appears to be external to the hospital environment, hospitals inevitably deal with the outbreak and are also a source risk due to their use of air conditioning and water storage.

In our efforts to reduce the risks associated

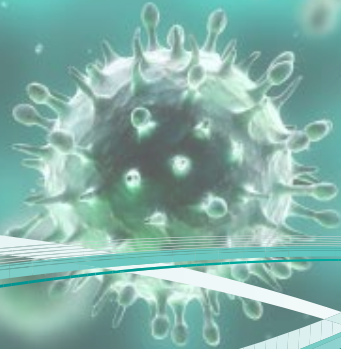
with hospital acquired infections, AquaVENT® heated breathing circuits are supplied with BioCote® antimicrobial additive to limit the spread of bacterium species between patients and users, should transferable bacteria come into contact with the surfaces of the breathing circuit.

BioCote® technology in AquaVENT® heated breathing circuits uses inorganic silver ion particles dispersed homogeneously throughout the polymer used to form components of the breathing circuit. For more information, contact Armstrong Medical or visit <http://www.biocote.com/about>.

For more information, contact Armstrong Medical or visit <http://www.biocote.com/about>.

H1N1 Virus Case Study

Proven reduction of the H1N1 influenza virus on BioCote treated materials



Background

Outbreaks of influenza caused by the H1N1 virus are a repeated threat. The contagious H1N1 virus spreads effectively between people and, due to the widespread international travel, between countries.

July 2009 saw the beginning of the most recent global H1N1 influenza pandemic with around 30,000 confirmed cases reported in 74 countries although unconfirmed cases make this outbreak undoubtedly more significant. The economic impact of influenza can be huge; the World Health Organisation estimated an H1N1 pandemic could cost the UK economy over £70 billion so a measure with the potential to limit the spread of viral infection is worthy of including in an infection control strategy. The evidence described here suggests the application of BioCote® antiviral technology has the potential to complement strategies aimed at inhibiting the spread of viruses responsible for influenza illness.

Viruses cause human disease by infecting cells of the body. Viral disease can be averted if the virus is rendered noninfectious before it enters the body's cells and establishes an infection. Antiviral vaccines typically operate by converting the virus from an infectious to noninfectious form. This study quantified the conversion of influenza A H1N1 virus from an infectious to non-infectious form because of its exposure to BioCote® containing materials.

Aim

To understand how effective BioCote® approved silver ion antimicrobial technology is against influenza A H1N1 virus when incorporated into various manufacturing materials.

Method

Known amounts of infectious H1N1 virus (Fig.1) were added to the surface of a variety of materials commonly used for manufacturing that contained BioCote® approved antimicrobial silver ions; specifically, acrylonitrile butadiene styrene (ABS), polycarbonate (PC), thermoplastic polyurethane (TPU), polyvinyl chloride (PVC) and polybutylene terephthalate (PBT) polymers, laminated wood board and wet and powder paints. Exposures were left overnight after which the virus was recovered from the test materials. Viruses still able to infect cells after exposure to BioCote® technology were counted using an immunological microplate plaque assay (Fig.2). Because controls were included in these experiments, the amount of virus inactivation directly attributable to the BioCote® silver technology was determined.

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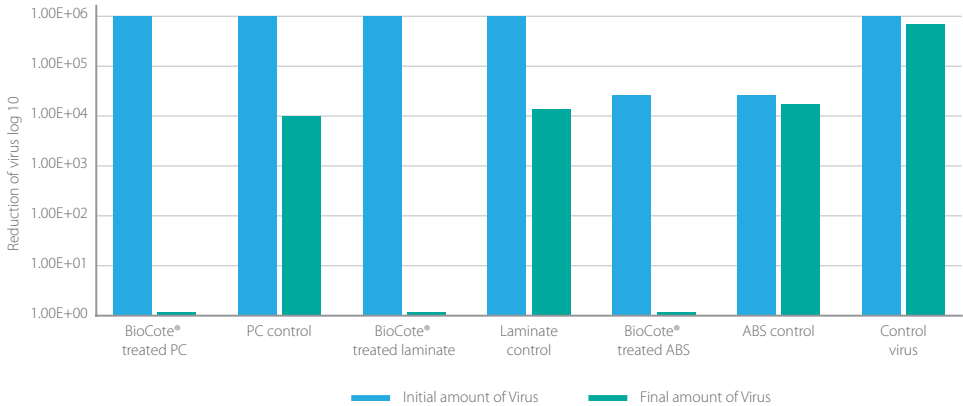
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Results

All BioCote® containing materials demonstrated significant antiviral activity compared to untreated and/or virus controls.

A selection of reductions in numbers of infectious H1N1 virus because of exposure to treated materials is presented in Fig.3 alongside corresponding reductions by untreated controls. The survival of the H1N1 virus under test conditions not exposed to any material was also determined.



