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Editorial

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Here's wishing you A very Happy New Year to all our readers! Let's explore yet another issue of JHS with loads of interesting information. Kindly flip a few pages to believe us............

Mini Review section - Glutaraldehyde is used in large volume in a variety of industries as a disinfectant, preservative, fixative and cross-linking agent, and as a chemical intermediate in the synthesis of pharmaceuticals and pesticides. It is widely used in the industrial, scientific and biomedical fields. Many adverse health effects on humans have been reported in association with biomedical uses of GA, with 2–3.5% aqueous GA solution generally used for cold sterilization and GA exposure ranges of 0.001 to 2.6 ppm for this type of use.

Current Trends section - Removal of preservative antimicrobial activity is clearly an important step when recovering test organisms from contaminated products, yet surprisingly little has been published which compare the effectiveness of the different neutralization methods. Nonionic surface-active agents, such as Tween 80 and Lubrol W, have been widely used as preservative neutralizers, sometimes in combination with Lecithin.

In Profile Section – Dr. Donald Low (May 2, 1945 - September 18, 2013) was a Canadian microbiologist noted for his role in battling the SARS outbreak of 2003. He was microbiologist-in-chief at Mount Sinai Hospital, Toronto, from 1985 to 2013.

Relaxed Mood section – All work & no play makes Jack a dull boy! We don't forget that ever. Each issue comes with its own bouquet of jokes & thoughts so enjoy..............

Bug of the Month section - Acinetobacter baumannii is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. An opportunistic pathogen, A. baumannii has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged (90 days) hospital stay. Commonly associated with aquatic environments, it has been shown to colonize the skin as well as being isolated in high numbers from the respiratory and oropharynx secretions of infected individuals. In recent years, it has been designated as a “red alert” human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum.

Did You Know section - One of the hardest parts of taking blood can be finding a suitable vein. Some patients are 'difficult sticks'; their veins are either very small, and/or deep, preventing health professionals from finding a site easily and quickly. Many companies now market 'Vein Finder' products. This tool works by using near-infrared wavelength LEDs to illuminate the flesh at the site. The veins will appear as dark bands because they are more absorbent of this spectrum of light than the surrounding tissue.

Best Practices section - Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. They are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant contains both a water insoluble (or oil soluble) component and a water soluble component.

Genetic toxicity & carcinogenicity studies of Glutraldehyde (GA)

Glutaraldehyde is used in large volume in a variety of industries as a disinfectant, preservative, fixative and cross-linking agent, and as a chemical intermediate in the synthesis of pharmaceuticals and pesticides. It is widely used in the industrial, scientific and biomedical fields. Many adverse health effects on humans have been reported in association with biomedical uses of GA, with 2–3.5% aqueous GA solution generally used for cold sterilization and GA exposure ranges of 0.001 to 2.6 ppm for this type of use. GA is metabolized extensively to CO_2, but urinary excretion of it is low. Sensory irritant effects, sensitization of skin and respiratory organs and other symptoms have been reported among endoscopy nurses and medical radiation technologists. The prevalence of chronic bronchitis and nasal symptoms in humans is significantly correlated with peak concentrations of GA exposure. The extent of primary skin irritation depends on the duration and site of contact, and the severity of symptoms is dose-related. Chronic inhalation affects the nose and respiratory tract, and lesions become severe with prolonged duration of exposure. Increases in neither mortality nor tumor incidence have been found in workers with less than 0.2 ppm GA exposure, no evidence of carcinogenic activity has been obtained in experimental animal studies. There has been no clear evidence of genetic toxicity of GA in either in vitro or in vivo studies, and neither developmental nor reproductive toxicity has been found in humans or animals.

Glutaraldehyde (GA) is a colourless liquid with a pungent odour. It has a wide spectrum of medical, scientific and industrial applications. GA is the best disinfectant for cold sterilization of medical equipment and is also used as a fixative in histochemistry and electron microscopy, a developer and fixer in X-ray film processing, a linking material, a leather tanning agent and as an ingredient in cosmetic, toiletry and chemical specialty products It is irritating and corrosive to the skin, eyes and respiratory tract and is recognized as a cause of health problems in those handling it.

Many regulatory organizations including the Japanese Ministry of Health, Labour and Welfare (MHLW) have therefore set limits on exposure to GA to prevent its irritating effects.

Chemical Formula: $C_5H_8O_2$

Molecular Weight: 100.13

Synonyms: 1,3-Diformylpropane; glutaral; glutardialdehyde; glutaric dialdehyde; 1,5-pentanedial; 1,5-pentanedione; potentiated acid glutaraldehyde.

Recently, not only irritation and sensitization but also darkroom disease (DRD) among radiographers, associated with various symptoms including indefinite complaints, has been reported to be related to GA exposure, though the relationship between DRD and GA exposure has not been clarified. In addition, onset of multiple chemical sensitivity (MCS) has been reported among nurses using GA, however, there was no description about work environmental conditions. Aldehydes are one of the major pollutants of indoor air and cause sick building syndrome (SBS) and sick house syndrome, the major symptoms of which are irritation and indefinite complaints. Since the symptoms of DRD are very similar to those of SBS, GA, one of the aldehydes, may contribute to the onset of DRD. Prolonged low exposure to formaldehyde affects regulation of hypothalamic-pituitary-adrenal axis activity in the female mouse, which may be a suitable animal model for SBS and/or MCS. Thus, not only formaldehyde but also GA may cause MCS.

Toxicity

Acute toxicity

There are several reports on accidental acute exposure to GA in humans. In a case in which approximately 100 ml of GA was spilled on a child's face by mistake during surgery, fever, vomiting, tachypnea and tachycardia were noted for 6 h after the accident, and chemical pneumonia was diagnosed. The child finally recovered without sequelae. It was reported that colitis was induced by retention of 2% GA disinfectant in endoscope channels. The acute toxicity of GA has been investigated in many studies with various animal species 2.

Genetic toxicology

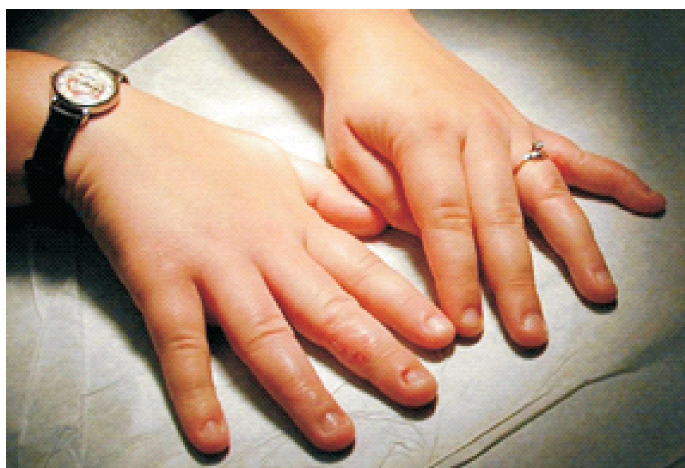
In genetic toxicity studies, glutaraldehyde was mutagenic with and without S9 metabolic activation in S. typhimurium strains TA100, TA102, and TA104. Glutaraldehyde was mutagenic in mouse L5178Y lymphoma cells in the absence of S9 and induced sister chromatid exchanges in cultured Chinese hamster ovary cells with and without S9. No increase in chromosomal aberrations was induced by glutaraldehyde in cultured Chinese hamster ovary cells with or without S9 at one laboratory; at another laboratory, chromosomal aberrations were induced in the absence of S9 only. Glutaraldehyde did not induce sex-linked recessive lethal mutations in germ cells of male D. melanogaster treated as adults by feeding or injection or treated as larvae by feeding. In vivo, glutaraldehyde induced a significant increase in chromosomal aberrations in mouse bone marrow cells 36 hours after a single intraperitoneal injection. In a subset of the 36-hour chromosomal aberrations test, there was a small increase in the number of micronucleated bone marrow polychromatic erythrocytes, which was judged to be equivocal. Additional short-term (3-day) and subchronic (13-week) micronucleus tests in mice, using the intraperitoneal or inhalation routes, respectively, yielded negative results.

Irritation and sensitization

1. Skin

GA has been used to treat hyperhidrosis because of its antiperspirant effect, and has been investigated in dermatological studies. The findings obtained indicated little irritation by and low sensitivity to GA. Although Juhlin and Hansson observed no allergic reactions to GA, even in patients sensitive to formaldehyde, they noted that evaluation of their findings concerning sensitivity was difficult because the dose used in their experiments was too small (1–10% with occlusion). GA is also used to treat warts. There were no cases of sensitization to buffered 10% GA solution, although a 20% solution produced necrosis.

Reaction to applied GA depends on the thickness of the skin. Irritation and sensitization were observed on the anterior ankle but not on the posterior ankle or medial, lateral or posterior heel.



