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Editorial

Contents

Sterilization is a term referring to any process that eliminates or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Chemicals are also used for sterilization. Although heating provides the most reliable way to rid objects of all transmissible agents, it is not always appropriate, because it will damage heat-sensitive materials such as biological materials, fiber optics, electronics, and many plastics. Liquid sterilants and high disinfectants typically include oxidizing agents such as hydrogen peroxide and peracetic acid and aldehydes such as glutaraldehyde and more recently o-phthalaldehyde may be used. The chemicals used as sterilants are designed to destroy a wide range of pathogens and typically the same properties that make them good sterilants makes them harmful to humans.

Urinary tract infection is defined as the microbial invasion of any tissue of tract from the renal cortex to urethral meatus also including prostate and epidymis. Normally, urine and all of the organs and structures of the urinary tract are sterile and contain no microorganisms. However, infectious microorganisms can get into the urinary tract from outside the body through the urethra and cause urinary tract infections. Our Current Trends section focuses on the Laboratory Diagnosis of UTI.

Our In Profile scientist of the month is Sir Frederick Gowland Hopkins - Nobel Prize winner in Physiology or Medicine for discovery of essential nutrient factors—now known as vitamins in 1929.

Candida Albicans is a yeast infestation, from a parasite that thrives in warm-blooded animals. It is an ovoid or spherical budding cell, which produces pseudomycelia in culture as well as in tissues. There are more than 20 species of *Candida*, the most common is *Candida albicans*. Candidal infections commonly occur in warm moist body areas, such as underarms.

The development of antifungal agents has lagged behind that of antibacterial agents. Fungi are eukaryotes, and consequently most agents toxic to fungi are also toxic to the host. Furthermore, because fungi generally grow slowly and often in multicellular forms, they are more difficult to quantify than bacteria. This difficulty complicates experiments designed to evaluate the *in vitro* or *in vivo* properties of a potential antifungal agent. An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host.

Laboratories require high levels of organisms to culture successfully for respiratory viruses such as Influenza, RSV & Para influenza virus. A properly taken nasopharyngeal swab will yield high level of organisms. Nasopharyngeal culture is a sample of secretions from the uppermost part of the throat, behind the nose, to detect organisms that can cause disease.

"Laughter is the best medicine" so relax your mind with light humour & also know some interesting facts about human body.

We are looking forward for your continuous support in making this journal a better effort each time. Feedback & suggestions are always welcomed.

Chemical Sterilization Techniques

DISINFECTANTS / STERILANTS

Introduction

Many disinfectants are used alone or in combinations in the health-care setting. These include alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, *ortho*-phthalaldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, biguanides and quaternary ammonium compounds. In most instances, a given product is designed for a specific purpose and is to be used in a certain manner. Therefore, users should read labels carefully to ensure the correct product is selected for the intended use and applied efficiently.

Disinfectants are not interchangeable, and incorrect concentrations and inappropriate disinfectants can result in excessive costs. Because occupational diseases among cleaning personnel have been associated with use of several disinfectants (e.g., formaldehyde, glutaraldehyde, and chlorine), precautions (e.g., gloves and proper ventilation) should be used to minimize exposure. Asthma and reactive airway disease can occur in sensitized persons exposed to any airborne chemical, including disinfectants. The preferred method to control this is elimination of the chemical (through engineering controls or substitution) or relocation of the worker.

The following overview of the performance characteristics of each provides users with sufficient information to select an appropriate disinfectant for any item and use it in the most efficient way.

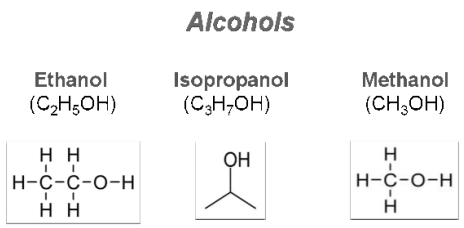
Chemical Disinfectants / Sterilants

Alcohol

Overview:

In the healthcare setting, "alcohol" refers to two water-soluble chemical compounds: ethyl alcohol and isopropyl alcohol. There is no liquid chemical sterilant or high-level disinfectant with alcohol as the main active ingredient. These alcohols are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria; they also are tuberculocidal, fungicidal, and virucidal but do not destroy bacterial spores. Their cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is 60% - 90% solutions in water (v/v)

Mode of Action:



The most feasible explanation for the antimicrobial action of alcohol is denaturation of proteins. This mechanism is supported by the observation that absolute ethyl alcohol, a dehydrating agent, is less bactericidal than mixtures of alcohol and water because proteins are denatured more quickly in the presence of water. Protein denaturation also is consistent with observations that alcohol destroys the dehydrogenases of *Escherichia coli*, and that ethyl alcohol increases the lag phase of *Enterobacter aerogenes*. The bacteriostatic action was believed caused by inhibition of the production of metabolites essential for rapid cell division.

Microbicidal Activity:

Methyl alcohol (methanol) has the weakest bactericidal action of the alcohols and thus seldom is used in healthcare. The bactericidal activity of various concentrations of ethyl alcohol (ethanol) was examined against a variety of microorganisms in exposure periods ranging from 10 seconds to 1 hour. *Pseudomonas aeruginosa* was killed in 10 seconds by all concentrations of ethanol from 30% to 100% (v/v), and *Serratiamarcescens, E. coli* and *Salmonella typhosa* were killed in 10 seconds by all concentrations of ethanol from 40% to 100%. The gram-positive organisms *Staphylococcus aureus* and *Streptococcus pyogenes* were slightly more resistant, being killed in 10 seconds by ethyl alcohol concentrations of 60%-95%. Isopropyl alcohol (isopropanol) was slightly more bactericidal than ethyl alcohol for *E. coli* and *S. aureus*.

Ethyl alcohol, at concentrations of 60%-80%, is a potent virucidal agent inactivating all of the lipophilic viruses (e.g., herpes, vaccinia, and influenza virus) and many hydrophilic viruses (e.g., adenovirus, enterovirus, rhinovirus, and rotaviruses but not hepatitis A virus (HAV) or poliovirus. Isopropyl alcohol is not active against the nonlipidenteroviruses but is fully active against the lipid viruses. Studies also have demonstrated the ability of ethyl and isopropyl alcohol to inactivate the hepatitis B virus (HBV) and the herpes virus, and ethyl alcohol to inactivate human immunodeficiency virus (HIV), rotavirus, echovirus, and astrovirus.

In 1964, Spaulding stated that alcohols were the germicide of choice for tuberculocidal activity, and they should be the standard by which all other tuberculocides are compared. For example, he compared the tuberculocidal activity of iodophor (450 ppm), a substituted phenol (3%), and isopropanol (70% v/v) using the mucin-loop test (10^6M. tuberculosis per loop) and determined the contact times needed for complete destruction were 120–180 minutes, 45–60 minutes, and 5 minutes, respectively. The mucin-loop test is a severe test developed to produce long survival times. Thus, these figures should not be extrapolated to the exposure times needed when these germicides are used on medical or surgical material.

Ethyl alcohol (70%) was the most effective concentration for killing the tissue phase of *Cryptococcus neoformans, Blastomyces dermatitidis, Coccidioides immitis,* and *Histoplasma capsulatum* and the culture phases of the latter three organisms aerosolized onto various surfaces. The culture phase was more resistant to the action of ethyl alcohol and required about 20 minutes to disinfect the contaminated surface, compared with <1 minute for the tissue phase.

Isopropyl alcohol (20%) is effective in killing the cysts of *Acanthamoeba culbertsoni* as are chlorhexidine, hydrogen peroxide, and thimerosal.

Uses:

Alcohols are not recommended for sterilizing medical and surgical materials principally because they lack sporicidal action and they cannot penetrate protein-rich materials. Fatal postoperative wound infections with *Clostridium* have occurred when alcohols were used to sterilize surgical instruments contaminated with bacterial spores. Alcohols have been used effectively to disinfect oral and rectal thermometers, hospital pagers, scissors, and stethoscopes. Alcohols have been used to disinfect fiberoptic endoscopes but failure of this disinfectant have lead to infection. Alcohol towelettes have been used for years to disinfect small surfaces such as rubber stoppers of multiple-dose medication vials or vaccine bottles. Furthermore, alcohol occasionally is used to disinfect external surfaces of equipment (e.g., stethoscopes, ventilators, manual ventilation bags), CPR manikins, ultrasound instruments or medication

preparation areas. Two studies demonstrated the effectiveness of 70% isopropyl alcohol to disinfect reusable transducer heads in a controlled environment. In contrast, three bloodstream infection outbreaks have been described when alcohol was used to disinfect transducer heads in an intensive-care setting.

The documented shortcomings of alcohols on equipment are that they damage the shellac mountings of lensed instruments, tend to swell and harden rubber and certain plastic tubing after prolonged and repeated use, bleach rubber and plastic tiles and damage tonometer tips (by deterioration of the glue) after the equivalent of 1 working year of routine use. Tonometer biprisms soaked in alcohol for 4 days developed rough front surfaces that potentially could cause corneal damage; this appeared to be caused by weakening of the cementing substances used to fabricate the biprisms. Corneal opacification has been reported when tonometer tips were swabbed with alcohol immediately before measurement of intraocular pressure. Alcohols are flammable and consequently must be stored in a cool, well-ventilated area. They also evaporate rapidly, making extended exposure time difficult to achieve unless the items are immersed.

Glutaraldehyde

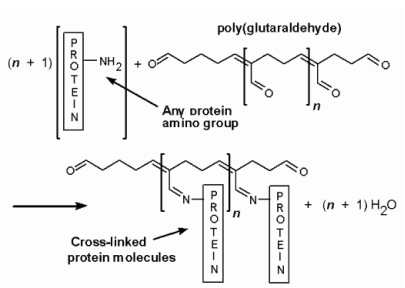
Overview:

Glutaraldehyde is a saturated dialdehyde that has gained wide acceptance as a high-level disinfectant and chemical sterilant. Aqueous solutions of glutaraldehyde are acidic and generally in this state are not sporicidal. Only when the solution is "activated" (made alkaline) by use of alkalinating agents to pH 7.5–8.5 does the solution become sporicidal. Once activated, these solutions have a shelf-life of minimally 14 days because of the polymerization of the glutaraldehyde molecules at alkaline pH levels. This polymerization blocks the active sites (aldehyde groups) of the glutaraldehyde molecules that are responsible for its biocidal activity.

