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Editorial

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We would like to wish all our readers "A very Happy & Prosperous New year".

Well to Jump start with this issue we have the 'Mini Review' section giving us a brief on **"Antibacterial Agents".** Each class of antibacterial agents has a unique mode of action that is the way in which an antibiotic affects the microbes at the cellular level. In this issue, we shall discuss other important cell-wall active agents which in addition play a vital role in defense mechanism of the immune system.

Our Current Trends section highlights about "Povidone iodine" 10% can be considered as a first-choice antiseptic for the prevention and treatment of superficial skin infections. The role of iodine in wound care is primarily as an antimicrobial agent. Povidone iodine has been used and tested in wound healing for many decades.

In Profile Scientist – **"Maurice Hilleman"** invented eight of the fourteen vaccines used in routine vaccination schedules today. In an unusually long and productive career in science he developed over 40 vaccines – an enormous number compared with more celebrated scientists, such as Pasteur, Sabin and Salk. In fact, Maurice Hilleman is the most prolific inventor of vaccines in history.

Bug of the month - Varicella-zoster virus (VZV) causes chickenpox and herpes zoster (shingles). Chickenpox follows initial exposure to the virus and is typically a relatively mild, self-limited childhood illness with a characteristic exanthem, but can become disseminated in immunocompromised children. Reactivation of the dormant virus results in the characteristic painful dermatomal rash of herpes zoster, which is often followed by pain in the distribution of the rash (postherpetic neuralgia).

Did You Know? That Bacterial Populations in Mother's GI Tract May Play a Central Role in Autism. In research on mice, the researchers found that the composition of bacterial populations in the mother's digestive tract can influence whether maternal infection leads to autistic-like behaviors in offspring.

Best Practices - Infection is the most common cause of hospitalization and the second most common cause of mortality among hemodialysis (HD) patients, after cardiovascular disease. HD patients as well as the dialysis staff are vulnerable to contracting health-care-associated infections (HAIs) due to frequent and prolonged exposure to many possible contaminants in the dialysis environment. The extracorporeal nature of the therapy, the associated common environmental conditions and the immune compromised status of HD patients are major predisposing factors. It is recommended to clean and disinfect the external surfaces of the HD machine after each dialysis session.

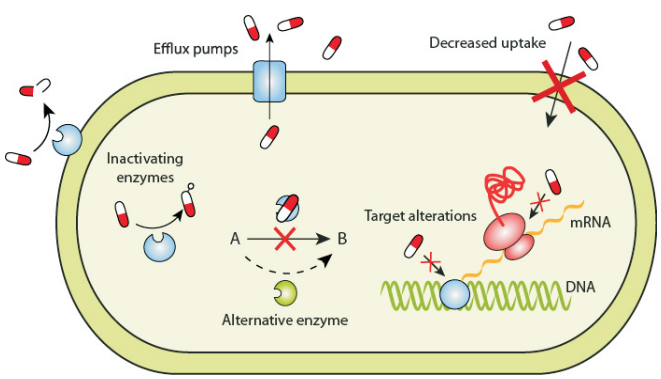
Our JHS team is thankful to all our readers for their immense support. Feedback & suggestions are always welcomed.

Common Antibacterial Agents Grouped by Mechanism of Activity (Issue 2)

Antimicrobial agents that target the cell membrane or bacterial cell wall have been used effectively for the past 70 years (Chambers, et al., 1998). In the previous article (issue1), we have discussed the agents and their mode of action on the bacterial cell-wall. This was further elaborated in the context of the antibiotics that inhibits the bacterial cell-wall synthesis, such as the β-lactam antibiotics that have emerged into broad spectrum agents that inhibit most pathogenic bacteria, which includes penicillins and cephalosporins.

In this current issue, we shall discuss other important cell-wall active agents which in addition play a vital role in defense mechanism of the immune system. The mechanism of their activity on the bacterial cell wall occurs in several ways such as inactivation of the cell wall binding enzymes, alterations of the cell-wall proteins, preventing the metabolic activities by decreased uptake or blocking the efflux pumps, and inhibition of the nucleic acid synthesis (Fig. 1).

Each class of antibacterial agents has a unique mode of action, that is the way in which an antibiotic affects the microbes at the cellular level, and these are summarized in the figure below:



Other Important Cell-Wall Active Agents

A diverse group of cell-wall active agents which can be grouped together as they all have a common mechanism of action are mostly referred as *Protein synthesis inhibitors*. The drug target and their mode of action of these different classes of antibiotics often differs, although some may overlap, however all affects the protein assembly at the ribosomal level (Chambers, et al., 1998).

Different classes of cell-wall active agents are as follows:

i.*Antibiotics that inhibit cell wall synthesis*
Vancomycin, bacitracin, cycloserine

ii.Antibiotics that alter cell membrane
Polymyxins, gramicidin

iii.Antibiotics that inhibit protein synthesis
Aminoglycosides (the only bactericidal class in this group), tetracyclines, chloramphenicol, macrolides and clindamycin

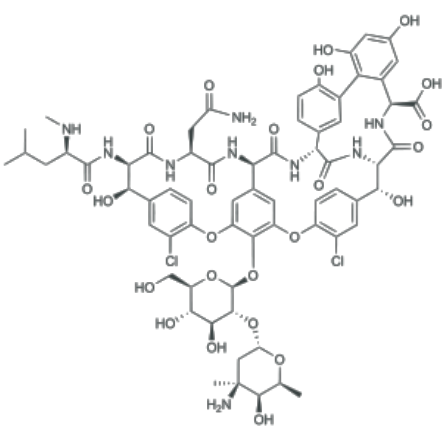
iv.Antibiotics that inhibit nucleic acid synthesis
Rifampin, Quinolones, Metronidazole

v.Antibiotics with anti-metabolic activity
Sulfonamides

I. Antibiotics that inhibit cell wall synthesis

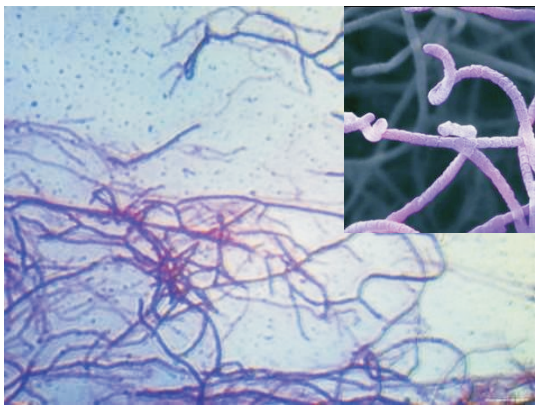
These antibiotics that inhibit cell wall synthesis are rarely the drugs of choice for either a particular infection or for a particular bacterial species; however, they continue to have an important role as antibacterial agents as they play an alternative for the hosts' defense mechanism.

Vancomycin is a glycopeptide. It is an antimicrobial agent that prevents the formation of the peptidoglycan by binding to the cell wall peptide precursors, that is, it prevents the peptidoglycan elongation and cross-linking of the bacterial cell wall.