Results for Laminate

>99.99%

reduction in viable H1N1 virus particles on BioCote treated laminate

Results for PC

>99.99%

reduction in viable H1N1 virus particles on BioCote treated PC

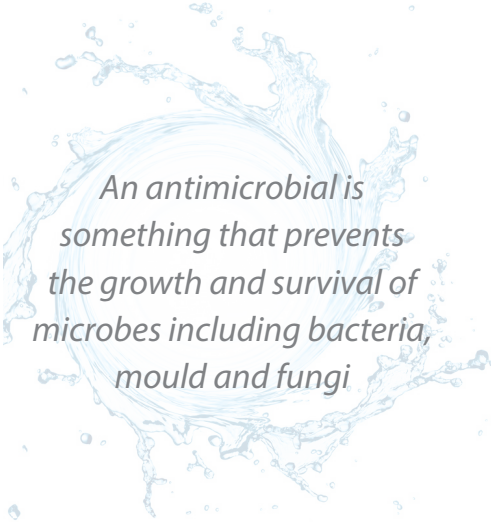
Conclusions

BioCote® approved silver ion technology is effective at significantly reducing numbers of infectious influenza A H1N1 virus. Antiviral activity was demonstrated by BioCote® containing ABS, PC, TPU, PVC and PBT polymers, laminated board and wet and powder paints.



Proven Antimicrobial Protection

The BioCote® Antimicrobial Technology Fact Sheet



An antimicrobial is something that prevents the growth and survival of microbes including bacteria, mould and fungi

BioCote technology protects surfaces from microbes resisting the potentially negative impact they can have on a product, resulting in a number of benefits. BioCote technology will:

- ① **Make a product more hygienic**, if it is likely to host microbes harmful to human health.
- ② **Keep a product fresher for longer**, if it is likely to host odour-causing or staining microbes.
- ③ **Extend the usable lifetime of a product**, if it is likely to host microbes which might degrade the surface.

BioCote: the antimicrobial brand you can trust

The BioCote Brand:

- Is a recognised sign of quality
- Represents superior product performance
- Is found on products around the world

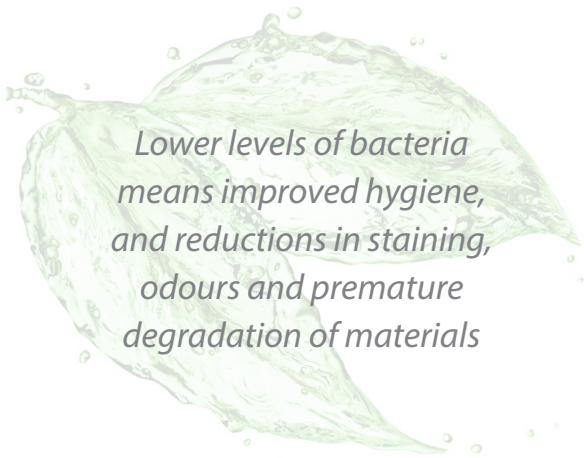
BioCote is the only antimicrobial supplier in the world to:

- Set global performance standards
- Prove customers' products really are antimicrobial
- Be HACCP International approved
- Prove the technology performs in the real world

The BioCote brand is globally recognised as a **guarantee of superior antimicrobial performance**. The BioCote trademark is the sign of a brand you can trust.



BioCote reduces the level of microbes on a surface



Lower levels of bacteria means improved hygiene, and reductions in staining, odours and premature degradation of materials

What does BioCote technology do?

BioCote technology provides long-lasting protection to a product by creating a surface upon which microbes cannot survive.

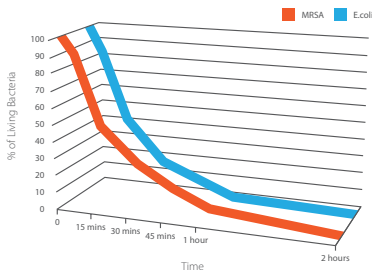
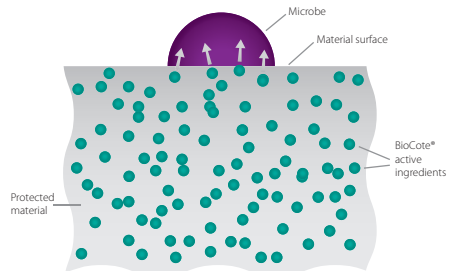
BioCote technology:

- Is proven effective against a wide range of bacteria, fungi & the H1N1 virus
- Reduces microbes by up to 99.99%
- Significant reductions within 15 minutes
- Up to 99.5% reduction in just 2 hours
- Works continuously for the expected lifetime of the product

How does BioCote® technology work?

BioCote technology protects products by binding with microbes contaminating its surface. Microbes do not survive being exposed to BioCote® technology.

BioCote technology is an ideal way maintaining a more hygienic product surface between cleans.



BioCote technology:

- Stops cells from reproducing
- Damages the cell wall
- Disrupts energy production and other cellular functions

Hospital Case Study

Proven reduction in bacterial contamination in a real-life hospital environment



Background

The control of healthcare-associated infections (HCAIs) remains a priority for healthcare providers, who are employing a combination of infection prevention and control strategies, including hand hygiene, cleaning, training and the adoption of new technologies, to tackle the problem.

As a result, a wide range of infection control products and technologies are emerging on the market, including antimicrobial technology.