New glutaraldehyde formulations (e.g., glutaraldehyde-phenol-sodium phenate, potentiated acid glutaraldehyde, stabilized alkaline glutaraldehyde) produced in the past 30 years have overcome the problem of rapid loss of activity (e.g., use-life 28–30 days) while generally maintaining excellent microbicidal activity. However, antimicrobial activity depends not only on age but also on use conditions, such as dilution and organic stress. Manufacturers' literature for these preparations suggests the neutral or alkaline glutaraldehydes possess microbicidal and anticorrosion properties superior tothose of acid glutaraldehydes, and a few published reports substantiate these claims. However, two studies found no difference in the microbicidal activity of alkaline and acid glutaraldehydes. The use of glutaraldehyde-based solutions in health-care facilities is widespread because of their advantages, including excellent biocidal properties; activity in the presence of organic matter (20% bovine serum); and noncorrosive action to endoscopic equipment, thermometers, rubber, or plastic equipment.

Mode of Action:

The biocidal activity of glutaraldehyde results from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis.



Microbicidal Activity:

The in vitro inactivation of microorganisms by glutaraldehydes has been extensively investigated and reviewed. Several investigators showed that ≥2% aqueous solutions of glutaraldehyde, buffered to pH 7.5–8.5 with sodium bicarbonate effectively killed vegetative bacteria in <2 minutes; *M. tuberculosis*, fungi, and viruses in <10 minutes; and spores of *Bacillus* and *Clostridium* species in 3 hours. Spores of *C. difficile* are more rapidly killed by 2% glutaraldehyde than are spores of other species of *Clostridium* and *Bacillus*. Microorganisms with substantial resistance to glutaraldehyde have been reported, including some mycobacteria (*M. chelonae, Mycobacterium avium-intracellulare, M. xenopi*), *Methylobacteriummesophilicum*, *Trichosporon*, fungal ascospores (e.g., *Microascus cinereus, Cheatomium globosum*), and *Cryptosporidium*. *M. chelonae* persisted in a 0.2% glutaraldehyde solution used to store porcine prosthetic heart valves.

Two percent alkaline glutaraldehyde solution inactivated *M. tuberculosis* cells on the surface of penicylinders within 5 minutes at 18°C. However, subsequent studies questioned the mycobactericidal competency of glutaraldehydes. Two percent alkaline glutaraldehyde has slow action (20 to >30 minutes) against *M. tuberculosis*. Suspensions of *M. avium, M. intracellulare*, and *M. gordonae* were more resistant to inactivation by a 2% alkaline glutaraldehyde (estimated time to complete inactivation: ~60 minutes) than were virulent *M. tuberculosis* (estimated time to complete inactivation ~25 minutes). The rate of kill was directly proportional to the temperature, and a standardized suspension of *M. tuberculosis* could not be sterilized within 10 minutes. Commercially available chemical sterilants containing ≥2% glutaraldehyde uses increased temperature (35°C) to reduce the time required to achieve high-level disinfection (5 minutes), but its use is limited to automatic endoscope reprocessors equipped with a heater. Several investigators have demonstrated that glutaraldehyde solutions inactivate 2.4 to >5.0 $\log_{10}$ of *M. tuberculosis* in 10 minutes (including multidrug-resistant *M. tuberculosis*) and 4.0–6.4 $\log_{10}$ of *M. tuberculosis* in 20 minutes. On the basis of these data and other studies, 20 minutes at room temperature is considered the minimum exposure time needed to reliably kill *Mycobacteria* and other vegetative bacteria with ≥2% glutaraldehyde.

Studies showed a glutaraldehyde concentration decline after a few days of use in an automatic endoscope washer. The decline occurs because instruments are not thoroughly dried and water is carried in with the instrument, which increases the solution's volume and dilutes its effective concentration. This emphasizes the need to ensure that semicritical equipment is disinfected with an acceptable concentration of glutaraldehyde. Data suggest that 1.0%–1.5% glutaraldehyde is the minimum effective concentration for >2% glutaraldehyde solutions when used as a high-level disinfectant. Chemical test strips or liquid chemical monitors are necessary for determining whether an effective concentration of glutaraldehyde is present despite repeated use and dilution. The frequency of testing should be based on how frequently the solutions are used (e.g., used daily, test daily; used weekly, test before use; used 30 times per day, test each 10th use), but the strips should not be used to extend the use life beyond the expiration date. The bottle of test strips should be dated when opened and used for the period of time indicated on the bottle. The results of test strip monitoring should be documented. The concentration should be considered unacceptable or unsafe when the test indicates a dilution below the product's minimum effective concentration (MEC) (generally to ≤1.0%–1.5% glutaraldehyde) by the indicator not changing colour.

A 2.0% glutaraldehyde–7.05% phenol–1.20% sodium phenate product that contained 0.125% glutaraldehyde–0.44% phenol–0.075% sodium phenate when diluted 1:16 is not recommended as a high-level disinfectant because it lacks bactericidal activity in the presence of organic matter and lacks tuberculocidal, fungicidal, virucidal, and sporicidal activity. The regulatory bodies issued an order to stop the sale of all batches of this product because of efficacy data showing the product is not effective against spores and possibly other microorganisms or inanimate objects as claimed on the label.

Uses:

Glutaraldehyde is used most commonly as a high-level disinfectant for medical equipment such as endoscopes spirometry tubing, dialyzers, transducers, anesthesia and respiratory therapy equipment, hemodialysis proportioning and dialysate delivery systems and reuse of laparoscopic disposable plastic trocars. Glutaraldehyde is noncorrosive to metal and does not damage lensed instruments, rubber or plastics.

Colitis believed caused by glutaraldehyde exposure from residual disinfecting solution in endoscope solution channels has been reported and is preventable by careful endoscope rinsing. It was found that residual glutaraldehyde levels after disinfection was <0.2 mg/L to 159.5 mg/L. Similarly, keratopathy and corneal decompensation were caused by ophthalmic instruments that were inadequately rinsed after soaking in 2% glutaraldehyde.

Healthcare personnel can be exposed to elevated levels of glutaraldehyde vapor when equipment is processed in poorly ventilated rooms, when spills occur, when glutaraldehyde solutions are activated or changed, or when immersion baths are used. Acute or chronic exposure can result in skin irritation or dermatitis, mucous membrane irritation (eye, nose, mouth), or pulmonary symptoms. Epistaxis, allergic contact dermatitis, asthma, and rhinitis also have been reported in healthcare workers exposed to glutaraldehyde.

Glutaraldehyde exposure should be monitored to ensure a safe work environment. Testing can be done by four techniques: a silica gel tube/gas chromatography with a flame ionization detector, dinitrophenylhydrazine (DNPH)-impregnated filter cassette/high-performance liquid chromatography (HPLC) with an ultraviolet (UV) detector, a passive badge/HPLC, or a handheld glutaraldehyde air monitor. The silica gel tube and the DNPH-impregnated cassette are suitable for monitoring the 0.05 ppm ceiling limit. The passive badge, with a 0.02 ppm limit of detection, is considered marginal at the Americal Council of Governmental Industrial Hygienists (ACGIH) ceiling level. The ceiling level is considered too close to the glutaraldehyde meter's 0.03 ppm limit of detection to provide confidence in the readings. A monitoring schedule is needed to ensure the level is less than the ceiling limit. For example, monitoring should be done initially to determine glutaraldehyde levels, after procedural or equipment changes, and in response to worker complaints. If the glutaraldehyde level is higher than the maximum limit of 0.05 ppm, corrective action and repeat monitoring would be prudent.

Work-practice controls that can be used to resolve these problems, which include air systems that provide 7–15 air exchanges per hour, ductless fume hoods with absorbents for the glutaraldehyde vapor, tight-fitting lids on immersion baths, personal protection (e.g., nitrile or butyl rubber gloves but not natural latex gloves, goggles) to minimize skin or mucous membrane contact. If engineering controls fail to maintain levels below the ceiling limit, institutions can consider the use of respirators (e.g., a half-face respirator with organic vapor cartridge or a type "C" supplied air respirator with a full face-piece operated in a positive pressure mode).

Disposal:

If glutaraldehyde disposal through the sanitary sewer system is restricted, sodium bisulfate can be used to neutralize the glutaraldehyde and make it safe for disposal.

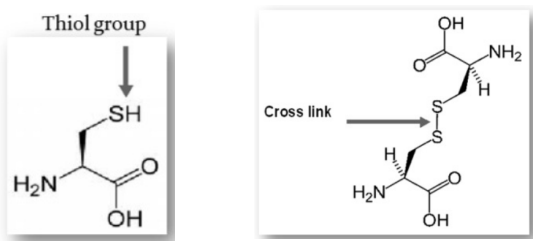
Hydrogen Peroxide

Overview:

The literature contains several accounts of the properties, germicidal effectiveness, and potential uses for stabilized hydrogen peroxide in the health-care setting. Published reports ascribe good germicidal activity to hydrogen peroxide and attest to its bactericidal, virucidal, sporicidal, and fungicidal properties.