Allergy to Glutaraldehyde

2. Eye

In reports by the National Institute for Occupational Safety and Health in the United States (US NIOSH), eye irritation was noted to occur in medical workers using GA. For instance, in one hospital, 28 of 44 workers (64%) using GA at least once a week complained of eye irritation while using GA solution. Cases of keratopathy and conjunctivitis were caused by use of medical equipment with incomplete washing and removal of 2% GA solution.

Genotoxicity and mutagenicity

Although there has been no report on genetic toxicity of GA to humans, it has been investigated extensively in animals. Both positive and negative results have been reported in in vitro mutagenicity studies, while almost all in vivo tests have yielded negative results. GA exhibited mild to strong mutagenic effects with and without S9 metabolic activation in S. Typhimurium strain TA102. In TA100, negative results were reported both with and without S9 81), while weakly positive results were reported with S9. GA was mutagenic without S9 in TA104, which exhibited higher sensitivity to carbonyl mutagenesis than TA100 did. GA was not mutagenic with or without S9 in TA98,TA1535, TA1537 and TA1538. GA was positive in the DNA repair test by liquid rec-assay and by umu test without S9 activation. GA was mutagenic in E. Coli WP2 tester strains, but yielded negative results in the SOS chromotest with E. Coli PQ37. GA did not induce mutation in in-vitro chromosomal aberration tests, in sister chromatid exchanges (SCE) tests, or forward gene mutation assays in cultured Chinese hamster ovary cells. SCE and a low frequency of chromosomal aberration were induced by high concentrations of GA, 3.6–16 mg/l, without metabolic activation. Gene mutation was induced by GA in L5178Y tk +/tk– mouse lymphoma cells and the humanTK6 lymphoblast cell line. Since GA induced a marginal increase in unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay (50, 100 μM), DNA-reactive genotoxic activity of GA was suggested to involve DNA-protein cross-linking.

Preventive Measures

GA is an eye, skin and respiratory tract irritant and skin and respiratory tract sensitizer. Generally, alkalinized 2–3.5% GA aqueous solution is used for cold sterilization of endoscopy instruments. GA concentrations of commercial products range from 3 to 20%, and a 20% GA solution is diluted to 2% at use. Since these levels of GA solution produce moderate to severe irritation of the skin, wearing gloves is essential to prevent hazards to the skin. When the permeability of gloves was tested with 2% or 3.4% GA solutions, nitrile rubber, butyl rubber, a synthetic surgical glove and polyethylene were each impermeable for at least 4 h, but latex gloves exhibited breakthrough at 45 min. With 50% GA, only butyl rubber and nitrile rubber were impermeable for 4h. When changing sterilization solutions, workers are exposed to high concentrations of GA solution, and should therefore wear butyl rubber or nitrile rubber gloves.

In addition, airborne GA concentrations can be high during the changing of GA solutions or dipping of instruments by hand. Since the vapor pressure of GA is low but its airborne concentration depends on the temperature of aqueous solution, the temperature of the solution should be kept low, and a respirator may be necessary.

Bibliography:

1) AA Stonehill, S Krop and MP Borick: Buffered glutaraldehyde, a new chemical sterilizing solution. Am J Hosp Pharm 20, 458–465 (1963).
2) RO Beauchamp Jr, MBG St Clair, TR Fennell, DOClarke, KT Morgan and FW Kari: A critical review of the toxicology of glutaraldehyde. Crit Rev Toxicol 22, 143–174 (1992).
3) International Programme on Chemical Safety (IPCS). Glutaraldehyde. International Chemical Safety Cards, ICSC number 0158. Geneva: IPCS, 2000.
4) IPCS. Glutaraldehyde (50% solution). International Chemical Safety Cards, ICSC number 0352. Geneva: IPCS, 2000.
5) HSE. Glutaraldehyde. HSE Review 1997. London: HSE, 1999.
6) ACGIH. Glutaraldehyde. In: Documentation of the

TLVs and BEIs with other world wide occupational exposure values CD-ROM 2002. Cincinnati: ACGIH, 2001.

7) DFG. List of MAK and BAT Values 2004. Weinheim: Wiley-VCH, 2004.

8) Labour Standards Bureau. Preventive measures against health hazard of workers caused by GA exposure in medical facilities. Tokyo: Japanese Ministry of Health, Labour and Welfare, 2005 (in Japanese).

9) PF Gannon, P Bright, M Campbell, SP O'Hickey and PS Burge: Occupational asthma due to glutaraldehyde and formaldehyde in endoscopy and x ray departments. Thorax 50, 156–159 (1995).

10) GM Liss, SM Tarlo, J Doherty, J Purdham, J Greene, L McCaskell and M Kerr: Physician diagnosed asthma, respiratory symptoms, and associations with workplace tasks among radiographers in Ontario, Canada. Occup Environ Med 60, 254–261 (2003) .

11) TO'Connor: Poisoned careers. Nurs NZ 3, 16–17 (1997)

12) G Ziem and J McTamney: Profile of patients with chemical injury and sensitivity. Environ Health Perspect 105 (Suppl 2), 417–436 (1997) .

13) Y Endo, H Ikeda, M Sasagawa, T Miyazaki, H Matsushige and H Uehara: Exposure and medical surveys of sick-house-syndrome patients. Jpn J Clin Ecol 10, 3–10 (2001) (in Japanese) .

14) T T akigawa, T Horike, Y Ohashi, H Kataoka, DH Wang and S Kira: Were volatile organic compounds the inducing factors for subjective symptoms of employees working in newly constructed hospitals? Environ Toxicol 19, 280–290 (2004).

15) H Nakazawa, H Ikeda, T Yamashita, I Hara, Y Kumai, G Endo and Y Endo: A case of sick building syndrome in a Japanese office worker. Ind Health 43, 341–345(2005).

16) DK Sari, S Kuwahara, Y Tsukamoto, H Hori, N Kunugita, K Arashidani, H Fujimaki and F Sasaki: Effect of prolonged exposure to low concentrations of formaldehyde on the corticotropin releasing hormone neurons in the hypothalamus and adrenocorticotropic hormone cells in the pituitary gland in female mice. Brain Res 1013, 107–116 (2004).

17) Chemical Evaluation and Research Institute (CERI). Glutaraldehyde. Hazard Data Book for Chemical Substances. Tokyo: CERI, 2002 (in Japanese).

18) NA Miner, JW McDowell, GW Willcockson, NI Bruckner, RL Stark and EJ Whitmore: Antimicrobial and other properties of a new stabilized alkaline glutaraldehyde disinfectant/sterilizer. Am J Hosp Pharm 34, 376–382 (1977).

19) DM Ranly and D Horn: Distribution, metabolism, and excretion of [14C] glutaraldehyde. J Endod 16, 135–139 (1990).

20) JA McKelvey, RH Garman, CM Anuszkiewicz, MJT allant and B Ballantyne: Percutaneous pharmacokinetics and material balance studies with glutaraldehyde. J Toxicol Cutan Ocul Toxicol 11, 341–367 (1992).

21) SW Frantz, JL Beskitt, MJ Tallant, JW Futrell and B Ballantyne: Glutaraldehyde; species comparisons of in-vitro skin penetration. J Toxicol Cutan Ocul Toxicol 12, 349–361 (1993).

22) D Anadol, U Ozcelik, N Kiper and A Gocmen: Chemical pneumonia caused by glutaraldehyde. Pediatr Int 43, 701–702 (2001).

23) AB West, SF Kuan, M Bennick and S Lagarde: Glutaraldehyde colitis following endoscopy: clinical and pathological features and investigation of an outbreak. Gastroenterology 108, 1250–1255 (1995).

24) R Zissin, G Gayer and Y Maor-Kendler: CT findings of glutaraldehyde colitis: a report of two cases. Clin Radiol 54, 123–125 (1999).

25) B Ballantyne and RC Myers: The acute toxicity and primary irritancy of glutaraldehyde solutions. Vet Hum Toxicol 43, 193–202 (2001).

27) National Industrial Chemicals Notification Assessment Scheme. Glutaraldehyde. Full Public Report. InPriority Existing Chemical No. 3. Canberra: Australian Government Publishing Service, 1994.

28) K Ohno, K Yasuhara, Y Kawasaki, Y Nakaji and Y Kurokawa: Comparative studies on acute toxicity of glutaraldehyde using young and old rats. Eisei Shikenjo Hokoku 109, 92–97 (1991) (in Japanese).

29) Greim H. Glutaraldehyde. Occupational toxicants, critical data evaluation for MAK values and classification of carcinogens. Weinheim: Wiley-VCH, 1997.

30) MB St Clair, EA Gross and KT Morgan: Pathology and cell proliferation induced by intra-nasal instillation of aldehydes in the rat: comparison of glutaraldehyde and formaldehyde. Toxicol Pathol 18, 353–361 (1990).

