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Editorial

Contents

It is interesting to note that resistance in microorganisms can be caused by a variety of mechanisms: the presence of an enzyme that inactivates the antimicrobial agent; the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent; mutation in the antimicrobial agent's target, which reduces the binding of the antimicrobial agent; post-transcriptional or post-translational modification of the antimicrobial agent's target, which reduces binding of the antimicrobial agent; reduced uptake of the antimicrobial agent; active efflux of the antimicrobial agent through efflux pump; and overproduction of the target of the antimicrobial agent. In addition, resistance may be caused by a previously unrecognized mechanism. So in this edition of Journal Of Hygiene Sciences, we are going to discuss about various modes of detection of antibacterial resistance, and also various anti-microbial agents, used for disinfection in medical and industrial segments. Also, various cleanroom techniques have been explained.

In the section mini review, Standard Methods for the Detection of Antibacterial Resistance is discussed. Antimicrobial resistance is a natural-biological phenomenon about response of microbes to the selective pressure of an antimicrobial drug.

Under Current Trend, in the previous edition we have discussed the importance of Pre-cleaning and Disinfection of Surgical Instruments. In this edition we are going to explore a wide range of Instrument Disinfectants available in the market, and learn about their pros and cons. There are some latest technologies released in the disinfection segment. The feasibility of these new technologies are discussed.

A small document on The Novel Laureate Axel Hugo Theodor Theorell is presented in the In Profile section.

Trichoderma species will be discussed in the Bug of the month segment, which are free-living fungi that are highly interactive in root, soil and foliar environments. It has been known for many years that they produce a wide range of antibiotic substances and that they parasitize other fungi. They can also compete with other microorganisms; for nutrients and/or space. Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surfaces.

Did You Know emphasizes on (Enterobacter) sakazakii : an Opportunistic Food Borne Pathogen. It is a gram-negative rod that causes severe illness in human infants, including necrotizing enterocolitis, septicemia, and meningitis.

Best Practices segment will instruct the various Clean-Room Techniques. It is a valuable tool to prevent particulate and bio contamination. A cleanroom is an environment used in manufacturing or scientific research. It has a low level of environmental pollutants such as dust, airborne microbes, aerosol particles and chemical vapors.

JHS team thanks all our readers for their support and contribution in making this journal a success. Feedback and suggestions are always invited.

Standard Methods for the Detection of Antibacterial Resistance

Antimicrobial resistance is a natural-biological phenomenon about response of microbes to the selective pressure of an antimicrobial drug. Resistance can be caused by a variety of mechanisms: (i) the presence of an enzyme that inactivates the antimicrobial agent; (ii) the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent; (iii) mutation in the antimicrobial agent's target, which reduces the binding of the antimicrobial agent; (iv) post-transcriptional or post-translational modification of the antimicrobial agent's target, which reduces binding of the antimicrobial agent; (v) reduced uptake of the antimicrobial agent; (vi) active efflux of the antimicrobial agent through efflux pump; and (vii) overproduction of the target of the antimicrobial agent. In addition, resistance may be caused by a previously unrecognized mechanism. On the other hand, a gene which is not expressed in vitro may be expressed in vivo.

Detection of *Oxacillin/Methicillin-resistant Staphylococcus aureus* (MRSA)

Strains that are oxacillin and methicillin resistant, historically termed methicillin-resistant *S. aureus* (MRSA), are resistant to all beta-lactam agents, including cephalosporins and carbapenems. MRSA isolates often are multiply resistant to commonly used antimicrobial agents, including erythromycin, clindamycin, and tetracycline. Since 1996, reports of MRSA strains with decreased susceptibility to vancomycin (minimum inhibitory concentration [MIC], >8 µg/ml) have been published.

Glycopeptides, vancomycin and Teicoplanin are the only drug of choice for treatment of severe MRSA infections, although some strains remain susceptible to fluoroquinolones, trimethoprim/sulfamethoxazole, gentamicin, or rifampicin. Because of the rapid emergence of rifampicin resistance, this drug should never be used as a single agent to treat MRSA infections.

The National Committee for Clinical Laboratory Standards (NCCLS) has recommended "Screening Test for Oxacillin-resistant *S. aureus*" and uses an agar plate containing 6 microg/ml of oxacillin and Müeller-Hinton agar supplemented with NaCl (4% w/v; 0.68 mol/L). These plates can be stored refrigerated for up to 2 weeks. The inoculum is prepared by matching a 0.5 McFarland tube. Two methods can be followed for inoculation: (1) Dilute the suspension 1:100, and inoculate 10 microL on the plate, to get an inoculum of 10^4 CFU. (2) Dip a swab in the suspension and express excess fluid by pressing swab against the wall of the tube. Streak swab over a 1-1.5 inch area. In both methods, any growth after 24 hours incubation at 35^0C denotes oxacillin resistance, if controls are satisfactory.

Accurate detection of oxacillin/methicillin resistance can be difficult due to the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture. All cells in a culture may carry the genetic information for resistance but only a small number can express the resistance in vitro. This phenomenon is termed heteroresistance and occurs in staphylococci resistant to penicillinase-stable penicillins, such as oxacillin.

Heteroresistance is a problem for clinical laboratory personnel because cells expressing resistance may grow more slowly than the susceptible population. This is why isolates being tested against oxacillin, methicillin, or nafcillin should be incubated at 35^0C for a full 24 hours before reading. The breakpoints for *S. aureus* are different from those for coagulase-negative staphylococci (CoNS).

MICs	Oxacillin Susceptible	Oxacillin Intermediate	Oxacillin Resistant
S. aureus	<2 µg/ml	no intermediate MIC	MIC>4 µg/ml
CoNS	<0.25µg/ml	no intermediate MIC	MIC>0.5 µg/ml

Zone sizes	Oxacillin Susceptible	Oxacillin Intermediate	Oxacillin Resistant
S. aureus	>13 mm	11-12 mm	<10 mm
CoNS	>18 mm	no intermediate zone	<17 mm

When used correctly, broth-based and agar-based tests usually can detect MRSA. Oxacillin screen plates can be used in addition to routine susceptibility test methods or as a back-up method. Amplification tests like those based on the polymerase chain reaction (PCR) detect the *mecA* gene. These tests confirm oxacillin/methicillin resistance caused by *mecA* in *Staphylococcus* species.

Detection of *Oxacillin-resistant Coagulase-negative Staphylococcus sp.*

Although there are about 20 CoNS species, they often are considered to be a single group. Some species are more resistant to commonly used antimicrobial agents than others. Identification to species level can aid in the recognition of outbreaks and in tracking resistance trends. *S. epidermidis* is the most common CoNS isolated in clinical laboratories. Usually, *S. epidermidis*, *S. haemolyticus* and *S. hominis* are more likely to be multiply resistant to antimicrobial agents than are other CoNS species. However, resistance patterns of CoNS may differ between hospitals and wards.

Oxacillin-resistant CoNS isolates are resistant to all beta-lactam agents, including penicillins, cephalosporins, and carbapenems. In addition, oxacillin-resistant CoNS isolates are often resistant to other commonly used antimicrobial agents, so vancomycin is frequently the drug of choice for treatment of clinically significant infections.

