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Editorial

Contents

We would like to thank all our valued readers for appreciating the previous efforts & our intention is to go still higher. With this issue starts the fifth year of existence of "Journal of Hygiene Sciences".

The term 'antisepsis' was first coined at the end of the 18th century by the English military physician John Pringle to apply to substances that were able to prevent putrefaction. Polyhexamethylene biguanide, better known as polihexanide or PHMB, is one of the modern antiseptics that combines a broad antimicrobial spectrum with low toxicity, high tissue compatibility, no reported adsorption and good applicability as solution, gel, ointment, foam and in wound dressing. It is actually one of the most promising antiseptic substances.

We all have heard about Sexually transmitted diseases (STD's) or rather better referred to as Sexually transmitted infections (STI's). These infections can be bacterial, fungal, viral, parasites or Protozoal. Trichomoniasis is a sexually transmitted infection caused by a protozoan (Trichomonas vaginalis). Let us see more about it in our Current Trends section.

Our In Profile segment Scientist of the month is Gerhard Domagk, a German pathologist and bacteriologist who was credited for the discovery of Sulfonamidochrysoidine (KI-730) – the first commercially available antibiotic (marketed under the brand name Prontosil) – for which he received the 1939 Nobel Prize in Physiology or Medicine.

Chlamydia infections are the most common bacterial sexually transmitted infections in humans and are the leading cause of infectious blindness worldwide. Chlamydia is known as a 'silent' infection because most infected people have no symptoms. Chlamydia can infect both men and women and can cause serious, permanent damage to a woman's reproductive organs. ***Chlamydia trachomatis***, an obligate intracellular human pathogen, is one of three bacterial species in the genus *Chlamydia* & is a Gram-Negative bacteria which causes numerous disease states in both men and women. *C. trachomatis* is also an important neonatal pathogen, where it can lead to infections of the eye (trachoma) and pulmonary complications. Chlamydia trachomatis is the single most important infectious agent associated with blindness.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is also called multidrug-resistant *Staphylococcus aureus* and oxacillin-resistant *Staphylococcus aureus* (ORSA). The detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in the clinical laboratory remains as challenging as does the need for new antibiotics to fight the bacterial resistance mechanisms. Historically, there has been a continuing battle to manufacture new antibiotics that are successful in inhibiting or killing *S. aureus*. Penicillin was effective against *S. aureus* for a very short period of time before resistance to it was seen.

Blood culture specimens are among the most critical specimens processed by the microbiology laboratory. The taking of blood cultures to detect bacteraemia is an important medical investigation affecting the diagnosis and treatment of our patients. Their value will be maximised by taking the cultures for the appropriate indications and with the correct technique in order to reduce the risk of contamination.

Relax & unwind yourself with our Relax Mood section

Our JHS team is thankful to all our readers for their ever increasing appreciation that has served as a reward & motivation for us. Looking forward towards your valuable inputs & suggestions.

Polihexanide (Polyhexamethylene Biguanide) : State-of-the-Art Antiseptic

Infected wounds are still one of the great challenges in medicine. In the last decade, it has become increasingly clear that antimicrobial chemotherapy is limited by the spread of antimicrobial resistance. Additionally, intensive use of antibiotics promotes resistance even further. With the availability of new antiseptic substances with a broad antimicrobial spectrum, provided in easy-to-use and well-tolerated formulations, local treatment is expected to become more and more important in wound therapy.

Polyhexamethylene biguanide, better known as polihexanide or PHMB, is one of the modern antiseptics that combines a broad antimicrobial spectrum with low toxicity, high tissue compatibility, no reported adsorption and good applicability as solution, gel, ointment, foam and in wound dressing. It is actually one of the most promising antiseptic substances. For over 20 years, it has now been used in medicine for many indications including not only the treatment of infected wounds, but *Acanthamoeba* keratitis, preservation and disinfection of contact lenses, decolonization of skin and mucosa, preoperative eye antisepsis and mouth rinses to name only a few.

Today, PHMB is one of the best investigated antiseptic substances. Its microbicidal effect is based on a strong interaction with negatively charged phospholipids in the bacterial membrane (leading to its disruption) and an inhibition of the bacterial cell metabolism. These two independent and non-specific mechanisms make the development of resistance to PHMB highly unlikely. Actually, no bacterial resistance has been described in vitro or from clinical or environmental samples.

Unlike other antiseptics, the antimicrobial efficacy of PHMB is not impaired in human wound fluid, human tissue or by high loads of blood or albumin. This is of atmost importance for the clinical use, because the antimicrobial effect is not limited in the wound. The presence of mucin and chondroitin, on the other hand, abolish the antibacterial effect of PHMB even in low concentrations, as found in nasal and joint fluid. Despite that, a first pilot study using only PHBM-based products for MRSA decolonization (including the nares) interestingly had a success rate of 37% after single decolonization for 7 days. Due to its mode of action, PHMB needs about 5 min after application for the full antiseptic effect to occur. Once applied and dispersed in the wound, PHMB binds to cellular surfaces and possesses a sustained effect for hours.

In recent years, it has become more and more evident that microbial biofilms play an important role in many chronic infections. PHMB blocks the microbial attachment to surfaces and has been shown to effectively remove biofilms in vitro and in vivo.

The most interesting feature of PHMB is its outstanding relation between antimicrobial efficacy and low cytotoxicity and exceptional tissue compatibility that has been repeatedly described by independent researchers in vitro, in animal models as well as in controlled clinical studies and case reports. In low concentrations, PHMB even seems to be not only non-toxic, but to have a positive effect on the proliferation of human keratinocytes. This goes well along with animal studies where complete wound closure was achieved significantly earlier using PHMB than placebo and clinical data reporting the same effect in patients with chronic wounds. Because non-healing chronic wounds were shown to have a much higher bacterial load with *Pseudomonads* (not detectable by cultural methods) compared to healing wounds, the reversal of protein degradation by PHMB could be one clue to its positive effects on wound healing.

PHMB is not only well tolerated locally, but has an LD 50 that makes intoxications unlikely and a therapeutic index that is more than 200-fold that of chlorhexidine. Chronic oral intake over 2 years was also tolerated without any adverse reactions. Finally, unlike its sibling chlorhexidine, PHMB seems to carry only a negligible allergic risk.

Mode of Action

Being a strong base, PHMB interacts with acidic, negatively charged phospholipids in the bacterial membrane, leading to increased fluidity, permeability and loss of integrity, followed by the death of the organism. The maximum activity occurs at a pH value of between 5 and 6. PHMB is also transferred to the cytoplasm, where it leads to disruption of the bacterial metabolism. Neutral phospholipids on the other hand are little or not affected by PHMB. This is commonly seen as the main reason for the low toxicity of PHMB against human cells and its high therapeutic range. Furthermore, presumably as a result of the interaction with the cell membrane, PHMB blocks microbial attachment to surfaces, as shown for dental plaque and for the preoperative eye antiseptic. PHMB is able to significantly eliminate artificial plaques of fibrin in vitro which is of clinical relevance, because plaques can impair the self-cleansing of the wound.

Interactions

PHMB is compatible with acids, quaternary ammonium compounds and neutral detergents but incompatible with anionic detergents, soaps and alkyl sulfates (e.g. ammonium lauryl sulfate). Strong inorganic bases and complex phosphates lead to precipitation. PHMB does not affect stainless steel or anodized aluminium, but copper and some types of rubber are delicate. Ansorg et al. were able to show that the antibacterial effect of PHMB is abolished by mucin in concentrations of 0.5 and 1%, which is even lower than the mucin concentrations in healthy nasal secretions. PHMB is completely neutralized in the presence of chondroitin sulfate which obstructs the use of polihexanide in situations in which mucin or chondroitin sulfate load is expected. Polihexanide has a greater antimicrobial efficacy than chlorhexidine under both clean and dirty conditions. Its antimicrobial effect is not impaired even with high loads of blood or albumin, but exceeds the efficacy of PVP-iodine in situations with high blood load. Most importantly, PHMB was shown to maintain its antibacterial effect in human wound fluid and human tissue.

Antimicrobial Efficacy and Resistance

Due to its nonspecific, strong interaction with negatively charged

phospholipids, PHMB has a broad antimicrobial spectrum, including Gram-positive and Gram negative bacteria, plaque-forming and biofilm-building bacteria, spore-forming bacteria (but not bacterial spores), intracellular bacteria such as chlamydiae and mycoplasma, and fungi including *Candida* spp. as well as *Aspergillus* spp. PHMB is able to inactivate HIV-1 and HSV in vitro.

The minimal microbiocidal concentrations of PHMB are reported as follows: *Staphylococcus aureus* : 0.1 g/ml, *Bacillus subtilis* : 0.5 g/ml, *Streptococcus faecalis* , *Streptococcus lactis* , *Escherichia coli* and *Enterobacter cloacae* : 5 g/ml, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* : 25 g/ml. In 10% fetal bovine serum, PHMB at a concentration of 100 g/ml was shown to achieve a 3 log 10 reduction of *S. aureus* and at a concentration of 90 g/ml a similar reduction of *E. coli* after 30 min contact times in each case. A reduction of 1 5 log 10 after 5 min contact time is achieved with a concentration of 0.02% (200 g/ml) against *S. aureus, E. coli, E. faecium, P. aeruginosa* and *C. albicans* under clean and dirty conditions (0.3% blood and 0.3% albumin load). Despite the incompatibility with chondroitin sulfate, a 0.005% (50 g/ml) concentration of PHMB was shown to achieve a 3 log 10 reduction against *E. coli* and *S. aureus* in the presence of cartilage.

Furthermore, PHMB was not only shown to completely eliminate elastase-expressing *P. aeruginosa* that degraded wound fluid proteins as well as human skin during infection ex vivo, but inhibited consequent protein degradation. A 0.02% solution of PHMB effectively removed an artificial *P. aeruginosa* biofilm from plastic slides in a contact time of 60 min in vitro. Compared to Ringer solution and saline, a surfactant PHMB solution achieved a significant reduction against a *P. aeruginosa* biofilm on silicone after a 24-hour exposure time.