31) WG Reifenrath, SD Prystowsky, JH Nonomura and PB Robinson: Topical glutaraldehyde-percutaneous penetration and skin irritation. Arch Dermatol Res 277, 242–244 (1985).

32) US NTP: Toxicology and carcinogenesis studies of glutaraldehyde (CAS No. 111-30-8) in F344/N rats and B6C3F1 mice (Inhalation studies). Natl Toxicol Program Tech Rep Ser 490, 1–234 (1999).

33) JS Vergnes and B Ballantyne: Genetic toxicology studies with glutaraldehyde. J Appl Toxicol 22, 45–60 (2002).

34) DE Levin, M Hollstein, MF Christman, EA Schwiers and BN Ames: A new Salmonella tester strain (TA102) with A X T base pairs at the site of mutation detects oxidative mutagens. Proc Natl Acad Sci USA 79, 7445–7449 (1982).

35) R Jung, G Engelhart, B Herbolt, R Jackh and W Muller: Collaborative study of mutagenicity with Salmonella typhimurium TA102. Mutat Res 278, 265–270 (1992).

36) P Wilcox, A Naidoo, DJ Wedd and DG Gatehouse: Comparison of Salmonella typhimurium TA102 with Escherichia coli WP2 tester strains. Mutagenesis 5, 285–291 (1990).

37) Wvon der Hude, C Behm, R Gurtler and A Basler: Evaluation of the SOS chromotest. Mutat Res 203, 81–94 (1888).

Neutralizing Media

An **antimicrobial** is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called *microbicidal*, while those that merely inhibit their growth are called *microbiostatic*.

The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living organisms but is now also applied to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones. The term also used to be restricted to antibacterials (and is often used as a synonym for them by medical professionals and in medical literature), but its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth.

NEUTRALIZATION OF ANTIMICROBIAL ACTIVITY

Removal of preservative antimicrobial activity is clearly an important step when recovering test organisms from contaminated products, yet surprisingly little has been published which compare the effectiveness of the different neutralization methods. Inactivation methods can involve the use of a specific neutralizer or simply the physical removal of an agent, by dilution to a sub inhibitory level or by washing using a membrane filtration technique. Most procedure rely upon the removal of residual-free inhibitors. Where inhibitors have already bound firmly onto cells, then these techniques will not be successful in removing antimicrobial activity.

Nonionic surface-active agents, such as Tween 80 and Lubrol W, have been widely used as preservative neutralizers, sometimes in combination with Lecithin. The detoxifying action of lecithin is particularly useful for cationic antimicrobials. These cause cell membrane damage and generally have a high affinity for the acidic phospholipids. Orth (1981) compared the recovery of *S.aureus* from a preserved lotion on three different media, standard method agar, Baird-Parker Agar and Tryptone Soya Agar with Lecithin and Polysorbate 80 (TSLA) containing 0.07% Lecithin and 0.5% Polysorbate 80. Although no difference was seen between the three media immediately after inoculation, the recovery of *S.aureus* after 3 hour exposure to the preservative was significantly higher on TSLA than with the other media. In addition certain media may have some nonspecific neutralization effect. Media containing serum on meat have been used successfully for detecting reasonably high numbers of phenol-exposed cells. Cook and Steel (1959) reported that the addition of serum, before but not after the addition of culture, would efficiently neutralize mercuric chloride. This is presumably because there is no residual-free inhibitor if the cells are added first.

DISINFECTANT CHALLENGE TESTING

Under FIFRA, the EPA requires companies that register public health antimicrobial pesticide products including disinfectants, sanitization agents, sporicidal agents, and sterilants to ensure the safety and effectiveness of their products before they are sold or distributed. Companies registering these products must address the chemical composition of their product, include toxicology data to document that their product is safe if used as directed on the label, include efficacy data to document their claims of effectiveness against specific organisms and to support the directions for use provided in the labeling, and provide labeling that reflects the required elements for safe and effective use. While these directions provide valuable information, they may not be helpful in terms of the products' use as disinfectants in a manufacturing environment.

In the United States, the official disinfectant testing methods are published by AOAC International and include the Phenol-Coefficient Test, Use-Dilution Method Test, Hard Surface Carrier Method, and Sporicidal Carrier Test. A scientific study submitted for EPA review in support of disinfectant registration must be conducted at a laboratory facility that follows the Good Laboratory Practices (GLP) regulations (21 CFR 58). To demonstrate the efficacy of a disinfectant within a pharmaceutical manufacturing environment, it may be deemed necessary to conduct the following tests: (1) use-dilution tests (screening disinfectants for their efficacy at various concentrations and contact times against a wide range of standard test organisms and environmental isolates); (2) surface challenge tests (using standard test microorganisms and microorganisms that are typical environmental isolates, applying disinfectants to surfaces at the selected use concentration with a specified contact time, and determining the log reduction of the challenge microorganisms); and (3) a statistical comparison of the frequency of isolation and numbers of microorganisms isolated prior to and after the implementation of a new disinfectant. This is considered necessary because critical process steps like disinfection of aseptic processing areas, as required by GMP regulations, need to be validated, and the EPA registration requirements do not address how

disinfectants are used in the pharmaceutical, biotechnology, and medical device industries. For the surface challenge tests, the test organisms are enumerated using swabs, surface rinse, or contact plate methods. Neutralizers that inactivate the disinfectants should be included in either the diluents or microbiological media used for microbial enumeration or both (see *Table* 1). Additional information on disinfectant neutralization may be found in *Validation of Microbial Recovery from Pharmacopeial Articles* 1227.

Table 1. Neutralizing Agents for Common Disinfectants

Disinfectant	**Neutralizing Agent**
Alcohols	Dilution or polysorbate 80
Glutaraldehyde	Glycine and sodium bisulfate
Sodium hypochlorite	Sodium thiosulfate
Chlorhexidine	Polysorbate 80 and lecithin
Mercuric chloride and other mercurials	Thioglycolic acid
Quaternary ammonium compounds	Polysorbate 80 and lecithin
Phenolic compounds	Dilution or polysorbate 80 and lecithin

Universal neutralizer broths may be formulated to contain a range of neutralizing agents.

For example, **Dey/Engley (D/E) Broth** contains 0.5% polysorbate 80, 0.7% lecithin, 0.1% sodium thioglycolate, 0.6% sodium thiosulfate, 0.25% sodium bisulfite, 0.5% tryptone, 0.25% yeast extract, and 1.0% dextrose; **Letheen Broth** contains 0.5% polysorbate 80, 0.07% lecithin, 1.0% peptamin, 0.5% beef extract, and 0.5% sodium chloride; and **Tryptone–Azolectin–Tween (TAT) Broth Base + Tween 20** contains 4.0% (v/v) polysorbate 20, 0.5% lecithin, and 2.0% tryptone.

In practice, sufficient organisms need to be inoculated on a 2-inch × 2-inch square of the surface being decontaminated, i.e., a coupon, to demonstrate at least a 2 (for bacterial spores) to 3 (for vegetative bacteria) log reduction during a predetermined contact time (i.e., 10 minutes over and above the recovery observed with a control disinfectant application). The efficacy of the neutralizers and their ability to recover inoculated microorganisms from the material should be demonstrated during the use-dilution or surface-challenge studies. Points to remember are that disinfectants are less effective against the higher numbers of microorganisms used in laboratory challenge tests than they are against the numbers that are found in clean rooms; that inocula from the log growth phase that are typically employed in laboratory tests are more resistant, with the exception of spores formed during the static phase, than those from a static or dying culture or stressed organisms in the environment; and that microorganisms may be physically removed during actual disinfectant application in the manufacturing area.

The measurement of microbial kill requires the ability to measure the number of surviving microorganisms with time after exposure to the antimicrobial agent. Bioburden determinations have the same requirement as they depend on the ability to recover viable microorganisms in the presence of potentially antimicrobial products or raw materials. However, carryover of residual disinfectant from the test could inhibit growth in the recovery medium, leading to poor microbial recovery. This potential residual activity must be neutralized and it is necessary to demonstrate the adequacy of neutralization for these tests. This demonstration of neutralization in compendial microbiological tests is known as demonstration of method suitability.

METHODS OF NEUTRALIZING ANTIMICROBIAL PROPERTIES

Three common methods are used to neutralize antimicrobial properties of a product: (1) chemical inhibition, (2) dilution, and (3) filtration and washing.

Chemical Inhibition

Table 2 shows known neutralizers for a variety of chemical antimicrobial agents and the reported toxicity of some chemical neutralizers to specific microorganisms. However, despite potential toxicity, the convenience and quick action of chemical inhibitors encourage their use. Chemical inhibition of bactericides is the preferred method for the antimicrobial efficacy test. The potential of chemical inhibitors should be considered in the membrane filtration and the direct transfer sterility tests. Antibiotics may not be susceptible to neutralization by chemical means, but rather by enzymatic treatment (e.g., penicillinase). These enzymes may be used where required.