Vancomycin is specifically bacteriocidal as it shows activity only against gram positive bacteria including ß-lactamase producing especially *staphylococci* and those resistant to nafcillin and methicillin (one exception is that it is active against *Flavobacterium).* This antibiotic elaborated by *Streptomyces orientalis*, an *actinomycete* found in soil samples from India and Indonesia.

Critical resistance to the antibacterial action of vancomycin is due to a modification of its peptidoglycan binding site, a modification that reduces binding affinity. Vancomycin kills only dividing cells and relatively slowly. Vancomycin acts synergistically with gentamicin and streptomycin (aminoglycosides) against *E. faecium* and *E. faecalis* isolates not resistant to aminoglycosides.



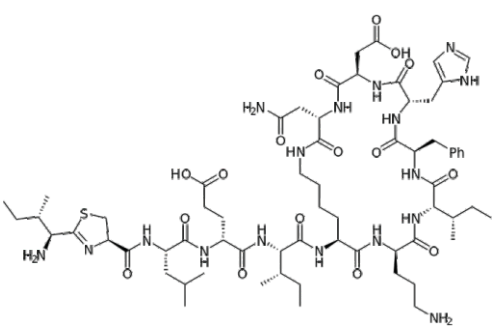
Streptomyces orientalis

Bacillus licheniformis

Vancomycin is poorly absorbed when administered orally; however, this property has proved to be useful for the treatment of gastrointestinal disease caused by *S.aureus* or *Clostridium difficile*. The drug is administrated intravenously for treatment of serious systemic infections in patients infected with methicillin-resistant *staphylococci* or with a history of allergic to the penicillins.

Major clinical uses of vancomycin are sepsis, endocarditis due to methicillin resistant *staphylococci.* Methicillin-susceptible *Staph* isolates would likely be more effectively treated with methicillin than vancomycin. Treatment alternative for enterococcal endocarditis is a mixture of vancomycin with gentamycin for patient allergic to penicillin. Vancomycin in combination with cefotaxime, ceftriaxone or rifampin has reported to be appropriate for treatment of meningitis when the suspected infecting agent is thought/known to be highly penicillin resistant.

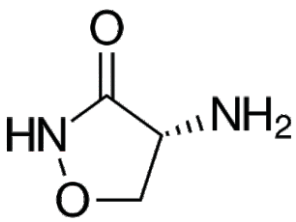
Bacitracin, which was isolated from *Bacillus licheniformis*, is a mixture of polypeptides used topically, for example: in creams, ointments, sprays, for skin infections caused by gram-positive bacteria, particularly, those caused by *Staphylococcus* and group A *Streptococcus*.



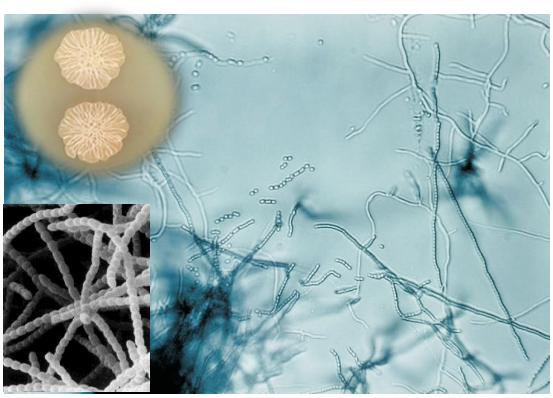
Bacitracin is a cyclic peptide mixture that is active against gram-positive microbes. Bacitracin inhibits cell wall formation by interfering with peptidoglycan transfer to the developing cell wall and exhibits no cross-resistance between bacitracin and other antimicrobials. Due to systemic toxicity, bacitracin is limited to topical use.

Major Clinical Use: Alone or in combination with polymyxin or neomycin: treatment of mixed skin, wound or mucous membrane infections.

Adverse Effects: Significant nephrotoxicity with systemic administration



Cycloserine, a structural analog of D-alanine, inhibits both Gram-positive and Gram-negative bacteria. Mechanism of action is inhibition of D-alanine incorporation into peptidoglycan by inhibiting alanine racemase (which converts L-alanine to D-alanine) and D-alanyl-D-analanine ligase. Cycloserine is similar in structure to the amino acid d-alanine and works by interfering with the formation of the bacteria's cell wall. Cycloserine, sold under the brand name '*Seromycin*', is an antibiotic used to treat tuberculosis (Gottlieb et al., 2012). Specifically it is used, along with other anti-tuberculosis medications, for active drug resistant tuberculosis.



Streptomyces venezuelae

Cycloserine works as an antibiotic by inhibiting cell-wall biosynthesis in bacteria (Prosser et al., 2013). As a cyclic analogue of D-alanine, cycloserine acts against two crucial enzymes important in the cytosolic

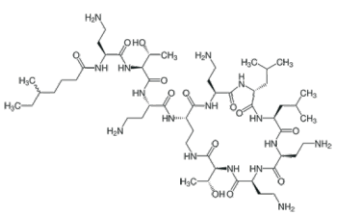
stages of peptidoglycan synthesis: alanine racemase (Alr) and D-alanine:D-alanine ligase (Ddl). The first enzyme is a pyridoxal 5'-phosphate-dependent enzyme which converts the L-alanine to the D-alanine form. The second enzyme is involved in joining two of these D-alanine residues together by catalyzing the formation of the ATP-dependent D-alanine-D-alanine dipeptide bond between the resulting D-alanine molecules. If both of these enzymes are inhibited, then D-alanine residues cannot form and previously formed D-alanine molecules cannot be joined together. This effectively leads to inhibition of peptidoglycan synthesis (Prosser et al., 2013).

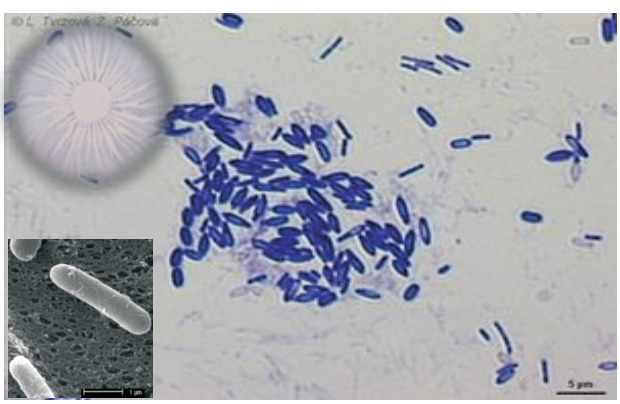
Major Clinical Use: Used almost exclusively for treating tuberculosis caused by *M. tuberculosis* isolates resistant to primary drugs.

Adverse Effects: CNS toxicity at higher than clinical doses.

II. Antibiotics that alter cell membrane

Polymyxins, are basic peptides (derived from *Bacillus polymyxa*) that act as cationic detergents to cause lysis of the lipoprotein in the cell membrane. Polymixins (polymixin E) are amphipathic (containing lipophilic and lipophobic groups) basic peptides which exhibit activity against gram-negative bacteria.