Accurate detection of oxacillin resistance can be difficult. Colony sizes of CoNS are often smaller than those of *S. aureus*, making growth more difficult to read. In addition, like *S. aureus*, two subpopulations (one susceptible and the other resistant) may coexist within a culture. When studies were performed to evaluate oxacillin breakpoints for CoNS, the current breakpoints for *S. aureus* failed to detect many CoNS that contained the *mecA* gene. In general, the new breakpoints for CoNS correlate better with *mecA* production for CoNS.

Detection of Extended-Spectrum Beta-Lactamases (ESBLs)

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem). ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. For example, one enzyme may actively hydrolyze ceftazidime, resulting in ceftazidime minimum inhibitory concentrations (MICs) of 256 microg/ml, but have poor activity on cefotaxime, producing MICs of only 4 microg/ml. If an ESBL is detected, all penicillins, cephalosporins, and aztreonam should be reported as resistant, even if in vitro test results indicate susceptibility.

There are standard broth microdilution and disc diffusion screening tests using selected antimicrobial agents. Each *K. pneumoniae*, *K. oxytoca*, or *Escherichia coli* isolate should be considered a potential ESBL-producer if the test results are as follows:

Disk diffusion		MICs	
cefpodoxime	< 22 mm	cefpodoxime	> 2 µg /ml
ceftazidime	< 22 mm	ceftazidime	> 2 µg /ml
aztreonam	< 27 mm	aztreonam	> 2 µg /ml
cefotaxime	< 27 mm	cefotaxime	> 2 µg /ml
ceftriaxone	< 25 mm	ceftriaxone	> 2 µg /ml

The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefpodoxime and ceftazidime show the highest sensitivity for ESBL detection.

Phenotypic confirmation of potential ESBL-producing isolates of *K. pneumoniae*, *K. oxytoca*, or *E. coli* can be done by testing both cefotaxime and ceftazidime, alone and in combination with clavulanic acid. Testing can be performed by the broth microdilution method or by disk diffusion. For MIC testing, a decrease of > 3 doubling dilutions in an MIC for either cefotaxime or ceftazidime tested in combination with 4 µg /ml clavulanic acid, versus its MIC when tested alone, confirms an ESBL-producing organism. For disc diffusion testing, a > 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL-producing organism.

Discs can be made by adding 10 µl of a 1000 µg /ml stock solution of clavulanic acid to cefotaxime and ceftazidime disks each day of testing. In future, commercial manufacturers of antimicrobial discs may produce discs containing cefotaxime and ceftazidime with clavulanic acid. Until commercial discs are available, SmithKline Beecham can provide clinical laboratories with clavulanic acid powder for routine use. *K. pneumoniae* ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) should be used for quality control of ESBL tests. Some organisms with ESBLs contain other beta-lactamases that can mask ESBL production in the phenotypic test, resulting in a false-negative test. These beta-lactamases include AmpCs and inhibitor-resistant TEMs (IRTs). Hyper-production of TEM and/or SHV beta-lactamases in organisms with ESBLs also may cause false-negative phenotypic confirmatory test results. Currently, detection of organisms with multiple beta-lactamases that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing, methods that are not usually available in clinical laboratories.

If an isolate is confirmed as an ESBL-producer by the NCCLS-recommended phenotypic confirmatory test procedure, all penicillins, cephalosporins, and aztreonam should be reported as resistant. This list does not include the cephamycins (cefotetan and cefoxitin), which should be reported according to their routine test results. If an isolate is not confirmed as an ESBL-producer, current recommendations suggest reporting results as for routine testing. Do not change interpretations of penicillins, cephalosporins, and aztreonam for isolates not confirmed as ESBLs. Other isolates of Enterobacteriaceae, such as *Salmonella* species and *Proteus mirabilis*, and isolates of *Pseudomonas aeruginosa* also produce ESBLs. However, at this time, methods for screening and phenotypic confirmatory testing of these isolates have not been determined.

Detection of Imipenem or Meropenem Resistance in Gram-negative Organisms

Within a health care setting, increases in species-specific carbapenem resistance should be monitored and sudden increases investigated to rule out an outbreak of resistant organisms or spurious test results.

Published reports indicate some resistance in a variety of clinical Gram-negative organisms, including *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Acinetobacter* species, *Proteus* species, *Serratia marcescens*, *Enterobacter* species, and *K. pneumoniae*. *Stenotrophomonas maltophilia* isolates are intrinsically resistant to imipenem. Organisms can produce more than one hydrolyzing enzyme and may show modifications in more than one porin, producing high-level resistance to the carbapenems (minimum inhibitory concentration [MIC] >16 µg/ml). Organisms with decreased susceptibility produced by porin changes alone often have lower MICs (2-8 microg/ml).

Organisms with MICs near interpretation breakpoints have greater potential for reporting errors. For example, isolates of *P. aeruginosa* often have MICs that are at or near the carbapenem intermediate (8 µg/ml) and resistant (>16 µg/ml) breakpoints. Some species, such as *P. mirabilis*, *P. vulgaris*, and *Morganella morganii*, often have MICs (1-4 µg/ml) just below the carbapenem intermediate breakpoint of 8 mg/ml. Most other species of Enterobacteriaceae are very susceptible (<0.5 µg/ml).

Broth microdilution methods usually detect carbapenem resistance when the tests are performed properly. However, studies have shown false resistance to imipenem in commercially prepared test panels due to degradation of the drug or to a manufacturing problem where concentrations of imipenem were too low. When performed properly, disc diffusion and agar gradient diffusion also are acceptable methods for carbapenem testing. Imipenem degrades easily. Studies suggest that meropenem may be more stable than imipenem. However, for either antimicrobial agent, storage conditions of susceptibility panels, cards, and discs must be monitored carefully and quality control results checked frequently. If possible, store supplies containing carbapenems at the coldest temperature range stated in the manufacturer's directions. An additional test method, such as agar gradient diffusion (i.e., E test), can be used to verify intermediate or resistant results.

Quinolones and resistance

The number and location of mutations affecting critical sites determine the level of resistance. Organisms may have alterations in more than one enzyme target site and, in gram-negative organisms, may contain more than one porin change. Many resistant organisms have multiple enzyme target site, porin, and efflux mutations, producing high-level resistance to quinolones. In contrast, organisms with decreased susceptibility produced only by porin changes usually have lower minimum inhibitory concentrations (MICs).

The fluoroquinolone susceptibility profile for each clinical isolate is determined by the number and location of mutational changes in specific enzyme target sites, porin proteins, and efflux mechanisms. The effect of each mutation in an isolate is not equivalent for all fluoroquinolones, due to variations of the chemical structures among this class of agents. Therefore, an organism with one or more mutations may have resistant MICs/zone sizes to one quinolone but have intermediate or susceptible MICs/zone sizes to another quinolone. During therapy, the potential exists for an organism with a single mutation to acquire a second mutation, leading to high-level resistance. After multiple mutations occur, an organism is generally highly resistant to all quinolones. Resistance to quinolones has been reported in a variety of important bacterial pathogens, including *E. coli*, *K. pneumoniae* and other enteric organisms; *P. aeruginosa*; *Chlamydia trachomatis*, *Mycoplasma pneumoniae*; *Campylobacter jejuni*, *B. cepacia*; *S. maltophilia*, *N. gonorrhoeae*, *S. aureus* (especially oxacillin-resistant strains), *Enterococcus faecium* and *S. pneumoniae*.