BioCote Ltd works with equipment manufacturers, engineering silver ion technology into a variety of healthcare related products, helping them to resist the growth of bacteria and mould on their surface. Silver is an ideal antimicrobial agent because it has a high efficacy against a wide range of medically-important microorganisms and is regarded as non-toxic. For the NHS to employ new technologies and products they need to show a demonstrable ability to contribute positively to infection control. The use of any product that claims it has antimicrobial efficacy should be supported by a robust evidence-base.

Aim

A pilot study, conducted at the Heart of England NHS Foundation Trust, investigated to what extent BioCote® antimicrobial products can reduce microbial contamination in a healthcare environment.

In laboratory tests, BioCote® antimicrobial materials regularly demonstrate reductions in counts of E. coli and S.aureus greater than 95%, compared with untreated samples. The aim of this study was to determine to what degree this high level of antimicrobial efficacy could be achieved in a real-life hospital environment.

Method

Two outpatient units provided the environments for this 18 month pilot study. Unit A was refurbished with BioCote® treated products including blinds, tiles, door handles, sack holders and light switches and also a number of untreated products. A similar, refurbished outpatient ward containing untreated items (unit B), served as a control. Both outpatient units were similar in terms of volume of people, layout and floor-surface area and were subjected to standard cleaning practice. Both were allowed to function for 12 months before swabbing commenced.

Swabs were collected over a five month period from BioCote® treated and untreated products in both outpatient units. Swabs were processed for total counts of viable bacteria and results expressed as average counts of colony-forming units (CFUs).

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Results

Table 1 shows that CFU counts from BioCote®-treated products in unit A were between 62% and 98% lower than from comparable, untreated products in unit B.

The products used in the trial were manufactured from a variety of materials e.g plastics and fabrics. CFU counts from these different materials were also compared and are shown at the bottom of Table 1. CFU counts from BioCote® treated materials were between 70% (fabrics) to 99% (laminates) lower than untreated equivalents.

CFU counts from BioCote® treated products in unit A were compared with CFU counts from untreated products in both unit A and unit B. CFU counts on untreated products in unit A were also compared to untreated products in unit B.

Table 1

% reduction of CFU counts from BioCote®-treated products / materials in unit A compared to nontreated products / materials in unit B

Product	% Reduction
Door	98%
Door handle	89%
Electrical Switch	95%
Curtains / blinds	73%
Chair	93%
Treatment Couch	62%
Sign	75%
Waste Bin	84%
Tiles	90%
Material	% Reduction
Powder Coating	94%
Plastic	98%
Wood Lacquer	98%
Fabric	70%
Laminate	99%

Discussion

Results suggest that BioCote® antimicrobial products will demonstrate the same high level of antimicrobial efficacy in a real-life environment as seen in laboratory tests, e.g an average bacterial reduction of 95.8%.

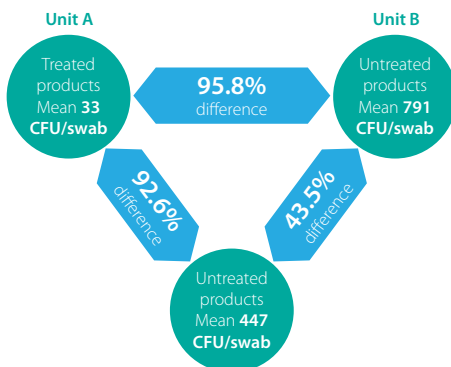
In addition to the effect of standard cleaning, BioCote® antimicrobial products showed sustained reductions in bacterial counts, compared to untreated products. Because BioCote® technology does not wear out or wipe off surfaces it can provide a continuous decontamination effect. Treated products can complement cleaning practices, helping

This three way comparison is shown in Figure 1 and provides the following results:

- The average CFU count from any BioCote® treated product was 95.8% lower than that from any untreated product in unit B.
- The average CFU count from any BioCote® treated product was 92.6% lower than that from any untreated product in the same environment (unit A)
- The average CFU count from any untreated product in unit A was 43.5% lower than that from any untreated product in unit B.

Figure 1

Inter-site comparison of average CFU counts from BioCote® treated and untreated products in units A and B.



to continually reduce levels of bacteria on surfaces and in the wider healthcare environment.

Bacterial contamination on untreated products in unit A was on average 43.5% lower compared with untreated products in unit B. This suggests that a reduction in bacteria on BioCote® antimicrobial surfaces results in lower numbers of bacteria on other surfaces because there are fewer bacteria being transferred. Using a number of antimicrobial objects in a healthcare environment may therefore help the decontamination of the wider environment.

Conclusions

This study, first published in the Journal of Infection Prevention¹, highlights the ability of BioCote®-treated antimicrobial products to reduce levels of bacteria contaminating healthcare settings.