Mode of Action:

Targets Cell wall, Cytoplasmic membrane, Cytoplasm. Interacts with the cell surface and break down into H_2O & free OH^- radicals. Free OH^- radicals oxidises all macromolecules (carbohydrates, lipids, proteins, amino acids & nucleic acid) Oxidises the thiol groups in cysteine residues (determinants of protein structure & function) present in proteins & vital microbial enzymes resulting in metabolic inhibition of the cell. Disruption of disulphide cross-links between cysteine residues present in the structural proteins in of cell wall, membrane &ribosomes. Disruption of cell membrane. Inactivation of enzymes. Metabolic inhibition. Cell death. Hydrogen peroxide works by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA, and other essential cell components. Catalase, produced by aerobic organisms and facultative anaerobes that possess cytochrome systems, can protect cells from metabolically produced hydrogen peroxide by degrading hydrogen peroxide to water and oxygen. This defence is overwhelmed by the concentrations used for disinfection.



$$2\ H_2O_2(aq) \rightarrow 2\ H_2O(l) + O_2(g)$$

Microbicidal Activity:

Hydrogen peroxide is active against a wide range of microorganisms, including bacteria, yeasts, fungi, viruses, and spores. A 0.5% accelerated hydrogen peroxide demonstrated bactericidal and virucidal activity in 1 minute and mycobactericidal and fungicidal activity in 5 minutes. Organisms with high cellular catalase activity (e.g., *S. aureus, S. marcescens*, and *Proteus mirabilis*) required 30–60 minutes of exposure to 0.6% hydrogen peroxide for a8 log reduction in cell counts, whereas organisms with lower catalase activity (e.g., *E. coli, Streptococcus* species, and *Pseudomonas* species) required only 15 minutes' exposure. In an investigation a complete kill of 10^6 spores (i.e., *Bacillus* species) occurred with a 10% concentration and a 60-minute exposure time. A 3% concentration for 150 minutes killed 10^6 spores in six of seven exposure trials. A 7% stabilized hydrogen peroxide proved to be sporicidal (6 hours of exposure), mycobactericidal (20 minutes), fungicidal (5 minutes) at full strength, virucidal (5 minutes) and bactericidal (3 minutes) at a 1:16 dilution when a quantitative carrier test was used. The 7% solution of hydrogen peroxide, tested after 14 days of stress (in the form of germ-loaded carriers and respiratory therapy equipment), was sporicidal (>7 log10 reduction in 6 hours), mycobactericidal (>6.5 log10 reduction in 25minutes),

fungicidal (>5 log10 reduction in 20 minutes), bactericidal (>6 log10 reduction in 5 minutes) and virucidal (5 log10 reduction in 5 minutes). The time required for inactivating three serotypes of rhinovirus using a 3% hydrogen peroxide solution was 6–8 minutes; this time increased with decreasing concentrations (18-20 minutes at 1.5%, 50–60 minutes at 0.75%).

The product marketed as a sterilant is a premixed, ready-to-use chemical that contains 7.5% hydrogen peroxide and 0.85% phosphoric acid (to maintain a low pH). The mycobactericidal activity of 7.5% hydrogen peroxide has been verified in a study showing the inactivation of $>10^5$ multidrug-resistant *M. tuberculosis* after a 10-minute exposure. Thirty minutes were required for >99.9% inactivation of poliovirus and HAV.

When the effectiveness of 7.5% hydrogen peroxide at 10 minutes was compared with 2% alkaline glutaraldehyde at 20 minutes in manual disinfection of endoscopes, no significant difference in germicidal activity was observed. No complaints were received from the nursing or medical staff regarding odour or toxicity. In one study, 6% hydrogen peroxide (unused product was 7.5%) was more effective in the high-level disinfection of flexible endoscopes than was the 2% glutaraldehyde solution. A new, rapid-acting 13.4% hydrogen peroxide formulation (that is not yet FDA-cleared) has demonstrated sporicidal, mycobactericidal, fungicidal, and virucidal efficacy. Manufacturer data demonstrate that this solution sterilizes in 30 minutes and provides high-level disinfection in 5 minutes. This product has not been used long enough to evaluate material compatibility to endoscopes and other semicritical devices, and further assessment by instrument manufacturers is needed.

Under normal conditions, hydrogen peroxide is stable when properly stored (e.g., in dark containers). The decomposition or loss of potency in small containers is less than 2% per year at ambient temperatures.

Uses:

Commercially available 3% hydrogen peroxide is a stable and effective disinfectant when used on inanimate surfaces. It has been used in concentrations from 3% to 6% for disinfecting soft contact lenses (e.g., 3% for 2–3 hrs), tonometer biprisms, ventilators, fabrics, and endoscopes. Hydrogen peroxide was effective in spot-disinfecting fabrics in patients' rooms. Corneal damage from a hydrogen peroxide-soaked tonometer tip that was not properly rinsed has been reported. Hydrogen peroxide also has been instilled into urinary drainage bags in an attempt to eliminate the bag as a source of bladder bacteriuria and environmental contamination. Although the instillation of hydrogen peroxide into the bag reduced microbial contamination of the bag, this procedure did not reduce the incidence of catheter-associated bacteriuria.

An epidemic of pseudomembrane-like enteritis and colitis in seven patients in a gastrointestinal endoscopy unit also has been associated with inadequate rinsing of 3% hydrogen peroxide from the endoscope.

As with other chemical sterilants, dilution of the hydrogen peroxide must be monitored by regularly testing the minimum effective concentration (i.e., 7.5%–6.0%).

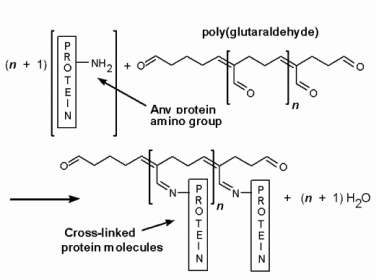
Ortho-phthalaldehyde (OPA)

Overview:

Ortho-phthalaldehyde is a high-level disinfectant that received acceptance from regulatory bodies since October 1999. It contains 0.55% 1,2 benzenedicarboxaldehyde (OPA). OPA solution is a clear, pale-blue liquid with a pH of 7.5.

Mode of Action:

Preliminary studies on the mode of action of OPA suggest that both OPA and glutaraldehyde interact with amino acids, proteins, and microorganisms. However, OPA is a less potent cross-linking agent. This is compensated for by the lipophilic aromatic nature of OPA that is likely to assist its uptake through the outer layers of mycobacteria and gram-negative bacteria. OPA appears to kill spores by blocking the spore germination process.



Microbicidal Activity:

Studies have demonstrated excellent microbicidal activity in vitro. For example, OPA has superior mycobactericidal activity (5-log10 reduction in 5 minutes) to glutaraldehyde. The mean times required to produce a 6-log10 reduction for *M. bovis* using 0.21% OPA was 6 minutes, compared with 32 minutes using 1.5% glutaraldehyde. OPA showed good activity against the mycobacteria tested, including the glutaraldehyde-resistant strains, but 0.5% OPA was not sporicidal with 270 minutes of exposure. Increasing the pH from its unadjusted level (about 6.5) to pH 8 improved the sporicidal activity of OPA. The level of biocidal activity was directly related to the temperature. A greater than 5-$\log_{10}$ reduction of *B. atrophaeus* spores was observed in 3 hours at 35°C, than in 24 hours at 20°C. Also, with an exposure time ≤5 minutes, biocidal activity decreased with increasing serum concentration. However, efficacy did not differ when the exposure time was ≥10 minutes. In addition, OPA is effective (>5-log10 reduction) against a wide range of microorganisms, including glutaraldehyde-resistant mycobacteria and *B. atrophaeus* spores.

The influence of laboratory adaptation of test strains, such as *P. aeruginosa*, to 0.55% OPA has been evaluated. Resistant and multiresistant strains increased substantially in susceptibility to OPA after laboratory adaptation (log10 reduction factors increased by 0.54 and 0.91 for resistant and multiresistant strains, respectively).

Uses:

OPA has several potential advantages over glutaraldehyde. It has excellent stability over a wide pH range (pH 3–9), is not a known irritant to the eyes and nasal passages, does not require exposure monitoring, has a barely perceptible odor, and requires no activation. OPA, like glutaraldehyde, has excellent material compatibility. A potential disadvantage of OPA is that it stains proteins gray (including unprotected skin) and thus must be handled with caution. However, skin staining would indicate improper handling that requires additional training and/or personal protective equipment (e.g., gloves, eye and mouth protection, and fluid-resistant gowns). OPA residues remaining on inadequately water-rinsed trans-oesophageal echo probes can stain the patient's mouth. Meticulous cleaning, using the correct OPA exposure time (e.g., 12 minutes) and copious rinsing of the probe with water should eliminate this problem. The results of one study provided a basis for a recommendation that rinsing of instruments disinfected with OPA will require at least 250 mL of water per channel to reduce the chemical residue to a level that will not compromise patient or staff safety (<1 ppm). Personal protective equipment should be worn when contaminated instruments, equipment, and chemicals are handled. In addition, equipment must be thoroughly rinsed to prevent discoloration of a patient's skin or mucous membrane.

Peracetic Acid

Overview:

Peracetic, or peroxyacetic, acid is characterized by rapid action against all microorganisms. Special advantages of peracetic acid are that it lacks harmful decomposition products (i.e., acetic acid, water, oxygen, hydrogen peroxide), enhances removal of organic material, and leaves no residue. It remains effective in the presence of organic matter and is sporicidal at low temperatures.

Peracetic acid can corrode copper, brass, bronze, plain steel, and galvanized iron but these effects can be reduced by additives and pH modifications. It is considered unstable, particularly when diluted; for example, a 1% solution loses half its strength through hydrolysis in 6 days, whereas 40% peracetic acid loses 1%–2% of its active ingredients per month.

Mode of Action:

Little is known about the mechanism of action of peracetic acid, but it is believed to function similarly to other oxidizing agents—that is, it denatures proteins, disrupts the cell wall permeability, and oxidizes sulfhydryl and sulfur bonds in proteins, enzymes, and other metabolites.

Microbicidal Activity:

Peracetic acid will inactivate gram-positive and gram-negative bacteria, fungi, and yeasts in $\leq$5 minutes at <100 ppm. In the presence of organic matter, 200–500 ppm is required. For viruses, the dosage range is wide (12–2250 ppm), with poliovirus inactivated in yeast extract in 15 minutes with 1,500–2,250 ppm. In one study, 3.5% peracetic acid was ineffective against HAV after 1-minute exposure using a carrier test. Peracetic acid (0.26%) was effective ($\log_{10}$ reduction factor >5) against all test strains of mycobacteria (*M. tuberculosis*, *M. avium-intracellul* are, *M. chelonae*, and *M. fortuitum*) within 20–30 minutes in the presence or absence of an organic load. With bacterial spores, 500–10,000 ppm (0.05%–1%) inactivates spores in 15 seconds to 30 minutes using a spore suspension test.