PHMB is effective against *Acanthamoeba* keratitis in concentrations as low as 0.025% (250 g/ml) as single substance as well as in combination with propamidine and neomycin. PHMB was found to be cysticidal against *Acanthamoeba* at 9.4, 5.6 and 2.4 g/ml after 8, 24 or 48 h contact times, respectively. When used to combat *Acanthamoeba* keratitis, the therapist should keep in mind that some primary resistances of *Acanthamoeba* to PHMB are reported in the literature. But until now, no bacterial resistances to PHMB have been reported and are not to be expected due to its nonspecific mode of activity. Because polihexanide binds to cellular surfaces, it also has a sustained effect over hours.

Toxicity

Acute Toxicity

PHMB is classified as 'practically nontoxic', based on the low oral toxicity of 5 g/kg in rat. The therapeutic index of PHMB, calculated as a quotient of the LD 50 (rat) and the MIC against *P. aeruginosa,* is more than 200-fold that of chlorhexidine (0.9). PHMB was shown to positively affect skin microcirculation.

Application of very high doses of PHMB can trigger fever and a generalized exanthema. The intraperitoneal application of a 0.04% solution of PHMB led to local vasodilatation and systemic hypotension in mice. The underlying principle assumed by the authors was the possible promotion of nitric oxide liberation, potassium channel activation and vasodilation that would result in hypotension. The minimum concentration for irritation for skin is reported to be well above 5% (rats) and over 25% for eye (rabbits). PHMB is compatible with nasal mucosa at a concentration of 0.02%.

Genotoxicity, Reproductive and Developmental Toxicity

No indication of any mutagenicity or carcinogenicity was found in vitro or in vivo. Administration of up to 40 mg/kg/body weight/day (p.o.) was not teratogenic in mice. Similarly, the administration of up to 8 mg/kg/body weight/day showed no teratogenicity or embryotoxic effects in rabbits, but the oral administration of a 0.04% solution of PHMB in combination with polyethylene glycol was embryotoxic at a dose of 32 mg/kg/body weight/day.

Oral administration of 100 mg/kg/body weight/day was embryotoxic in rats, and intraperitoneal application of 10 mg/kg/body weight/day showed teratogenicity. There is no evidence of relevant adverse effects on the male or female reproductive organs from chronic carcinogenicity studies, subacute toxicity studies and 2-year treatment studies with PHMB. Chronic oral toxicity studies in dogs showed reduced testis weights and testicular tubular degeneration in individual animals at the highest dose, producing overt signs of toxicity only.

In 2004, the US Environmental Protection Agency (EPA) reviewed the database on mice, rats and rabbits for prenatal developmental toxicity as well as reproductive toxicity of PHMB. No evidence of reproductive and developmental toxicity from any of the publicly available and well-controlled animal studies submitted for PHMB to the Agency was described. Nevertheless, the teratogenic effect in rats was mentioned in the patient information of Lavasept, but the reliability of the source could not be confirmed by the EPA.

Polihexanide – State-of-the-Art Antiseptic

In the past decade, increasing outbreaks of hospital acquired infections caused by multidrug-resistant bacteria have become a serious problem worldwide. Selection of superbugs like multidrug-resistant *Clostridium difficile, Mycobacterium tuberculosis, Serratia marcescens, Acinetobacter,* and methicillin-resistant *S. aureus* or vancomycin-resistant *Enteroccocus* in hospital and nonhospital health care facilities was provoked by misuse of antibiotics either prophylactically or for treatment of minor ailments not shown to be bacterial. A prerequisite for containing the spread of superbugs is the rational use of antibiotics, which can be replaced in many cases by antiseptics, i.e. in wound care for treatment of contaminated wounds and infection prophylaxis. Polihexanide is a broad-spectrum biocide not only effective against Grampositive and Gram-negative bacteria, but also against *Saccharomyces cerevisiae.* fungal (*C. albicans, Aspergillus niger, Fusarium solani)* and protozoal (*Acanthamoeba* spp.) pathogens of infective keratitis, and against the enveloped virus HIV. In treatment of chronic wounds, new products containing polihexanide (wound rinsing solutions, wound dressings, wound gels, and antiseptics) were used successfully to reduce infection rates and it is recommended by medical experts as a state-of-the-art antiseptic for chronic wounds.

The EPA (US Environmental Protection Agency) has classified

polihexanide for general use. The LD 50 (lethal dose for 50% of the population) range of acute oral toxicity in 3 rat studies was 1,049–2,747 mg/kg. There is no evidence of mutagenic, genotoxic, and neurotoxic effects of polihexanide in the EPA's database. In utero exposure studies did not indicate increased susceptibility of the fetus, and when adults were exposed in a two-generation reproductive study, there was no evidence of increased susceptibility of the offspring.

The safety margin or therapeutic index of an agent describes the risk-benefit ratio for the patient. For an antibacterial, it is defined as the quotient of LD 50 in rats and the minimum inhibitory concentration for a pathogen. The greater the difference between LD 50 and minimum inhibitory concentration, the better the risk-benefit ratio. Considering the specific action of polihexanide on microbial envelopes, it is not surprising that the safety margin of polihexanide is far ahead of widely used antibiotics (table 2). Also the toxicity profile is excellent and superior to common antiseptics. Due to its effectiveness, broad-spectrum activity and excellent tolerance, polihexanide is considered to be an antiseptic of first choice, but it is contraindicated for treatment of cartilage and central nervous system.

Table 2. Safety margin of some common antiseptics compared to polihexanide

Antiseptic	Oral LD_{50} rat/minimum inhibitory concentration, mmol/kg or mmol/l	
	S. aureus	*P. aeruginosa*
Benzalkonium chloride	8.0	2.0
Chlorhexidine	0.9	0.9
Octenidine	3.2	3.2
Polihexanide	25,000	200
PVP-iodine	500	1,000

For comparison of the tolerability of wound antiseptics, the BI is suitable. A condition of this is that the testing for microbicides and cytotoxicity must be carried out under identical test conditions. Cell culture media with a protein content of 6–7 g/l serum albumin and a physiological electrolyte concentration are largely equivalent in composition to the protein and electrolyte contents of wound fluids. The BI is obtained from the quotient of IC 50, i.e. the molar concentration at which 50% of the test cells in the cytotoxicity test are no longer vital, and the molar concentration that in the

Clinical Applications:

Products that are suitable for eye antiseptics are also known to be effective and tolerable for wound treatment without inhibiting the healing process and vice versa. Therefore, some results regarding eye antisepsis are briefly presented. 0.02% PHMB is effective against *Acanthamoeba* keratitis as pure active agent as well as in combination with other substances like propamidine, hexamidine, or neomycin. Therefore, PHMB is considered to be the first-choice therapy for *Acanthamoeba* keratitis. Numerous studies proved acanthamoebicidal efficacy and good clinical outcome with prompt local treatment. On the other hand, treatment failures have been reported, particularly when presentation was late, in cases of deep stromal infection, or in rare cases of primary PHMB resistance of *Acanthamoeba* strains. 0.02% polihexanide is also effective against *Nocardia asteroides* keratitis (MIC 0.01%) and *Fusarium* keratomycosis in rabbits. In addition, polihexanide has shown superior efficacy and a sustained effect compared to povidone iodine when used as preoperative antiseptic for cataract surgery.

Wound Antisepsis.

Different authors have published data on the clinical use of PHMB on wounds either as case reports or as controlled studies.

Summaries of some case reports & uncontrolled studies

CASE	SUMMARY
Infected hip prostheses	Patients with infected total hip prostheses were treated with PHMB in addition to debridement and systemic antibiotic therapy. Treatment with PHMB was reported to be useful supplement to through surgical revision.
Infected hip prostheses	Instead of local antibiotic therapy, a PHMB solution was used for jet lavage of bone and to rinse the surrounding soft tissue. Re-infection rate was 6.3%.
Oesophageal carcinoma	A patient with distal oesophageal carcinoma presented with dysphagia, dyspnoea, tachycardia, hypotension. Purulent pericardial and bilateral pleural effusions were successfully treated with antibiotics, repeated pleurocentesis and pericardial drainage with daily PHMB lavage.
MRSA	The first publication that described the complete eradication of MRSA after debridement and application of PHMB gel on the whole circumference of the lower leg within 2 days.

References:

1. www.ncbi.nlm.nih.gov/pubmed
2. Review on the Efficacy, safety & clinical applications of Polihexanide, amodern wound antiseptic.
3. Polihexanide - A safe & effective Biocide
4. Clinical use of Polihexanide on acute & chronic wounds for antisepsis & decontamination.

Trichomoniasis

Trichomoniasis is a sexually transmitted infection (or sexually-transmitted disease, STD) caused by a protozoan (*Trichomonas vaginalis*), usually found in the vagina and urethral tissues. Women are most often affected by this disease, although men can become infected and pass the infection to their partners through sexual contact. Trichomoniasis is primarily an infection of the urogenital tract; the most common site of infection is the urethra and the vagina in women.

The average size of a trichomonad is 15 mm (they are not visible with the naked eye). Reproduction of the parasites occurs every 8 to 12 hours. *Trichomonas vaginalis* was isolated in men in 14% to 60% of male partners of infected women and in 67% to 100% of female partners of infected men. ***Trichomonas vaginalis*** is an anaerobic, flagellated protozoan, a form of microorganism. The parasitic microorganism is the causative agent of trichomoniasis, and is the most common pathogenic protozoan infection of humans in industrialized countries. Infection rates between men and women are the same with women showing symptoms while infections in men are usually asymptomatic. Transmission takes place directly because the trophozoite does not have a cyst. The WHO has estimated that 160 million cases of infection are acquired annually worldwide. The estimates for North America alone are between 5 and 8 million new infections each year, with an estimated rate of asymptomatic cases as high as 50%.