Stability of Biocote® Antimicrobial Silver Additive in Armstrong Medical respiratory tubing during active humidification

Independent laboratory Wardell Armstrong (Truro, UK) analysed samples of condensed water recovered from the patient-end of an active humidification breathing circuit, intended for neonatal use; the tubing of which contained antimicrobial silver (Ag) additive Biocote® in the dispersion rate of 1.5% by weight of polymer used to form the tubing. The analysis aimed to establish the extent to which Ag could be released from the tubing and become part of the inhalatory gas stream, whether as gas or as water vapour. Analysis of water condensate was deemed the most likely indicator of stability of the Ag additive in the tubing. The study period of 10-days combined with the temperatures under test, simulate the most rigorous conditions of clinical use. Our breathing circuits are indicated for use over a maximum of 7-days, from date of initial use.

Method to produce water condensate samples for analysis

- Breathing circuit design: Aquavent Neo heated circuit for nasal CPAP used on >24-weeks gestational weight neonates
- Gas flow (air and oxygen mix at 30% O₂) maintained at 10L.min. at 3cmH₂O PEEP
- Sterile water for injection used as water supply to the humidification chamber of the heated circuit
- Heater humidifier set to intubated mode to achieve 37°C chamber temperature and 40°C patient-end temperature. Note that in clinical use of this system, the heater humidifier is set to 'face mask mode' which achieves 31°C chamber temperature and 34°C patient-end temperature.
- >6mL condensed water taken daily over 10 consecutive days from a collection point in the unheated portion of the circuit, proximal to the patient interface
- Water samples analysed individually for silver element using sterile water for injection as control. Analyses performed and reported by independent laboratory Wardell Armstrong

Summary of results

Wardell Armstrong report 0115 of 20th May 2013 confirms that presence of Ag, in samples day 1 to day 10 inclusive, was on or below the detection limit and was no greater than Ag content analysed in the control specimen. The data show zero or negligible potential for Ag to be added to water vapour for inhalation during this clinical simulation.

A handwritten signature in black ink, appearing to read 'Ciarán Magee', is shown within a light grey rectangular box.

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encl. Wardell Armstrong report 0115

Analytical Report

Client Biocote Limited

Sample: Condensed Water Samples

Sample Received: 16 May 2013 **Report Number:** 0115

<i>Biocote Ref</i>	<i>WAI lsn</i>	<i>Description</i>	<i>Ag (mg/l)</i>	<i>Ag (mmol/l)</i>
90/091	0969	Control	0.01	0.0002
90/092	0970	Day 1	<0.01	<0.0002
90/093	0971	Day 2	0.01	0.0002
90/094	0972	Day 3	<0.01	<0.0002
90/095	0973	Day 4	<0.01	<0.0002
90/096	0974	Day 5	0.01	0.0002
90/097	0975	Day 6	<0.01	<0.0002
90/098	0976	Day 7	0.01	0.0002
90/099	0977	Day 8	0.01	0.0002
90/100	0978	Day 9	<0.01	<0.0002
90/101	0979	Day 10	<0.01	<0.0002

Key of Abbreviations

- < - Less than the detection limit
- mg/l - Milligrams per litre
- mmol/l- Millimoles per litre
- lsn - Laboratory sample number

Signed:.....

Date: 20 May 2013

Andrea E Semmens MRSC
 Senior Chemist





Live/dead imaging of *Pseudomonas* species on BioCote silver ion treated Armstrong Medical products

Abstract

Antibacterial performance and associated marketing claims are often anchored in international standards results such as ISO22196:2011 ‘Measurement of antibacterial activity on plastics and other non-porous surfaces’.

Good performance to this standard can be considered the first step in taking an antimicrobial product to market. However, percentage and LOG₁₀ reductions may need additional explanation and context when an understanding of the potential of antibacterial or antimicrobial properties are made available to interested parties who perhaps do not have a scientific or technical background.

With this goal in mind, we investigated how we might demonstrate antibacterial performance via other means. In collaboration with Birmingham University Technology Hub Imaging Core, it was determined that current methods for wide field epi-fluorescent microscopy with a live/dead staining system could image a number of microorganisms on replicated material, technically equivalent to Armstrong Medical’s AquaVENT® Breathing circuits.

We assessed Armstrong Material’s antibacterial performance via ISO22196:2011 and achieved excellent levels of reduction of test organisms: summarized in the tables below.

Reductions against Control

Organism	% Reduction of treated material, compared with control
<i>Pseudomonas aeruginosa</i>	99.67%
<i>Escherichia coli</i>	>99.99%
<i>Staphylococcus aureus (MRSA)</i>	>99.89

The above table displays efficacy of silver ion treated material as percentages when compared with control or untreated material. Bacteria were inoculated onto treated and untreated material and allowed to grow for 24 hours. The reported percentage difference is the reduction of organism numbers on the treated material when compared with any growth on the control, during the same time period.

Reductions against initial

Organism	% Reduction of treated material, compared with the initial population
<i>Pseudomonas aeruginosa</i>	97.46%
<i>Escherichia coli</i>	>99.91%
<i>Staphylococcus aureus (MRSA)</i>	>99.93%

The above table displays the efficacy of the silver ion treated material when compared with the initial or starting population loaded onto the tested sample. Test organisms were inoculated onto treated material and allowed to grow for 24 hours. Reported percentage difference is the reduction of bacteria following exposure to the treated surface, when compared with the number of organisms initially loaded onto the sample.