Uses:

An automated machine using peracetic acid to chemically sterilize medical (e.g., endoscopes, arthroscopes), surgical, and dental instruments is used in the United States. As previously noted, dental handpieces should be steam sterilized. The sterilant, 35% peracetic acid, is diluted to 0.2% with filtered water at 50°C. Simulated-use trials have demonstrated excellent microbicidal activity, and three clinical trials have demonstrated both excellent microbial killing and no clinical failures leading to infection. The high efficacy of the system was demonstrated in a comparison of the efficacies of the system with that of ethylene oxide. Only the peracetic acid system completely killed 6 $\log_{10}$ of *M. chelonae, E. faecalis,* and *B. atrophaeus* spores with both an organic and inorganic challenge. An investigation that compared the costs, performance, and maintenance of urologic endoscopic equipment processed by high-level disinfection (with glutaraldehyde) with those of the peracetic acid system reported no clinical differences between the two systems.

Furthermore, three clusters of infection using the peracetic acid automated endoscope reprocessor were linked to inadequately processed bronchoscopes when inappropriate channel connectors were used with the system. These clusters highlight the importance of training, proper model-specific endoscope connector systems, and quality-control procedures to ensure compliance with endoscope manufacturer recommendations and professional organization guidelines. An alternative high-level disinfectant available in the United Kingdom contains 0.35% peracetic acid. Although this product is rapidly effective against a broad range of microorganisms, it tarnishes the metal of endoscopes and is unstable, resulting in only a 24-hour use life.

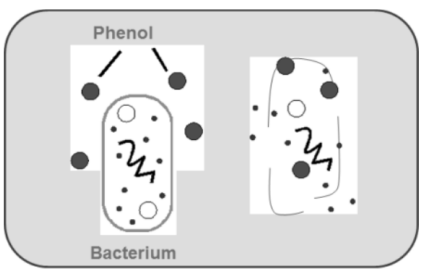
Phenolics

Overview:

Phenol has occupied a prominent place in the field of hospital disinfection since its initial use as a germicide by Lister in his pioneering work on antiseptic surgery. In the past 30 years, however, work has concentrated on the numerous phenol derivatives or phenolics and their antimicrobial properties. Phenol derivatives originate when a functional group (e.g., alkyl, phenyl, benzyl, halogen) replaces one of the hydrogen atoms on the aromatic ring. Two phenol derivatives commonly found as constituents of hospital disinfectants are *ortho*-phenylphenol and *ortho*-benzyl-*para*-chlorophenol. The antimicrobial properties of these compounds and many other phenol derivatives are much improved over those of the parent chemical.

Phenolics are absorbed by porous materials, and the residual disinfectant can irritate tissue. In 1970, depigmentation of the skin was reported to be caused by phenolic germicidal detergents containing para-tertiary butylphenol and para-tertiary amylphenol.

Mode of Action:



In high concentrations, phenol acts as a gross protoplasmic poison, penetrating and disrupting the cell wall and precipitating the cell proteins. Low concentrations of phenol and higher molecular-weight phenol derivatives cause bacterial death by inactivation of essential enzyme systems and leakage of essential metabolites from the cell wall.

Microbicidal Activity:

Published reports on the antimicrobial efficacy of commonly used phenolics showed they were bactericidal, fungicidal, virucidal, and tuberculocidal. One study demonstrated little or no virucidal effect of a phenolic against coxsackie B4, echovirus, and poliovirus. Similarly, 12% *ortho*-phenylphenol failed to inactivate any of the three hydrophilic viruses after a 10-minute exposure time.

Manufacturers' data demonstrate that commercial phenolics are not sporicidal but are tuberculocidal, fungicidal, virucidal, and bactericidal at their recommended use-dilution.However, results from these same studies have varied dramatically among laboratories testing identical products.

Uses:

Many phenolic germicides are registered as disinfectants for use on environmental surfaces (e.g., bedside tables, bedrails, and laboratory surfaces) and noncritical medical devices. Phenolics are not FDA-cleared as high-level disinfectants for use with semicritical items.

The use of phenolics in nurseries has been questioned because of hyperbilirubinemia in infants placed in bassinets where phenolic detergents were used. In addition, bilirubin levels were reported to increase in phenolic-exposed infants, compared with nonphenolic-exposed infants, when the phenolic was prepared according to the manufacturers' recommended dilution.

Phenolics (and other disinfectants) should not be used to clean infant bassinets and incubators while occupied. If phenolics are used to terminally clean infant bassinets and incubators, the surfaces should be rinsed thoroughly with water and dried before reuse of infant bassinets and incubators.

We will discuss some more chemical disinfectants in the next issue.....

Laboratory Diagnosis of Urinary Tract Infection

Urinary tract infection is defined as the microbial invasion of any tissue of tract from the renal cortex to urethral meatus also including prostate and epidymis. Normally, urine and all of the organs and structures of the urinary tract are sterile and contain no microorganisms. However, infectious microorganisms can get into the urinary tract from outside the body through the urethra and cause urinary tract infections. Infectious microorganisms can also travel through the bloodstream from other parts of the body to cause urinary tract infections.

Risk of urinary tract infection is higher in female than male because urethra is much shorter and closer to anus in female due to which it makes easier for microorganisms to enter the tract and cause infection. Other risk factors for urinary tract infections includes patients with indwelling catheters, history of kidney stones and kidney diseases, immunocompromised patients, prolonged antibiotic use, vesicoureteral reflux, diabetes mellitus etc. UTI may be symptomatic- presence of clinical features along with the significant growth of uropathogens or asymptomatic- absence of any detectable clinical features with critical number of potential uropathogens. Bacteria most commonly associated with the UTI are *E. coli*, *Klebsiella* spp, *Proteus* spp, *Citrobacter* spp, *Proteus* spp, *Pseudomonas* spp, *Providencia* spp, *Morganella* spp, *Staphylococcus aureus*, Cogulase negative staphylococci (CONS) *Enterococcus* spp, *Acinetobacter* spp etc.

In many clinical laboratories, urine cultures are the most common type of culture, accounting for 24%-40% of submitted cultures; as many as 80% of these urine cultures are submitted from the outpatient setting. Making a diagnosis of a urinary tract infection begins with taking a thorough personal and family medical history, including symptoms, and completing a physical examination. It also includes performing a urinalysis test, which checks for the presence of pus, white blood cells, and bacteria in the urine, which point to a urinary tract infection. A urine culture and sensitivity is usually performed to find the exact microorganism that is causing the infection and to determine the most effective antibiotic to treat it.

In order to confirm the laboratory diagnosis of UTI with full confidence following three criteria should meet: Clinical features, Pyuria and Bacteriuria.

Clinical features:

Symptoms of infection include feeling the need to urine in a hurry (urgency) and frequent urination (frequency). Other symptoms include difficulty urinating and burning or pain with urination (dysuria), cloudy foul-smelling urine, and bloody urine (hematuria). Lower abdominal pain or cramping and fever may also occur.

Pyuria

Pyuria is defined as presence of pus in urine and is detected by microscopy. Presence of increased number of polymorphs is an indication of infection in the tract and is important specially when bacteria are being killed as a result of antibiotic therapy or infecting organism is unable to grow on routinely used media for example: tubercle bacilli, chlamydia or a ureaplasma. Sometime polymorphs are usually present in the urine of healthy and uninfected person and it is only if the number is clearly greater than the normal values that the finding of pus cells is indicative of UTI.

***Detection of pyuria*:** Centrifuge 5 ml urine at 3000 rpm for 5 minutes and remove the supernatant. Put one or two drops of pellet in clean slide, cover with the coverslip and observe under high power field (× 40). If more than 3 WBC and 5 WBC are seen per high power field in male and female respectively it is taken as significant pyuria. Pyuria may also be seen during pyelonephritis or other kidney diseases, pregnancy, high fever etc and care should be taken to avoid confusing tubular epithelial cells with leucocytes. Another microscopic method for quantifying pyuria is to measure the urinay leucocyte excretion rate. Patient with symptomatic UTI have urinary leucocyte excretion rate of $\geq$ 400,000 leucocytes/hr however this is an impractical method. Similarly hemocytometer count of urine can be done and hemocytometer count of $\geq$10 leucocytes/mm^3 correlates with a urinary leucocyte excretion rate of $\geq$400,000 leucocytes/hr and bacterial concentration of >10^{5}cfu/ml.

Significant Bacteriuria: Occasionally urethral contaminates flushed out along with the urine may give the growth positive in culture therefore it is necessary to determine whether organisms are actually multiplying in the urine and present in a count. To detect bacteriuria by quantitative culture method, pour plate method can be used but is a too laborious for routine laboratory purpose. Filter paper method, dip-slide method serves as semi quantitative method but quicker and most commonly used semi quantitative method is standard loop streak method.

Sample: Clean catch midstream urine in sterile container is the most commonly used and widely accepted technique and is simple, inexpensive and can be performed in any clinical settings. The major disadvantage with this method is that, urine may get contaminated as it passes through the distal urethra. The advantage of mid-stream urine is that initial urinary passes flushed out the contaminants. Similarly cleansing of skin and mucous membrane adjacent to urethral orifice before micturation significantly reduces the contamination. The optimum timing for specimen collection is at the first voiding of urine in the morning. Urine can also be collected by the use of catheter by inserting a catheter through urethra but this technique has the disadvantage that insertion of catheter may introduce bacteria into the bladder and thereby cause UTI. To collect sample from the indwelling catheter, clamp the catheter tubing above the port and clean the catheter part with 70% alcohol and collect 10 ml urine using a needle.

Suprapubic aspiration can be done when urine can't be collected by any other methods by the trained physician which includes draining the full bladder by inserting a sterile needle through the skin above the pubic arch directly into the bladder. This method is particularly useful for isolation from infants or children and recovery of anaerobes. Even a single count of bacteria is taken as significant growth when the sample is collected by this method.

Sample transportation:

Preferably the sample should be processed within half an hour of collection as bacteria tend to multiply in urine thereby giving the false positive results. In unavoidable circumstances, it may be refrigerated or preservatives like 1.8% boric acid can be used which is a bacteriostatic agent and limits the overgrowth during the transport.

Sample acceptance criteria: Mid stream urine, catheterized urine, suprapubic bladder apirates and cytoscopy specimen that is collected properly, labeled and received within 2 hours of collection.

Rejection criteria: Bedpan urine, condom urines, specimen received in non sterile container, bag urine, unlabelled and improperly labeled specimen and specimen that have leaked out of container, urine received greater than 24 hours after collection (specimens should be refrigerated if submitted greater than 2 hours after collection). Inform the related person when the sample is rejected.