New research indicates that the incidence of trichomoniasis, caused by the parasite *Trichomonas vaginalis*, is higher than chlamydia and gonorrhea in both high school and college students. In addition, trichomoniasis continues to be under-diagnosed and if left untreated, it can contribute to reduced fertility and may enhance the acquisition of HIV. Further, it has been linked to reproductive complications such as preterm birth.

In a recent issue of *Infectious Disease News*, three studies on trichomoniasis prevalence were reported on from the annual meeting of the International Society for Sexually Transmitted Disease Research. In a study performed by Thornton et al. at Indiana University and the University of Kentucky, 4.8% of 145 sexually active women examined had trichomoniasis compared to 2.8% with chlamydia and 1.4% with gonorrhea.

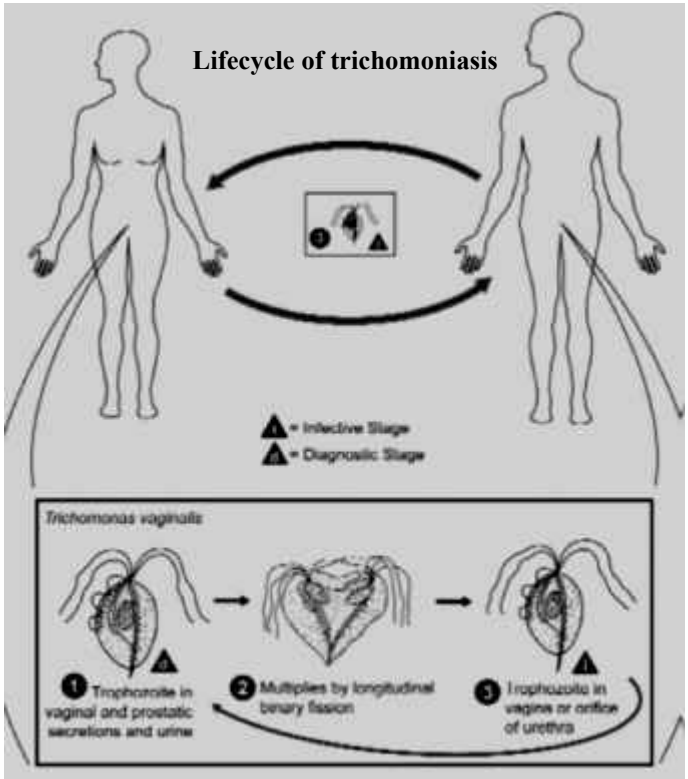
In a separate study of high school men and women performed by Gaydos et al. at Johns Hopkins University, the incidence was 16.2%, 13.3% and 5.5%, respectively, for trichomoniasis, chlamydia and gonorrhea. A third study from the University of North Carolina found that up to 75% of male sexual partners of women with trichomoniasis were themselves infected; however, 55% of the men were asymptomatic.

How common is trichomoniasis?

Trichomoniasis is considered the most common curable STD. In the United States, an estimated 3.7 million people have the infection, but only about 30% develop any symptoms of trichomoniasis. Infection is more common in women than in men, and older women are more likely than younger women to have been infected. It is unclear why women are infected more often than men. One possibility is that prostatic fluid contains zinc and other substances that may be harmful to trichomonads.

How do people get trichomoniasis?

The parasite is passed from an infected person to an uninfected person during sex. In women, the most commonly infected part of the body is the lower genital tract (vulva, vagina, or urethra), and in men, the most commonly infected body part is the inside of the penis (urethra). During sex, the parasite is usually transmitted from



a penis to a vagina, or from a vagina to a penis, but it can also be passed from a vagina to another vagina. It is not common for the parasite to infect other body parts, like the hands, mouth, or anus. It is unclear why some people with the infection get symptoms while others do not, but it probably depends on factors like the person's age and overall health. Infected people without symptoms can still pass the infection on to others.

Signs & Symptoms of Trichomoniasis

Men often do not have symptoms of trichomoniasis and usually do not know they are infected until their partners need treatment. But when symptoms do occur, they include:

- Irritation inside the penis
- Mild discharge
- Slight burning after urination or ejaculation

Many women do have signs or symptoms of infection. Symptoms in women can include:

- Greenish-yellow, frothy vaginal discharge with a strong odor
- Painful urination
- Vaginal itching and irritation
- Discomfort during intercourse
- Lower abdominal pain (rare)

Symptoms usually appear within five to 28 days of exposure in women.

How is Trichomoniasis Diagnosed?

The diagnosis is made by directly observing the trichomonads on a sample of vaginal or urethral discharge through a microscope (they are too small to be seen by the naked eye). Trichomonads are pear-shaped and have several flagella (whiplike tails) at one end. This lab test is usually ordered only if the doctor suspects trichomoniasis as a possible diagnosis.

Trichomoniasis is diagnosed by visually observing the trichomonads via a microscope. In women, the examiner collects

the specimen during a pelvic examination by inserting a speculum into the vagina and then using a cotton-tipped applicator to collect the sample. The sample is then placed onto a microscopic slide and sent to a laboratory to be analyzed.

Trichomonads are seen rarely during urine testing.

A diagnosis of trichomoniasis usually prompts a search for other sexually transmitted diseases, such as syphilis, HIV, gonorrhea, or Chlamydia.

TREATMENT:

Topical vaginal medications (creams and gels) and pessaries can be prescribed for the treatment of *T. vaginalis* in women. Modern preparations include clotrimazole, povidone-iodine, nonoxynol-9, and arsenical pessaries. These preparations provide local symptom relief, but documentation on their effectiveness as cures has been inconsistent. There are no topical treatments for trichomoniasis in men.

The only curative treatment currently available for *T. vaginalis* infection in the United States is **metronidazole**. Usually prescribed as a single or multiple oral doses, metronidazole can also be administered intravenously. Vaginal metronidazole creams and pessaries have also been available but are no longer favored due to their poor rate of cure compared to oral metronidazole.

Current Centers for Disease Control and Prevention guidelines recommend that metronidazole be administered orally, with dose regimens of 250 mg three times a day for 7 days, 500 mg twice a day for 7 days, or a single 2-g dose. The 2-g dose is usually favored because patient compliance is better and less total drug is required for successful treatment. However, there may be a slightly increased risk of side effects with this larger dose. Additionally, patients treated over 7 days are protected for this period from immediate reinfection whereas this protection is not as reliable with a single dose unless the sexual partner(s) is treated simultaneously.

Metronidazole can also be administered intravenously, with a dose of 500 mg to 2 g of metronidazole administered over 20 min. Intravenous administration, although rarely used, is associated with less severe side effects than oral dosing. Cure rates for oral and intravenous regimens are similar, at 85 to 95%, and increase if the sexual partner(s) is treated simultaneously. Therefore, given the high incidence of asymptomatic trichomoniasis, concurrent treatment of sexual partner(s) is highly recommended to prevent recurrent infections.

Treatment of Pregnant Women and Children

Use of metronidazole during pregnancy has been debated because the drug is known to cross the placenta. Trichomoniasis in pregnant women may cause premature rupture of the membranes that protect the baby, and early delivery. The damage caused by *Trichomonas vaginalis* to the vaginal endometrium increases a woman's susceptibility to an HIV infection. In addition to inflammation, the parasite also causes lysis of epithelial cells and RBCs in the area leading to more inflammation and disruption of the protective barrier usually provided by the epithelium. Having *Trichomonas vaginalis* also may increase the chances of the infected woman transmitting HIV to her sexual partner. This has led the Food and Drug Administration to classify metronidazole as a class B risk factor for pregnancy, a possible but unconfirmed risk to the fetus. Therefore, the drug has been contraindicated in the first trimester and is considered a second line of therapy in the latter stage of pregnancy.

More recent retrospective analyses, however, have failed to establish a link between metronidazole exposure during pregnancy and birth defects. This fact, coupled with the known risk of pregnancy complications associated with *T. vaginalis* infection, has led many clinicians and researchers to propose that the risk to the fetus from a maternal trichomoniasis is far greater than any risk related to metronidazole exposure. As such, a single 2-g dose of metronidazole is the treatment currently recommended by the Centers for Disease Control and Prevention for women at any stage of pregnancy. Alternatively, a daily intravaginal dose of 100 mg of clotrimazole for 6 days can provide temporary relief in the first trimester, followed by standard metronidazole therapy in the second trimester.

Metronidazole is secreted in breast milk in small quantities. It is recommended that lactating mothers be treated with a single 2-g dose of metronidazole, followed by a 24-h interruption in breast feeding to prevent neonatal exposure to the drug. This recommendation has become controversial, with some authors citing the lack of evidence of risk to the neonate and the small amount of drug exposure as a poor reason to interrupt breast feeding.

Neonatal trichomoniasis is the only nonsexually transmitted form of the disease. It is usually asymptomatic or mildly symptomatic and is dependent on maternal estrogen levels. Spontaneous resolution of the condition is common as the level of estrogen wanes in the third to sixth week of an infant's life. If infection persists after the sixth week or is symptomatic, the infant may be treated with metronidazole as a single 50-mg/kg dose or a 10- to 30-mg/kg dose daily for 5 to 8 days. Canadian guidelines for the treatment of prepubescent *T. vaginalis* infections with metronidazole recommend 15 to 20 mg/kg divided into three doses daily for 7 days, or a single dose of 40 mg/kg to a maximum of 2 g.

METRONIDAZOLE RESISTANCE

Resistance of *T. vaginalis* to metronidazole is classified as either aerobic or anaerobic. In aerobic resistance, oxygen-scavenging pathways and possibly ferredoxin are involved. These pathways are not implicated in anaerobic resistance, which is driven instead by a reduction or cessation of activity of PFOR and hydrogenase.