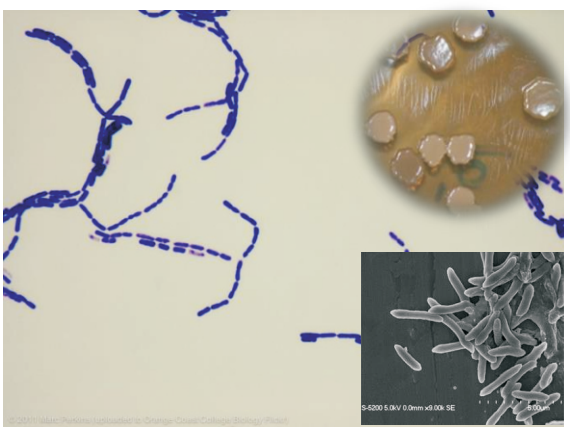




Bacillus polymyxa

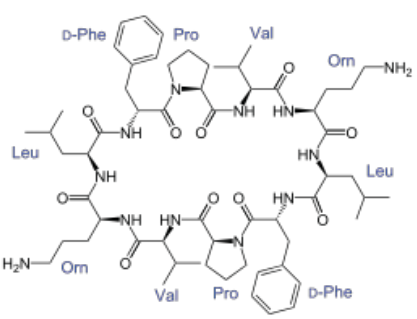
Although polymyxin B and colistin are active against the gram-negative bacteria (including *Pseudomonas* species), serious nephrotoxicity has limited their internal use. They are used chiefly to treat local infections such as external otitis, eye infections, and skin infections with sensitive organisms. They are bacteriocidal for many gram-negative rods including *Pseudomonas*. Polymyxins disrupt the bacterial cell membranes through strong interactions with phospholipid components. Gram-positive bacteria, *Proteus*, *Neisseria* are resistant to polymyxins. Polymyxin B sulfate used topically for treatment of external otitis and corneal ulcers due to *Pseudomonas aeruginosa*. Systemic use of polymyxins is not recommended because of poor tissue distribution, significant nephrotoxicity and neurotoxicity and also due to the availability of more effective other antibacterial drugs. Polymyxin E is active against *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter*, *Klebsiella*.

Clinical Applications of Polymyxin B: Skin, mucous membrane, eye and ear infections (for sensitive organism), external otitis (*Pseudomonas*) or corneal ulcers (*Pseudomonas aeruginosa*). Sometimes used by aerosol as an adjunct to other antibiotics in difficult cases of *Pseudomonas pneumonia*.



Bacillus brevis

Gramicidin is a heterogeneous mixture of three antibiotic compounds, Gramicidin A, B and C, making up 80%, 6%, and 14%, respectively, all of which are obtained from the soil bacterial species *Bacillus brevis* and collectively called as Gramicidin D.



Gramicidin D contains linear penta-deca-peptides, that is chains made up of 15 amino acids. It is a peptide antibiotic which alters membrane permeability-effective against gram-positive organisms. Gramicidin may be used in combination with neomycin, polymyxin B or both. It is available only for topical usage.

Gramicidin is active against: *Streptococci, Pneumococci, Staphylococci,* most anaerobic cocci *Neisseriae, Tetanus bacilli,* and *Diphtheria bacilli.*

Gramicidin is active against Gram-positive bacteria, except for the Gram-positive bacilli, and against select

Gram-negative organisms, such as *Neisseria* bacteria. Gramicidin's bactericidal activity is a result of increasing the permeability of the bacterial cell membrane, allowing inorganic monovalent cations (e.g. Na+) to travel through unrestricted and thereby destroying the ion gradient between the cytoplasm and the extracellular environment.

Its therapeutic use is limited to topical application, as it induces hemolysis in lower concentrations than bacteria cell death, so it cannot be administered internally. Since the exterior epidermis is composed of dead cells, applying it to the surface of the skin will not cause harm. It is used primarily as a topical antibiotic and is one of the three constituents of consumer antibiotic polysporin ophthalmic solution.

In general, gramicidin channels are completely selective for monovalent cations and the single-channel conductance for the alkali cations are ranked in the same order as the aqueous mobility of these ions. Divalent cations like Ca^{2+} block the channel by binding near its mouth so it is essentially impermeable to divalent cations and also excludes anions. Cl^- in particular is excluded from the channel because its hydration shell is thermodynamically stronger than that of most monovalent cations. The channel is permeable to most monovalent cations, which move through the channel in single file. The channel is filled with about six water molecules, almost all of which must be displaced when an ion is transported. Thus, ions moving through the gramicidin pore carry along a single file of water molecules. Such a flux of ion and water molecules is known as flux coupling. In the presence of a second type of permeable ion, the two ions couple their flux, as well. Like valinomycin and nonactin, the gramicidin channel is selective for potassium over sodium, but only slightly so. It has a permeability ratio of 2.9. Though it is impermeable to anions, conditions exist under which some anion permeation may be observed. Its ability to bind and transport cations is due to the presence of cation-binding sites, one strong and the other weak, in the channel.

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Povidone Iodine and its resistance overtime

Povidone iodine 10% can be considered as a first-choice antiseptic for the prevention and treatment of superficial skin infections.

Small molecules (e.g., diiodine, also referred to as 'free iodine', from povidone iodine) readily penetrate bacterial membrane channels (porins) and cause oxidation of proteins within the bacterial cytoplasm.

In the case of povidone iodine, diiodine is released gradually from a neutral polymer base (polyvinylpyrrolidone), and subsequent microbial membrane penetration of free iodine and intracytoplasmic protein oxidation cannot be stopped. Thus, povidone iodine has a particularly broad spectrum of antimicrobial activity and a lack of chromosome- or plasmid-mediated bacterial resistance.However, povidone iodine has variable activity against Actinobacteria (e.g., Corynebacterium spp., Mycobacterium spp. and Nocardia spp., among others), since thesemicroorganisms have cell walls with a high mycolic acid content, which makes it difficult for free iodine to penetrate.

In vitro, in the absence of organic stress, the antimicrobial action of povidone iodine is usually rapid (i.e., within 30 s) .Povidone iodine scrub 4 and 7.5%, when tested at four different exposure times (0.25, 0.5, 2.5 and 5 min), has also demonstrated virucidal activity against porcine influenza H1N1 virus in the presence or absence of interfering proteins (fetal calf serum); thus, at an exposure time of 15 s, povidone iodine scrub 4% reduced viral titer by $\geq$4.64–4.65 log10; the corresponding decrease in viral titer with povidone iodine scrub 7.5% was $\geq$ 4.43–4.64 log10.Moreover, several comparative studies have shown that, irrespective of exposure time or dilution, povidone iodine 10% is considerably more effective than chlorhexidine against methicillin-resistant Staphylococcus aureus (MRSA). Also, based on currently available literature, povidone iodine appears to be the only antiseptic with demonstrated activity against dermatophyte fungal infections (e.g., caused by species in the Microsporum orTrichophyton genera)

Povidone iodine, for instance, is inactivated to a lesser degree than chlorhexidine, since the iodophor reacts weakly with proteins.