Table 2. Some Common Neutralizers for Chemical Biocides

Neutralizer	**Biocide Class**	**Potential Action of Biocides**
Bisulfate	Glutaraldehyde, Mercurials	Non-Sporing Bacteria
Dilution	Phenolics, Alcohol, Aldehydes, Sorbate	—
Glycine	Aldehydes	Growing Cells
Lecithin	Quaternary Ammonium Compounds (QACs), Parabens, Bis-biguanides	Bacteria
Mg^{+2} or Ca^{+2} ions	EDTA	—
Polysorbate	QACS, Iodine, Parabens	—
Thioglycollate	Mercurials	Staphylococci and Spores
Thiosulfate	Mercurials, Halogens, Aldehydes	Staphylococci

Dilution

A second approach to neutralizing antimicrobial properties of a product is by dilution, because the concentration of a chemical bactericide exerts a large effect on its potency. The relationship between concentration and antimicrobial effect differs among bactericidal agents but is constant for a particular antimicrobial agent. This relationship is exponential in nature, with the general formula:

$C\eta t = k$

in which *C* is the concentration; *t* is the time required to kill a standard inoculum; *k* is a constant; and the concentration exponent, η, is the slope of the plot of log *t* versus log *C*. Antimicrobial agents with high η values are rapidly neutralized by dilution, whereas those with low η values are not good candidates for neutralization by dilution.

Membrane Filtration

An approach that is often used, especially in sterility testing, is neutralization by membrane filtration. This approach relies upon the physical retention of the microorganism on the membrane filter, with the antimicrobial agent passing through the filter into the filtrate. The filter is then incubated for recovery of viable microorganisms. However, filtration alone may not remove sufficient quantities of the bactericidal agent to allow growth of surviving microorganisms. Adherence of residual antimicrobial agents to the filter membrane may cause growth inhibition. Filtration through a low-binding filter material, such as polyvinylidene difluoride, helps to minimize this growth inhibition. Additionally, the preservative may be diluted or flushed from the filter by rinsing with a benign fluid; such as diluting *Fluid A* (see *Diluting and Rinsing Fluids for Membrane Filtration* under *Sterility Tests* <71> for diluting fluid compositions). Chemical neutralizers in the rinsing fluid can ensure that any antimicrobial residue on the membrane does not interfere with the recovery of viable microorganisms.

Methods of Neutralizing Antimicrobial Properties

Chemical Inactivation

Many antimicrobials can be chemically inactivated. *USP* <1227> provides a listing of some of the more popularly used neutralizers (*USP* <1227>). Several neutralizing and dilution broth media have been formulated to take advantage of these neutralizers. Among the more popular of these broths are Dey-Engley (D/E), Letheen, and microbial content test agar (MCTA). Many beta-lactam antibiotics can be inactivated using a sterile solution of a beta-lactamase to degrade the chemical structure of the beta-lactam ring.

Demonstration of chemical inactivation requires the establishment of two characteristics: neutralizer efficacy and neutralizer toxicity. These can be demonstrated using comparison among three populations. Ideally, these comparisons can be done in some manner allowing quantification of the growth in the populations, allowing for comparisons among the populations.

Dey-Engley Neutralizing Broth (D/E Broth Disinfectant Testing)

Dey-Engley Neutralizing Agar (D/E Agar Disinfectant Testing)

Dey-Engley Neutralizing Broth/Agar is used in disinfectant testing where neutralization of the antiseptics and disinfectants is important for determining its bactericidal activity.

Composition**

Ingredients	Gms / Litre
Casein enzymichydrolysate	5.000
Yeast extract	2.500
Dextrose	10.000
Sodium thiosulphate	6.000
Sodium thioglycollate	1.000
Sodium bisulphite	2.500
Lecithin	7.000
Polysorbate 80	5.000
Bromocresol purple	0.020
Agar	15.000
Final pH (at 25°C)	7.6±0.2

Dey-Engley Neutralizing Agar neutralizes a broad spectrum of antiseptics and disinfectants including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde.

Sodium bisulfite neutralizes aldehydes; sodium thioglycollate neutralizes mercurials; sodium thiosulfate neutralizes iodine and chlorine; lecithin neutralizes quaternary ammonium compounds; and polysorbate 80, a non-ionic surface-active agent, neutralizes substituted phenolics.

Dey -Engley Neutralizing Agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. Incubate the plates, by covering the lids, at an appropriate temperature. The presence of microorganism is determined by the appearance of colonies on the surface of agar medium.

Bromocresol purple is an indicator for dextrose utilization. Due to the high concentration of lecithin in the broth medium, turbidity cannot be used to detect growth. Therefore, bromocresol purple and dextrose are added to the medium. Those organisms that ferment dextrose will turn the medium from purple to yellow. Growth of *Pseudomonas* species, which do not ferment dextrose, can be detected by the formation of a pellicle on the surface of the broth.

Neutralization Test

For testing disinfectants, prepare two sets of test tubes, one containing 9 ml Dey-Engley Neutralizing Broth and other

with 9 ml Dey-Engley Neutralizing Broth Base. Add 1 ml of disinfectant under test. Mix well and allow it to stand for 15 minutes. Inoculate 0.1 ml of 1:100,000 dilution of overnight broth cultures and incubate at 37°C for 48 hours. Growth is indicated by a colour change from purple to yellow or pellicle formation. Growth in Neutralizing Broth and no growth in Neutralizing Broth Base indicate neutralization of disinfectant. To check bactericidal activity, both broth tubesare inoculated on D/E Neutralizing Agar. Positive growth from negative tubes of Neutralizing Broth Base indicates bacteriostatic substance while negative growth indicates a bactericidal disinfectant. All positive tubes should show growth on Dey-Engley Neutralizing Agar.

Tryptone Soya Agar with Lecithin and Polysorbate 80

Tryptone Soya Agar with Lecithin and Polysorbate 80 is used validation of cleanliness on surfaces of containers, equipment's surfaces and water miscible cosmetics.

Composition**

Ingredients	Gms / Litre
Casein enzymichydrolysate	15.0
Papaic digest of soyabean meal	5.0
Sodium chloride	5.0
Lecithin	0.70
Polysorbate 80 (Tween 80)	5.0
Agar	15.0
Final pH (at 25°C)	7.3±0.2

Tryptone Soya Agar with Lecithin and Polysorbate 80 is used in RODAC (Replicate Organism Detection and Counting) plates for the detection and enumeration of microorganisms present on surfaces of sanitary importances.

Lecithin and polysorbate 80 (Tween 80) are neutralizers reported to inactivate residual disinfectants from where the sample is collected. Lecithin neutralizes quaternary ammonium compounds and polysorbate 80 neutralizes phenolic disinfectants, hexachlorophene, formalin and with lecithin ethanol.

Collection of samples from areas before and after the treatment with disinfectant evaluates cleaning procedures in environmental sanitation. The presence and number of microorganisms is determined by the appearance of colonies on the agar surface.

1. A.D. Russell, 1979. Microbiological Applications of the Inactivation of Antibiotics and Other Antimicrobial Agents,"*Journal of Applied Bacteriology* **46**, 207-245.
2. AOAC International Official Methods of Analysis, 15, 16, and 17th editions. Arlington, VA.
3. Cook, A. M. and Steel, K. J. 1959. The antagonism of the antibacterial action of mercury compounds. Journal of Pharmacy.
4. Denny, V.F.; Marsik, F.J. **1997.** Current Practices in the Use of Disinfectants within the Pharmaceutical Industry. *PDA J. of Pharmaceutical Sci. and Tech.*, *51*, (6), 227–228.
5. Orth, D. S. 1981. Principles of preservative efficacy testing. Cosmetics and Toiletries 96: 43-52.
6. S. Sutton, 1996. "Neutralizer Evaluations as Control Experiments for Antimicrobial Efficacy Tests," in *Handbook of Disinfectants & Antiseptics*, J Ascenzi (ed), Marcel Dekker, pp. 43-62.
7. *USP* <51> Antimicrobial Effectiveness Testing
8. *USP* <71> *Sterility Tests*.
9. *USP* <1227> *Validation of Microbial Recovery from Pharmacopeial Articles.*

Dr. Donald Low

(May 2, 1945 - September 18, 2013) was a Canadian microbiologist noted for his role in battling the SARS outbreak of 2003. He was microbiologist-in-chief at Mount Sinai Hospital, Toronto, from 1985 to 2013. He was an international authority on public health, emerging infectious diseases and antimicrobial resistance whose work at the hospital has left a lasting legacy.

SARS?

Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV). SARS was first reported in Asia in February 2003. Over the next few months, the illness spread to more than two dozen countries in North America, South America, Europe, and Asia before the SARS global outbreak of 2003 was contained.