Imaging of *Pseudomonas aeruginosa* on control and silver ion containing material was successful, and images are contained within this report. Clear distinction between silver ion treated and control material was observed, with cell counts of approximately 93.7% dead on the treated sample, compared with 57% on control material.

Imaging performed by Dr. Robert K Shaw, Imaging Specialist, Technology Hub Imaging Core, College of Medical and Dental Sciences, University of Birmingham.

Introduction

Silver ion technology

Silver belongs to the oligodynamic group of metals, those having a toxic effect on microorganisms at relatively low concentrations. This general mechanism is common amongst heavy and/or noble metals such as silver, zinc, copper and lead. Oligodynamic metals have similar effects on microorganisms; however the primary mode of action of each metal may be different. For example, copper ions are often reported to cause damage to cell membranes but do not cause damage to DNA (Christophe Espírito Santo *et al.*, 2012). Synergy is displayed between silver and copper ions, with an increase in efficacy demonstrated against pathogenic bacteria (Huang *et al.*, 2008).

Silver and its ionic state

In order for silver to display antimicrobial properties, it must be in its ionized form (Lok *et al.*, 2007; Rai *et al.*, 2009). Silver in its non-ionized form is inert (Guggenbichler *et al.*, 1999); however any contact with moisture will result in the formation of silver ions. Environmental moisture is considered sufficient for efficacy (Radheshkumar and Munstedt, 2005), thus any silver containing compound or additive in contact with moisture will display antimicrobial properties. The speed by which silver ions are released will depend on the antimicrobial compound.

BioCote technology is based on silver ions held within a phosphate glass matrix. Ionic release from phosphate glasses and zeolites (as ion exchangers) can be considered relatively controlled when compared with nanosilver particles or elemental silver (Nagy *et al.*, 2011). This is also true for silver based medicinal creams and/or lotions which can be tailored to have fast or slow ionic release

depending on the target application of the medicine.

Ions entering the cell

The silver cation may gain entry to the cell via transmembrane proteins that normally function to transport ions other than silver ions. Transmembrane proteins such as CopB-ATPase from *Enterococcus hirae* have been shown to transport silver ions although its putative function is a copper transporter (Solioz and Odermatt, 1995). The CUS silver and copper ion efflux transporters are also able to transport silver and copper ions in *E. coli* and other organisms (Mealman *et al.*, 2012).

DNA association

Klueh (2000) proposed the silver cations may enter the microbial cell and intercalate between the purine and pyrimidine base pairs. This intercalation disrupts the hydrogen bonding between the two anti-parallel strands and denatures the DNA molecule, suggesting no interaction with phosphate groups.

The significance of any DNA interaction by the silver cation when considering all other potential biocidal mechanisms is unclear, although interaction between silver ions has been reported multiple times (Izatt *et al.*, 1971; Rahn *et al.*, 1973; Thurman *et al.*, 1989; Zavriev *et al.*, 1979; Woo kyung jung, *et al.*, 2008).

Silver ions binding and denaturing proteins

Silver ions bind to thiol groups (-SH) in proteins which can lead to deactivation of enzymes. Silver forms stable S-Ag bonds with thiol-containing compounds in the cell membrane that are involved in transmembrane energy generation and ion transport (Klueh *et al.*, 2000). Amino acids such as cysteine and other thiol group containing compounds neutralize the activity of silver ions and their effectiveness against bacteria (Liau *et al.*, 1997). In contrast, disulphide bond containing amino acids and non-sulphur containing amino acids and were unable to neutralize silvers antibacterial activity. These and other findings strongly imply the interactions of silver ions and thiol groups in enzymes play an essential role in silver's antimicrobial activity although other cellular components such as hydrogen bonding may also be involved (Furr *et al.*, 1994). Silver ions are also implicated in the release of potassium ions from microorganisms, further suggesting interactions between enzymes and proteins associated with cytoplasmic or plasma membranes contribute to the biocidal activity of silver (Feng *et al.*, 2000; Miller *et al.*, 1957; Schreurs and Rosenberg, 1982).

Protein interaction

Silver ions take part in catalytic oxidation reactions that result in the formation of disulfide bonds (R-S-S-R). This occurs via reactions between oxygen molecules in the cell and hydrogen atoms of thiol groups, water is released as a product and two thiol groups become covalently bonded to one another through a disulfide bond (Davies and Etris, 1997).

The silver-catalyzed formation of disulfide bonds can lead to changes in protein structure and the inactivation of key enzymes, such as those needed for cellular respiration (Davies and Etris, 1997). A number of proteins such as the 30S ribosomal subunit protein, succinyl coenzyme A synthetase,

maltose transporter (MalK), and fructose bisphosphate adolase were identified as having decreased expression when treated with a 900 ppb Ag⁺ solution (Yamanaka *et al.*, 2005)

Recent investigation into silvers biocidal mode of action

Morones-Ramirez *et al.*, (2013) reported a number of experiments detailing potential mechanisms by which silver ions exert antibacterial action, centering on the production of free radicals and their downstream effects. A brief summary of these mechanisms is shown below. It is worth noting that the author reports several incidences where silver ions have re-potentiated antibiotics against *E. coli* which had previously displayed resistance.