Treatment of Infections Caused by Metronidazole-Resistant *Trichomonas vaginalis*

When metronidazole is the only approved treatment of trichomoniasis, a treatment regimen for refractory infection involves increased doses of oral metronidazole (often double doses) for extended periods. Extended therapy is effective in only about 80% of these patients (in contrast to a 95% cure rate in compliant, nonrefractory patients). In cases in which trichomonad drug resistance is very high and toxic levels of metronidazole are required, administration of the drug intravenously or in combination with oral and vaginal therapy may minimize side effects. One treatment protocol is available that recommends regimens for marginal, low, moderate, and high levels of metronidazole resistance. This highlights the need for metronidazole susceptibility testing in refractory *T. vaginalis* infection, as well as the need for alternate therapies to avoid increasing resistance in wild-type strains as a result of increased metronidazole pressure.

Tinidazole

Tinidazole is a 5-nitroimidazole currently in use for the treatment of trichomoniasis in countries outside the United States and under Food and Drug Administration review. It has a longer half-life than metronidazole and is eliminated at a significantly lower rate. It shows superior tissue distribution to that of metronidazole, and concentrations of the drug found in vaginal secretions are close to the levels found in serum, showing that it is delivered more effectively to this area than is metronidazole MLCs of tinidazole

for various *T. vaginalis* strains are consistently lower than MLCs for metronidazole, and this is reflected clinically: tinidazole is curative at lower doses then metronidazole. The therapeutic doses of tinidazole result in fewer and milder side effects.

Since tinidazole is a nitroimidazole, however, its mode of action is similar to that of metronidazole, and cross-resistance to *T. vaginalis* is a concern. Studies have shown that cross-resistance among nitroimidazoles does occur but is incomplete. Both in vitro and in vivo assays have shown that MLCs of tinidazole for metronidazole-resistant trichomonads are generally significantly lower than the MLCs of metronidazole. Thus, metronidazole-resistant trichomoniasis may be treated with tinidazole, but rapid development of tinidazole resistance due to the similarity of metabolic pathways should be a concern.

Other Nitroimidazoles

A number of nitroimidazole derivatives other than metronidazole and tinidazole have been investigated for the treatment of *T. vaginalis* infection. The modes of action of these derivatives are similar, but the pharmacokinetics, tissue distribution, levels in serum, trichomonicidal activity, and toxicity are variable.

Ornidazole and secnidazole are similar to tinidazole in that they have longer half-lives and lower rates of elimination than metronidazole. In contrast, nimorazole is rapidly absorbed and metabolized. It retains significant antiprotozoal activity, however, since its two major metabolites are much more active than the metabolites of metronidazole. More recently, the nitroimidazole EU11100 was developed in an attempt to obtain a drug with the trichomonicidal activity of metronidazole but without the side effects. In vitro testing has shown that EU11100 has a very low MLC compared to other nitroimidazoles, but no clinical trials have been published.

Comparative studies of metronidazole and other nitroimidazoles have shown that most drugs in this family are effective at similar dosages (1.5 to 2 g one-time dose) and that the majority of patients suffer similar, usually mild, side effects. Severe adverse reactions (especially related to single high doses) and hypersensitivity are usually related to nitroimidazoles in general and not to one drug in particular. The one exception to this is misonidazole, which has consistently been shown to cause more serious side effects (peripheral neuropathy). Interest in misonidazole has therefore shifted away from a possible role in the treatment of trichomoniasis and is focusing on its radiosensitizing properties.

Other Chemotherapeutic Agents

A number of studies have been published demonstrating the in vitro trichomonicidal activities of nonimidazole drugs. These compounds include both agents currently in use in industry or for the treatment of other infectious diseases and drug derivatives synthesized specifically for the treatment of *T. vaginalis* infection.

Hamycin is an aromatic polyene related to amphotericin B. It can induce cell death in *T. vaginalis* and other eukaryotic cells by binding to ergosterols in the plasmalemma and causing the formation of pores, leading to cytoplasmic leakage and cell death. Studies have shown that hamycin at low concentrations effectively kills both metronidazole-sensitive and -resistant strains of *T. vaginalis*. The drug is currently in use in India as a topical treatment for trichomoniasis. Unfortunately, reported side effects in patients and laboratory animals, along with in vitro studies with mammalian tissue cultures, indicate that the toxicity of hamycin may limit future clinical applications.

Intravaginal application of paromomycin has been successfully used to treat recurrent trichomoniasis. However, severe side effects, including pain and mucosal ulceration, make it an unlikely candidate for clinical therapy.

Sodium nitrite, sodium nitroprusside, and Roussin's black salt, traditionally used to prevent food contamination, exhibit trichomonicidal activity. The mode of action of these compounds is unknown, but they are active against both metronidazole-sensitive and -resistant strains of *T. vaginalis*.

The nitrothiazole derivative niridazole has a broad spectrum of antimicrobial activity and is active against *T. vaginalis*. Both metronidazole-sensitive and -resistant strains are inhibited by this drug. Chemical analyses have shown that niridazole may have multiple modes of action, accounting for its wide range of inhibitory effects, but specific mechanisms are unknown.

Nitazoxanide is a 5-nitrothiazolyl that has a broad spectrum of activity against protozoan parasites in vivo. In vitro studies have shown that the compound is active against both metronidazole-sensitive and -resistant strains of *T. vaginalis*, with some formulations being about five times more active against trichomonads than is metronidazole.

An in vitro comparison study of antimicrobial drugs established that of the 50 compounds tested, only metronidazole and tinidazole (no other nitroimidazoles were tested) and three others possessed significant trichomonicidal activity. Both metronidazole-sensitive and -resistant strains of *T. vaginalis* were inhibited by the heterocyclic antibiotic anisomycin; an antigiardial nitrofuran, furazolidone; and mebendazole, a microtubular inhibitor commonly used to treat helminth infections. Unfortunately, the level of toxicity exhibited by all three of these compounds is cause for concern and may limit their potential as intravaginal preparations; clearly, more research is required.

An in vitro comparison of two synthetic derivatives of benzoizothiazolinone showed that the drugs exhibited significantly higher trichomonicidal activities than metronidazole. The antimicrobial mechanisms of the derivatives remain largely undefined.

A partial list of other drugs investigated for antitrichomonal activities include sulfimidazole, a 5-nitroimidazole with a functional sulfonamide group, nifuratel, a nitrofuran derivative, berberine sulfate, a plant alkaloid; MDL 63,604, a derivative of the antibiotic purpuromycin; lipophilic tetracyclines; thiadiazine derivatives; some 4-nitrobenzimidazole derivatives; specific benzimidazole derivatives; acetylated derivatives of sugar hydrazones; spiroarsoranes, "spiranized" arsonic acids; and disulfiram, a drug often used to treat alcoholism. These compounds have all shown some promise in the treatment of trichomoniasis, but research either is in the preliminary stages or is not being systematically pursued at this time.

VACCINES

With an increasing incidence of refractory infection and the lack of a safe and effective alternative to nitroimidazoles, disease prevention with a vaccine would clearly be desirable. As with many sexually transmitted diseases, infection with *T. vaginalis* does not induce long-term immune protection. A complicated host defense system restricts *T. vaginalis* infection to the genitourinary tract and may play a role in limiting the immune response and preventing long-term immunity.

The host defense network has three components: nonimmunologic factors such as zinc concentration and iron availability; nonspecific innate mechanisms including complement, natural antibodies, and phagocytes; and specific adaptive B- and T-cell responses. The antibody response to *T. vaginalis* infection is well documented and includes circulating serum and cervicovaginal immunoglobulin A (IgA), IgG, and IgM. However, these antibodies appear to provide only limited protection from invading

parasites, and antibody titers progressively dwindle after infection is eradicated by treatment. At 6 to 12 months after infection, neither *T. vaginalis*-specific antibodies nor memory B cells are present in the circulation, leaving the host with no defense against subsequent infection. A T-cell-mediated response has also been demonstrated in trichomoniasis, but its mechanisms remain largely undefined and it is likely to be too limited to provide sustained protective immunity.

Research into the development of a vaccine for *T. vaginalis* has shown some promise, elucidating a number of mechanisms by which protection could potentially be achieved. One study has shown that many women develop an immune response to the 115-kDa α-actin protein of *T. vaginalis*. Elevating this response through the use of an adjuvant or other costimulation might contribute to a stronger immune reaction. It has also been shown that *T. vaginalis* strains possess both unique and common antigenic epitopes. The presence of shared epitopes suggests that it may be possible to provide protection to a cross section of *T. vaginalis* strains in a single vaccine. Potential antigen candidates could include a 100-kDa protein that was found to be immunogenic across a broad sampling of *T. vaginalis* isolates. Essential adherence molecules, including adhesins, mucinases, and cysteine proteinases, are also potential targets, given the importance of adherence for the pathogenicity of the organism.

Two distinct *T. vaginalis* vaccine candidates have progressed to the stage of human clinical trials. The first was studied in the 1960s and involved a trial of 100 women with refractory trichomoniasis receiving intravaginal inoculations with increasing numbers of heat-killed *T. vaginalis* cells. A 100% improvement in clinical symptoms was reported, but the procedure has not been repeated and this method of vaccination has been left unpursued.

In the late 1970s, another *T. vaginalis* vaccine, under the commercial name SolcoTrichovac or Gynatren, became available. The vaccine was derived from heat-inactivated "abnormal strains of lactobacilli" that had been isolated from the vaginal secretions of women with trichomoniasis. These lactobacilli were reported to have lost the ability to produce lactic acid, and their morphology was described as shortened or coccoidal rather than the traditional elongated bacillus.

A diverse spectrum of mechanisms were proposed to explain the activity of SolcoTrichovac. It was proposed that aberrant lactobacilli associated with *T. vaginalis*, leading to the formation of bacterial-trichomonad immune complexes which immobilized the parasites and induced cytolysis and/or stimulated macrophage activity. SolcoTrichovac-induced production of lymphokines, leading to the activation and attraction of T cells to the infected area, was another proposed mechanism. Since the antibody response is strong during *T. vaginalis* infection, enhanced trichomonicidal activity was hypothesized to be the result of either antilactobacillus antibodies cross-reacting with *T. vaginalis* or trichomonad uptake of Lactobacillus membrane components, leading to antibody recognition and destruction of the parasites. It was also suggested that the abnormal lactobacilli in some way reestablished a "normal" vaginal flora, which would lead to a reduction of pH that would inhibit the growth of *T. vaginalis*. None of these mechanisms of action were experimentally proven to be the mode of action of the vaccine.