Processing: Most often microbiologist use a calibrated loop designed to deliver a known volume, either 0.01 or 0.001 ml or urine. Routine culture media are plated using calibrated loop (4 mm diameter loop = 0.001 ml approx). Calibrated loop that delivers larger volume of urine (0.01 ml) is recommended to detect lower number of organisms in certain specimens for example urine collected from catheterization, suprapubic aspirations, nephrostomies etc. Standard loop streak method has an added advantage of providing information regarding number of colony forming unit per milliliter (cfu/ml) and also provides isolated colonies for biochemical tests and antibiotic susceptibility testing. Commonly used media are **MacConkey agar, blood agar and CLED agar**. CLED is less inhibitory to *Staphylococcus saprophyticus* while blood agar promotes growth of nutritionally deficient organisms. Use of CLED and MacConkey also helps to distinguish lactose fermenter from non –lactose fermenter.

Mix the urine thoroughly before plating and remove the top of container. Insert the calibrated loop vertically into the container and inoculate it on the Blood agar, MacConkey agar and CLED agar plates separately each time by using streak plate method and then plates are incubated at 37°C for 24 hours aerobically. Sometime Enterococci and other streptococci may be obscured by heavy growth of Enterobacteriaceae therefore additional selective plate of columbia colisitn-nalidixic acid agar or phenylethyl agar can be used. In recent years, **chromogenic media** has been introduced and become commercially available that allows for more specific detection and differentiation of urinary tract pathogens on primary plate. Specific enzymes in the bacterial isolates breakdown the chromogenic substrates, producing different colored colonies so that predominant uropathogens like *E. coli* and *Enterococcus* can be identified without additional confirmatory testing as well as providing presumptive identification of *S saprophyticus*, *Streptococcus agalactiae*, *Klebsiella-Enterobacter-Serratia* and *Proteus-Morganella-Providentia* group.

Reporting: On day 2 observe for the growth of bacteria on each plate and if there is no growth in any plate, report as 'no growth seen after 24 hours of aerobic incubation'. Some specimen inoculated late in the day can't be read accurately the next morning. They should be either reincubated until the next day or possibly interpreted later in the day when full 24-hour incubation has been completed. If growth is present, notice count the number of colony and express in cfu/ml as follows: For example if 100 colonies are obtained on the plate using calibrated loop of 0.001 ml then,

Number of cfu/ml = Number of colonies × 1000
= 100 × 1000
= 10^5 cfu/ml

Kass, Marple and Sandford have proposed a criterion to interpret significant bacteriuria and are as follows:

$<10^5$ cfu/ml: contaminant,

$\geq 10^5$ cfu/ml: significant bacteriuria,

10^4 - 10^5 cfu/ml: low count significant bacteriuria,

10^2 cfu/ml is significant for the catheter collected urine sample.

But these criteria may not be true for those organisms which grow in urine at a slower rate and also for anaerobic organisms causing infection. So it is suggested that low count significant bacteriuria should also be considered. Low count may be due to different reasons like patients may be under antibiotic treatment, urine was collected before the organisms has reached the log phase of growth, infection with relatively slow growing organism like *Staphylococcus saprophyticus, Streptococci* or *Enterococci* etc.

Identification of Bacteria:

The gram negative isolates can be identified by using different biochemical tests like catalase, oxidase, indole production, H_2S production, other gas production, motility, MR/VP, urease, triple sugar iron utilization test while for gram positive isolates catalase, oxidase and coagulase and other different sugar fermentation tests can be done.

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Sir Frederick Gowland Hopkins

Born	20 June 1861 Eastbourne, Sussex, England
Died	16 May 1947 (aged 85) Cambridge, Cambridgeshire, England
Nationality	United Kingdom
Fields	Biochemistry
Institutions	University of Cambridge
Known for	Vitamins, tryptophan, glutathione
Notable awards	Nobel Prize (1929) Royal Medal (1918) Copley Medal (1926) Order of Merit (1935)

Sir Frederick Gowland Hopkins, (born June 20, 1861, Eastbourne) British biochemist, who received the 1929 Nobel Prize for Physiology or Medicine for discovery of essential nutrient factors—now known as vitamins—needed in animal diets to maintain health.

Hopkins was educated at the City of London School completing his further study with the University of London External Programme and the medical school at Guy's Hospital which is now part of King's College London School of Medicine. He then taught physiology and toxicology at Guy's Hospital from 1894 to 1898.

In 1898 he married Jessie Anne Stephens; they had two daughters, one of whom, Jacquetta Hawkes, was married to J.B. Priestley, the author.

Hopkins had for a long time studied how cells obtain energy via a complex metabolic process of oxidation and reduction reactions. His study in 1907 with Sir Walter Morley Fletcher of the connection between lactic acid and muscle contraction was one of the central achievements of his work on the biochemistry of the cell. He and Fletcher showed that oxygen depletion causes an accumulation of lactic acid in the muscle. Their work paved the way for the later discovery by Archibald Hill and Otto Fritz Meyerhof that a carbohydrate metabolic cycle supplies the energy used for muscle contraction.

In 1912 Hopkins published the work for which he is best known, demonstrating in a series of animal feeding experiments that diets consisting of pure proteins, carbohydrates, fats, minerals, and water fail to support animal growth. This led him to suggest the existence in normal diets of tiny quantities of as yet unidentified substances that are essential for animal growth and survival. These hypothetical substances he called "accessory food factors", later renamed vitamins. It was this work that led his being awarded (together with Christiaan Eijkman) the 1929 Nobel Prize in Physiology for Medicine.

During World War I, Hopkins continued his work on the nutritional value of vitamins. His efforts were especially valuable in a time of food shortages and rationing. He agreed to study the nutritional value of margarine and found that it was, as suspected, inferior to butter because it lacked the vitamins A and D. As a result of his work, vitamin-enriched margarine was introduced in 1926.

In 1901 Hopkins discovered the amino acid tryptophan, isolated it from protein, and eventually (1906–07) showed that it and certain other amino acids (known as essential amino acids) cannot be manufactured by certain animals from other nutrients and must be supplied in the diet. By so doing, he laid the foundation for the concept of the essential amino acids necessary for proper body functioning.

Noticing that rats failed to grow on a diet of artificial milk but grew rapidly when a small quantity of cow's milk was added to their daily ration, Hopkins realized that no animal can live on a mixture of pure protein, fat, and carbohydrate, even when mineral salts are added, and termed the missing factors—later called vitamins—"accessory substances."

In 1907 Hopkins and Sir Walter Fletcher laid the foundations for a modern understanding of the chemistry of muscular contraction when they demonstrated that working muscle accumulates lactic acid. Fifteen years later, Hopkins isolated from living tissue the tripeptide (three amino acids linked in sequence) glutathione and showed that it is vital to the utilization of oxygen by the cell.

Hopkins spent most of his career at Cambridge University (1898–1943). He was knighted in 1925 and received many other honours, including the presidency of the Royal Society (1930) and the Order of Merit (1935).

Timeline:

Father:	(bookseller)
Wife:	Jessie Anne Stevens (nurse)
Daughter:	Barbara Hopkins
Son:	Frederick Hopkins Jr. ("Hopper")
Daughter:	Jacquetta Hopkins Hawkes (archaeologist)
High School:	City of London School (expelled)
University:	Imperial College London University College London BS Chemistry, University of London (1888)
Medical School:	MD, King's College London (1894)
Teacher:	Physiology, Cambridge University (1898-1914)
Scholar:	Biochemistry, Cambridge University (1910-14)
Professor:	Biochemistry, Cambridge University (1914-43)

Honours:

Royal Society 1905 (President, 1930-35)
Nobel Prize for Medicine 1929 (with Christiaan Eijkman)
Royal Medal 1918
Knight of the British Empire 1925
Copley Medal 1926
Order of Merit 1935

References:

1) www.nobelprize.org
2) www.nnbd.com
3) www.discoveriesinmedicine.com

Enjoy the humour

An elementary school teacher sends this note to all parents on the first day of school.
"If you promise not to believe everything your child says happens at school, I will promise not to believe everything your child says happens at home.

It is said that Husband is the head of D family, But Remember that wife is D Neck of D family. & the Neck can turn the Head exactly D way she wants.

A white couple gets a black child.
Angry husband asks- You white, Me white. Why is baby black?
Wife- You hot, Me hot. Baby burnt!

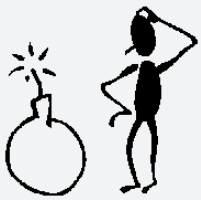
Possible Reasons When A Man Opens a car door for wife
1) The Car Is New.
2) The Wife Is New
3) (Most Imp) She Is Not His Wife

Three FASTEST means of Communication :
1. Tele-Phone
2. Tele-Vision
3. Tell to Woman
Need still FASTER - Tell her NOT to tell ANY ONE.

A pregnant lady went to an astrologer.
Astrologer: When u deliver a baby, baby's father will die.
Lady: Thank god! My husband is safe!

God:"What do you want ??
Boy:"A very beautiful girl..
God:"If you are muslim, I'll give you "Katrina".
If you are hindu, I'll give you "Kareena"
If you are sikh, I'll give you "Anushka" and
if you are christian, I'll give you "Genelia".
What's your name ??
Boy:" Abdul Vijay Singh Fernandes..
God to his angel: "Mayawati" de saale ko, jyada smart ban raha hai...