- General increased hydroxyl radical production.
- Increased membrane permeability.
- Disruption of internal and external iron homeostasis.
- Disruption of the electron transport chain (ECT).
- Disruption of the TCA (tricarboxylic acid) cycle.
- Morphological damage viewable via Electron Microscopy.
- Induction of aggregated and mis-folded proteins.
- Potentiation of antibiotics to resistant strains.

Methods

Antibacterial analysis

Control and silver ion containing material was assessed via the international standard ISO22196:2011 (Measurement of antibacterial activity on plastics and other non-porous surfaces). Assessment of the material was performed against three organisms, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Briefly, three samples of control and three samples of treated material were prepared for each organism. Samples were then inoculated with known quantities of test organism and incubated at 37°C for 24 hours. After incubation remaining cells were washed from the surface of the assessed material, diluted as appropriate and enumerated on agar plates. Results were then expressed as percentage and LOG₁₀ reductions against initial (total number of organisms loaded onto the material) and the control (against organisms recovered from non-treated, control material).

Fluorescence microscopy imaging

Preparation of samples

Polyethylene was dosed with BioCote antimicrobial masterbatch at appropriate rate and plaques manufactured. Plaques were cut into 10mm x 10mm squares using a hacksaw. These squares were filed to remove edges and burrs. Approximately 25 silver ion containing and 25 control squares were generated.

Growth of test organisms and inoculation

Test bacteria were grown in overnight broth culture and diluted to 1:100 in Luria Broth. Cultures were incubated under agitation for 2 hours at 37°C.

Treated and untreated polyethylene samples were attached to the bases of 12 well multiwall plates and test bacteria applied. These samples were then incubated for a further 3 hours at 37°C. Samples were washed by inverting onto water wells for 2 minutes. Bacteria on samples were then stained with Baclight kit Live/dead kit (Life Technologies) for 15 minutes at room temperature. Samples were then washed in water for two minutes to remove excess stain.

For cell staining, solution B was used from the Molecular probes kit L7007 at 1.50 dilution, the viable cells are stained green with 1.67mM Cyto9 and dead cells (red) with 18.3mM propidium iodide.

Cells were mounted with the supplied mounting oil under a square coverslip and imaged on a Leica DMRE widefield epi-fluorescence microscope with a 100x plan apo oil immersion objective.

Results

Antibacterial ISO22196:2011

A summary of the results of antibacterial analysis are shown below in Table 1. Broad spectrum antibacterial performance can be considered validated due to efficacy against standard test organisms *E. coli* and *S. aureus*, as representatives of Gram negative and Gram positive bacteria respectively. Good efficacy was also demonstrated against the target organism in this application, *Pseudomonas aeruginosa*.

Species	LOG ₁₀ Reduction (control)	% Reduction (control)	LOG ₁₀ Reduction (initial)	% Reduction (initial)
<i>P. aeruginosa</i>	2.48	99.67%	1.59	97.46%
<i>E. coli</i>	≥ 3.94	≥ 99.99%	≥ 3.03	≥ 99.91%
<i>S. aureus</i> (MRSA)	≥ 2.98	≥ 99.89%	≥ 3.13	≥ 99.93%

Table 1. ISO22196:2011 antibacterial performance of BioCote treated material against three test organisms, *P. aeruginosa*, *E. coli*, *S. aureus* (MRSA). Results show log₁₀ and percentage reductions against control (reduction when compared with organisms recovered from non-treated control material) and initial (reduction of the total number of organisms loaded onto the material).

Fluorescence microscopy imaging

Figure 1 displays the results of the imaging of *Pseudomonas aeruginosa* on control (left) and silver ion containing (right) polyethylene plaques. Green indicates living cells, stained with Cyto9, whilst red shows dead, stained with propidium iodide.

Of the three organisms tested, *Pseudomonas* was able to adhere to the surface sufficiently for imaging in the 3 hour time frame.

Via the use of ImageJ, a public domain imaging tool, and the cell counter plugin we approximated in the control sample field a total of 91 cells, of which 27 (57%) were dead. In contrast, the treated sample displayed approximately 209 cells of which 196 (93.37%) were dead.

Discussion

We were then able to successfully demonstrate via microscopy, the antibacterial properties of silver ion treated polyethylene, technically equivalent to Armstrong Medical AquaVENT® material. Significant differences between silver biocide treated and control material was observed, demonstrating comparable levels of efficacy to the ISO22196:2011 results.

It was reported during the imaging that although every attempt had been made to provide flat, burr free surfaces for imaging, due to the non-uniform surface of the sawn polyethylene square raised edges still caused problems in adherence of the coverslip to the plastics surface.

Feedback from the University of Birmingham's Technology Hub Imaging Core outlined technical challenges in imaging bacteria on material of this type. Dr. Shaw commented that in order to reliably and consistently image similar material significant time would need to be invested in developing a robust method, of both sample preparation of microscopy technique. It was suggested this could form the basis of an MSc research project.