Although SolcoTrichovac was initially purported to both cure existing infection and provide protection from reinfection, subsequent studies cast doubt on the actual efficacy of the vaccine against *T. vaginalis*. Clinical trial results were positive, but these studies often were not very thorough or lacked the proper control groups. The fundamental theory behind the vaccine was tenuous, since no particular antigenic similarity between lactobacilli and *T. vaginalis* was ever shown. Given these facts, it is unlikely that any trichomonidical activity of SolcoTrichovac was the result of a direct immune or antibody response to *T. vaginalis*. It is more likely that vaccination stimulated an increase in nonspecific immune factors capable of alleviating symptoms and eliminating infection.

Given that the immune response to vaginal *T. vaginalis* infection is comparatively weak and does not elicit protection, introduction of a vaccine via a systemic route has been considered as a method of inducing a stronger and more lasting immune response. A murine model of *T. vaginalis* infection was established, in which mice were immunized with two subcutaneous injections of whole trichomonads, first in Freund's complete adjuvant and later in Freund's incomplete adjuvant. The mice were then estrogenized and vaginally preinoculated with *Lactobacillus acidophilus* to establish a vaginal milieu comparable to that found in humans. Infection with *T. vaginalis* was then performed, and vaginal washes were used to compare infection rates between immunized and control mice. Results showed that immunized mice either were protected or cleared the infection faster than sham-vaccinated or naive mice did. Nonvaccinated mice cured with metronidazole were not protected from vaginal infection and showed no immune response, whereas vaccinated mice had elevated levels of anti-*T. vaginalis* antibodies in both serum and vaginal secretions.

How Can I Prevent Trichomoniasis Infection?

- Use condoms correctly every time you have sex.
- Limit the number of sex partners, and do not go back and forth between partners.
- Practice sexual abstinence, or limit sexual contact to one uninfected partner.
- If you think you are infected, avoid sexual contact and see a doctor.

Any genital symptoms such as discharge or burning during urination or an unusual sore or rash should be a signal to stop having sex and to consult a doctor immediately. If you are told you have trichomoniasis or any other STD and receive treatment, you should notify all of your recent sex partners so that they can see a doctor and be treated.

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Gerhard Domagk

Born:	30 October 1895 Lagow, Brandenburg
Died:	24 April 1964 (aged 68) Burgberg
Nationality:	Germany
Fields:	Bacteriology
Known for:	Prontosil
Notable awards:	1939, Nobel Prize in Medicine

Gerhard Johannes Paul Domagk (30 October 1895 – 24 April 1964) was a German pathologist and bacteriologist credited with the discovery of Sulfonamidochrysoidine (KI-730) – the first commercially available antibiotic (marketed under the brand name Prontosil) – for which he received the 1939 Nobel Prize in Physiology or Medicine.

Domagk was born in Lagow, Brandenburg, the son of a school headmaster. Until he was 14, he attended school in Sommerfeld (now Lubsko, Poland). Domagk studied medicine at the University of Kiel, but volunteered to serve as a soldier in World War I. After the war, he finished his studies, and worked at the University of Greifswald, where he researched infections caused by bacteria.

In 1923 he moved to Greifswald and there became, in 1924, University Lecturer in Pathological Anatomy. In 1925 he held the same post in the University of Münster and in 1958 became professor of this subject. During the years 1927-1929 he was, however, given leave of absence from the University of Münster to do research in the laboratories of the I.G. Farbenindustrie, at Wuppertal. In 1929 a new research institute for pathological anatomy and bacteriology was built by the I.G. Farbenindustrie and there, in 1932, Domagk made the discovery for which his name is so well known, the discovery that earned him the Nobel Prize in Physiology or Medicine for 1939, namely, the fact that a red dye-stuff, to which the name «prontosil rubrum» was given, protected mice and rabbits against lethal doses of staphylococci and haemolytic streptococci. Prontosil was a derivative of sulphanilamide (*p*-aminobenzenesulphonamide) which the Viennese chemist, Gelmo, had synthesized in 1908.

Domagk was, however, not satisfied that prontosil, so effective in mice, would be equally effective in man, but it so happened that his own daughter became very ill with a streptococcal infection, and Domagk, in desperation, gave her a dose of prontosil. She made a complete recovery, but Domagk omitted mentioning the recovery of his daughter from the report on the effect of the drug, waiting until 1935 when results were available from clinicians who had tested the new drug on patients. During subsequent years much work was done in various countries on this class of antibacterial compound and some thousands of derivatives of sulphanilamide have been produced and tested for their antibacterial properties. Domagk's work has thus given to medicine, and also to surgery, a whole new series of weapons that are effective against many infectious diseases.

He was appointed the director of Bayer's Institute of Pathology and Bacteriology, where he continued the studies of Josef Klarer and Fritz Mietzsch, based on works by Paul Ehrlich, to use dyes, at that time a major product of IG Farben, as antibiotics. He found the sulfonamide Prontosil to be effective against streptococcus, and treated his own daughter with it, saving her the amputation of an arm.

In 1939, Domagk received the Nobel Prize in Medicine for this discovery, the first drug effective against bacterial infections. He was forced by the Nazi regime to refuse the prize and was arrested by the Gestapo for a week. Sulfonamides became a revolutionary weapon at the time, surpassing phage therapy, but were later replaced by penicillin, which showed both better effects and fewer side effects (sulfonamides can cause kidney stones and changes in bone marrow). Domagk's work on sulfonamides eventually led to the development of the antituberculosis drugs thiosemicarbazone and isoniazid, which helped to curb the epidemic of tuberculosis which swept Europe after World War II.

After the war, in 1947, Domagk was finally able to receive his Nobel Prize, but not the monetary portion of the prize due to the time that had elapsed.

The discovery of the antibacterial action of the sulphonamides was not, however, Domagk's only contribution to chemotherapy. He also discovered the therapeutic value of the quaternary ammonium bases and he also extended, in collaboration with Klarer and Mietzsch, his work on the sulphonamides. Later, he attacked the problem of the chemotherapy of tuberculosis, developing for this the thiosemicarbazones (Conteben) and isonicotinic acid hydrazide (Neoteben). His work has undoubtedly resulted in more effective control of many infectious diseases which nowadays have lost the terrors they formerly caused. The supreme aim of chemotherapy is, in Domagk's opinion, the cure and control of carcinoma and he was convinced that this will be, in the future, achieved.

Personal Life:

Father: (assistant headmaster)
Mother: Martha Reimer Domagk
Wife: Gertrud Strübe Domagk (m. 1925, three sons, one daughter)
Son: Götz Domagk (author, b. 1926)
Daughter: Hildegard Domagk (b. 1929)
Son: Wolfgang Domagk (b. 1930)
Son: Jörg Domagk (b. 1932)

Honours:

Domagk held honorary doctorates of the Universities of Bologna, Münster, Cordoba, Lima, Buenos Aires, and Giessen. He was made Knight of the Order of Merit in 1952, was awarded the Grand Cross of the Civil Order of Health of Spain in 1955. Other honours and distinctions bestowed upon him were: Paul Ehrlich Gold Medal and Paul Ehrlich Prize, University of Frankfurt (1956); Foreign Member of the British Academy of Science and of the Royal Society (1959); Honorary Member of the German Dermatological Society (1960); Japanese Order of Merit of the Rising Sun (1960). His short biography was published by the Royal Society in 1964.

Retiring to his old university of Münster, when laboratory work was no longer possible for him, he had devoted himself to the experimental (chemotherapeutic) study of carcinoma and to the dissemination of modern knowledge about it among the students and others interested in it. His recreation was painting.

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Enjoy the humour

Funny Quotes

1. Before I got married I had six theories about bringing up children; now I have six children and no theories.
—John Wilmot

2. Always forgive your enemies; nothing annoys them so much.
—Oscar Wilde

3. Laughing at our mistakes can lengthen our own life. Laughing at someone else's can shorten it.
—Cullen Hightower

4. We learn something every day, and lots of times it's that what we learned the day before was wrong.
—Bill Vaughan

5. Don't ever wrestle with a pig. You'll both get dirty, but the pig will enjoy it.
—Cale Yarborough

6. An inventor is simply a fellow who doesn't take his education too seriously.
—Charles F. Kettering

7. Some people like my advice so much that they frame it upon the wall instead of using it.
—Gordon R. Dickson

great thoughts

Great thoughts By Great People

Napoleon said
"The world suffers a lot...Not because of the violence of bad people, But because of the silence of good people!"

Michael Paul Said
I wrote on the door of heart, "Please do not enter it"
Love came smiling and said: "Sorry I am an illiterate"

Einstein said
"I am thankful to all those who said NO to me
Its Because of them I did it myself...."

Abraham Lincoln said
"If friendship is your weakest point then you are the strongest person in the world"

Shakespeare said
"Laughing faces do not mean that there is absence of sorrow!
But it means
That they have the ability to deal with it."

Shakespeare said
"In the time of crisis I was not hurt by the harsh words of my enemies, but by the silence of my FRIENDS"

William Arthur said
"Opportunities are like sunrises, if you wait too long you miss them"

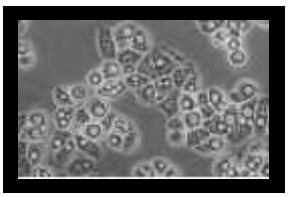
Shakespeare said
"Never play with the feelings of others because you may win the game but the risk is that you will surely loose the person for life time"

Hitler said
"When you are in the light, everything follows you. But when you enter into the dark, even you own shadow doesn't follow you."