Detection of Resistant Enterococci

Penicillin/Ampicillin Resistance

Enterococci may be resistant to penicillin and ampicillin because of production of low-affinity, penicillin-binding proteins (PBPs) or, less commonly, because of the production of ß-lactamase. The disc diffusion test can accurately detect isolates with altered PBPs, but it will not reliably detect ß-lactamase producing strains. The rare ß-lactamase- producing strains are detected best by using a direct, nitrocefin-based, ß-lactamase test. Certain penicillin- ampicillin-resistant enterococci may possess high-level resistance (i.e., penicillin MICs > 128 µg/ml or ampicillin MICs > 64 µg/ml). The disc test will not differentiate those with normal resistance from this high-level resistance. For enterococci recovered from blood and CSF, the laboratory should consider determining the actual MIC for penicillin or ampicillin since *E. faecium* strains with normal lower level resistance (penicillin MICs < 64 µg/ml and ampicillin < 32 µg/ml) should be considered potentially susceptible to synergy with an aminoglycoside (in the absence of high-level aminoglycoside resistance) whereas strains with higher level resistance may be resistant to such synergy.

Vancomycin Resistance

Accurate detection of vancomycin-resistant enterococci by the disc diffusion test requires that plates be incubated for a full 24 hours (rather

than 16 to 18 hours) and that any zone surrounding the vancomycin disc be examined carefully with transmitted light for evidence of small colonies or a light film growing within the zone. An intermediate category result by the disc diffusion test should be verified by determining the vancomycin MIC.

Clindamycin Resistance

There has been increasing awareness that inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative Staphylococcus species may not be detected by standard tests. Macrolide resistance in staphylococci may be due to ribosomal target modification that affects the activities of both macrolides and clindamycin, called macrolide-lincosamide-streptogramin B (MLSB) resistance, which is encoded by either erm(A) or erm(C). While strains that demonstrate constitutive resistance to clindamycin can normally be detected by standard susceptibility testing methods, inducible resistance (MLSBi) present in some strains is not routinely detected by standard broth- or agar-based susceptibility test methods. It is important to distinguish the MLSBi strains from macrolide-resistant strains that contain the gene msr(A), encoding an efflux pump that affects only macrolides, not clindamycin.

Clindamycin resistance can be easily detected by disk induction testing on standard sheep blood agar plates used for verification of inoculum purity in conjunction with an automated susceptibility test system. It is important to note that clindamycin zone flattening, not zone size, is assessed on the purity plate. Thus, the use of standard sheep blood agar rather than Mueller-Hinton agar suffices for recognition of inducible resistance. Routine detection of clindamycin resistance among clinically significant staphylococcal isolates is twofold. First, prior investigations have demonstrated the potential for clinical failures when patients infected with MLSBi strains are treated with clindamycin for various types of infections. However, to categorically regard all macrolide-resistant staphylococci as clindamycin resistant would deny potentially safe and effective therapy for patients infected with isolates that carry only the macrolide efflux mechanism. The percentage of clinical staphylococcal isolates that demonstrate macrolide efflux compared to MLSB resistance may vary widely by geographic location or patient group. Therefore, the second benefit of routine testing for inducible clindamycin resistance is to clearly identify those strains that remain susceptible to clindamycin despite macrolide resistance. For these reasons, routine testing of significant staphylococcal isolates for inducible clindamycin resistance is now advocated by the National Committee for Clinical Laboratory Standards.

High-level Aminoglycoside Resistance

High-level resistance to aminoglycosides is an indication that an enterococcal isolate will not be affected synergistically by a combination of a penicillin or glycopeptide plus an aminoglycoside. Special, high-content gentamicin (120 µg) and streptomycin (300 µg) discs can be used to screen for this type of resistance. No zone of inhibition indicates resistance, and zones of > 10 mm indicate a lack of high-level resistance. Strains that yield zones of 7 to 9 mm should be examined using a dilution screen test. Other aminoglycosides need not be tested, because their activities against enterococci are not superior to gentamicin or streptomycin.

Aminoglycoside Resistance in Enterobacteriaceae and *Pseudomonas aeruginosa*

Resistance to one aminoglycoside may not predict resistance to the others. In general, resistance is relatively common in *P. aeruginosa* but less common in Enterobacteriaceae. Enterobacteriaceae resistant to gentamicin and tobramycin can be susceptible to amikacin or netilmicin because these drugs are not affected by many of the aminoglycoside modifying enzymes (AMEs). Therefore, the prevalence of amikacin resistance can be lower than the prevalence of resistance to gentamicin and tobramycin, depending upon the resistance mechanisms present at a healthcare facility. Aminoglycosides are not clinically effective against *Salmonella* species and *Shigella* species, although they may appear susceptible, aminoglycosides should not be tested or reported.

MOLECULAR TECHNIQUES USED IN CLINICAL MICROBIOLOGY

Molecular techniques have been used to detect antimicrobial resistance determinants , Hybridization is one of the oldest molecular techniques and is based on the fact that in nucleic acids a cytosine forms base pairs with a guanine and an adenine forms base pairs with either a thymidine (in DNA) or a uracil (in RNA). PCR is well known and involves cycles of heating the sample for denaturing, annealing of the primers, and elongation of the primers by a thermo stable DNA polymerase.

Besides these molecular techniques above, a number of other amplification techniques are used in the clinical microbiology laboratory, although not to detect antibiotic resistance determinants. These techniques include the DNA amplification techniques of strand displacement amplification and ligase chain reaction and the RNA amplification techniques of Q replication and self-sustain sequence replication or nucleic acid-based sequence amplification. This latter method can be modified to amplify DNA. The choice of a particular technique is also dependent on the information required or the targets under consideration, but some techniques are more favored than others. New techniques continue to be developed that involve a new approach to amplification, hybridization, formats, and labels.