Pseudomonas aeruginosa was the sole organism to adhere sufficiently to the polymer surface, allowing imaging. It is believed that after three hours of growth the organism had initiated biofilm formation, which aided or possibly allowed adherence to the necessary levels for visualization. Both enteropathogenic *E. coli* and *Salmonella* strains used in this work failed to adhere sufficiently for imaging.

To achieve good a representative image a balance was sought between contact or incubation time of test organism and substrate, and the desire to demonstrate a timely bactericidal action. The three hour incubation time of the test organism and material has presumably allowed *P. aeruginosa* time to adhere (possibly via the formation of a biofilm), prior to accumulating sufficient cellular damage via the biocidal action of silver, resulting in the death of the cell and accumulating propidium iodide stain.

The required balance of adherence, growth and required time for biocidal effect should be taken into account when considering the required times to visualize dead cells on treated material. BioCote has demonstrated that significant effects can be observed within 15 minutes (approximately 80%) with reductions of approximately 99.5% observed after 2 hours under laboratory conditions.

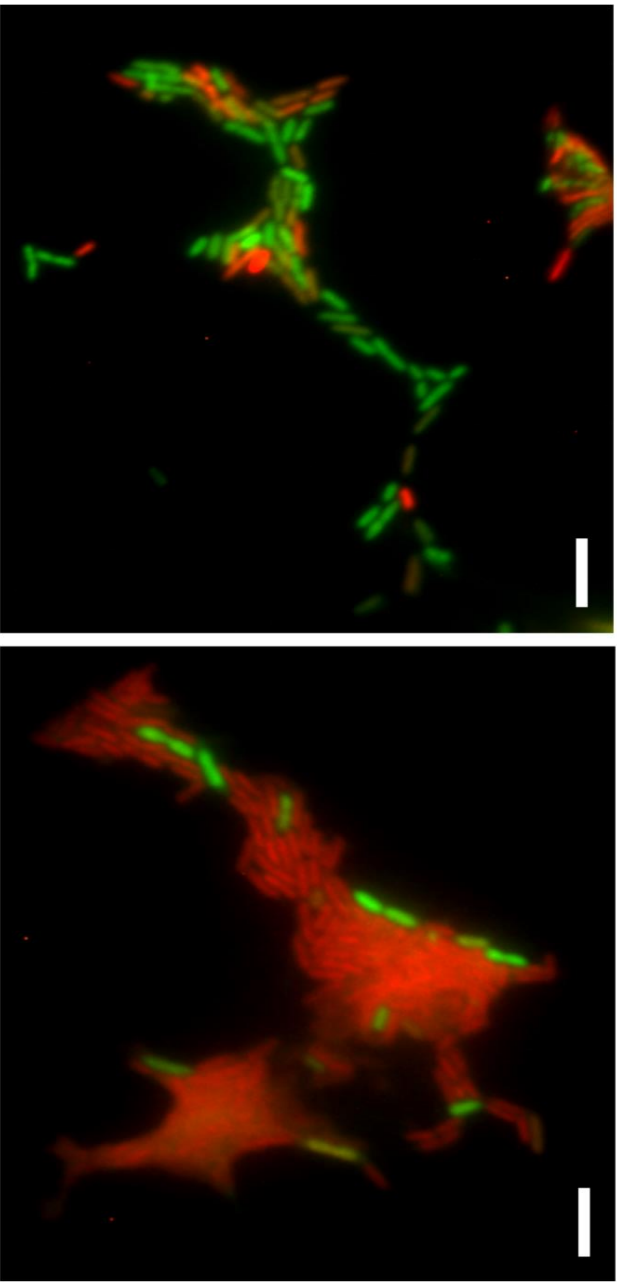


Figure 1. *Pseudomonas aeruginosa* imaged on a Leica DMRE widefield epi-flourescence microscope with a 100x plan apo oil immersion objective. Solution B was used from the Molecular probes kit L7007 at 1.50 dilution, the viable cells are stained green with 1.67mM Cyt9 and dead cells,(red), with 18.3mM propidium iodide. The left hand image is control material without biocide; approximately 57% of cells are red stained. The right hand image, silver ion biocide treated, displays approximately 93.7% dead cells (stained red).

References

Santo CE, Quaranta D and Grass G. Antimicrobial metallic copper surfaces kill *Staphylococcus haemolyticus* via membrane damage. *Microbiology open*. 2012 Mar, 1 (1) 46-52.

Huang HI, Shih HY, Lee CM, Yang TC, Lay JJ, Lin YE. In vitro efficacy of copper and silver ions in eradicating *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii*: implications for on-site disinfection for hospital infection control. *Water Res*. 2008 Jan;42(1-2):73-80. Epub 2007 Jul 12.

Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Che CM. Silver nanoparticles: partial oxidation and antibacterial activities. *J Biol Inorg Chem*. 2007 May;12(4):527-34. Epub 2007 Feb 16.

Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv*. 2009 Jan-Feb;27(1):76-83

Guggenbichler JP, Böswald M, Lugauer S, Krall T. A new technology of microdispersed silver in polyurethane induces antimicrobial activity in central venous catheters. *Infection*. 1999;27 Suppl 1:S16-23. Review.