The SARS outbreak of 2003

According to the World Health Organization (WHO), a total of 8,098 people worldwide became sick with SARS during the 2003 outbreak. Of these, 774 died. In the United States, only eight people had laboratory evidence of SARS-CoV infection. All of these people had traveled to other parts of the world with SARS. SARS did not spread more widely in the community in the United States.

Symptoms of SARS

In general, SARS begins with a high fever (temperature greater than 100.4°F [>38.0°C]). Other symptoms may include headache, an overall feeling of discomfort, and body aches. Some people also have mild respiratory symptoms at the outset. About 10 percent to 20 percent of patients have diarrhea. After 2 to 7 days, SARS patients may develop a dry cough. Most patients develop pneumonia.

How SARS spreads?

The main way that SARS seems to spread is by close person-to-person contact. The virus that causes SARS is thought to be transmitted most readily by respiratory droplets (droplet spread) produced when an infected person coughs or sneezes. Droplet spread can happen when droplets from the cough or sneeze of an infected person are propelled a short distance (generally up to 3 feet) through the air and deposited on the mucous membranes of the mouth, nose, or eyes of persons who are nearby. The virus also can spread when a person touches a surface or object contaminated with infectious droplets and then touches his or her mouth, nose, or eye(s). In addition, it is possible that the SARS virus might spread more broadly through the air (airborne spread) or by other ways that are not now known.

Donald Low graduated from medical school at the University of Manitoba. Low became a familiar face to the Canadian public during 2003's SARS crisis; although he had no official role, he was seen as calm and effective in press conferences about the response to the outbreak. He was one of several physicians who were required to quarantine themselves at home during part of the outbreak. After the 2003 breakout of severe acute respiratory syndrome in Toronto, Low oversaw regular updates to the public about the syndrome, which eventually killed 44 people in Canada and nearly 800 worldwide. In 2005 he took on the role of medical director of public health laboratory of the Ontario Agency for Health Protection and Promotion. Low was also a noted expert in necrotizing fasciitis due to Group A. streptococcus. Under Dr. Low's direction, the Mount Sinai/UHN shared Department of Microbiology was created, and services expanded to ten health-care institutions. He played a vital role in Ontario's management of the 2003 SARS outbreak, and in the revitalization of the Ontario Public Health Laboratory, which he led from 2005-2012. He was also the Head of the Division of Microbiology in the Department of Laboratory Medicine and Pathobiology at the University of Toronto.

Dr. Allison McGeer, director of infectious disease control at Mount Sinai hospital, worked with Low and knew him for 25 years. "He was the face and a good piece of the brains behind our response to SARS," McGeer told host Matt Galloway. "What many of us in Toronto don't recognize is the loss he leaves behind to microbiology and infectious diseases in Canada, and to all of his research work in emerging diseases around the world. It's a big loss to all of us in microbiology. "With Don, no problem is ever too large," said McGeer. "You simply lay it out, you put it in its pieces, you figure out how to deal with it and you move on. That may be his biggest legacy." Dr. Michael Gardam, from the University Health Network, told CBC News that Low's commitment to keeping the public informed during the outbreak was a unique trait. "The thought you would have a world renowned expert really seeing it as one of his major jobs … to go directly to the public and actually talk about what's going on… I can't tell you how unusual that is," he said.

Low's wife was CBC News reporter Maureen Taylor. He had three children from a previous marriage. Low was diagnosed with a brain tumour in February 2013, and died September 18, 2013, at age 68. “Dr. Low's many friends and colleagues here at Mount Sinai are profoundly saddened by this loss, and will remember him not only for his many outstanding contributions, including the significant role that he played here at Mount Sinai and all of Toronto during the 2003 SARS crisis, but for his kindness, good humour and commitment to patient care,” said Joseph Mapa, President and CEO, Mount Sinai Hospital.

References:

(1) Perry, Ann (December 27, 2003). "Donald Low: Life was 'put on hold' during SARS outbreak". *Toronto Star*. p. B4. (2) Branswell, Helen (September 19, 2013). "Dr. Donald Low, the face of Toronto's response to SARS, dies at 68. *The Globe and Mail*. Retrieved September 21, 2013. (3) Marchione, Marilyn (April 3, 2003). "Toronto doctor finds himself on front line In battle against SARS". *Milwaukee Journal Sentinel*. p. 6A. (4) (September 19, 2013). "Dr. Donald Low, public face of Toronto SARS crisis, dies". CBC News. Retrieved September 21, 2013. (5) McDiarmid, Jessica (September 24, 2013). "Dr. Donald Low, face of the SARS crisis, pleads for 'dying with dignity'. Toronto Star. Retrieved September 24, 2013

Funny Quotes

Car se takra kar kabootar behosh ho gaya.

Aadmi usse doctor ke paas le gaya,

phir ghar le aaya aur pinjre mein rakkha.

Kabootar ko hosh aaya toh bola -

"Aai sala jail ho gai,

woh car wala mar gaya kya"?

Doctor:-

Aapka weight kitna hai?

Gajodhar:-

Chasme ke sath 75kgs.

Doctor:-

Aur without chasme ke?

Gajodhar:-

Woh mujhe dikhta hi nahi...

Best customer service reply:

Customer -

Agar mai aaj cheque jama karu toh wo kab clear hoga?

Clerk -

3 din mein.

Customer -

Mera cheque toh samne wali bank ka hai....

Dono bank amne-samne hai fir bhi itna samay kyun ?

Clerk -

Sir PROCEDURE toh FOLLOW karna padta hai na.

Socho aap kahi jaa rahe ho...

aur baaju mein hi Shamshaan hai...

Agar aap Shamshan ke bahar hi mar gaye,

toh aapko pehle ghar lekar jayenge ya wahin nipta denge?

Customer behosh...

Words of Wisdom

The biggest challenge after success is shutting up about it." — Criss Jami

Voice is not just the sound that comes from your throat, but the feelings that come from your words." — Jennifer Donnelly

look for a long time at what pleases you, and longer still at what pains you..." — Colette

We need to learn how to let it wash over us, without drowning in it. Our life doesn't have to end where the pain begins, but rather, it is where we start to mend." — Jaeda DeWalt

"A person who could muster the courage to remove from his daily life the products that he basically doesn't need would automatically delete the negative thoughts and the toxic people in his life." — Anuj Somany

If I disagree with you sometimes, it's because I have a mind of my own." — Emma Paul

"People may like a person's thought for its touching words, but they like him truly for his own deeds touching their heart." — Anuj Somany

Facts do not lie within biased opinions." — Dawn Stewart Field

Acinetobacter

Scientific classification

Domain: Bacteria
Kingdom: Eubacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Pseudomonadales
Family: Moraxellaceae
Genus: *Acinetobacter*
Species: ***A. baumannii***

Acinetobacter baumannii is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. An opportunistic pathogen, A. baumannii has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged (90 days) hospital stay. Commonly associated with aquatic environments, it has been shown to colonize the skin as well as being isolated in high numbers from the respiratory and oropharynx secretions of infected individuals. In recent years, it has been designated as a "red alert" human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum.

This phenomenon of multidrug-resistant (MDR) pathogens has increasingly become a cause for serious concern with regard to both nosocomial and community-acquired infections. Indeed, the World Health Organization (WHO) has recently identified antimicrobial resistance as one of the three most important problems facing human health.6 **The most common and serious MDR pathogens have been encompassed within the acronym "ESKAPE," standing for Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.**

While in the 1970s A. baumannii is thought to have been sensitive to most antibiotics, today the pathogen appears to exhibit extensive resistance to most first-line antibiotics. More recently, A. baumannii has become a major cause for concern in conflict zones, and has gained particular notoriety in the resent desert conflicts in Iraq, earning it the moniker "Iraqibacter." In particular, high incidences of MDR bacteremia (bloodstream infections) have been noted among US Army service members following Operation Iraqi Freedom (OIF). Interest from the scientific community over the past 15 years has led to significant advances of our understanding of this organism.

Natural Habitat:

Organisms belonging to the genus Acinetobacter are often considered to be ubiquitous in nature given that they can be recovered from almost all soil and surface water samples.

As a pathogen, A. baumannii specifically targets moist tissues such as mucous membranes or areas of the skin that are exposed, either through accident or injury. Skin and soft tissues infected with A. baumannii initially present with a "peau d'orange" appearance (similar to the skin of an orange) followed by a sandpaper-like presentation which eventually gives way to clear vesicles on the skin. In areas of skin disruption hemorrhagic bullae can be seen, with a visible necrotizing process followed by bacteremia. If left untreated, this infection can lead to septicemia and death. Although it is likely that A. baumannii is responsible for these recognizable features, copathogens, such as Klebsiella pneumoniae, Candida albicans and Enterococcus faecalis, are thought to be a contributing factor. These co-pathogens may cause necrotizing infection and may create a nidus of entry into the bloodstream for A. baumannii.