Shakespeare said
"Coin always makes sound but the currency notes are always silent. So when your value increases keep yourself calm & silent"

John Keats said
"It is very easy to defeat someone, but it is very hard to win someone"

Chlamydia



Classification:

Domain:	Bacteria
Class:	Chlamydiae
Order:	Chlamydiales
Family:	Chlamydiaceae
Genus:	Chlamydia

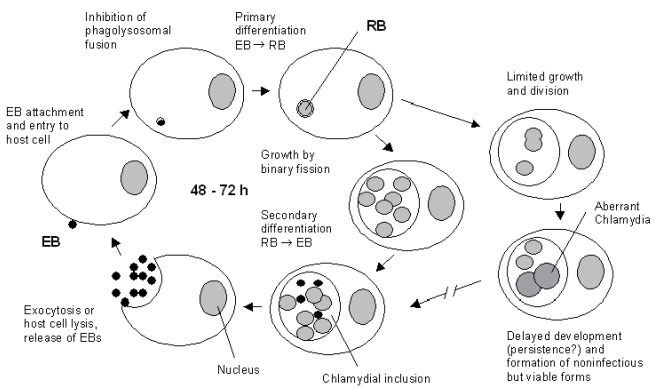
3 Species: *Chlamydia muridarum, Chlamydia suis, Chlamydia trachomatis*

Introduction:

Chlamydia is a genus of bacteria that are obligate intracellular parasites **which are 300 nm in length**. Chlamydia infections are the most common bacterial sexually transmitted infections in humans and are the leading cause of infectious blindness worldwide.

There are three *Chlamydia* species include *Chlamydia trachomatis* (a human pathogen), *Chlamydia suis* (affects only swine), and *Chlamydia muridarum* (affects only mice and hamsters).

Reproductive Cycle:



The infectious form is elementary bodies. After entry of host cell these bodies are transformed into metabolically active reticulate or inclusion bodies, capable to replicate. Most of these replicate by binary fission followed by transformation into elementary bodies again. These elementary bodies which are infectious are released by rupture of host cell & turn capable of infecting other cells.

Pathology:

Most commonly, chlamydial infection do not cause symptoms. *Trachomatisis* is an exclusively human pathogen. This Chlamydia species cause different diseases such as trachoma, lymphogranuloma venereum, urogenital and ocular infections and newborn pneumonia.

Detection:

Chlamydia can be detected through culture tests or non-culture tests. The main non-culture tests include Fluorescent Monoclonal Antibody Test, enzyme immunoassay, DNA probes, rapid Chlamydia tests and leukocyte esterase tests. Whereas the first test can detect the major outer membrane protein or the LPS, the second detects a colored product converted by an enzyme linked to an antibody. The rapid Chlamydia tests use antibodies against the LPS, the leukocyte esterase tests detect enzymes produced by leukocytes containing the bacteria in urine.

Culture Tests:

C. trachomatis **Culture:** Cell culture for *C. trachomatis* involves inoculating a confluent monolayer of susceptible cells with an appropriately collected and transported specimen. After 48–72 hours of growth, infected cells develop characteristic intracytoplasmic inclusions that contain substantial numbers of *C. trachomatis* elementary and reticulate bodies. These unique inclusions are detected by staining with a fluorescein-conjugated monoclonal antibody that is specific for the major outer membrane protein (MOMP) of *C. trachomatis*.

Cell culture methods vary among laboratories, leading to probable substantial interlaboratory variation in performance. For example, because of a larger inoculum and reduced risk of cross-contamination, the shell vial method of culture is more sensitive and specific than the 96-well microtiter plate method. In certain laboratories, higher sensitivities are obtained by performing a blind pass in which an inoculated cell monolayer is allowed to incubate for 48–72 hours, after which the monolayer is disrupted and used to inoculate a fresh monolayer that is stained after another cycle of growth.

Tissue culture detection of *C. trachomatis* is highly specific if a *C. trachomatis*-MOMP-specific stain is used, because stained *C. trachomatis* inclusions have a unique appearance. Less specific inclusion-detection methods using EIA, iodine, and Giemsa are not recommended. Certain CDC consultants believe that commercial stains employing monoclonal antibodies directed against lipopolysaccharide (LPS), which are genus-specific rather than species-specific, are more sensitive and more economical than species-specific monoclonal antibody stains directed against MOMP. Such stains might be suitable for routine use, but a species-specific stain would be preferable in situations requiring increased specificity.

The high specificity of cell culture and ability to retain the isolate make cell culture the first choice when the results will be used as evidence in legal investigations. In addition, cell culture is the only method by which a clinical isolate can be obtained for antimicrobial susceptibility testing. The relatively low sensitivity, long turnaround time, difficulties in standardization, labor intensity, technical complexity, stringent transport requirements, and relatively high cost are the primary disadvantages of cell culture isolation of *C. trachomatis*.

Non-Culture Tests:

Non-culture tests for the detection of *C trachomatis* in clinical specimens were first introduced in the 1980s. These tests generated a great deal of enthusiasm, as they did not require viable organisms, thus circumventing some of the collection and transport problems associated with tissue culture. These tests also offered the possibility of "rapid" diagnosis, i.e results could be obtained while the patient was waiting.

The first of the non-culture tests introduced was the direct fluorescein-conjugated monoclonal antibody (DFA) test, which detects a species-specific epitope. Although the results can be obtained within 30 minutes of collection, the DFA is not considered to be clinically practical because most doctors' offices do not have the required fluorescent microscope or a very experienced individual to read the smears. Furthermore, a pelvic examination is still required to obtain an endocervical specimen. The DFA test is less sensitive than culture (70% to 90%, depending on the site and clinical status) but is highly specific (>95%). Thus, the DFA test is considered to be best suited for high-prevalence populations when culture is not available, although the very nature of the test makes it difficult to use for screening large numbers of specimens.

Enzyme immunoassays (EIAs) were subsequently introduced a technology that has produced the greatest number of commercial tests available today. These tests generally use a polyclonal or a monoclonal antibody directed against the genus-specific lipopolysaccharide antigen. Unlike the DFA, EIAs are semiautomated and suitable for processing large numbers of specimens. Since their introduction in the late 1980s, EIA performance has improved. The sensitivity for cervical specimens is about 75% to 80%, and the specificity is approximately 98%. Blocking tests are available for confirmation of positive results, which can improve the EIA specificity to >99%.

Perhaps the most common non-culture test for detection of *C trachomatis*

is PACE2 which is used in many US hospitals and public health laboratories. The test uses a direct nucleic acid probe that hybridizes to a species-specific sequence of chlamydial 16S rRNA and detects *C trachomatis* by chemiluminescence. The performance of the probe assay is similar to that of available EIAs, with sensitivities of 75% to 80% and specificities >99%.

Despite improvements in sensitivities, these non-culture tests are not useful in low-prevalence populations; furthermore, they require invasive specimen collection. However, in recent years there has been considerable interest in the use of EIA tests with urine, predominantly from men, although the urine must be centrifuged and the sediment (including chlamydia-infected cells) resuspended before the test is performed. In general, EIAs have been reported to perform best with urine samples from symptomatic men. When compared to culture of the urethra with or without non-culture tests of urethral specimens as a standard, EIAs of urine have had sensitivities ranging from 55% to 86%, with specificities >94%. This relatively low-level performance makes them unacceptable for use as a screening test in low-prevalence populations. In addition, EIAs cannot be performed on urine samples from women because of low sensitivity.

The development of nucleic acid amplification tests has resulted in a significant increase in sensitivity. The most widely used of the nucleic amplification technologies is polymerase chain reaction (PCR), although another DNA amplification technology now in wide use is the ligase chain reaction (LCR). PCR and LCR both use primers directed at the *C trachomatis* cryptic plasmid, which is usually present at 10 copies per cell.

A third available nucleic acid amplification test is transcription-mediated amplification (TMA), an RNA amplification method. TMA uses reverse transcriptase and a T7 RNA polymerase to produce anywhere from 1 million to 1 billion copies of an RNA target. TMA is also isothermal--that is, it does not require the use of a thermocycler, which is a necessity for performing PCR or LCR. As shown in Table 1, EIAs can detect a minimum of 10^4 to 10^5 organisms, culture can detect 10 to 100 organisms, and DNA amplification tests can detect one to 10 organisms.

The introduction of nucleic acid amplification tests has been the most important advance in the field of chlamydia diagnostics since tissue culture replaced inoculation of eggs for culture and isolation of *C trachomatis* from clinical specimens. Because nucleic acid amplification is exquisitely sensitive and highly specific, it offers the opportunity to use noninvasive sampling (ie, urine) to screen for infection in asymptomatic women as well as men.

Symptoms:
As many as 1 in 4 men with chlamydia have no symptoms. In men, chlamydia may produce symptoms similar to gonorrhea. Symptoms may include:

- Burning sensation during urination
- Discharge from the penis or rectum
- Testicular tenderness or pain
- Rectal discharge or pain

Only about 30% of women with chlamydia have symptoms. Symptoms that may occur in women include:

- Burning sensation during urination
- Painful sexual intercourse
- Rectal pain or discharge
- Symptoms of PID, salpingitis, liver inflammation similar to hepatitis
- Vaginal discharge

If Untreated Causes….

- **For women.** If left untreated, chlamydia infection can cause pelvic inflammatory disease which can lead to damage of the fallopian tubes (the tubes connecting the ovaries to the uterus) or even cause infertility (the inability to have children). Untreated chlamydia infection could also increase the risk of ectopic pregnancy (when the fertilized egg implants and develops outside the uterus.) Furthermore, chlamydia may cause premature births (giving birth too early) and the infection can be passed along from the mother to her child during childbirth, causing an eye infection, blindness, or pneumonia in the newborn.

- **For men.** Chlamydia can cause a condition called nongonococcal urethritis (NGU) -- an infection of the urethra (the tube by which men and women pass urine), epididymitis -- an infection of the epididymis (the tube that carries sperm away from the testes), or proctitis -- an inflammation of the rectum.