Reference: (1) Drinkovic, D., E. R. Fuller, K. P. Shore, D. J. Holland, and R. Ellis-Pegler. 2001. Clindamycin treatment of Staphylococcus aureus expressing inducible clindamycin resistance. J. Antimicrob. Chemother. 48:315-316. (2) Eady, E. A., J. I. Ross, J. L. Tipper, C. E. Walters, J. H. Cove, and W. C. Noble. 1993. Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci. J. Antimicrob. Chemother. 31:211-217. (3) Fiebelkorn, K. R., S. A. Crawford, M. L. McElmeel, and J. H. Jorgensen. 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. J. Clin. Microbiol. 41:4740-4744. (4) Frank, A. L., J. F. Marcinak, P. D. Mahgat, J. T. Tjhio, S. Kelkar, P. C. Schreckenberger, and J. P. Quinn. 2002. Clindamycin treatment of methicillin-resistant Staphylococcus aureus infections in children. Pediatr. Infect. Dis. J. 21:530-534. (5) Hamilton-Miller, J. M. T., and S. Shah. 2000. Patterns of phenotypic resistance to the macrolide-lincosamide-ketolide-streptogramin group of antibiotics in staphylococci. J. Antimicrob. Chemother. 46:941-949. (6) Jenssen, W. D., S. Thakker-Varia, D. T. Dubin, and M. P. Weinstein. 1987. Prevalence of macrolide-lincosamide-streptogramin B resistance and erm gene classes among clinical strains of staphylococci and streptococci. Antimicrob. Agents Chemother. 31:883-888. (7) National Committee for Clinical Laboratory Standards. 2004. Performance standards for antimicrobial susceptibility tests. Supplement M100-S14. National Committee for Clinical Laboratory Standards, Wayne, Pa. (8) Nicola, F. G., L. K. McDougal, J. W. Biddle, and F. C. Tenover. 1998. Characterization of erythromycin-resistant isolates of Staphylococcus aureus recovered in the United States from 1958 through 1969. Antimicrob. Agents Chemother. 42:3024-3027. (9) Sanchez, M. L., K. K. Flint, and R. N. Jones. 1993. Occurrence of macrolide-lincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. Diagn. Microbiol. Infect. Dis. 16:205-213. (10) Siberry, G. K., T. Tekle, K. Carroll, and J. Dick. 2003. Failure of clindamycin treatment of methicillin-resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. Clin. Infect. Dis. 37:1257-1260. (11) John D.T and James H.J Antimicrobial Susceptibility testing: General Considerations. Manual of Clinical Microbiology 7th edition, Murray P.R, Baron E.J, Pfaller M.A, Tenover F.C, Yolken R, American Society for Microbiology, Washington DC, 1999, P. 1469-1473. (12) National Committee for Clinical Laboratory Standards. Performance Standards for antimicrobial susceptibility testing. 8th Informational Supplement. M100 S12. National Committee for Clinical Laboratory Standards, 2002. Villanova, Pa. (13) Lalitha M.K, .Manayani D.J, Priya L, Jesudason M.V , Thomas K, Steinhoff M.C. E test as an alternative to conventional MIC determination for surveillance of drug resistant *S.pneumoniae*. *Indian J. Med Res.* 1997: 106; P. 500-503. (14) A guide to sensitivity testing: Report of working party on antibiotic sensitivity testing of the British Society for Antimicrobial Chemotherapy. *J. Antimicrob Chemothrap* 1991; 27: Supplement D, P. 1-50.

Pros and Cons of Various Instrument Disinfectants and Technologies

In the previous edition we have discussed the importance of Pre-cleaning and Disinfection of Surgical Instruments. In this edition we are going to explore a wide range of Instrument Disinfectants available in the market, and learn about their pros and cons. There are some latest technologies released in the disinfection segment. The feasibility of these new technologies will be discussed at the end.

A measurement for effectiveness is the United States Environmental Protection Agency (EPA) classification which categorizes disinfectants according to level of disinfection. High-level disinfectants kills all organisms, except some high levels of bacterial spores, and is cleared for marketing as a sterilant by the U.S. Food and Drug Administration (FDA). Intermediate-level disinfectants kill mycobacteria, most viruses, and bacteria and are registered as a "tuberculocide" by the EPA. Low-level disinfectants kill some viruses and bacteria and are registered as a hospital disinfectant by the EPA. Sterilization means complete eradication of all the forms of microorganisms along with the spores.

Disinfection and Sterilization Agents

Alcohols

Usually ethanol, propanol and isopropanol, are used as a disinfectant. Longer carbon chains with higher number of branching make alcohols more effective.

PROS

Fast acting.
Leaves no residue.
They are non-corrosive.
Good Compatibility, (combine with other disinfectants (quaternaries, phenolics, and iodine) to give synergistic effect.
Fairly inexpensive.
They also have limited residual activity due to evaporation.

CONS

Flammable, not to be used near a flame or electrical socket / appliance.
Less that 50% solution not very effective.
Not active when organic matter present.
Not active or partly effective against most non-enveloped viruses (such as hepatitis A), and is not effective against fungal and bacterial spores.
Evaporates quickly.
Contact time not sufficient for killing.
Toxic and an eye irritant.

Glutaraldehyde (2-2.45 % solutions)

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.

Activated Glutaraldehyde 2% solution is necessary for efficient disinfection. Since glutaraldehyde polymerizes and deactivates quickly with time, 2.45% solution is necessary to maintain the Minimum Effective Concentration (MEC) for longer duration.

PROS

Effective against wide spectrum of bacteria and viruses.
Sporicidal when used properly (10 hour contact period).
Bactericidal, Fungicidal, Tuberculocidal, Virucidal.
Good activity against vegetative bacteria, spores and viruses.
Non-staining.
Relatively non-corrosive.
Useable as a sterilant on plastics, rubber, lenses, stainless steel and other items that can’t be autoclaved.

CONS

Not stable in the solution, limited stability after activation.
Requires alkaline solution for longer life.
Polymerize with time and lose its activity.
Inactivated by organic material.
Glutaraldehyde fixes cells and proteins on the surface of medical instruments and devices.
Toxic properties (Potent carcinogen).
Throat and lung irritation, asthma, asthma-like symptoms, breathing difficulty, nose irritation, sneezing, and wheezing, nose-bleed, burning eyes and conjunctivitis, rash-contact and/or allergic dermatitis, staining of the hands (brownish or tan) hives, headaches, nausea.
Sensitizer (allergen that causes atopic dermatitis on the second exposure following the initial sensitizing exposure).
Some bacteria have developed resistance to glutaraldehyde.

Ortho-phthalaldehyde (OPA)

PROS

Excellent stability over a wide pH range (pH 3–9).
OPA has superior mycobactericidal activity (5-log10 reduction in 5 minutes) to glutaraldehyde.
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 is effective (>5-log10 reduction) against a wide range of microorganisms, including glutaraldehyde-resistant mycobacteria and *B. atrophaeus* spores.
Does not require exposure monitoring, has a barely perceptible odor, and requires no activation.
Excellent material compatibility.
OPA lasts longer in an automatic endoscope reprocessor before reaching its MEC limit (MEC after 82 cycles) than glutaraldehyde (MEC after 40 cycles).

CONS

Repeated exposure may result in hypersensitivity in some patients with bladder cancer.
More expensive than glutaraldehyde.
Eye irritation with contact.
Slow sporicidal activity, 0.5% OPA is not sporicidal at its natural pH 6.5. Increasing the pH to 8 improves the sporicidal activity of OPA.

Biocidal activity decreases with increasing serum concentration
It stains proteins gray (including unprotected skin) and thus must be handled with caution.
Stains skin, mucous membranes, clothing, and environmental surfaces.
Meticulous cleaning and copious rinsing of the instruments with water prior to store and use is necessary to remove the residue of OPA. As OPA residue stains skin grey.
OPA must be disposed in accordance with local and state regulations. If OPA disposal through the sanitary sewer system is restricted, glycine (25 grams/gallon) can be used to neutralize the OPA and make it safe for disposal.

Hydrogen Peroxide

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 patient's rooms.

PROS
Published reports ascribe good germicidal activity to hydrogen peroxide and attest to its bactericidal, virucidal, sporicidal, and fungicidal properties.
A 0.5% accelerated hydrogen peroxide demonstrated bactericidal and virucidal activity in 1 minute and mycobactericidal and fungicidal activity in 5 minutes.
6% hydrogen peroxide was more effective in the high-level disinfection of flexible endoscopes than was the 2% glutaraldehyde solution.
No activation required.
May enhance removal of organic matter and organisms.
No disposal issues.
No odor or irritation issues.
Does not coagulate blood or fix tissues to surfaces.
Inactivates *Cryptosporidium.*

CONS
Material compatibility concerns (brass, zinc, copper, and nickel/silver plating), but no corrosion effect was observed on medical grade steel (SS304 and higher).