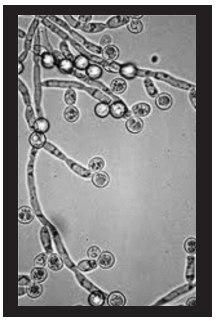
Interesting Facts



Scientific Facts about Human Body

1. The stomach's digestive acids are strong enough to dissolve zinc. Fortunately for us, the cells in the stomach lining renew so quickly that the acids don't have time to dissolve it.
2. The lungs contain over 300,000 million capillaries (tiny blood vessels). If they were laid end to end, they would stretch 2400km (1500 miles).
3. A mans testicles manufacture 10 million new sperm cells each day – enough that he could repopulate the entire planet in only 6 months!
4. Human bone is as strong as granite in supporting weight. A block of bone the size of a matchbox can support 9 tonnes – that is four times as much as concrete can support.
5. Each finger and toe nail takes six months to grow from base to tip.
6. The largest organ in the body is the skin. In an adult man it covers about 1.9m2 (20sq ft). The skin constantly flakes away – in a lifetime each person sheds around 18kg (40 lb) of skin.
7. When you sleep, you grow by about 8mm (0.3in). The next day you shrink back to your former height. The reason is that your cartilage discs are squeezed like sponges by the force of gravity when you stand or sit.
8. The average person in the West eats 50 tonnes of food and drinks 50,000 liters (11,000 gallons) of liquid during his life.
9. Each kidney contains 1 million individual filters. They filter an average of around 1.3 liters (2.2 pints) of blood per minute, and expel up to 1.4 liters (2.5 pints) a day of urine.
10. The focusing muscles of the eyes move around 100,000 times a day. To give your leg muscles the same workout, you would need to walk 80km (50 miles) every day.
11. In 30 minutes, the average body gives off enough heat (combined) to bring a half gallon of water to boil.
12. A single human blood cell takes only 60 seconds to make a complete circuit of the body.
13. The eyes receive approximately 90 percent of all our information, making us basically visual creatures.
14. The female ovaries contain nearly half-a-million egg cells, yet only 400 or so will ever get the opportunity to create a new life.

Candida albicans



Scientific Classification:

Kingdom:	Fungi
Phylum:	Ascomycota
Subphylum:	Saccharomycotina
Class:	Saccharomycetes
Order:	Saccharomycetales
Family:	Saccharomycetaceae
Genus:	Candida
Species:	C. albicans

Candida albicans is an ovoid or spherical budding cell, which produces pseudomycelia in culture as well as in tissues. There are more than 20 species of *Candida*, the most common is *Candida albicans*. *Candida* species are normal inhabitants of skin. Candidal infections commonly occur in warm moist body areas, such as underarms.

Candida albicans is a yeast infestation, from a parasite that thrives in warm-blooded animals. In the allopathic world of medicine it is referred as a fungus. This fungus can cause thrush and vaginal infections and spread to any part of the body that is weakened. We all have intestinal candida and when in balance it helps maintain and aid our immune system by controlling the unfriendly organisms. However, *Candida albicans* takes advantage of circumstances in the body. This single cell fungi multiplies and develops toxins which circulate in the blood stream which cause an array of maladies.

C. albicans is commensal and a constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestinal tract. *C. albicans* lives in 80% of the human population without causing harmful effects, although overgrowth of the fungus results in candidiasis (candidosis). Candidosis is an infection caused by *Candida albicans*. It is also known as candidiasis or moniliasis. It is an infection of the skin, mucosa and rarely of internal organs. The most common predisposing factor is being diabetes. In women, pregnancy, menstruation, and birth control pills also can contribute to infection. Candidal infections are more common after menopause.

Candidal infections can occur throughout the entire body and can be life-threatening, in people who have weak immune system or suffering from diseases such as AIDS. *Candida* is a major cause of esophagitis (inflammation in the swallowing tube) in people with AIDS. A common form of candidiasis restricted to the mucosal membranes in mouth or vagina is thrush, which is usually easily cured in people who are not immunocompromised.

Candida produces an alcohol called ethanol producing an intoxicating effect in the blood if the count level is too high. Ethanol grows rapidly when yeast has a food source like white sugar or white floor products. In severe cases it produces much more than the liver can oxidize and eliminate. It can produce a false estrogen and make the body think it has enough, which signals the body to cease production. Or send messages to the thyroid, making it think it has enough stopping the production of thyroxin. The cause of this is menstrual problems and hypothyroid problems.

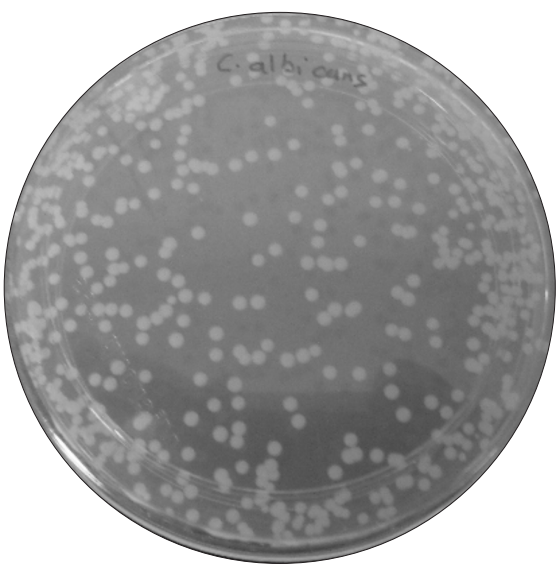
Another byproduct is acetaldehyde and it is related to formaldehyde this disrupts collagen production, fatty acid oxidation and blocks normal nerve functions. Basically it interferes with the normal functions of the entire body and is a severe problem. One way to get an overdose of *candida* in the system is by taking antibiotics and birth control pills, and consuming sugar products. *Candida* feeds on antibiotics (it is their food source). Other causes: cortisone, progesterone suppositories, faulty diets, diet, too much meat, weakened immune systems.

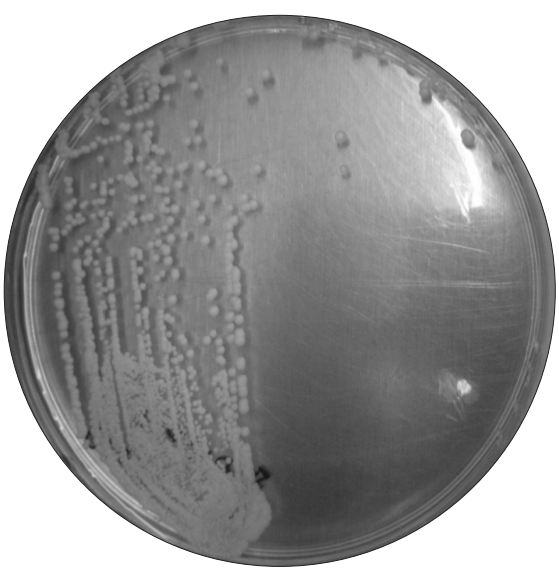
Laboratory Examination:

Commonly used medium for isolation of *candida albicans* are Sabouraud Dextrose Agar (SDA). Colonies are creamy white and smooth. *C.albicans* form chlamydospores on corn meal agar at 20 °C. It can be also diagnosed by Gram-Staining & Wet Films.

Laboratory Examination is done as follows:-

1. Wear sterile gloves and with a sterile cotton swab collect the sample from the infected area.
2. Take the swab and streak it on Sabouraud Dextrose Agar.
3. Incubate the plates at 20-25°C for 2-5days.
4. Plates should be incubated in inverted position.
5. Observe the results after completion of incubation time.





Treatment:

Antifungal Drugs

The first line of defense of most doctors for candidiasis is the use of antifungal drugs. For most health practitioner, they prescribe them to provide immediate relief from the symptoms of thrush. The drugs are available either as over-the-counter or as prescription medication. Usually, it only takes one week to completely clear the infection. The *Candida albicans* treatment comes in a variety of forms. You can choose the lotion, cream, pills, troches and vaginal suppositories. You just have to talk to your doctor to determine what is right for you.

If you go for the self-medication, just be sure to read the labels on its proper use and finish the whole course of treatment. Do not stop the treatment even when the symptoms disappear on the first few days of using antifungal drugs. Otherwise, the possibility of re-infection is very high. Examples of home medications available as over-the-counter include the following:

- Miconazole
- Tioconazole
- Butoconazole
- Clotrimazole

Detoxification

Another *Candida albicans* treatment is the use detoxification diet. Detoxification is a powerful way to cleanse the body from the inside. It helps you get rid of harmful chemicals and toxins that have accumulated over time. Detox diet helps flush out the *Candida albicans* and its by-products. There are different plans available to detoxify your body. You can either choose the fasting, colon cleansing method, wheatgrass, fresh vegetable juice, herbs, water and exercise. All of these methods help extract the toxins in the body.

Detoxification is not a single process. It is true that you clean your body on the first phase but you have to find an effective maintenance plan to avoid the recurrence of *Candida albicans*.

Use of Probiotics

Probiotics are friendly bacteria that help kill unwanted organisms in the body. They are also called as the normal flora and they are found in the intestines, female genitals and in the mouth. Probiotics inhibit the growth of bad bacteria, promote good digestion, boost the immune system and increase your resistance against infection. Lactobacilli and bifidobacteria and acidophilus found in yogurt fight the growth of disease causing bacteria. They do this by balancing the number of organisms in the intestines. Aside from the direct effect, they also produce substances like the bacteriocins that act as natural antibiotics and kill undesirable microorganisms.

Natural Remedy

The next *Candida albicans* treatment for you is to try the natural remedies to control the yeast population. Examples of natural remedies are garlic, olive leaf extract, caprylic acid, oil of oregano and taheebo. For sure, they can provide you with more remedies to treat your recurrent thrush.

Oil of oregano and garlic has potent antifungal properties. You can eat the garlic raw or use drops of the oil to a liter of water and use it as your regular washing solution. Garlic capsules are also available if you do not like the idea of preparing raw garlic. Other natural remedies to treat thrush are the following:

- Healthy fats like fish oil, cod liver oil, extra virgin oil and extra virgin coconut oil
- Grapefruit and berry juices
- Plenty of purified water with herbal tea and barley grass

Dietary Changes

Since the diet is one of the major reasons why you have a recurrent *candida* infection, then you have to do something about it to free you from the problem. Most health experts recommend that you reduce your carbohydrate intake especially simple crabs like sweets. Instead of going for a high carbohydrate rich diet, you can have a high protein diet. Protein coming from fish, meat, poultry products and beans provide your body with essential nutrients.

Foods and Drinks to Avoid:

- Aged cheeses
- Alcoholic and carbonated beverages
- Dried fruits
- Fermented foods
- Mushroom
- Honey, syrup and all sugars
- Foods with yeast or mold like baked goods, muffins and cakes
- Fruit juices
- Pickles and salad dressing
- Peanuts

The rule of thumb for the *Candida albicans* treatment is to eat fresh and organically grown food.

Foods to Eat

The following foods are known to have anti-fungal property or proven to reduce the population of fungi in your body. As much as possible have at least one to two servings of these foods daily to improve your condition.