When are iodine dressings contraindicated? Iodine dressings must be used under medical supervision in patients with thyroid diseases, known or suspected iodine sensitivity, in pregnant or breastfeeding women or in newborn babies and up to the age of six months. Long-term use of PVP-I has been loosely associated with mild hyperthyroidism and long-term use is not recommended for patients with impaired thyroid function. However, a number of studies have monitored thyroid function during PVP-I clinical trials and have reported that it remains unchanged. To avoid toxicity or the hypothetical risk of thyroid-related complications, iodine products should be used with caution in children, in those with large burn areas, and where prolonged treatment of large open wounds is required. The use of iodine dressings should also be avoided before and after the use of radio-iodine diagnostic tests (until permanent healing). Reports of systemic effects following short-term PVP-I treatment are extremely rare. Iodine absorption has been found to be dependent on the size of the wound and the duration of treatment. Hunt et al31 also discovered a relationship between wound area and iodine levels in serum and urine following the treatment of burn wounds with PVP-I, but it was proposed that renal function was a factor in the determination of this. Iodine should, therefore be avoided in patients with significant renal disease.

Povidone-iodine stock solution is 10%, comprising 90% water, 8.5% povidone-iodine, 1% available iodine, and iodide. Previous studies have shown that 5% povidone-iodine effectively decreases the bacterial flora of the ocular surface and adnexae, and thus theoretically decreases the risk of endophthalmitis, while other large studies have demonstrated 5% povidone-iodine to directly decrease the incidence of endophthalmitis.

Higher povidone-iodine concentrations and longer exposure times were more effective than lower povidone-iodine concentrations or shorter exposure in preventing growth of bacterial isolates.

Data on povidone iodine safety

Data on the systemic absorption of antiseptics are scant. Iodine seems to be absorbed from the skin, but more so from mucosa . However, the condition of the skin barrier will determine transdermal iodine absorption. The absorption will be increased if the skin barrier is broken as in wounds and also dependent on skin age and surface area of application (Fig. 2). Safety information in povidone iodine product labeling includes general warnings against use in patients allergic to povidone iodine or excipients, thyroid disorders, in very low birth weight infants, and in patients receiving radio-iodine therapy .

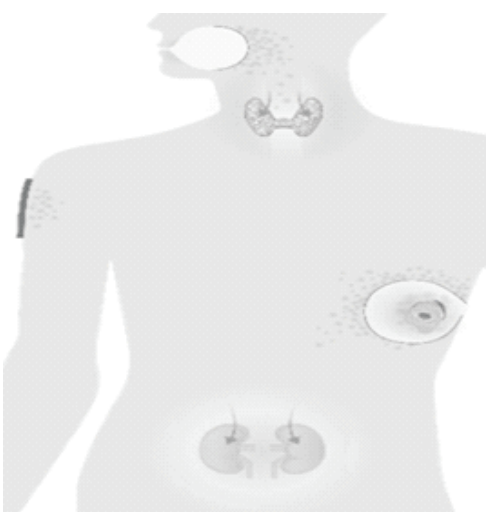

Fig. 2. Schematic representation of iodine pharmacokinetics after application of povidone.

Absorption: Depends on site the site and area of application, with an intact stratum corneum being the major barrier (thus higher absorption from mucosa and wounds).

Metabolism: Iodine is rapidly converted into iodide. Iodide is

incorporated into thyroxine (thyroid produces approx.100 µg/dL).

Excretion: 97% are excreted renally with a half-life of 2 days

Povidone iodine

The role of iodine in wound care is primarily as an antimicrobial agent. Povidone iodine has been used and tested in wound healing for many decades. In povidone iodine, iodine forms a complex with the synthetic carrier polymerpovidone, which itself has no microbicidal activity. In an aqueous medium, free iodine is released into solution from the povidone iodine complex and an equilibrium is established, with more free iodine being released from the povidone iodine reservoir as iodine-consuming germicidal activity proceeds (Fig. 1).

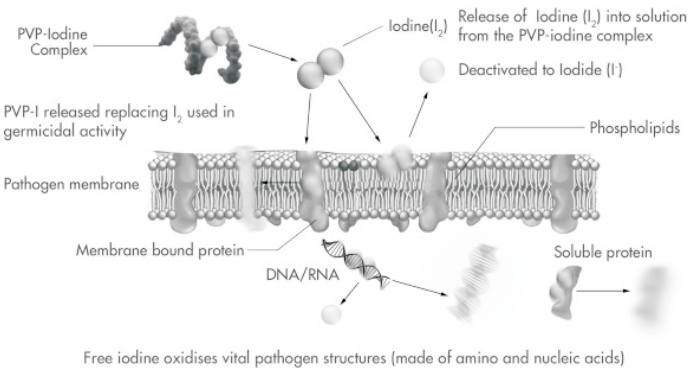

Fig. 1. Mechanism of action of povidone iodine in equilibrium with free iodine.

The active moiety is iodine, oxidising pathogen nucleotides and fatty/amino acids and thus deactivates proteins as well as DNA/RNA.

The formulation-, concentration- and temperature-dependent equilibrium of povidone-bound iodine to free iodine serves to minimize safety and tolerability issues associated with skin exposure to earlier elemental iodine formulations, and appears to protect against inhibition of granulation tissue formation.

Mode of action

The microbicidal activity of iodine appears to involve the inhibition of vital bacterial cellular mechanisms and structures, and oxidizes nucleotidesfatty/amino acids in bacterial cell membranes, in addition to cytosolic enzymes involved in the respiratory chain, causing them to become denatured and deactivated (Fig.1). However, the precise sequence of events occurring at the molecular level has yet to be fully elucidated. Cytotoxicity studies have shown that the bactericidal effect occurs even before individual human cells are affected.

In vitro evidence suggests that iodine not only has broad spectrum antibacterialeffects, but also counteracts inflammation elicited by both pathogens and the host response .

"Free" iodine contributes to the bactericidal activity of iodophors and dilutions of iodophors demonstrate more rapid bactericidal action than does a full-strength povidone-iodine solution. The reason for the observation that dilution increases bactericidal activity is unclear, but dilution of povidone-iodine might weaken the iodine linkage to the carrier polymer with an accompanying increase of free iodine in solution. Therefore, iodophors must be diluted according to the manufacturers' directions to achieve antimicrobial activity.

Mode of Action. Iodine can penetrate the cell wall of microorganisms quickly, and the lethal effects are believed to result from disruption of protein and nucleic acid structure and synthesis.