Peracetic Acid

Used to chemically sterilize medical (e.g., endoscopes, arthroscopes), surgical, and dental instruments. The sterilant, 35% peracetic acid, is diluted to 0.2% with water at 50 ºfor effective sterilization.

PROS
Rapid sterilization, cycle time (30-45 minutes).
Environmental friendly by-products (acetic acid, O_2, H_2O).
May enhance removal of organic material and endotoxin.
Does not coagulate blood or fix tissues to surfaces.
Sterilant flows through scope facilitating salt, protein, and microbe removal.
Rapidly sporicidal.

CONS
Potential material incompatibility (e.g., aluminum anodized coating becomes dull), incompatible with many materials and instruments. Peracetic acid can corrode copper, brass, bronze, plain steel, and galvanized iron.
It tarnishes the metal of endoscopes and is unstable, resulting in only a 24-hour use life.
It is considered unstable, particularly when diluted; for example, a 1% solution loses half its strength through hydrolysis in 6 days.
High temperature (50-55ºC) liquid immersion sterilization.
Adverse health effects to operators, serious eye and skin damage with contact.
Used for immersible instruments only.
Biological indicator may not be suitable for routine monitoring.
One scope or a small number of instruments can be processed in a cycle.
More expensive (endoscope repairs, operating costs, purchase costs) than high-level disinfection.
Point-of-use system, no sterile storage.

Peracetic Acid and Hydrogen Peroxide

0.23% peracetic acid plus 7.35% hydrogen peroxide is commercially available. The combination of peracetic acid and hydrogen peroxide has been used for disinfecting hemodialyzers.

PROS
High level disinfection in 15 minutes at 20 ºC.
Sterilization in 3 hours at 20 ºC.
No activation required.
Odor not significant.

CONS
Materials compatibility concerns (lead, brass, copper, zinc) both cosmetic and functional.
Limited clinical experience.
Potential for eye and skin damage.
After testing the 7.35% hydrogen peroxide and 0.23% peracetic acid product, Olympus America concluded it was not compatible with the company's flexible gastrointestinal endoscopes; this conclusion was based on immersion studies where the test insertion tubes had failed because of swelling and loosening of the black polymer layer of the tube (Olympus America, personal communication, September 13, 2000).

Phenol

Many phenolic germicides are EPA-registered as disinfectants for use on environmental surfaces and noncritical medical devices. Phenolics are not FDA-cleared as high-level disinfectants for use with semi-critical items but could be used to pre-clean or decontaminate critical and semi-critical devices before terminal sterilization or high level disinfection.
Two phenol derivatives commonly found as constituents of hospital disinfectants are *ortho*phenylphenol and *ortho*benzyl-*para*chlorophenol.

PROS
They were bactericidal, fungicidal, Virucidal, and Tuberculocidal.

CONS
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.
Manufacturers' data using the standardized AOAC methods demonstrate that commercial phenolics are not sporicidal but are tuberculocidal, fungicidal, virucidal, and bactericidal at their recommended use-dilution.
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.

Quaternary Ammonium Compounds
EPA-registered quaternary ammonium compounds are appropriate to use for disinfecting medical equipment that contacts intact skin. Some of the chemical names of quaternary ammonium compounds used in healthcare are alkyl dimethyl benzyl ammonium chloride, alkyl dodecyl dimethyl ammonium chloride, and dialkyl dimethyl ammonium chloride. The newer quaternary ammonium compounds (i.e., fourth generation) are referred to as twin-chain or dialkyl quaternaries, (e.g. dodecyl dimethyl ammonium bromide and dioctyl dimethyl ammonium bromide).

PROS
The quaternaries are good cleaning agents.
Remain active in hard water and are tolerant of anionic residues.
Quaternaries sold as hospital disinfectants are generally fungicidal, bactericidal, and virucidal against lipophilic (enveloped) viruses.

CONS
Health-care–associated infections have been reported from contaminated quaternary ammonium compounds used to disinfect patient-care supplies or equipment, such as cystoscopes or cardiac catheters.
They are not sporicidal and generally not tuberculocidal or virucidal against hydrophilic (non-enveloped) viruses.
BUT combination of Quaternary ammonium compounds with Polyhexamethylene Biguanide or 70% isopropyl alcohol or phenolic or a chlorine-containing wipe [80 ppm]) effectively (>95%) remove and/or inactivate contaminants (i.e., multidrug-resistant *S. aureus*, vancomycin-resistant *Entercoccus*, *P. aeruginosa*).

Disinfection and Sterilization Technologies

Steam Sterilization
The two basic types of steam sterilizers (autoclaves) are the gravity displacement autoclave and the high-speed prevacuum sterilizer. Steam sterilization should be used whenever possible on all critical and semicritical items that are heat and moisture resistant (e.g., steam sterilizable respiratory therapy and anesthesia equipment), even when not essential to prevent pathogen transmission.

PROS
Moist heat in the form of saturated steam under pressure is the most widely used and the most dependable.
Nontoxic to patient, staff, environment.
Cycle easy to control and monitor.
Rapidly microbicidal.
Least affected by organic/inorganic soils among sterilization processes listed.
Rapid cycle time.
Penetrates medical packing, device lumens.

CONS
Heat labile materials cannot be autoclaved.
Steam sterilization has deleterious effects on some materials, including corrosion and combustion of lubricants associated with dental handpieces; reduction in ability to transmit light associated with laryngoscopes; and increased hardening time (5.6 fold) with plaster-cast.
Microsurgical instruments damaged by repeated exposure.
May leave instruments wet, causing them to rust.
Potential for burns.

Flash Sterilization
Sterilization of an unwrapped object at 132ºfor 3 minutes at 27-28 lbs of pressure in a gravity displacement sterilizer. Flash sterilization is a modification of conventional steam sterilization in which the flashed item is placed in an open tray or is placed in a specially designed, covered, rigid container to allow for rapid penetration of steam.

PROS
Machine could be placed for flash sterilization in close proximity to operating rooms to facilitate aseptic delivery to the point of use.
Flash sterilization is considered acceptable for processing cleaned patient-care items that cannot be packaged, sterilized, and stored before use.

CONS
When sterile items are open to air, they will eventually become contaminated. Thus, the longer a sterile item is exposed to air, the greater the number of microorganisms that will settle on it.
Therefore some rigid, reusable sterilization container systems is designed and validated by the container manufacturer for use with flash cycles.
There is a need to develop policies and educate staff to prevent the use of instruments hot enough to cause clinical burns.
Because of the potential for serious infections, flash sterilization is not recommended for implantable devices (i.e., devices placed into a surgically or naturally formed cavity of the human body).

Low-Temperature Sterilization Technologies

Ethylene Oxide (ETO) "Gas" Sterilization
It has been the most commonly used process for sterilizing temperature- and moisture-sensitive medical devices and supplies in healthcare institutions.
Two types of ETO sterilizers are available, mixed gas and 100% ETO. Until 1995, ethylene oxide sterilizers combined ETO with a chlorofluorocarbon (CFC) stabilizing agent, most commonly in a ratio of 12% ETO mixed with 88% CFC (referred to as 12/88 ETO). CFCs were phased out in December 1995 under provisions of the Clean Air Act. CFCs were classified as a Class I substance under the Clean Air Act because of scientific evidence linking them to destruction of the earth's ozone layer.