- Vegetables especially raw garlic
- Live yogurt
- Non-glutinous grains like millet, rice bran and oat bran
- Lentils
- Avocado
- Chia seeds
- Coconut oil
- Green leafy salads
- Fresh lemons

Recent Advances:

A new, rapid test for *Candida* infections of the bloodstream may cut patient mortality from 40% to 11% by diagnosing candidemia 25 times faster than blood culture can and quickly identifying the *Candida* species that is causing the infection. The new test, T2Candida, uses polymerase chain reaction (PCR) assay to amplify *Candida* DNA in blood, with the genetic material hybridizing to superparamagnetic nanoparticles coated with complementary DNA. The nanoparticles aggregate into "microclusters," which greatly alter a T2 magnetic resonance (T2MR) signal.

Antifungal Agents

Introduction:

The development of antifungal agents has lagged behind that of antibacterial agents. This is a predictable consequence of the cellular structure of the organisms involved. Bacteria are prokaryotic and hence offer numerous structural and metabolic targets that differ from those of the human host. Fungi, in contrast, are eukaryotes, and consequently most agents toxic to fungi are also toxic to the host. Furthermore, because fungi generally grow slowly and often in multicellular forms, they are more difficult to quantify than bacteria. This difficulty complicates experiments designed to evaluate the *in vitro* or *in vivo* properties of a potential antifungal agent.

Despite these limitations, numerous advances have been made in developing new antifungal agents and in understanding the existing ones. This chapter summarizes the more common antifungal agents. Three groups of drugs are emphasized: the polyenes, the azoles, and one antimetabolite.

Definition:

An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host.

Polyene Antifungal Drugs

The polyene compounds are so named because of the alternating conjugated double bonds that constitute a part of their macrolide ring structure. The polyene antibiotics are all products of *Streptomyces* species. These drugs interact with sterols in cell membranes (ergosterol in fungal cells; cholesterol in human cells) to form channels through the membrane, causing the cells to become leaky. The polyene antifungal agents include nystatin, amphotericin B, and pimaricin.

Amphotericin B is the mainstay antifungal agent for treatment of life-threatening mycoses and for most other mycoses, with the possible exception of the dermatophytoses. Discovered by Gold in 1956, it can truly be said to represent a gold standard. Its broad spectrum of activity includes most of the medically important moulds and yeasts, including dimorphic pathogens such as *Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis,* and *Paracoccidioides brasiliensis*. It is the drug of choice in treating most opportunistic mycoses caused by fungi such as *Candida* species, *Cryptococcus neoformans, Aspergillus* species, and the Zygomycetes. Resistance to this agent is rare, but is noteworthy for *Pseudallescheria boydii*, *Fusarium* spp., *Trichosporon* spp., certain isolates of *Candida lusitaniae* and *Candida guilliermondii*.

The drug must be administered intravenously and is associated with numerous side effects, ranging from phlebitis at the infusion site and chills to renal toxicity, which may be severe. A major advance in the use of this agent has resulted from an understanding of the mechanism of its renal toxicity, which is presumed to involve tubuloglomerular feedback. The suppression of glomerular filtration can be reduced by administering sodium chloride.

Nystatin was the first successful antifungal antibiotic to be developed, and it is still in general use. It is representative of the polyene antifungal agents developed later. The promise of its broad-spectrum antifungal activity is offset by host toxicity. Therefore, it is limited to topical use, where it has activity against yeasts such as the *Candida* species.

Pimaricin (natamycin), another polyene, is used topically to treat superficial mycotic infections of the eye. It is active against both yeasts and moulds.

Azole Antifungal Drugs

The azole antifungal agents have five-membered organic rings that contain either two or three nitrogen molecules (the imidazoles and the triazoles respectively). The clinically useful imidazoles are clotrimazole, miconazole, and ketoconazole. Two important triazoles are itraconazole and fluconazole. In general, the azole antifungal agents are thought to inhibit cytochrome P_{450}-dependent enzymes involved in the biosynthesis of cell membrane sterols.

Ketoconazole set the stage for the orally administered antifungal azoles. It can be administered both orally and topically and has a range of activity including infections due to *H capsulatum* and *B dermatitidis*, for which it is often used in nonimmunocompromised patients. It is also active against mucosal candidiasis and a variety of cutaneous mycoses, including dermatophyte infections, pityriasis versicolor, and cutaneous candidiasis. It is not indicated for treatment of aspergillosis or of systemic infections caused by yeasts.

The triazoles (fluconazole, itraconazole) have become the standard for the azoles, and have replaced amphotericin B for managing certain forms of the systemic mycoses. Fluconazole is now routinely used to treat candidemia in non-neutropenic hosts, and is gaining acceptance for use in cryptococcosis and selected forms of coccidioidomycosis. Itraconazole has proven to be effective for histoplasmosis, blastomycosis, sporotrichosis, coccidioidomycosis, consolidation treatment for cryptococcosis, and certain forms of aspergillosis. Fluconazole can be administered either orally, or intravenously. The licensed formulation for itraconazole is oral, but an intravenous formulation is under study, and could be a significant addition directed at bioavailability problems relating to absorption of the oral formulation.

Side effects are not as common with the azoles as with amphotericin B, but life-threatening liver toxicity can arise with long-term use. Liver toxicity noted with ketoconazole has been less problematic with the triazoles. Other side effects include nausea and vomiting. Drug interactions are a potential problem between azoles and other drug classes and include cyclosporin, certain antihistamines, anticoagulants, and antiseizure, oral hypoglycemic and other medications that are metabolized via similar pathways in the liver.

5-Fluorocytosine

In contrast to the situation with antibacterial agents, few antimetabolites are available for use against fungi. The best example is 5-fluorocytosine, a fluorinated analog of cytosine. It inhibits both DNA and RNA synthesis via intracytoplasmic conversion to 5-fluorouracil. The latter is converted to two active nucleotides: 5-fluorouridine triphosphate, which inhibits RNA processing, and 5-fluorodeoxyuridine monophosphate, which inhibits thymidylate synthetase and hence the formation of the deoxythymidine triphosphate needed for DNA synthesis. As with other antimetabolites, the emergence of drug resistance is a problem. Therefore, 5-fluorocytosine is seldom used alone. In combination with amphotericin B it remains the treatment of choice for cryptococcal meningitis and is effective against a number of other mycoses, including some caused by the dematiaceous fungi and perhaps even by *C albicans*.

Other Antifungal Agents

Griseofulvin is an antifungal antibiotic produced by *Penicillium griseofulvum*. It is active *in vitro* against most dermatophytes and has been the drug of choice for chronic infections caused by these fungi (e.g., nail infections with *Trichophyton rubrum*) since it is orally administered and presumably incorporated into actively growing tissue. It is still used in such instances but is being challenged by some of the newer azole antifungal agents. The drug inhibits mitosis in fungi.

Potassium iodide given orally as a saturated suspension is uniquely used to treat cutaneous and lymphocutaneous sporotrichosis. This compound, interestingly, is not active against *Sporothrix schenckii in vitro*. It appears to act by enhancing the transepidermal elimination process in the infected host.

Two other classes of antifungal agents represent new additions to topical treatment of the dermatomycoses in Europe. The two allylamines (naftifine and terbinafine) inhibit ergosterol synthesis at the level of squalene epoxidase; one morpholene derivative (amorolfine) inhibits at a subsequent step in the ergosterol pathway.

Nasopharyngeal swab specimen collection & culture methodology

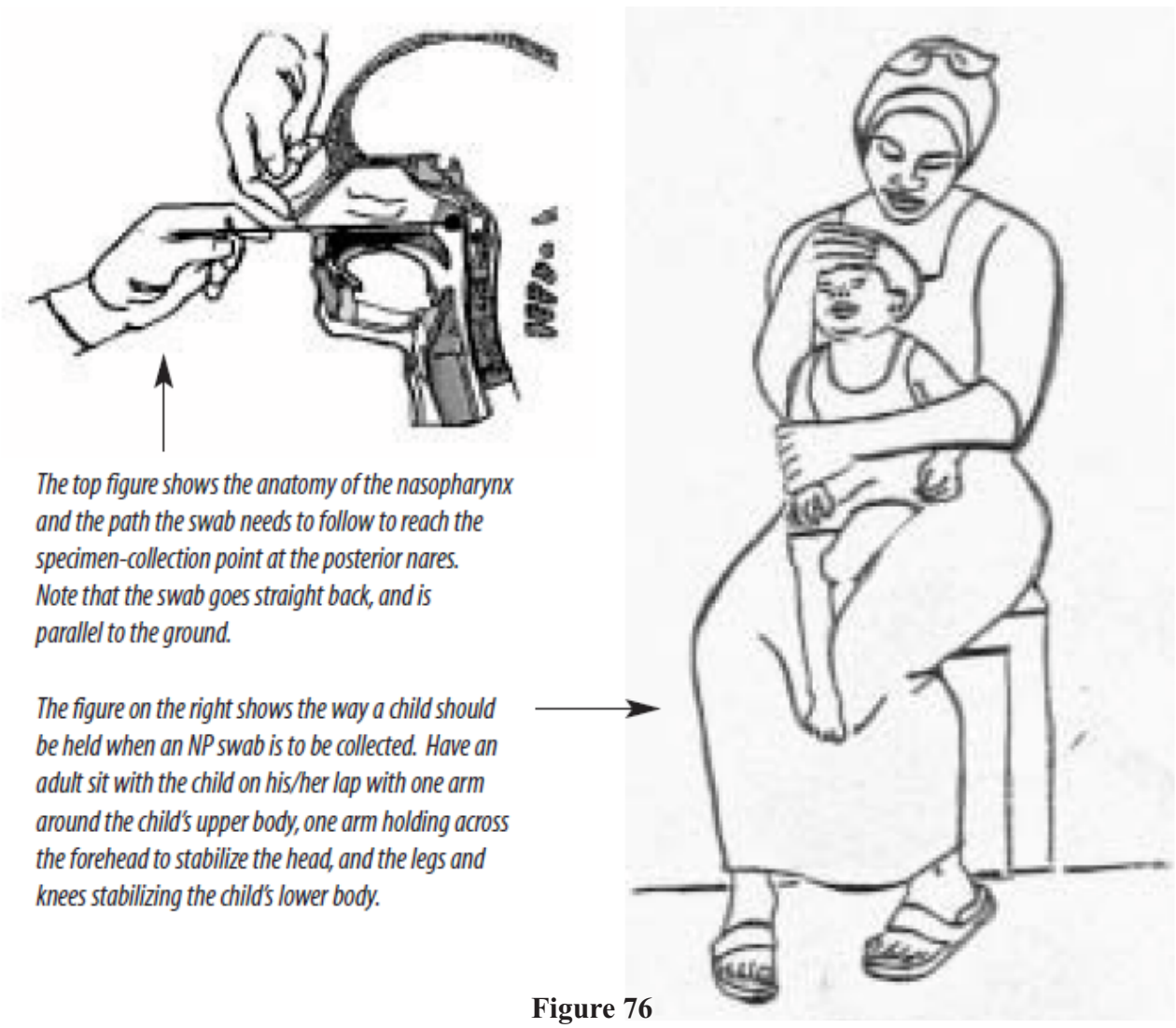
Laboratories may receive nasopharyngeal (NP) swabs in the course of prevalence surveys and carriage studies of respiratory organisms. Culture methods for this type of specimen are included below.