Despite its association with skin infections, A. baumannii is found only rarely as part of the normal skin microflora, with one study estimating that only 3% (at most) of the population are colonized by the bacterium. Interestingly, Acinetobacter was recovered from 22% of body lice sampled from homeless people, suggesting another potentially important reservoir for the pathogen.

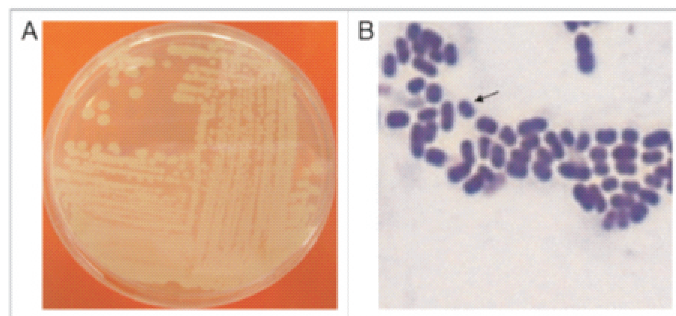


Figure 1 (A) Complex streak of Acinetobacter baumannii following overnight growth on Luria Bertani agar at 37°C (B) gram stain of log phase A. baumannii cells grown on Luria Bertani broth. Arrow indicates an individual A. baumannii cell.

Clinical Symptoms:

A. baumannii infections are implicated across a wide range of anatomical regions and with varying severity and patient outcomes. There is considerable debate relating to the actual clinical impact of infection and its relationship to patient mortality. While a number of studies have concluded that infection with Acinetobacter has a detrimental effect on patient outcome,other similar studies implied little or no effect on patient outcome as a result of infection.

The lack of consensus is most likely due to the difference in the approaches of the various studies; some being prospective while others have been of retrospective samples. The results generated by some studies have also only identified the organism to genus level but not to species level, with many referring to infection with Acinetobacter calcoaceticus-baumannii complex which

could conceivably indicate colonization with the environmental species Acinetobacter calcoaceticus coupled with a polymicrobial infection, rather than a monomicrobial infection with a virulent Acinetobacter species such as MDR Acinetobacter.

Hospital-acquired pneumonia

Ventilator associated pneumonia (VAP) is commonly linked to infection.55 Longer periods of hospitalization, longer time on mechanical ventilation and prior use of antibiotics are the recognized factors increasing the risk of VAP due to Acinetobacter. Nosocomial outbreaks have also been described due to health care professionals with colonized hands and poor personal hygiene; such individuals may act as opportunist carriers of an epidemic stain. Contaminated ventilators or respiratory care equipment as well as intra-hospital transmission may also contribute to the beginning of an outbreak.

Community-acquired pneumonia

Pneumonia acquired outside of the hospital setting and caused by Acinetobacter has beennoted in Australia and Asia. The source of infection may be throat carriage, which occurs in up to 10% of community residents with excessive alcohol consumption.57 It is characterized by a severe and sudden onset coupled with secondary bloodstream infection and has a mortality rate of between 40% and 60%.

Bloodstream infections

In a seven year review (1995–2002) of nosocomial bloodstream infections in the United States, Acinetobacter accounted for 1.3% of all monomicrobial bloodstream infections. Acinetobacter was a more common cause of CU-acquired bloodstream infection than of non-ICU-ward infection (1.6% vs. 0.9% of bloodstream infections, respectively, in those locations). Crude mortality figures overall from Acinetobacter bloodstream infection was 34.0% to 43.4% in the ICU and 16.3% outside the ICU. Acinetobacter bloodstream infection had the third highest crude mortality rate in the ICU, exceeded only by P. aeruginosa and Candida spp infections. It is notable that 102 patients had bloodstream infections at sites treating US military personnel injured in Iraq or Afghanistan from January 1, 2002 and August 31, 2004.

Battlefield trauma and other wounds

Acinetobacter is a welldocumented pathogen of burns units and is difficult to treat in patients with severe burns.60 However, infection of the skin and soft tissue outside of a military environment is uncommon.61 A retrospective review of 57 patients with SSTI revealed that eight cases were infected with Acinetobacter.3 In this instance all patients were male, ranging in age from 13 to 55 and of both American and Iraqi nationality. The median time from trauma to diagnosis with Acinetobacter infection was 15 d. All eight patients had a similar clinical presentation of SSTI; characteristic cellulitis with "peau d'orange" appearance, severe infection resulted in formation of bullae on the skin surface. The mortality rate in this instance was 12.5% (i.e., one of the eight died; however given that the patient was admitted with a gunshot wound to the groin, mortality cannot be solely assigned to infection).

Meningitis

Nosocomial, post-neurosurgical Acinetobacter meningitis is becoming increasingly more common with many other Gram-negative bacteria also becoming problematic in postoperative care. Installation of an external ventricular drain becomes a site for opportunistic infection. The mortality rate may be as high as 70%; however it is not possible to discern the definitive cause of mortality

Therapeutic Strategies

Existing antimicrobials

As mentioned previously, one of the distinguishing features of A. baumannii is its impressive array of acquired antibiotic resistance mechanisms, which although beyond the scope of this review, includes degradation enzymesc against β-lactams, modification enzymes against aminoglycosides, altered binding sites for quinolones, and a variety of efflux mechanisms and changes in outer membrane proteins (see Peleg et al. for a detailed overview). Any and all of these elements can be combined to result in a highly drug-resistant pathogen; making selection of an appropriate empirical antimicrobial agent extremely difficult. Indeed, given the probability that A. baumannii would be most likely resistant to one of the common first line antibiotics, treatment of the infection should be performed following sound consideration of antimicrobial susceptibility testing. Nevertheless, as a delay in accessing correct treatment may have adverse consequences for a patient's health, carbapenems such as Imipenem are often given as a drug of preference for serious and suspected Acinetobacter infections. However, despite its utility short-term, this prescription method jeopardizes future efficacy of such drugs as effective antimicrobial agents.

Future therapies

Given the rapid and extensive development of antibiotic resistance, several attempts have been made to develop alternative control strategies for dealing with A. baumannii including, but not limited to the following:

Bacteriophage.

Recently renewed interest in the area of antibacterial phage therapy has gained some traction.67 Due to the high specificity of phage and their ability to work quickly, bacteriophage therapy is being re-examined as an alternative treatment to help counteract the phenomenon of antibiotic Resistance. Indeed, a recent study by Yang et al. has resulted in the isolation and characterization of the virulent AB1 bacteriophage which has been shown to be effective against A. baumannii and as such represents a

novel therapeutic of some potential.

Bactericidal gene transfer therapy.

The design and delivery of vectors containing bactericidal genes that can be introduced into recipient pathogenic organisms by conjugation using attenuated donor cells is referred to as bactericidal gene transfer therapy. While the therapeutic potential of this approach is limited by the requirement for donor cells to be in contact with the pathogen (to facilitate vector transfer), positive effects have nonetheless been observed using murine burn infection models. Using this approach, Shankar et al.65 have shown that mice treated with a single dose of 1010 CFU of donor cells containing bactericidal genes had lower levels of A. baumannii in burn wounds compared with untreated mice.

Cathelicidins.

Marsupials give birth to immunologically naïve, altricial young that reside in the maternal pouch for 9–10 mo while being supported by a sophisticated lactation system. In the pouch, cathelicidins interact with and destroy Gram-positive and Gram-negative bacteria, protozoa and fungi via electrostatic interactions between their positively charged peptides and the negatively charged molecules found in the cell membranes of their targets. The best studied cathelicidin is human LL-37; the only human cathelicidin, it exhibits both anti-tumor and anti-HIV activity. The Tammar Wallaby cathelicidin WAM1 has been shown to be effective against Acinetobacter, and is 3–80 times more potent than LL-37 against a host of bacterial pathogens. WAM1 was not hemolytic against human red blood cells indicating potential for parenteral use in humans.70 Indeed, WAM1's anti-microbial activity and tolerance to salt concentrations similar to those found in the human body make it seem a likely candidate for further in vivo studies.

Radioimmunotherapy.

Although not yet not exploited as a therapeutic antimicrobial strategy in the clinic, radioimmunotherapy can target microorganisms as quickly and efficiently as cancer cells. This approach takes advantage of the specificity of antigen-antibody interactions to deliver radionuclides that emanate lethal doses of cytotoxic radiation directly to the target cell. Producing only transient hematological toxicity in experimental animals, radioimmunotherapy has been successfully adapted for the treatment of bacterial, fungal and viral infections. Given that previous studies have already described the development of antibodies against A. baumannii, the application of radioimmunotherapy as a novel therapeutic strategy for A. baumannii is a definite possibility.

Photodynamic therapy.