C. Radheshkumar, & H. Münstedt. Antimicrobial polymers from polypropylene/silver composites—Ag⁺ release measured by anode stripping voltammetry. *Reactive & Functional Polymers*. 2006. Volume 66. p. 780-788.

Amber Nagy, Alistair Harrison, Supriya Sabbani, Robert S Munson, Jr, Prabir K Dutta, and W James Waldman. Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action. *Int J Nanomedicine*. 2011;6:1833-52.

Soliz M & Odermatt A. Copper and silver transport by CopB-ATPase in membrane vesicles of *Enterococcus hirae*. *J Biol Chem*. 1995 Apr 21;270(16):9217-21.

Mealman TD, Blackburn NJ, McEvoy MM. Metal export by CusCFBA, the periplasmic Cu(I)/Ag(I) transport system of *Escherichia coli*. *Curr Top Membr*. 2012;69:163-96

Klueh U, Wagner V, Kelly S, Johnson A, Bryers JD. Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. *J Biomed Mater Res*. 2000;53(6):621-31.

Izatt RM, Christensen JJ, Rytting JH. Sites and thermodynamic quantities associated with proton and metal ion interaction with ribonucleic acid, deoxyribonucleic acid, and their constituent bases, nucleosides, and nucleotides. *Chem Rev*. 1971 Oct;71(5):439-81

Rahn RO, Landry LC. Ultraviolet irradiation of nucleic acids complexed with heavy atoms. II. Phosphorescence and photodimerization of DNA complexed with Ag. *Photochem Photobiol*. 1973 Jul;18(1):29-38.

Thurman, R. B.; Gerba, C. P. The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. 1989. CRC Crit. Rev. Environ. Control 18:295-315.

Zavriev, S. K., L. E. Minchenkova, M. Vorlickova, A. M. Kolchinsky, M. V. Volkenstein, and V. I. Ivanov. 1979. Circular dichroism anisotropy of DNA with different modifications at N7 of guanine. Biochim. Biophys. Acta 564:212-224

Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH. Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. Appl Environ Microbiol. 2008 Apr;74(7):2171-8

Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. Lett Appl Microbiol. 1997 Oct;25(4):279-83.

Furr JR, Russell AD, Turner TD, Andrews A. Antibacterial activity of Actisorb Plus, Actisorb and silver nitrate. J Hosp Infect. 1994 Jul;27(3):201-8.

Fuhrmann GF, Rothstein A. The mechanism of the partial inhibition of fermentation in yeast by nickel ions. Biochim Biophys Acta. 1968 Nov 5;163(3):331-8.

Miller, L.P. and S.E.A McCallan. 1957. Toxic action of metal ions to fungus spores. Food Chem 5:116-122.

Rayman MK, Lo TC, Sanwal BD. Transport of succinate in *Escherichia coli*. II. Characteristics of uptake and energy coupling with transport in membrane preparations. J Biol Chem. 1972 Oct 10;247(19):6332-9.

Schreurs WJ, Rosenberg H. Effect of silver ions on transport and retention of phosphate by *Escherichia coli*. J Bacteriol. 1982 Oct;152(1):7-13.

Davies, R.L. and Etris S.F. The Development and Functions of Silver in Water Purification and Disease Control." Catalysis Today. 1997. Volume 36. p. 107-114.

Yamanaka M., Hara, K., Kudo, J. Bactericidal Actions of a Silver Ion Solution on *Escherichia coli*, Studied by Energy-Filtering Transmission Electron Microscopy and Proteomic Analysis. Applied and Environmental Microbiology. 2005. Volume 71, No. 11. p. 7589-7593.

Fuhrmann GF, Rothstein A. The mechanism of the partial inhibition of fermentation in yeast by nickel ions. Biochim Biophys Acta. 1968 Nov 5;163(3):331-8.

Miller, L.P. and S>E.A McCallan. 1957. Toxic action of metal ions to fungus spores. Food Chem 5:116-122.

Rayman MK, Lo TC, Sanwal BD. Transport of succinate in *Escherichia coli*. II. Characteristics of uptake and energy coupling with transport in membrane preparations. J Biol Chem. 1972 Oct 10;247(19):6332-9.

Schreurs WJ, Rosenberg H. Effect of silver ions on transport and retention of phosphate by *Escherichia coli*. J Bacteriol. 1982 Oct;152(1):7-13.

Davies, R.L. and Etris S.F. The Development and Functions of Silver in Water Purification and Disease Control. Catalysis Today. 1997. Volume 36. p. 107–114.

Yamanaka M., Hara, K., Kudo, J. Bactericidal Actions of a Silver Ion Solution on *Escherichia coli*, Studied by Energy-Filtering Transmission Electron Microscopy and Proteomic Analysis. Applied and Environmental Microbiology. 2005. Volume 71, No. 11. p. 7589-7593.

Morones-Ramirez JR, Winkler JA, Spina CS, Collins JJ. Silver enhances antibiotic activity against gram-negative bacteria. Sci Transl Med. 2013 Jun 19;5(190)



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