Treatment:
Chlamydia can be easily treated and cured with antibiotics. A single dose of azithromycin or a week of doxycycline (twice daily) are the most commonly used treatments. HIV-positive persons with chlamydia should receive the same treatment as those who are HIV negative.

All sex partners should be evaluated, tested, and treated. Persons with chlamydia should abstain from sexual intercourse for 7 days after single dose antibiotics or until completion of a 7-day course of antibiotics, to prevent spreading the infection to partners.

Women whose sex partners have not been appropriately treated are at high risk for re-infection. Having multiple infections increases a woman's risk of serious reproductive health complications, including infertility. Women and men with chlamydia should be retested about three months after treatment of an initial infection, regardless of whether they believe that their sex partners were treated.

Dual Therapy for Gonococcal and Chlamydial Infections:
Patients infected with *N. gonorrhoeae* frequently are coinfected with *C. trachomatis*; this finding has led to the recommendation that patients treated for gonococcal infection also be treated routinely with a regimen that is effective against uncomplicated genital *C. trachomatis* infection Because most gonococci in the United States are susceptible to doxycycline and azithromycin, routine cotreatment might also hinder the development of antimicrobial-resistant *N. gonorrhoeae*. Limited data suggest that dual treatment with azithromycin might enhance treatment efficacy for pharyngeal infection when using oral cephalosporins.

In case of Pregnancy:
Doxycycline, ofloxacin, and levofloxacin are contraindicated in pregnant women. However, clinical experience and published studies suggest that azithromycin is safe and effective. Repeat testing to document chlamydial eradication (preferably by NAAT) 3 weeks after completion of therapy with the following regimens is recommended for all pregnant women to ensure therapeutic cure, considering the severe sequelae that might occur in mothers and neonates if the infection persists. Women aged <25 years and those at increased risk for chlamydia (i.e., women who have a new or more than one sex partner) also should be retested during the third trimester to prevent maternal postnatal complications and chlamydial infection in the infant. Pregnant women diagnosed with a chlamydial infection during the first trimester should not only receive a test to document chlamydial eradication, but be retested 3 months after treatment.

The frequent gastrointestinal side effects associated with erythromycin can result in noncompliance with the alternative regimens. Although erythromycin estolate is contraindicated during pregnancy because of drug-related hepatotoxicity, the lower dose 14-day erythromycin regimens can be considered if gastrointestinal tolerance is a concern.

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Laboratory detection of Methicillin/Oxacillin - Resistant S. Aureus

INTRODUCTION:

The detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in the clinical laboratory remains as challenging as does the need for new antibiotics to fight the bacterial resistance mechanisms. Historically, there has been a continuing battle to manufacture new antibiotics that are successful in inhibiting or killing *S. aureus*. Penicillin was effective against *S. aureus* for a very short period of time before resistance to it was seen. The first penicillinase – stable penicillin, methicillin, was introduced in England as an effective agent against penicillin resistant *S. aureus* in 1959; by 1961, the first cases of methicillin (oxacillin) resistance appeared and slowly spread across the United States. Today 35% of hospital strains of *S. aureus* are found to be resistant to methicillin (methicillin has been replaced *in vivo* and *in vitro* by oxacillin or nafcillin or other penicillinase- resistant antibiotics; some articles refer to these strains as ORSA). These same strains are usually resistant to a variety of other non-ß-lactam agents, such as quinolones, trimethoprim-sulfamethoxazole (SXT), macrolides, etc. The acronym, MRSA, may more appropriately represent "multiply" resistant *S. aureus*. In addition to hospital-associated MRSA, in recent years, community acquired strains of MRSA (CA-MRSA) are appearing throughout the world. These strains tend to be more susceptible to non-ß lactam agents, as compared to the hospital acquired MRSA isolates, and appear to carry a unique staphylococcal chromosome in relation to the resistant gene.

Analysis of data from many laboratories throughout the United States, submitted to the Focus Technologies' database, showed recently that the prevalence of MRSA in hospitals increased from 30% in 1996 to 45% in 2001 in inpatient isolates and from 17% to 29% in outpatient isolates. Factors that might aid in reducing the chance for colonization with MRSA and thus aid in preventing disease include improvements in antimicrobial therapy, consistent use of infection control policies such as hand-washing and use of anti-infective devices. In addition, when the Microbiology Laboratory and Infection Control departments work together to rapidly and accurately report MRSA cases, the effects of the other control measures can be only positively enhanced.

When *S. aureus* is isolated in a laboratory from any site, susceptibility testing is usually performed that includes oxacillin and a variety of other agents. Many phenotypic methods are available for detection of oxacillin resistance. Many strains of *S. aureus* are homogeneous in their resistance to oxacillin. However, it has long been known that phenotypic heterogeneity is common and, as in any infection, resistant and susceptible strains may be mixed together, creating some difficulty in detection of resistance. **Addition of 2% NaCl to the Mueller Hinton media, use of a lower temperature for incubation (33-35ºC) and the need for a full 24 hours of incubation are some of the recommendations for increasing the chances of detecting resistance by the agar and broth dilution methods.**

Cells expressing hetero resistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C. This is why CLSI recommends incubating isolates being tested against oxacillin, methicillin, or nafcillin at 33-35° C (maximum of 35°C) for a full 24 hours before reading.

Resistance to the penicillinase-stable antibiotics, oxacillin and nafcillin, in staphylococci is caused by expression of a new penicillin binding protein, PBP2a (PBP2′), which is encoded by the *mecA* gene. *MecA* is located on a genetic element called the staphylococcal cassette chromosome (SCC) in *S. aureus*. The new PBP has a very low affinity for ß-lactam antibiotics and hence the resistance that is seen phenotypically to oxacillin. Molecular detection of this *mecA* gene has become the "gold standard" for identification of MRSA, although commercially available methods for its detection in clinical laboratories are lacking today.

Most laboratories use one of the non-molecular methods, in addition, there are strains of *S. aureus* that hyperproduce beta-lactamases (BORSA) and, while they appear oxacillin resistant, do not possess the usual genetic mechanism for such resistance. There are also strains of *S. aureus*, known as MODSA, which possess a modification of existing penicillin-binding proteins, rather than acquisition of a new PBP as is the mechanism for classic MRSA. Neither BORSA nor MODSA possess the *mecA* gene and it is felt that reporting them as MRSA is probably an overcall of resistance.

More recently, there have been publications describing the use of a disk diffusion method using cefoxitin, a cephamycin, to predict oxacillin resistance. In one study by Felten et al, all classes of oxacillin resistance were detected 100% of the time when the zone around the cefoxitin disk was < 27 mm.

Skov et al. evaluated the 30 µg cefoxitin disk on Iso-Sensitest Agar (ISA, Oxoid Limited), with overnight incubation at 35-36ºC, against 457 strains of *S. aureus*, of which 190 strains were MRSA. They defined a zone size of ≥ 29 mm as susceptible and < 29 mm as resistant; 100% sensitivity and 99% specificity were achieved in this study. The mm gap between the resistant and susceptible strains was, however, very small and although they recommended the test as a replacement for the oxacillin screen, they did suggest that a lower disk concentration of cefoxitin might prove more useful. The most recent NCCLS supplement (M100-S14) suggests the use of a 30 µg cefoxitin disk to predict presence of *mecA*, using a breakpoint of ≤ 19 mm as indicative of resistance of *S. aureus* to oxacillin.

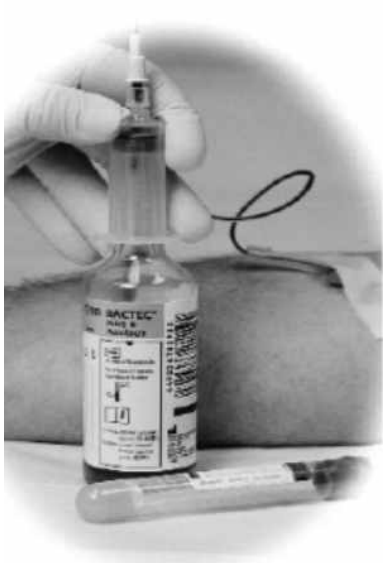
To increase sensitivity and potentially decrease turnaround time of detection of true MRSA, a number of molecular methods for detection of *mecA* gene have begun to appear in the literature. Use of real-time PCR assays directly in clinical specimens probably offers the most rapid approach. A multiplex PCR assay was reported on by Vannuffeel et al. in 1995 that was able to detect *mecA* gene as well as *femA* gene, the latter a regulator gene unique to *S. aureus* that is essential for the expression of oxacillin resistance. Applying their assay to whole blood, they were able to achieve a sensitivity of 50 organisms per ml of whole blood. Since coagulase-negative staphylococci do not possess this regulator, the potential is there for a very specific and rapid assay; however, no further work has been published on this to our knowledge.

Use of chromogenic media for the detection of many bacteria and fungi has begun to reappear in the literature. There is a new medium, BBL CHROMagar MRSA that has been shown to detect *S. aureus* directly from clinical specimens. Further evaluation will be needed to demonstrate its use in the clinical laboratory, but hopefully, it will prove comparable to methods such as the oxacillin screen or cefoxitin disk test. Recognition of isolates of *S. aureus* as MRSA continues to be a task that clinical microbiologists should pursue diligently and report as accurately and rapidly as possible.

REFRENCES:

www.cdc.gov/mrsa
www.bd.com
www.ncbi.nim.nih.gov

Best Practices in Blood Culturing



Blood culture specimens are among the most critical specimens processed by the microbiology laboratory. Positive blood cultures reveal the identity of the pathogenic microorganism and help guide therapeutic choices with subsequent antimicrobial susceptibility testing. This has been shown to decrease morbidity and mortality. However, if blood cultures are collected under sub-optimal conditions, positivity rates will be low and patient care will suffer.

When is the best time to draw blood cultures from a patient with suspected sepsis?