Involves the combination of nontoxic photosensitizers (PSs) with oxygen and visible to produce reactive oxygen species that oxidize biomolecules thereby killing cells. The use of photodynamic therapy (PDT) to treat localized bacterial infections generally involves the topical application of a PS into the infected tissue, followed by illumination with red (or near-infrared) which is capable of penetrating the infected tissue. Using a murine burn wound model, this technique has previously been shown to be effective against A. baumannii while having no obvious effects on wound healing. Recently, Tsai et al. investigated the effect of chitosan, a polycationic biopolymer, on increasing the efficacy of PDT against a number of pathogens including A. baumannii. Under conditions in which hematoporphyrin- PDT exhibited a bacteriocidal effect on a 2- to 4-log scale, subsequent treatment with chitosan (0.025%) for a further 30 min completely eradicated the bacteria (at a starting inoculums of 108 CFU/ml). Chitosan alone did not exert significant antimicrobial activity, without prior PDT, suggesting that the potentiated effect of chitosan worked only after the bacterial damage induced by PDT. Furthermore, the potentiated PDT effect of chitosan appears to be related to the level of PDT damage and the deacetylation level of the chitosan.

Nanoparticle technology.

Nitric oxide (NO) has been shown to exhibit potent antimicrobial activity as well as playing an important role in modulating immunity and regulating wound healing. Using nanotechnology based on a silane hydrogel, Friedman et al. have designed a stable nitric oxide (NO)-releasing nanoparticle (NO-np) platform. With the potential to serve as a novel, inexpensive and easily applied topical class of antimicrobials, this technology has been shown to be effective for the treatment of complex cutaneous infections such as those caused by A. baumannii. Indeed, Mihu et al. recently demonstrated the effect of NO-np against A. baumannii using murine wound and soft tissue models. Compared with control animals, NO-np-treated mice exhibited significant reductions in bacterial burden, enhanced wound healing rates and less collagen degradation by bacterial collagenases.

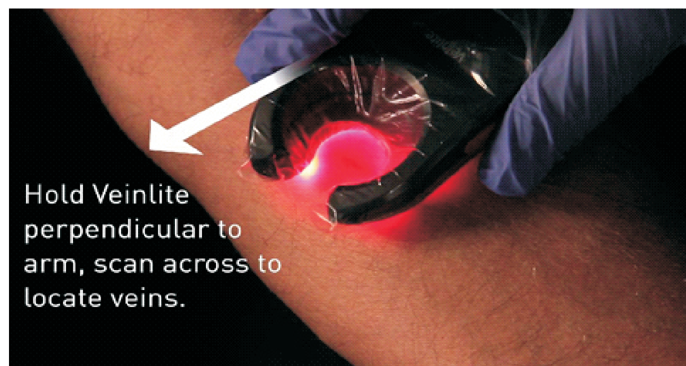
Conclusions:

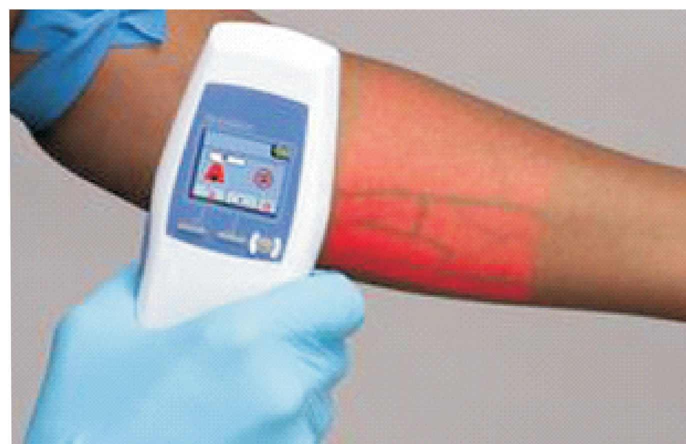
In conclusion, A. baumannii is an important opportunistic and emerging pathogen that can lead to serious nosocomial infections. Its pathogenic potential includes the ability to adhere to surfaces, form biofilms, display antimicrobial resistance and acquire genetic material from unrelated genera, making it a versatile and difficult adversary to control and eliminate.57 The optimal treatment for A. baumannii, especially nosocomial infections resulting from multiple resistant strains, remains to be established. It is thus a clinical imperative that well-designed procedures are put in place to help guide clinicians on decisions regarding the current best therapeutic practice.86 Furthermore, new experimental approaches are warranted to develop and evaluate novel therapeutic strategies for dealing with A. baumannii infections.

Vein Finder

One of the hardest parts of taking blood can be finding a suitable vein. Some patients are 'difficult sticks'; their veins are either very small, and/or deep, preventing health professionals from finding a site easily and quickly. Repetitive needle sticks are painful for the patient and may also lower their confidence in the ability of the phlebotomist performing the stick.

As you know venipuncture can be particularly challenging in some patients. Those with difficult venous access (DVA) can include the elderly, dark-skinned and obese patients. In fact, finding a suitable vein may pose a challenge on any patient. Many companies now market 'Vein Finder' products. This tool works by using near-infrared wavelength LEDs to illuminate the flesh at the site. The veins will appear as dark bands because they are more absorbent of this spectrum of light than the surrounding tissue. It is similar in principle to holding your hand over a flashlight (something we all did as kids). Once put into use, the benefits of the device will become so evident it will become part of the standard of care for all blood draws and IV starts.





The Cost of Failed Attempts

Of all invasive medical procedures, venipuncture is the most common and these venipuncture attempts can fail. As a result:

- Blood tests that facilitate diagnosis and patient management may be delayed;
- Intravenous therapy may not begin promptly;
- Physician intervention to access a difficult vein can erode productivity;
- Patients may endure unnecessary needlesticks and additional discomfort;
- Stress may increase for both patients and staff.

What Does The Data Say?

A study of patients reveals a strong desire for clinicians to use vein illumination.

But by using vein illumination device, many veins that might be otherwise undetectable without a vein locator, can be located and mapped on the patient's skin.

How Does It Work?

Hemoglobin in the blood absorbs infrared light. When the device is held above the skin, veins appear noticeably different than the surrounding tissue. The vasculature shows up clearly on the skin's surface, aiding in vein location to collect a blood sample or administer IV medications.

Vein Locator Features

Features of vein illumination device:

- **Easy to learn and use** – No pre-use calibration or adjustments are necessary- it can be used immediately.
- **Small size** –The device fits in your hand and weighs only 10 ounces.
- **Hands-free option** – In situations that require hands-free use, the device can be placed in a wheeled hands-free accessory or one that quickly attaches to a chair or bedrail.
- **No patient contact** – Because the device has been designed to be non-contact, it may not have to be sterilized after every use.
- **Works in light or dark** – Use the device in light or darkly lit environments.
- **Rechargeable battery** – The device doesn't need to be plugged into an electrical outlet.
- **Real world ruggedness** – Designed to take the wear and tear of hospital and field applications.
- **Movement tolerant** – Because the device shows the veins in real time, when operated properly, the device can accommodate patient movement.

Surfactants: Surface active agents

Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants.

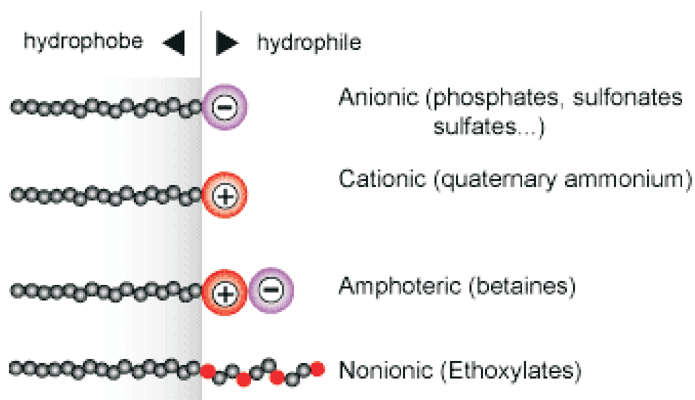
Surfactants lower the surface tension of an aqueous solution and are used as wetting agents, detergents, emulsifiers, antiseptics, and disinfectants. As antimicrobials, they alter the energy relationship at interfaces. Based on the position of the hydrophobic moiety in the molecule, surfactants are classified as anionic or cationic.

Composition and structure

Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant contains both a water insoluble (or oil soluble) component and a water soluble component. Surfactants will diffuse in water and adsorb at interfaces between air and water or at the interface between oil and water, in the case where water is mixed with oil. The water-insoluble hydrophobic group may extend out of the bulk water phase, into the air or into the oil phase, while the water-soluble head group remains in the water phase.

World production of surfactants is estimated at 15 Mton/y, of which about half are soaps. Other surfactants produced on a particularly large scale are linear alkylbenzenesulfonates (1700 kton/y), lignin sulfonates (600 kton/y), fatty alcohol ethoxylates (700 ktons/y), and alkylphenol ethoxylates (500 kton/y).