PROS
It can sterilize heat- or moisture-sensitive medical equipment without deleterious effects on the material used in the medical devices.
ETO inactivates all microorganisms, although bacterial spores (especially *B. atrophaeus*) are more resistant than other microorganisms and is the recommended biological indicator.

CONS
ETO is a colorless gas that is flammable and explosive.
Lengthy disinfection cycle time.
Very expensive disinfection setup.
ETO is absorbed by many materials, so following sterilization the item must undergo aeration to remove residual ETO.
Acute exposure to ETO may result in irritation (e.g., to skin, eyes, gastrointestinal or respiratory tracts) and central nervous system depression.
The effectiveness of ETO sterilization can be altered by lumen

length, lumen diameter, inorganic salts, and organic materials. Several studies have shown failure of ETO in inactivating contaminating spores in endoscope channels or lumen residual ETO levels averaging 66.2 ppm even after the standard degassing time.
Failure of ETO also has been observed when dental handpieces were contaminated with *Streptococcus mutans* exposed to ETO.
Chronic inhalation has been linked to the formation of cataracts, cognitive impairment, neurologic dysfunction, and disabling polyneuropathies.
Occupational exposure in healthcare facilities has been linked to hematologic changes and an increased risk of spontaneous abortions and various cancers. ETO is considered as a human carcinogen.

Hydrogen Peroxide Gas Plasma

PROS
Safe for the environment .
The by-products of the cycle (e.g., water vapor, oxygen) are nontoxic and eliminate the need for aeration.
The sterilized materials can be handled safely, either for immediate use or storage.
The process operates in the range of 37-44 °C and has a cycle time of 28-75 minutes.
Better than liquid state disinfectant as plasma can reach and disinfect all the surfaces easily.
This process has the ability to inactivate a broad range of microorganisms, including resistant bacterial spores.
Materials and devices that cannot tolerate high temperatures and humidity, such as some plastics, electrical devices, and corrosion-susceptible metal alloys, can be sterilized by hydrogen peroxide gas plasma.
This method has been compatible with most (>95%) medical devices and materials tested.
Simple to operate, install (208 V outlet), and monitor.
Only requires electrical outlet.

CONS
If any moisture is present on the objects the vacuum will not be achieved and the cycle aborts.
Effectiveness can be altered by lumen length, lumen diameter, inorganic salts, and organic materials.
Cellulose (paper), linens and liquids cannot be processed.
Sterilization chamber size from 1.8-9.4 ft3 total volume (varies with model type).
Requires synthetic packaging (polypropylene wraps, polyolefin pouches) and special container tray.
Hydrogen peroxide may be toxic at levels greater than 1 ppm TWA.

Peracetic Acid Sterilization
This automated machine is used to chemically sterilize medical (e.g., GI endoscopes) and surgical (e.g., flexible endoscopes) instruments. Lumened endoscopes must be connected to an appropriate channel connector to ensure that the sterilant has direct contact with the contaminated lumen.

PROS
Peracetic acid is a highly biocidal oxidizer that maintains its efficacy in the presence of organic soil.
Peracetic acid removes surface contaminants (primarily protein) on endoscopic tubing.
Peracetic acid will inactivate gram-positive and gram-negative bacteria, fungi, and yeasts in <5 minutes at <100 ppm. In the presence of organic matter, 200-500 ppm is required.
Bacterial spores in suspension are inactivated in 15 seconds to 30 minutes with 500 to 10,000 ppm (0.05 to 1%).

CONS
Low-level bacterial contamination may follow the use of filtered water in an AER to dilute Peracetic acid.
Used for immersible instruments only.
The system can only sterilize surfaces that can be contacted by the sterilant.
Bronchoscopy-related infections occurred when bronchoscopes were processed using the wrong connector.
Use of peracetic acid vapor, was removed from the marketplace because of reports of corneal destruction to patients when ophthalmic surgery instruments had been processed in the sterilizer.
Point-of-use system, no sterile storage.
Biological indicator may not be suitable for routine monitoring.
Some material incompatibility (e.g., aluminum anodized coating becomes dull).
One scope or a small number of instruments processed in a cycle.
Potential for serious eye and skin damage (concentrated solution) with contact.

References:
(1) Garner JS, Favero MS. CDC Guideline for handwashing and hospital environmental control, 1985. Infect. Control 1986;7:231-43. (2) Uttley AH, Simpson RA. Audit of bronchoscope disinfection: a survey of procedures in England and Wales and incidents of mycobacterial contamination. J. Hosp. Infect. 1994;26:301-8. (3) Weber DJ, Rutala WA. Lessons from outbreaks associated with bronchoscopy. Infect. Control Hosp. Epidemiol. 2001;22:403-8. (4) Weber DJ, Rutala WA, DiMarino AJ, Jr. The prevention of infection following gastrointestinal endoscopy: the importance of prophylaxis and reprocessing. In: DiMarino AJ, Jr, Benjamin SB, eds. Gastrointestinal diseases: an endoscopic approach. Thorofare, NJ: Slack Inc., 2002:87-106. (5) Spaulding EH. Chemical disinfection of medical and surgical materials. In: Lawrence C, Block SS, eds. Disinfection, sterilization, and preservation. Philadelphia: Lea & Febiger, 1968:517-31. (6) Terleckyj B, Axler DA. Quantitative neutralization assay of fungicidal activity of disinfectants. Antimicrob. Agents Chemother. 1987;31:794-8. (7) Rutala WA, Cole EC. Ineffectiveness of hospital disinfectants against bacteria: a collaborative study. Infect. Control 1987;8:501-6. (8) Sagripanti JL, Eklund CA, Trost PA, et al. Comparative sensitivity of 13 species of pathogenic bacteria to seven chemical germicides. Am. J. Infect. Control 1997;25:335-9. (9) Sykes G. Disinfection and sterilization. London: E & FN Spon Ltd, 1965. (10) Petrocci AN. Surface active agents: quaternary ammonium compounds. In: Block SS, ed. Disinfection, sterilization, and preservation. Philadelphia: Lea & Febiger, 1983:309-29.Rutala WA, Weber DJ. New disinfection and sterilization methods. Emerg. Inf. Dis. 2001;7:348-53. (11) Schneider PM. New technologies for disinfection and sterilization. In: Rutala WA, ed. Disinfection, sterilization and antisepsis: Principles, practices, challenges, and new research. Washington DC: Association for Professionals in Infection Control and Epidemiology, 2004:127-39. (12) Bar W, Marquez de Bar G, Naumann A, Rusch-Gerdes S. Contamination of bronchoscopes with Mycobacterium tuberculosis and successful sterilization by low-temperature hydrogen peroxide plasma sterilization. Am. J. Infect. Control 2001;29:306-11. (13) Centers for Disease Control and Prevention. Corneal decompensation after intraocular ophthalmic surgery-Missouri, 1998. MMWR 1998;47:306-9.

Axel Hugo Theodor Theorell

Born: 6 July 1903, Linköping, Sweden
Died: 15 August 1982, Stockholm, Sweden
Affiliation at the time of the award: Karolinska Institutet, Nobel Medical Institute, Stockholm, Sweden
Prize motivation: "for his discoveries concerning the nature and mode of action of oxidation enzymes"

Axel Hugo Theodor Theorell was born at Linköping, Sweden, on July 6, 1903. He was the son of Thure Theorell, surgeon-major to the First Life Grenadiers practicing medicine in Linköping, and his wife Armida Bill.