Draw blood cultures as close as possible to the episode of chills or fever. Do NOT delay, as recovery of microorganisms diminishes with time after the fever spike.

How many blood culture sets do I need to draw?

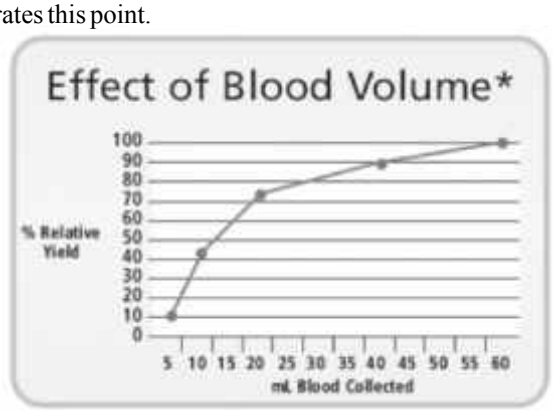
In the majority of circumstances, drawing 2 – 3 blood culture sets will detect ~99% of septic episodes. NEVER draw only 1 blood culture set during the initial evaluation of a septic patient.

In the evaluation of most patients with suspected sepsis, blood culture sets should be obtained within 5 minutes of each other, since the reticuloendothelial system will clear both transient and intermittent bacteremias within 15 – 30 minutes.

In cases of suspected sub acute infective endocarditis, urgent institution of empiric antimicrobial therapy is *usually* not required. It is far more important to establish the identity of the causative agent. Therefore, 3 sets of blood cultures should be drawn one hour apart, which helps document an endovascular source of infection. If those cultures are negative at 24 hours, two additional sets should be obtained. If urgent empiric antimicrobial therapy will be given, then obtain 3 blood culture sets, as above; i.e. within 5 minutes of each other.

How much blood should I draw from the patient?

The volume of blood is the single most critical factor in optimizing the sensitivity of blood culture. The graph below illustrates this point.



For most 2 bottle sets, at least 10 mL, and preferably 20 mL of blood should be obtained and divided between the 2 bottles of the set. The 2 sets (4 bottles) should therefore have between 20 – 40 mL total blood inoculated.

Recommendations:

A: Take blood for culture when there is a clinical need to do so and not as routine

Blood cultures are taken to identify patients with bacteraemia. There are many signs and symptoms in a patient which may suggest bacteraemia and clinical judgement is required, but the following indicators (which may be subtle in the very young, the elderly, those on steroids or immuno compromised) should be taken into account when assessing a patient for signs of bacteraemia or sepsis:

- Pyrexia > 38ºC
- focal signs of infection
- abnormal heart rate (raised), blood pressure (low or raised) or respiratory rate (raised)
- chills or rigors
- raised or very low white blood cell count
- new or worsening confusion.

Please note: signs of sepsis may be minimal or absent in the very young and the elderly.

Blood cultures should be taken after identification of possible bacteraemia or sepsis and before the administration of antibiotics. If a patient is on antibiotics, blood cultures should ideally be taken immediately before the next dose, with the exception of paediatric patients.

All blood cultures should be documented in the patient's notes, including date, time, site and indications.

B. Competence

Blood cultures should only be collected by members of staff (medical, nursing, healthcare assistant, phlebotomist or technician) who have been trained in the collection procedure and whose competence in blood culture collection has been assessed and maintained.

C. Always make a fresh stab

In patients with suspected bacteraemia, **do not** use existing peripheral lines/cannulae or sites immediately above peripheral lines. If a central line is present, blood **may** be taken from this **and** from a separate peripheral site when investigating potential infection related to the central line; the peripheral vein sample should be collected first. Identify a suitable venepuncture site before disinfecting the skin. Avoid femoral vein puncture because of the difficulty in adequate skin cleansing and disinfection.

D. Thoroughly disinfect the skin before inserting the needle

Thoroughly cleanse the patient's skin before venepuncture. Use soap and water to clean visibly soiled skin and then clean your own hands using the correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the National Patient Safety Agency (NPSA)

'Clean you hands campaign' is recommended). Use 2% chlorhexidine in 70% isopropyl alcohol to disinfect the patient's skin and allow to dry.

E. Once disinfected, do not touch the skin again

To avoid cross-contamination from the collector's fingers (even when gloved), it is vitally important not to palpate the site again once it has been disinfected.

F. Disinfect the culture bottle cap before transferring the sample

Ideally, remove the plastic cover immediately before collecting the sample; the top of the bottle will be clean but not sterile. Disinfect the tops of the culture bottles with a 2% chlorhexidine in 70% isopropyl alcohol impregnated swab. Allow the alcohol to fully evaporate for 30 seconds before proceeding with bottle inoculation.

Please note: the use of blood collection adapter caps without winged blood collection sets is not recommended. It is not possible to accurately judge sample volume and there is the potential for possible backflow of blood culture media into patient veins.

Procedure for Blood Culture Sampling:

1) Skin preparation

- Clean hands using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended).
- Clean any visibly soiled skin on the patient with soap and water then dry.
- Apply a disposable tourniquet and palpate to identify vein.
- Clean skin with 2% chlorhexidine in 70% isopropyl alcohol and allow to dry for 30 seconds.
- Do not repalpate skin following cleaning.
- If a culture is being collected from a central venous catheter, disinfect the access port with a 2% chlorhexidine in 70% isopropyl alcohol impregnated swab.

2) Kit preparation

- Have sharps disposal container available in immediate vicinity.
- Clean the tops of culture bottles with a 2% chlorhexidine in 70% isopropyl alcohol impregnated swab and allow to dry for 30 seconds.

3) Sample collection – Use method A as outlined below. (Method B should only be used where method A is not available)

A: WINGED BLOOD COLLECTION SET METHOD

- Clean hands again using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended) or use alcohol hand rub and apply clean examination gloves (sterile gloves are not necessary).
- Gloves and apron are worn (in line with local policy)
- Personal protective equipment (PPE) is disposed of correctly (in line with local policy) after use.
- Attach winged blood collection set to blood collection adapter cap.
- Insert needle into prepared site. Do not palpate again after cleaning.
- Place adapter cap over blood collection bottle and pierce septum.
- Hold bottle upright and use bottle graduation lines to accurately gauge sample volume and collect sample; inoculate aerobic culture first.
- If blood is being collected for other tests, always collect the blood culture first.
- Cover the site with an appropriate sterile dressing.
- Discard winged blood collection set in a sharps container.
- Clean hands using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended) after removing gloves.
- Document date, reason for sample, site of venepuncture, operator undertaking procedure and if procedure was high risk with signature.

B: NEEDLE AND SYRINGE METHOD

- Clean hands again using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended) or use alcohol hand rub and apply clean examination gloves (sterile gloves are not necessary).
- Gloves and apron are worn (in line with local policy).
- Personal protective equipment (PPE) is disposed of correctly (in line with local policy) after use.
- Insert needle. Do not palpate again after cleaning.
- Collect sample and release tourniquet.
- Cover the puncture site with an appropriate dressing.
- If blood is being collected for other tests, always inoculate the blood culture bottles first.
- Inoculate blood into culture bottles; do not change the needle between sample collection and inoculation; inoculate anaerobic culture first.
- Discard needle and syringe in a sharps container.
- Clean hands again using correct hand hygiene technique (use the World Health Organisation's '5 moment of hand hygiene' or the NPSA 'Clean Your Hands Campaign are recommended).
- Document date, reason for sample, site of venepuncture, operator undertaking procedure and if procedure was high risk with signature.

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Chromogenic media's are designed for quick detection of bacteria from water samples, food samples and clinical & non-clinical specimens.

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Clear differentiation	Superior performance
Improved colours aid interpretation	Easy to identify
Minimises Confirmatory testing	Reduces costs

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Cat No.	Product Name	Application
AM10251 AM50251	Chromogenic Coliform Agar	For detection of *Escherichia coli* and total coliforms in water & food samples.
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AM10253 AM50253	Chromogenic Enterococci Broth	For differentiation and identification of *Enterococci* from water samples
AM10254 AM50254	Chromogenic UTI Agar	For presumptive identification of microorganisms causing urinary Tract Infection.
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ACTIVITY : Broad spectrum: Bactericidal, Fungicidal & Virucidal.

CONTACT TIME : 1 min (undiluted & 10% v/v solution), 5 min (5% v/v solution), 10 min (2.5% v/v solution).

APPLICATIONS :

Medical: In Hospitals, Nursing homes, Medical colleges, Pathological laboratories for Inter-operative irrigation. Pre & post surgery skin and mucous membrane disinfection. Post-operative dressings. Surgical & non-surgical wound dressings. Surgical Bath/Sitz bath. Routine antisepsis during minor incisions, catheterization, scopy etc. First aid. Surface disinfection.

Industrial: In Pharmaceutical industry, Food & beverage industry, Hotel industry etc. General surface disinfection. Eliminating biofilms.

USAGE	DOSAGE AND ADMINISTRATION
Pre & post-surgery skin cleaning & disinfection	Use undiluted
Surgical, post operative, non surgical dressing	Use undiluted, once a day/alternate day
Surgical bath/Sitz bath	Add 50 mL of NUSEPT™ in 1 L of water & use
Antisepsis during minor incisions, Scopy, Catheterization, first aid, cuts, bites, stings etc	Use undiluted
Chronic wound management (diabetic foot, pressure and venous leg ulcers)	Use undiluted
Burn wound management (Only for 1st and 2nd Degree burns, chemical burns)	Use 100 mL NUSEPT™ in 1 L sterile water for both washing (with 1 minute contact time) and dressing of burn wound (Dressing must be changed everyday/ alternate days or as directed)
Midwifery, nursery & sickroom	Use undiluted
Intra-operative irrigation	Use 50 mL NUSEPT™ in 1 L sterile water
General hard surface disinfection	Add 100 mL of NUSEPT™ in 1 L of water and gently mop the floor or surfaces

Highlights of the coming issue

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