Types of Surfactants



Anionic Surfactants

Soaps are dipolar anionic detergents with the general formula RCOONa/K, which dissociate in water into hydrophilic K^+ or Na^+ ions and lipophilic fatty acid ions. Because NaOH and KOH are strong bases (whereas most fatty acids are weak acids), most soap solutions are alkaline (pH 8–10) and may irritate sensitive skin and mucous membranes. Soaps emulsify lipoidal secretions of the skin and remove, along with most of the accompanying dirt, desquamated epithelium and bacteria, which are then rinsed away with the lather. The antibacterial potency of soaps is often enhanced by inclusion of certain antiseptics, eg, hexachlorophene, phenols, carbanilides, or potassium iodide. They are incompatible with cationic surfactants.

Cationic Surfactants

Cationic detergents are a group of alkyl- or aryl-substituted quaternary ammonium compounds (eg, benzalkonium chloride, benzathonium chloride, cetylpyridinium chloride) with an ionizable halogen, such as bromide, iodide, or chloride. The major site of action of these compounds appears to be the cell membrane, where they become adsorbed and cause changes in permeability. Their activity is reduced by porous or fibrous materials (eg, fabrics, cellulose sponges) that adsorb them. They are inactivated by anionic substances (eg, soaps, proteins, fatty acids, phosphates). Therefore, they are of limited value in the presence of blood and tissue debris. They are effective against most bacteria, some fungi (including yeasts), and protozoa but not against viruses and spores. Aqueous solutions of 1:1,000 to 1:5,000 have good antimicrobial activity, especially at slightly alkaline pH. When applied to skin, they may form a film under which microorganisms can survive, which limits their reliability as antiseptics. Concentrations >1% are injurious to mucous membranes.

Among the classical cationic surfactants, quaternary ammonium compounds (QACs) are the most useful antiseptics and disinfectants. QACs are membrane active agents and cause lysis of spheroplasts and protoplasts suspended in sucrose. The cationic agents hypothetically react with phospholipid components in the cytoplasmic membrane, thereby producing membrane distortion and protoplast lysis under osmotic stress. On the other hand, the positive charge on microbial cells has often been correlated to the biocidal action. The deposition of organic monolayers onto solid surfaces containing quaternary ammonium groups has been shown to prevent deposition and growth of bacterial biofilms. Molecules with a net positive charge are able to kill microorganisms both in solution and upon attachment or adsorption to surfaces, particles, liposomes or bilayers. Various cationic architectures have been tested such as polyelectrolyte layers and hyper branched dendrimers.

Nonionic surfactants

Nonionic surfactants are also found in many cleaning

products, including carpet products. Nonionics have no charge on their hydrophilic end, which helps make them superior oily soil emulsifiers.

Some nonionics are high foamers (like anionics), while others do not generate much foam. Because of their lower foam profile and strong emulsifying potential, these surfactants are the preferred choice when formulating extraction cleaners and pre sprays.

However, unlike anionic surfactants, nonionics are thick liquids or syrups that are sticky or "gooey" to the touch. When left in the carpet, nonionic surfactants are the primary contributors to rapid resoiling.

Even with that being the case, their importance as cleaners outweighs this negative, and the cleaner or technician must take care to remove as much of the detergent residue as possible from the carpet in order to get the cleaning benefits of nonionics without their negatives.

Nonionic surfactants include:
- Ethoxylates
- Alkoxylates
- Cocamide

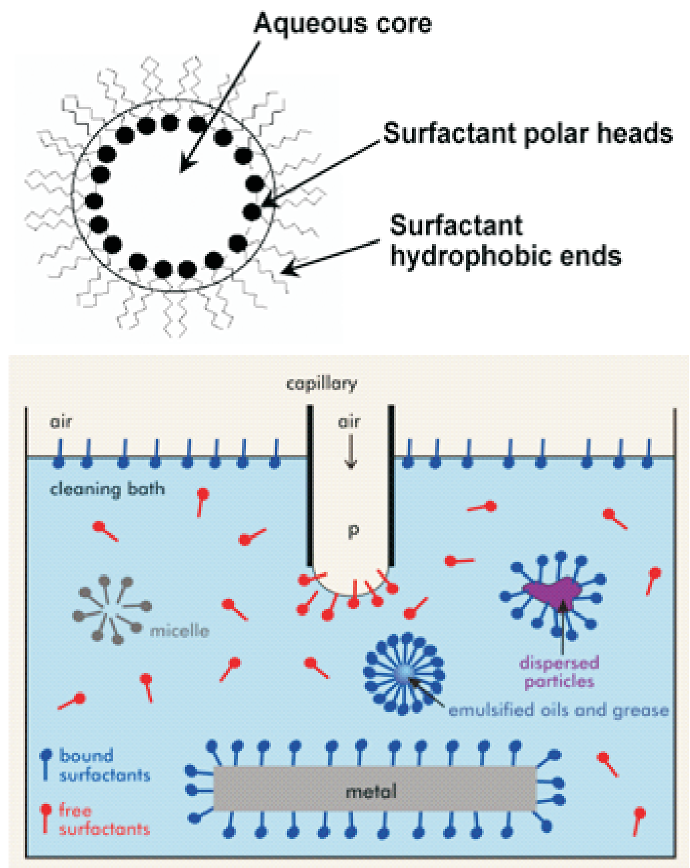
Amphoteric surfactants

Probably the least talked about surfactants are the amphoterics. These unique molecules possess both a positive and a negative charge on their hydrophilic end, giving them a net charge of zero.

Amphoteric surfactants have little utility on their own, but work extremely well in enhancing the cleaning effect of both anionic and nonionic surfactants. They can serve as "coupling agents," which hold the surfactants, solvents and inorganic salt components of a formula together.

Amphoterics are usually named in some way to indicate that they are amphoterics, as in amphoterge. Other examples of amphoterics are betaines and amine oxides.

Schematic of how surfactants work:



Microxpress Introduces

ULTRA PAP™

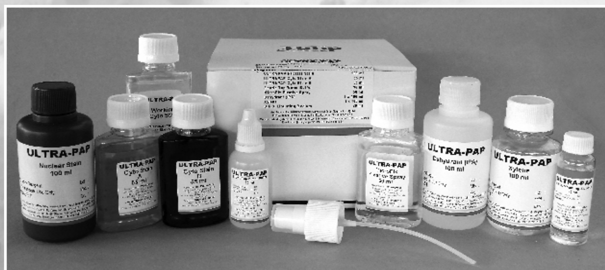
ULTRA-PAP Kit is modification of the classical PAP staining, formulated to give fast PAP staining of specimen smear with a simplified procedure thereby aiding clear nuclear and cytoplasmic staining.

Kit Contents :

ULTRAPAP – Nuclear Stain (100 ml), ULTRAPAP – Cyto-Stain A (55 ml), ULTRAPAP – Cyto-Stain B (55 ml), Scotts Tap Water Buffer (30 ml), Micro-Fix Fixative Spray (50 ml), Dehydrant (IPA) (3 x 100 ml), Xylene (2 x 100 ml), D. P. X. Mounting Medium (20 ml) and empty bottle (50ml) for preparing working cyto stain reagent.

Reagent Preparation :

As required make a Working Cyto - Stain by mixing equal amounts of ULTRAPAP Cyto - Stain A & B (An empty bottle is provided for the same). The Working Cyto - Stain is stable for at least 3 Months, provided contamination and hydration are avoided. The other contents are ready to use.



Ultra Fast Papanicolaou Staining Kit !

Presents

BIOSPRAY™

"Ideally, hand hygiene should be an automated behavior…"
WHO guidelines on hand hygiene in health care. ISBN 9789241597906, 2009, pg91

Product description:

BIOSPRAY™ is a state of art, touch-free and wall mounted dispenser to dispense handrub / handwash in medical and industrial settings. BIOSPRAY™ automatically dispenses both liquids and gels at a prefixed dose. This ensures adequate disinfection of hands without contaminating the environment.

FEATURES	BENEFITS
Touch-free	Prevents cross contamination
1 year warranty	Highly reliable
After sales service	Peace of mind
ABS plastic	Rust free, Durable and easily cleanable
Fixed dose dispensing	Adequate disinfection Reduced wastage of handrub / handwash
AC adapter provided	No need of battery
Compatible with liquids and gels	Versatile

Compatible with

- ALCONOX® : Colourless & odourless alcoholic handrub with moisturizer
- ECOMAX™ : Alcoholic handrub with moisturizer
- PURELLIUM™ GEL : Alcoholic handrub with moisturizer
- STERIMAX® : Liquid handrub antiseptic with triple action
- TRIOSEPT™ : Colourless & odourless liquid handrub with triple action
- BIOSCRUB™ : Antiseptic surgical scrub
- HITMAX® : Liquid microbial handwash soap

Highlights of the coming issue

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