Theorell was educated for nine years at a State Secondary School in Linköping and passed his matriculation examination there on May 23, 1921. In September, 1921, he began to study medicine at the Karolinska Institute and in 1924 he graduated as a Bachelor of Medicine. He then spent three months studying bacteriology at the Pasteur Institute in Paris under Professor Calmette.

In 1930 he obtained his M.D. degree with a thesis on the lipids of the blood plasma, and was appointed lecturer in physiological chemistry at the Karolinska Institute.

Since 1924, however, Theorell had been on the Staff of the Medico-Chemical Institution, first as an associate assistant and during the years 1928-1929 as a temporary Associate Professor. Here, under Professor Einar Hammarsten, he carried out his first work on the influence of the lipids on the sedimentation of the blood corpuscles. In 1931 he studied in Svedberg's institute at Uppsala University, the molecular weight of myoglobin with the aid of the ultracentrifuge.

In 1932 he was appointed Associate Professor in Medical and Physiological Chemistry at Uppsala University, and here he continued and extended his work on myoglobin. From 1933 until 1935 Theorell held a Rockefeller Fellowship and worked with Otto Warburg at Berlin-Dahlem, and here he became interested in oxidation enzymes, a subject to which he has given his attention ever since. At Berlin-Dahlem he produced, for the first time, the oxidation enzyme called «the yellow ferment» and he succeeded in splitting it reversibly into a coenzyme part, which was found to be flavinmononucleotide, and a colourless protein part.

Returning to Sweden in 1935, Theorell worked at the Karolinska Institute and in 1936 he was appointed Head of the newly established Biochemical Department of the Nobel Medical Institute, which was opened in 1937. For ten years this Institute was housed in the Karolinska Institute, but in 1947 it was able to occupy its own building.

Since 1935, Theorell has, with Swedish and other collaborators, carried out researches on various oxidation enzymes, and he has contributed especially to our knowledge of cytochrome *c*, peroxidases, catalases, flavoproteins, and «pyridine»-proteins, particularly the alcohol dehydrogenases. For his work on the nature and effects of oxidation enzymes he was awarded the Nobel Prize for Physiology or Medicine for 1955.

Theorell was a member of learned societies in Sweden, Denmark, Norway, Finland, the U.S.A., France, Italy, Poland, Belgium and India. He was Chairman of the Swedish Medical Society in 1947-1948 and 1957-1958, and served as Secretary of that Society during 1940-1946, he was a member of the Swedish Society for Medical Research during 1942-1950, the State Research Council for the Natural Sciences during 1950-1954, and the State Medical Research Council as from 1958.

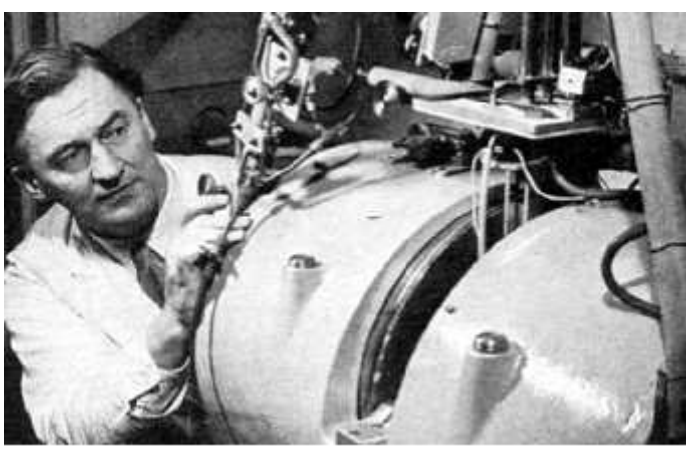
He was also Chairman of the Association of Swedish Chemists from 1947-1949. Since 1954 he has been Chief Editor of the journal *Nordisk Medicin*. He has been a member of many Government Committees and is Chairman of the Swedish National Committee for Biochemistry, of the Board of the Wenner-Gren Society and of the Wenner-Gren Center Foundation.

His interest in music is shown by the facts that he is also a Member of the Swedish Royal Academy of Music and Chairman of the Stockholm Symphony Society.

Theorell holds honorary doctorates of the Universities of Paris, Pennsylvania, Louvain, Brussels and Rio de Janeiro, and is a Foreign Member of the Royal Society of London, and the National Academy of Sciences of Washington.

In 1931 he married Elin Margit Elisabeth Alenius. They had one daughter, Eva Kristina, who died in 1935, end three sons: Klas Thure Gabriel (b. 1935), Henning Hugo (b. 1939), and Per Gunnar Töres (b. 1942).

Hugo Theorell died on August 15, 1982.



Nobel Laureate Hugo Theorell with the "home-made" magnetic weighing machine that was constructed at Karolinska Institutet.

Enjoy the humour

Chemical Formula Of Water

Teacher: What is chemical formula of Water??

Student: H I J K L M N O

Teacher: What the hell you are telling me????

Student: Yesterday you told it is H to O.

This Is My Father

The telephone rings in the principal's office at a school.

"Hello, this is Dunn Elementary," answers the principal.

"Hi. Jimmy won't be able to come to school all next week," replies the voice.

"Well, what seems to be the problem with him?"

"We are all going on a family vacation," says the voice, "I hope it is all right."

"I guess that would be fine," says the principal. "May I ask who is calling?"

"Sure. This is my father!"

Worms & Whiskey

A professor of chemistry wanted to teach his 5th grade class a lesson about the evils of liquor, so he produced an experiment that involved a glass of water, a glass of whiskey and two worms.

"Now, class. Observe closely the worms," said the professor putting a worm first into the water. The worm in the water writhed about, happy as a worm in water could be.

The second worm, he put into the whiskey. It writhed painfully, and quickly sank to the bottom, dead as a doornail.

"Now, what lesson can we derive from this experiment?" the professor asked.

Johnny, who naturally sits in back, raised his hand and wisely, responded, "Drink whiskey and you won't get worms."

Sun vs Moon

Teacher : Which is more important to us, the sun or the moon?

Pupil: Moon...

Teacher : Why?

Pupil : The moon gives us light at night when we need it but the sun gives us light only in the day time when we don't need it.

Quotes from Legends

- The essence of life is statistical improbability on a colossal scale.
  - *Richard Dawkins (English biologist,1941-)in The Blind Watchmaker (1986)*

- Trying to determine the structure of a protein by UV spectroscopy was like trying to determine the structure of a piano by listening to the sound it made while being dropped down a flight of stairs.
  - *Francis Crick [British molecular biologist]*

- A curious aspect of the theory of evolution is that everybody thinks he understands it.
  - *Jacques Monod (1910-1979)_On the Molecular Theory of Evolution_ (1974) (French Biochemist, Nobel Prize Medicine 1965)*

- They are in you and me; they created us, body and mind; and their preservation is the ultimate rationale for our existence ... they go by the name of genes, and we are their survival machines.
  - *Richard Dawkins (English biologist,1941-)in The Selfish Gene (1976)*

- Teleology is a lady without whom no biologist can live. Yet he is ashamed to show himself with her in public.
  - *Ernst Wilhem von Brücke (German physiologist, 1819-1892)*

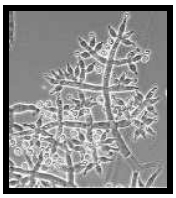
JUST FACTS

What's A Google ?