Microbicidal Activity. Published reports on the in vitro antimicrobial efficacy of iodophors demonstrate that iodophors are bactericidal, mycobactericidal, and virucidal but can require prolonged contact times to kill certain fungi and bacterial spores. Three brands of povidone-iodine solution have demonstrated more rapid kill (seconds to minutes) of *S. aureus* and *M. chelonae*at a 1:100 dilution than did the stock solution 683. The virucidal activity of 75–150 ppm available iodine was demonstrated against seven viruses. Other investigators have questioned the efficacy of iodophors against poliovirus in the presence of organic matter 685and rotavirus SA-11 in distilled or tapwater. Manufacturers' data demonstrate that commercial iodophors are not sporicidal, but they are tuberculocidal, fungicidal, virucidal, and bactericidal at their recommended use-dilution.

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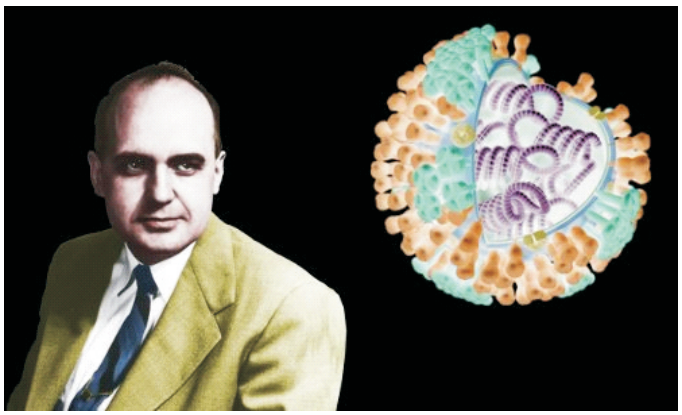
Maurice Hilleman

Lived 1919 – 2005



Maurice Hilleman invented eight of the fourteen vaccines used in routine vaccination schedules today. In an unusually long and productive career in science he developed over 40 vaccines – an enormous number compared with more celebrated scientists, such as Pasteur, Sabin and Salk. In fact, Maurice Hilleman is the most prolific inventor of vaccines in history.

Countless millions of people owe their lives to his work. An even greater number of people have been spared permanent disabilities such as blindness and deafness. His vaccines may account for as many as eight million lives saved every year.

Maurice Hilleman's Contributions to Science

Vaccines

Maurice Hilleman was the greatest inventor of vaccines the world has ever known. His vaccines were based on the idea, used by earlier scientists such as Louis Pasteur, that you could take a virus and weaken it. The virus would then be too weak to cause disease, but would push people's immune systems into producing natural antibodies. These antibodies would give people natural immunity to the full-strength, disease-causing virus.

Hilleman's First Vaccine

Hilleman joined the pharmaceutical company E. R. Squibb in New Jersey in 1944. There he developed an effective vaccine against Japanese B encephalitis. He also worked on the mass production of influenza vaccine.

Preventing an Influenza Pandemic

In 1948, aged 28, Hilleman moved to Washington, D.C. to join the Department of Respiratory Diseases at Army Medical Center.

There he became an authority on mutation in influenza viruses, observing two different mechanisms for genetic changes in influenza: *drift*, a gradual annual change in the virus; and *shift*, a less frequent but more dramatic change in the virus. If shift happened in a highly virulent variant of the virus, deadly pandemics could result.

In 1957 Hilleman was the first person to identify that a new strain of influenza first seen in Hong Kong had the potential to cause millions of deaths worldwide. He mass-produced a vaccine in just four months and 40 million doses of it were distributed around the USA. The resulting large-scale vaccination program disrupted the spread of the virus and limited the number of American deaths to 69,000 – many fewer than would have taken place in the absence of the vaccine. Hilleman was awarded the Distinguished Service Medal for his work.

Dozens of New Vaccines

In 1957, aged 38, Hilleman was recruited by the pharmaceutical company Merck & Co. He moved to West Point, Pennsylvania, from where he would lead Merck's virus and vaccination research programs for the next 45 years.

At Merck he had unprecedented success, inventing a series of highly effect new vaccines to protect people against measles, mumps, rubella, chickenpox, hepatitis B, meningitis, and pneumonia.

He never missed an opportunity to develop a new vaccine. In 1963 his daughter Jeryl Lynn got mumps. Hilleman promptly swabbed her throat for a sample of the virus. He isolated the virus and developed a vaccine against it. This vaccine, licensed in 1967, is part of the standard MMR vaccine used today. The MMR vaccine was also Hilleman's creation.

New Discoveries

Hilleman was the discoverer or co-discoverer of a number of viruses. These included hepatitis A, SV40, and a number of adenoviruses and rhinoviruses.

He was the first scientist to purify the drug interferon and he discovered that interferon's expression is induced by double-stranded RNA.

Some Personal Details and the End

In 1943, aged 23, Hilleman married Thelma Mason with whom he had two daughters, Jeryl Lynn and Kirsten. Thelma died in 1962. In 1963 Hilleman married Lorraine Witmer.

Hilleman had a reputation as a tough man and at times ill-tempered. He worked seven-day weeks and expected people he directed at Merck to do likewise. Anyone who didn't measure up to his expectations or the tough discipline he imposed on workers was quickly fired.

At one time in his career he kept a display in his office of models of shrunken heads of the workers he had fired.

His behavior as a manager was unusual but his research programs were amazingly productive, and he was idolized by the workers who had lived up to his expectations.

Hilleman retired from Merck at the age of 65 as required by company policy, but the company rehired him to continue as a 'consultant' rather than employee. He worked for a further 20 years as director of the Merck Institute for Vaccinology.

In 1988, aged 68, Hilleman received America's highest award for science – the National Medal of Science – presented by President Ronald Reagan, adding to many other awards Hilleman had received.

An inveterate workaholic, Hilleman continued working until his death.

Maurice Hilleman died of cancer at the age of 85 on April 11, 2005 in Philadelphia. He was buried near his home in Chestnut Hill, Pennsylvania.

He was survived by his second wife, Lorraine, his two daughters, Jeryl Lynn and Kirsten, and five grandchildren.

Microbiology Quiz

1. **Which of the following is not the characteristic of a growth curve?**
   A. Shows development of microbial population under relatively stable environmental conditions
   B. Plotted with logarithmic numbers
   C. Graphs numbers of microbes versus time
   D. Each growth curve consists of four distinct phases

2. **Generation time of Escherichia coli is**
   A. 20 minutes
   B. 20 hours
   C. 20 days
   D. 200 hours

3. **The organism which obtain their energy from chemicals are designated as**
   A. prototrophs
   B. chemotrophs
   C. organotrophs
   D. autotrophs

4. **The organism which grows best above 45°C are called**
   A. psychrophilic
   B. mesosphilic
   C. thermophilic
   D. any of these

5. **Lag phase is also known as**
   A. period of initial adjustment
   B. transitional period
   C. generation time
   D. none of these

6. **Which of the following is used to grow bacterial cultures continuously?**
   A. Chemostat
   B. Coulter Counter
   C. Hemostat
   D. Petroff-Hausser chamber

7. **Some organisms can use reduced inorganic compounds as electron donors and are termed as**
   A. lithotrophs
   B. phototrophs
   C. chemotrophs
   D. photo-organotrophs