Use swabs taken from the upper respiratory tract (*e.g.*, the nasopharynx) to inoculate the primary culture medium; the nasopharyngeal swab should be rolled over one-fourth of the plate (*i.e.*, one quadrant). Because bacteria other than *S. pneumoniae* and *H. influenzae* are generally present, selective media are used. For *S. pneumoniae*, the selective medium is a tryptone soy agar (TSA) plate containing 5% sheep or horse blood and 5 µg/ml of gentamicin sulfate; for *H. influenzae,* a chocolate agar plate containing 300 µg/ml of bacitracin is used. If one swab is being collected for recovery of both *S. pneumoniae* and *H. influenzae,* the blood agar and gentamicin plate should be inoculated first, followed by the inoculation of the chocolate agar and bacitracin plate (because *S. pneumoniae* is more susceptible to the antibacterial activity of the bacitracin than *H. influenzae* is to the antibacterial activity of gentamicin). After direct plating with the swab, use a bacteriological loop to streak the plate.

In areas where overgrowth of contaminants occurs in <10% of cultures, culture media without antibiotics may be used. However, in this case the primary plates must be streaked very carefully to allow separation of individual colonies.

Collection of nasopharyngeal (NP) swabs:

NP swab collection is a clinical procedure and should therefore be performed by trained health-care workers. A specifically designed swab with a flexible wire shaft and a small calcium alginate tip should be used; calcium alginate is inert and non toxic to *Neisseria* and other sensitive bacteria.



The top figure shows the anatomy of the nasopharynx and the path the swab needs to follow to reach the specimen-collection point at the posterior nares. Note that the swab goes straight back, and is parallel to the ground.

The figure on the right shows the way a child should be held when an NP swab is to be collected. Have an adult sit with the child on his/her lap with one arm around the child's upper body, one arm holding across the forehead to stabilize the head, and the legs and knees stabilizing the child's lower body.

Figure 76

Figure 76 depicts the proper method of collecting an NP swab. The patient's head should be tipped slightly backward, as shown, and immobilized. For young infants, a good way to collect NP swabs is for the person taking the specimen to hold his/her hand behind the neck of the infant while the infant is sitting in the lap of the parent or other adult. For children, the adult should lightly hold the child's head against his/her chest with a hand on the child's forehead; the adult's other arm should be used to restrain the child's arms. Sometimes it is also helpful if the adult's legs are used to stabilize the child's legs; this reduces body movement and kicking during the collection of the NP swab.

When the child's head is immobilized and body is restrained, the NP swab can be collected using the following procedures:

a) Unwrap the swab.
b) Insert the swab into a nostril and pass the swab parallel to the ground, back to the posterior nares. Do not use force. The swab should travel smoothly with minimal resistance; rotating the swab during the insertion will help the swab move. If resistance is encountered, remove the swab and try the other nostril.
c) Once in place, rotate the swab, leave in place approximately five seconds to saturate the tip, and remove slowly.
d) Use the swab to inoculate the appropriate (selective) medium (sheep blood with gentamicin to isolate *S. pneumoniae*; chocolate agar with bacitracin to isolate *H. influenzae;* blood or chocolate with no antimicrobial for *N. meningitidis*) by direct plating, or place the swab in STGG transport medium for transportation to the laboratory.

Skim-milk tryptone glucose glycerol (STGG) transport medium for nasopharyngeal secretions

Skim-milk tryptone glucose glycerol (STGG) transport medium is a tryptone broth with skim (nonfat) milk, glucose, and glycerol that can be used to transport NP swabs to the laboratory when the swabs cannot be plated directly from the patient. (The preparation of STGG medium is described in Appendix 2.) Culturing from the STGG as soon as possible is preferred, though STGG can also be used for storage and transport (for a several hours at room temperature; for up to 8 weeks at -20°C; and, for at least 2 years at -70°C).

Inoculation of STGG with an NP swab

a) Thaw frozen tubes of STGG before use.
b) Label the tube with appropriate patient and specimen information.
c) Using a calcium alginate swab, collect an NP swab from the patient.
d) Insert swab to the bottom of the STGG medium in thawed tube.
e) Raise the swab slightly and cut the wire portion (*i.e.*, the shaft) of the swab at the top level of the container. Allow the bottom portion of the swab (*i.e.*, the tip) containing the calcium alginate material to drop into the tube. Discard the remaining shaft into disinfectant solution or a sharps container.
f) Tighten the screw-cap top securely.

   Optional: If desired, after tightening the cap, vortex on high speed for 10–20 seconds.
g) Freeze specimen immediately in upright position at -70°C, if possible.

In some cases, the inoculated STGG medium has been placed on ice for several hours before placing the STGG medium at -70°C without loss of viable *S. pneumoniae*. Extended storage of inoculated STGG stored at -20°C for 8 weeks results in minimal loss of viability of *S. pneumoniae*, and indications are that *H. influenzae* survive as well as *S. pneumoniae* in STGG.

Short term storage of STGG is best at -70°C although a freezer at -20°C may also be used.

Recovery of bacteria from STGG

a) Remove the inoculated STGG medium from the freezer.
b) Allow the tube to thaw at room temperature.
c) Vortex each tube for a full 10 seconds.
d) Using a sterile loop, aseptically remove a 50–100 µl sample of inoculated STGG to streak onto a plate for culture. (If attempting isolation of *S. pneumoniae*, a 100-µl inoculum is preferable.)
   1) **5% sheep (or horse) blood + 5 µg/ml gentamicin sulfate agar** is the appropriate plated medium for the recovery of *S. pneumoniae* from a nasopharyngeal swab specimen stored in STGG.

      (If a gentamicin-containing medium is not available, attempt recovery from a standard blood agar plate.)
   2) **Chocolate + 300 µg/ml bacitracin agar** is the appropriate plated medium for recovery of *H. influenzae* from a nasopharyngeal swab specimen stored in STGG.

      (If a bacitracin-containing medium is not available, attempt recovery from a standard supplemented chocolate agar plate.)
   3) 5% sheep or chocolate agar is the appropriate plated medium for recovery of *N. meningitidis*.
e) Re-freeze the specimen (*i.e.*, the STGG) as soon as possible; keep it cool (in an ice water bath if necessary) if the time is extended beyond a few minutes at room temperature.
f) Avoid multiple freeze-thaw cycles whenever possible. One way to decrease risk of freeze-thaw cycles within the freezer is to make sure the cryo tubes are kept in the back of the freezer shelf and not the front or in the door.

Microxpress
Presents Chromogenic Media's.....

Chromogenic Medium

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

The chromogenic mixture consists of chromogenic substrates, which release differently coloured compounds upon degradation by specific enzymes. This helps in differentiation of certain species with minimum confirmatory tests.

Features	Benefits
Quick & fast identification of organisms	Saves time
Clear differentiation	Superior performance
Improved colours aid interpretation	Easy to identify
Minimises Confirmatory testing	Reduces costs

Product Range

Cat No.	Product Name	Application
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AM10252 AM50252	Chromogenic E.coli Agar	For detection & enumeration of *Escherichia coli* in foods without further confirmation on membrane filter or by indole reagent.
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.
AM10255 AM50255	Chromogenic Improved Salmonella Agar	For differentiation and identification of *Salmonella* from water samples

Pack Size Available- 100gms & 500gms

BioShields Presents Nusept

Composition - 1% w/v Poly (hexamethylene biguanide) hydrochloride, Perfume, Fast green FCF as color.

Description: NUSEPT™ is a new generation, powerful, non stinging, safe, highly effective and resistance-free microbicidal antiseptic solution. NUSEPT™ is an ideal antiseptic for use in medical settings. The main active ingredient of NUSEPT™ is poly (hexamethylenebiguanide) hydrochloride (PHMB). PHMB is a polymeric biguanide. There is no evidence that PHMB susceptibility is affected by the induction or hyper expression of multi-drug efflux pumps, neither there have been any reports of acquired resistance towards this agent.

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).

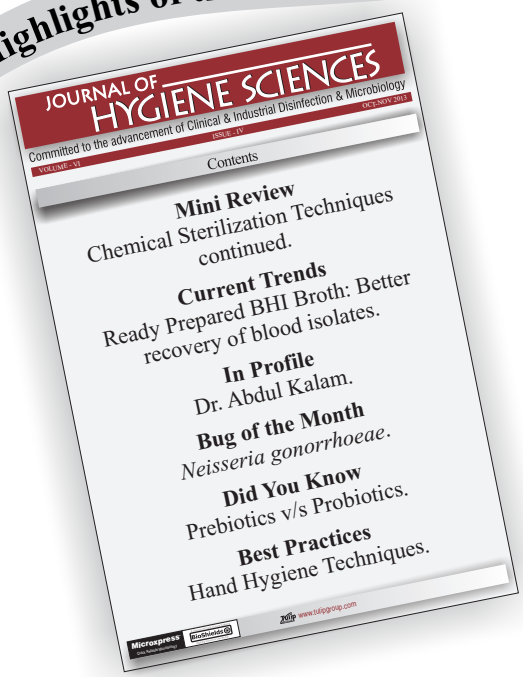
APPLICATIONS :

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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