8. **The growth is normally expressed as _____ in turbidimetric measurement**
   A. cells per ml
   B. cfu/ml
   C. optical density
   D. mg N2 /ml

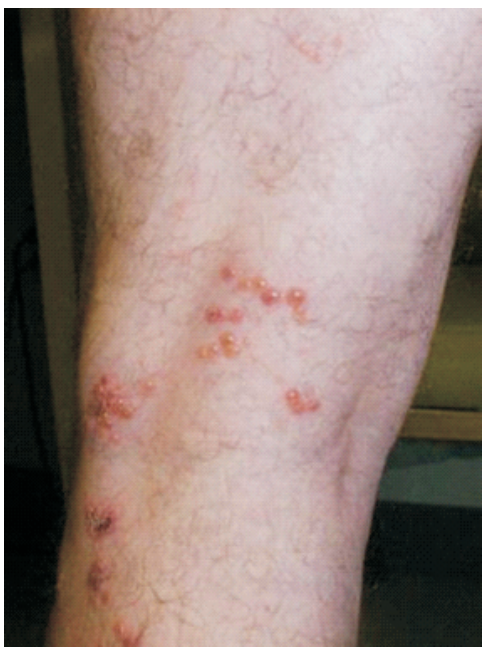
9. **Which of the following organisms typically get their carbon for biosynthesis from carbon dioxide?**
   A. Glucose-fermenting bacteria (fermentation)
   B. Anaerobic, glucose-respiring bacteria (anaerobic respiration)
   C. Aerobic, glucose-respiring bacteria (aerobic respiration)
   D. Ammonia-oxidizing bacteria (chemolithotrophic bacteria)

10. **The straightforward method of binary fission explains how bacteria**
    A. grow in nutrient agar
    B. evolve
    C. move
    D. reproduce

Answers:
1. D, 2. A, 3. B, 4. C, 5. A, 6. A, 7. A, 8. C, 9. D, 10. D.

Varicella zoster

Varicella-zoster virus (VZV) causes chickenpox and herpes zoster (shingles). Chickenpox follows initial exposure to the virus and is typically a relatively mild, self-limited childhood illness with a characteristic exanthem, but can become disseminated in immunocompromised children. Reactivation of the dormant virus results in the characteristic painful dermatomal rash of herpes zoster, which is often followed by pain in the distribution of the rash (postherpetic neuralgia). See the image below.



Typical zoster in the vicinity of right popliteal fossa in a vertebral nerve L4 distribution.

Signs and symptoms:

Pain and paresthesia are typically the first symptoms of VZV infection. Until the characteristic vesicular rash erupts, diagnosis may be difficult. A prodromal period during which symptoms may vary is common. Pain occurs in 41% of patients, itching in 27%, and paresthesias in 12%.

During the acute illness, patients may experience the following:

? Pain (90%)

? Helplessness and depression (20%)

? Flulike symptoms (12%)

Herpes zoster (shingles)

? The most common presentation is the shingles vesicular rash, which most commonly affects a thoracic dermatome

? After a prodromal illness of pain and paresthesias, erythematous macules and papules develop and progress to vesicles within 24 hours

? The vesicles eventually crust and resolve

? Pain and sensory loss are the usual symptoms

? Motor weakness also occurs and is frequently missed on examination

? Cases of actual monoplegia due to VZV brachial plexus neuritis have been reported

Zoster multiplex

? Shingles may appear in multiple dermatomes, both contiguous and noncontiguous, on either side of the body

? Immunocompromised individuals are more susceptible

? Terminology depends on the number of involved dermatomes and on whether the condition is unilateral or bilateral (eg, zoster duplex unilateralis refers to the involvement of 2 unilateral dermatomes)

? Cases of zoster simultaneously occurring in 7 noncontiguous dermatomes have been reported

Zoster sine herpete

VZV infection may reactivate without causing cutaneous vesicles. These patients have severe dermatomal pain, possible motor weakness and possible hypesthesia, but no visible rash or vesicles.

VZV infection may present as acute peripheral facial palsy in 8-25% of patients who have no cutaneous vesicles. This is more common in immunosuppressed patients who use acyclovir (or other agents) as zoster prophylaxis. [1]

Central nervous system deficits

? More common in immunocompromised individuals, but do occur in the general population

? CNS involvement may become apparent 3 weeks after the onset of the initial rash

? The manifestations are usually bilateral

? The physical findings may progress

? The underlying pathology typically progresses for 3 or more weeks

? Progression for 6 months in immunocompromised individuals has been reported

? Recurrence is rare but has been reported

? Zoster encephalitis is also rare but is reported in otherwise healthy individuals

Ramsay-Hunt syndrome

This syndrome occurs when the geniculate ganglion is involved. The clinical presentation includes the following:

? A peripheral facial palsy

? Pain in the ear and face

? Vesicles in the external ear canal (not always present)

? Additional auditory and vestibular symptoms in some cases

Bacterial Populations in Mother's GI Tract May Play a Central Role in Autism

Two new studies from MIT and the University of Massachusetts Medical School reveal that mothers who experience an infection severe enough to require hospitalization during pregnancy are at higher risk of having a child with autism. These new studies shed more light on this phenomenon and identify possible approaches to preventing it.

In research on mice, the researchers found that the composition of bacterial populations in the mother's digestive tract can influence whether maternal infection leads to autistic-like behaviors in offspring. They also discovered the specific brain changes that produce these behaviors.

"We identified a very discrete brain region that seems to be modulating all the behaviors associated with this particular model of neuro developmental disorder," says Gloria Choi, the Samuel A. Goldblith Career Development Assistant Professor of Brain and Cognitive Sciences and a member of MIT's McGovern Institute for Brain Research.

If further validated in human studies, the findings could offer a possible way to reduce the risk of autism, which would involve blocking the function of certain strains of bacteria found in the maternal gut, the researchers say.

Choi and Jun Huh, formerly an assistant professor at UMass Medical School who is now a faculty member at Harvard Medical School, are the senior authors of both papers, which appear in *Nature* on September 13. MIT postdoc Yeong Shin Yim is the first author of one paper, and UMass Medical School visiting scholars Sangdoo Kim and Hyunju Kim are the lead authors of the other.

Reversing symptoms

A 2010 study that included all children born in Denmark between 1980 and 2005 found that severe viral infections during the first trimester of pregnancy translated to a threefold risk for autism, and serious bacterial infections during the second trimester were linked with a 1.42-fold increase in risk. These infections included influenza, viral gastroenteritis, and severe urinary tract infections. Similar effects have been described in mouse models of maternal inflammation, and in a 2016 *Science* paper, Choi and Huh found that a type of immune cells known as Th17 cells, and their effector molecule, called IL-17, are responsible for this effect in mice. IL-17 then interacts with receptors found on brain cells in the developing fetus, leading to irregularities that the researchers call "patches" in certain parts of the cortex.

In one of the new papers, the researchers set out to learn more about these patches and to determine if they were responsible for the behavioral abnormalities seen in those mice, which include repetitive behavior and impaired sociability.

The researchers found that the patches are most common in a part of the brain known as S1DZ. Part of the somatosensory cortex, this region is believed to be responsible for proprioception, or sensing where the body is in space. In these patches, populations of cells called interneurons, which express a protein called parvalbumin, are reduced. Interneurons are responsible for controlling the balance of excitation and inhibition in the brain, and the researchers found that the changes they found in the cortical patches were associated with overexcitement in S1DZ.

When the researchers restored normal levels of brain activity in this area, they were able to reverse the behavioral abnormalities. They were also able to induce the behaviors in otherwise normal mice by overstimulating neurons in S1DZ.

The researchers also discovered that S1DZ sends messages to two other brain regions: the temporal association area of the cortex and the striatum. When the researchers inhibited the neurons connected to the temporal association area, they were able to reverse the sociability deficits. When they inhibited the neurons connected to the striatum, they were able to halt the repetitive behaviors.

Microbial factors

In the second *Nature* paper, the researchers delved into some of the additional factors that influence whether or not a severe infection leads to autism. Not all mothers who experience severe infection end up having child with autism, and similarly not all the mice in the maternal inflammation model develop behavioral abnormalities.

"This suggests that inflammation during pregnancy is just one of the factors. It needs to work with additional factors to lead all the way to that outcome," Choi says.

A key clue was that when immune systems in some of the pregnant mice were stimulated, they began producing IL-17 within a day. "Normally it takes three to five days, because IL-17 is produced by specialized immune cells and they require time to differentiate," Huh says. "We thought that perhaps this cytokine is being produced not from differentiating immune cells, but rather from pre-existing immune cells."

Previous studies in mice and humans have found populations of Th17 cells in the intestines of healthy individuals. These cells, which help to protect the host from harmful microbes, are thought to be produced after exposure to particular types of harmless bacteria that associate with the epithelium.

The researchers found that only the offspring of mice with one specific type of harmless bacteria, known as segmented filamentous bacteria, had behavioral abnormalities and cortical patches. When the researchers killed those bacteria with antibiotics, the mice produced normal offspring.

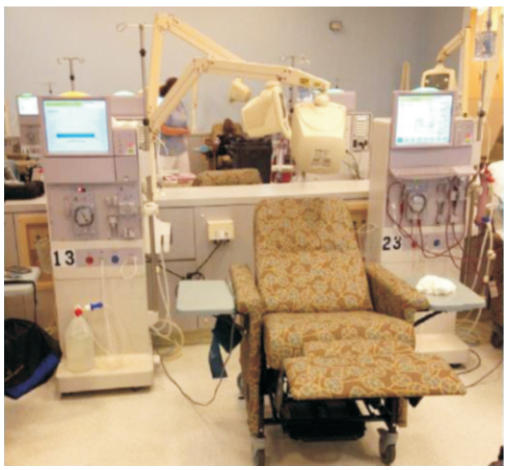
"This data strongly suggests that perhaps certain mothers who happen to carry these types of Th17 cell-inducing bacteria in their gut may be susceptible to this inflammation-induced condition," Huh says.

Humans can also carry strains of gut bacteria known to drive production of Th17 cells, and the researchers plan to investigate whether the presence of these bacteria is associated with autism.

Sarah Gaffen, a professor of rheumatology and clinical immunology at the University of Pittsburgh, says the study clearly demonstrates the link between IL-17 and the neurological effects seen in the mice offspring. "It's rare for things to fit into such a clear model, where you can identify a single molecule that does what you predicted," says Gaffen, who was not involved in the study.

The research was funded by the Simons Foundation Autism Research Initiative, the Simons Center for the Social Brain at MIT, the Howard Hughes Medical Institute, Robert Buxton, the National Research Foundation of Korea, the Searle Scholars Program, a Pew Scholarship for Biomedical Sciences, the Kenneth Rainin Foundation, the National Institutes of Health, and the Hock E. Tan and K. Lisa Yang Center for Autism Research.

Best practices in dialysis unit disinfection



CDC Approach to BSI Prevention in Dialysis Facilities (i.e., the core Interventions for Dialysis Bloodstream infection (BSI) Prevention)

1. Surveillance & Feedback
2. Hand hygiene & glove use observations
3. Catheter and vascular access observations
4. Patient education & engagement
5. Staff education & competency
6. Catheter reduction
7. CHG/alcohol for skin antisepsis
8. Catheter hub cleansing (aka scrub-the-hub)
9. Catheter exit site care: antimicrobial ointment or disk
10. Environmental surface disinfection

Common Themes from Outbreaks

- Patient overlaps in space and time (i.e., transmission from)
- One patient to the next at the same station
- One patient to another at adjacent stations
- Breaches in medication preparation and administration practices
- Preparing medications in potentially contaminated areas
- Mobile medication carts
- Not wiping injection ports prior to accessing
- Breaches in environmental cleaning and disinfection practices
- Surfaces wiped down with patient still at station
- Rushed turnover processes

Recommendations for disinfection of the HD machine:

- After an episode of blood leak in to the dialysate.
- If surveillance cultures show high cfu or endotoxin levels
- Regular disinfection at least once a week.
- After each dialysis session or once a day (optional).
- Bleach or Citrosteril (Combination of Citric, maleic and oxalic acid) or heat or a combination may be used for disinfection of HD machine

Disinfection of the HD machine is mandatory to prevent transmission of infections between patients. The disinfection of the machine may be performed using either bleach or citrosteril or heat. Disinfection with bleach is recommended after each blood leak in to the dialysate or at a regular interval of at least one week. Disinfection with citrosteril may be performed after each dialysis session or at least once daily. The steps for sterilization are detailed below. With standard disinfectant fitted to the rear of the machine, bleach must be administered via the pickup stick (Concentrate connectors) at the front of the machine. The disinfection procedure is performed by the designated personnel in the dialysis unit. Gloves and protective glasses must be worn during the procedure by the operator.

Steps of bleach disinfection:

1. Bleach (Sodium hypochlorite 5%) is used for disinfecting the machine.
2. Bleach should not be heated.
3. If bleach disinfection is required for the blood leak, rinse machine for 15 minutes.
4. Ensure that power and water supply to the machine are operational.
5. Turn on the machines.
6. Press cleaning key.
7. Use up/down arrow keys to select "Cleaning (font supplied)"
8. Select Treatment/Rinse – select chemical mode – confirm.
9. Machine alarm "! Connect disinfectant" displayed.
10. Place PICKUP STICK Concentrate connectors into sodium hypochlorite at the front of the machine.
11. Press conf key, "Please Wait" displayed.
12. On completion "Mandatory rinse end" displayed.
13. Test for residual bleach using Chlorine test strips on completion of cycle.

Steps of citrosteril disinfection:

1. Following conditions/reminder must be fulfilled before activating the cleaning program:
   a. The dialysate lines are connected to the shunt (Rinse bridge).
   b. The shunt door is closed.
   c. The concentrate suction tubes are in the appropriate rinse ports.
   d. The interlock plate of the bigbag® connector (option) is closed.
   e. The optical detector does not sense blood.
2. Citrosteril should be fitted to the rear of the machine
3. Citrosteril should be heated ($\geq$60oC) for efficient results.
4. Ensure that power and water supply to the machine are operational.
5. Turn on the machines.
6. Ensure the basic conditions/reminder (mentioned above) has been reviewed.
7. Press cleaning key.
8. Use up/down key to select the desired program- "Hot Disinfection".
9. Press conf key, "-F-HDIS- or –F-HDIS-M-HR-"displayed.
10. On completion "Mandatory Rinse End" will be displayed.
11. It is not necessary to test residual citric acid if Citrosteril is used, since it is a decaying agent which is formulated in a non-toxic solution.

Recommendation for once monthly evaluation and monitoring:

Microbiological monitoring: water for production of dialysate and actual dialysate proportioned and exiting the dialyzer should be monitored for bacterial levels on no less than a monthly basis. Microbiological monitoring is performed to establish ongoing validation of proper disinfection protocols. The sampling should be done at the termination of dialysis at the point where dialysate exits the dialyzer. Results for total microbial counts shall not exceed 2,000 colony forming units per ml.

Assessing trends: Pertinent information, i.e., bacterial levels, conductivity and pH readings, etc., should be logged on a chart across a page so that readings can be examined and compared over an extended period of time. This tool makes it possible to compare current readings to those taken during the past several days/weeks/months.

Cleaning and disinfection of external surfaces of HD machines

It is recommended to clean and disinfect the external surfaces of the HD machine after each dialysis session. A low-level disinfectant or any EPA-registered disinfectant solution labeled for use in a health-care setting is recommended to be used on non-critical items (including HD machines), and should also be in accordance with the machine manufacturer's recommendations. The presence of bio-burden will reduce the killing/inactivating effect of disinfectants. Therefore, if visible blood spills or other infectious material is present on the external surface of an HD machine, it should be cleaned separately (not to spread) before applying the disinfectant solution. In such cases, it is recommended to use an intermediate-level disinfectant or tuberculocidal agent (with specific label claims for HBV and HIV) or a 1:100 dilution of a hypochlorite solution (500-600 ppm free chlorine). If using disinfectant wipes, one wipe should be used to exclusively clean the blood stain followed by another wipe(s) for disinfection. All external surfaces of the machine, especially the frequently touched front panel, including the intravenous pole, the side, back and base, should be thoroughly cleaned and disinfected using *friction* and be allowed to air dry. All used towels or wipes and gloves that are contaminated with blood should be discarded in a biohazard waste container, and hand hygiene performed after glove removal.

WATER TREATMENT FOR HEMODIALYSIS

Rationale: The average hemodialysis patient is exposed to approximately 25 times the amount of water normally ingested by an individual. In addition he is deprived of the protective barrier of the gastrointestinal tract and the detoxification function of the kidneys, increasing the risk several fold of toxicity caused by the numerous chemical and microbiological contaminants in the water.

The final quality of the water is dependent on the configuration of the treatment system and the quality of the feed water which itself may be highly variable. As processes of hemodialysis evolve with use of high flux dialyzers and hemodiafiltration probably becoming increasingly used many countries in the world have made the use of ultrapure water the goal of every dialysis unit. This requires a unit to design a system capable of delivering this very high quality from the worst feed water and a monitoring system and quality assurance to prevent breakdown of the system. The following guidelines describe the setting up, maintenance and monitoring of a system designed to reliably provide ultrapure water

1. Water treatment to achieve the following water quality (AAMI standards) is mandatory for All hemodialysis units.
2. It is recommended that the hemodialysis units should try to achieve European Standards of purity of water (Table)

Maximum levels of the different water purity grades

Maximum levels	AAMI Water	European Pharmacopoeia		
		Regular water	Ultrapure water	Sterile water
Microbial contamination (CFU/ml)	200	<100	<0.1	<0.000001
Bacterial endotoxins (IU/ml)	< 2	<0.25	<0.03	<0.03

Tracking infections

Surveillance for infections (outcome measures) and monitoring adherence to recommended infection prevention practices (process measures) are important components of an infection prevention program. To enable accurate comparison and analyses of monthly rates within the same facility or meaningful bench-marking with other units/centers, it is important that a standardized and validated surveillance protocol be used uniformly by all dialysis facilities. A centralized surveillance system for health-care-associated infections like the CDC's national health-care safety network (NHSN), which requires all participating facilities to strictly follow every specific surveillance criteria, can provide accurate and reliable data that can be used to identify problem areas as well as measure progress of prevention efforts. Implementation of the CDC's NHSN Dialysis Event Protocol (accessible online: www.cdc.gov/nhsn/dialysis) by other dialysis facilities outside the United States have been demonstrated to be feasible. Dialysis events that should be reported include (a) intravenous antimicrobial starts, (b) positive blood cultures and (c) evidence of local access site infection (pus, redness or increased swelling at the vascular access site), and data collected from these three events can generate four other types of dialysis events: Blood-stream infection (BSI), local access site infection (LASI), access-related bloodstream infection (ARB) and vascular access infection (VAI). The number of maintenance HD out-patients who received HD in the unit/center during the first two working days of the month (including transient HD patients but excluding inpatients and PD patients) should be reported on a monthly basis and according to their vascular access type. This will serve as the denominators for rate calculation. Each patient is counted only once; if the patient has multiple vascular accesses, that patient is counted with the vascular access type of highest infection risk. Rates are calculated by dividing the number of events by the number of patient-months and multiplying the result by 100.

As a means to reduce infection transmission, each dialysis facility should also monitor other parameters like dialysis water and dialysis fluid cultures and endotoxin results, incidence of drug-resistant infections, hospitalizations, as well adherence to standard precautions (hand hygiene, glove use and other PPE, equipment and environmental cleaning, safe injection practices, etc.) and other recommended practices (screening for HBV, HCV, HIV and tuberculosis infections and immunizations). Regular feedback of surveillance results to everyone involved in the health-care delivery (especially the frontline staff) would help to stimulate and encourage active engagement and improve compliance with infection prevention efforts. At least one designated person with training in infection control and epidemiology (infection preventionist) should be responsible for over-sight of the program as well as education of staff and patients related to infection prevention and control.

Steps that should be taken to control spread of infection, especially if there is an incidence of a positive seroconversion or outbreak in the HD unit, include the following: (a) review of the laboratory test results of all patients dialyzing in the same unit to identify any additional case(s), (b) performance of additional tests (c) determination/tracking of potential sources for infection, which includes (i) revision of newly infected patients' recent history of blood transfusion, invasive procedure(s) and/or hospitalization and (ii) high-risk behavior such as history of injection drug use and sexual activity, and (d) revision of HD unit's practices and procedures of infection control.

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Highlights of the coming issue

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