When we hear the word Google the first image that comes to mind is the famous internet search engine but does the word actually mean anything? Well the answer is a firm yes because it comes from the term "Googol" which represents a number (written as a 1 followed by 100 zeros).

As you might imagine the number is rarely used except maybe in theoretical and computational calculations, however the big question is whether a number bigger than a Googol exists? Well funnily enough there is and it's called a googolplex which would be a number 1 followed by googol zeroes. This number is so large that it's almost impossible to imagine and also impossible to write.

Lastly for those of you that are interested in where this term actually came from, well it was popularized in the 1940's by the american mathematician Edward Kasner who created it as a useful number when comparing unimaginably large numbers with infinity. The actual term "googol" was coined by his then 9 year old nephew Milton Sirotta, not bad for a 9 year old!



Trichoderma species

Trichoderma spp. are free-living fungi that are highly interactive in root, soil and foliar environments. It has been known for many years that they produce a wide range of antibiotic substances and that they parasitize other fungi. They can also compete with other microorganisms; for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil and, more generally, compete with soil microorganisms for nutrients and/or space. Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surfaces.

These direct effects on other fungi are complex and remarkable and, until recently, were considered to be the bases for how *Trichoderma* spp. exert beneficial effects on plant growth and development. Research on these topics has generated a large body of knowledge, including the isolation and cloning of a range of genes that encode proteins of which some have antimicrobial activity. In addition to the ability of *Trichoderma* spp. to attack or inhibit the growth of plant pathogens directly, recent discoveries indicate that they can also induce systemic and localized resistance to a variety of plant pathogens. Moreover, certain strains also have substantial influence on plant growth and development. Their enhancement of plant growth has been known for many years and can occur in both AXENIC systems and natural field soils. These new findings are dramatically changing our knowledge of the mechanisms of action and uses of these fungi. We now consider that the direct effects of these fungi on plant growth and development are crucially important for agricultural uses and for understanding the roles of *Trichoderma* in natural and managed ecosystems. Recent research has also generated data regarding the direct interactions of *Trichoderma* spp. with other microorganisms, including several molecular studies of the roles of specific genes, but these data are outside the scope of this paper.

Trichoderma cultures are typically fast growing at 25-30°C, but will not grow at 35°C. Colonies are transparent at first on media such as cornmeal dextrose agar (CMD) or white on richer media such as potato dextrose agar (PDA). Mycelium are not typically obvious on CMD, conidia typically form within one week in compact or loose tufts in shades of green or yellow or less frequently white. A yellow pigment may be secreted into the agar, especially on PDA. Some species produce a characteristic sweet or 'coconut' odor.

Conidiophores are highly branched and thus difficult to define or measure, loosely or compactly tufted, often formed in distinct concentric rings or borne along the scant aerial hyphae. Main branches of the conidiophores produce lateral side branches that may be paired or not, the longest branches distant from the tip and often phialides arising directly from the main axis near the tip. The branches may rebranch, with the secondary branches often paired and longest secondary branches being closest to the main axis. All primary and secondary branches arise at or near 90° with respect to the main axis.

The mechanism of antibiosis was demonstrated in several studies. An antibiotic, gliovirin, from *Trichoderma virens* demonstrated strong inhibition of *Pythium ultimum* and the *Phytophthora* species. More recent research indicated that certain strains of *Trichoderma* can induce systemic and localized resistance to several plant pathogens. Plants treated with *Trichoderma* in the root zone can produce higher levels of peroxidase, chitinase activity, deposition of callose-enriched wall appositions on the inner surface of cell walls and pathogenesis-related proteins. Moreover, some strains may enhance plant growth and development. These phenomena was observed by several researchers who treated plants with *T. harzianum* resulting in large increases in root area and cumulative root length, as well as significant increases in dry weight, shoot length, and leaf area over that of the untreated control. Due to effective control of plant diseases, several commercial biological products based on *Trichoderma* species are manufactured and marketed in Asia, Europe and USA for use on a wide range of crops. These can be efficiently used as conidia, mycelium and chlamydospores which are produced in either solid state or liquid fermentation.

Trichoderma spp. parasitize a range of other fungi. The events leading to mycoparasitism are complex, and take place as follows: first, *Trichoderma* strains detect other fungi and grow tropically towards them; remote sensing is at least partially due to the sequential expression of cell-wall-degrading enzymes.

Different strains can follow different patterns of induction, but the fungi apparently always produce low levels of an extracellular exochitinase. Diffusion of this enzyme catalyses the release of cell-wall oligomers from target fungi, and this in turn induces the expression of fungitoxic endochitinases, which also diffuse and begin the attack on the target fungus before contact is actually made. Once the fungi come into contact, *Trichoderma* spp. attach to the host and can coil around it and form APPRESSORIA on the host surface. Attachment is mediated by the binding of carbohydrates in the *Trichoderma* cell wall to lectins on the target fungus. Once in contact, the *Trichoderma* produce several fungitoxic cell-wall-degrading enzymes, and probably also peptaibol antibiotics. The combined activities of these compounds result in parasitism of the target fungus and dissolution of the cell walls. At the sites of the appressoria, holes can be produced in the target fungus, and direct entry of *Trichoderma* hyphae into the lumen of the target fungus occurs. There are at least known genes, proteins and other metabolites that are directly involved in this interaction, which is typical of the complex systems that are used by these fungi in their interactions with other organisms.

Most *Trichoderma* strains have no sexual stage but instead produce only asexual spores. However, for a few strains the sexual stage is known, but not among strains that have usually been considered for biocontrol purposes. The sexual stage, when found, is within the Ascomycetes in the genus Hypocrea. Traditional taxonomy was based upon differences in morphology, primarily of the asexual sporulation apparatus, but more molecular approaches are now being used. Consequently, the taxa recently have gone from nine to at least thirty-three species.

Most strains are highly adapted to an asexual life cycle. In the absence of meiosis, chromosome plasticity is the norm, and different strains have different numbers and sizes of chromosomes. Most cells have numerous nuclei, with some

vegetative cells possessing more than 100. Various asexual genetic factors, such as parasexual recombination, mutation and other processes contribute to variation between nuclei in a single organism (thallus). Thus, the fungi are highly adaptable and evolve rapidly. There is great diversity in the genotype and phenotype of wild strains.

While wild strains are highly adaptable and may be heterokaryotic (contain nuclei of dissimilar genotype within a single organism) (and hence highly variable), strains used for biocontrol in commercial agriculture are, or should be, homokaryotic (nuclei are all genetically similar or identical). This, coupled with tight control of variation through genetic drift, allows these commercial strains to be genetically distinct and nonvariable. This is an extremely important quality control item for any company wishing to commercialize these organisms.

Uses of Trichoderma

These versatile fungi are used commercially in a variety of ways, including the following:

Foods and textiles:

Trichoderma spp. are highly efficient producers of many extracellular enzymes. They are used commercially for production of cellulases and other enzymes that degrade complex polysaccharides. They are frequently used in the food and textile industries for these purposes. For example, cellulases from these fungi are used in "biostoning" of denim fabrics to give rise to the soft, whitened fabric--stone-washed denim. The enzymes are also used in poultry feed to increase the digestibility of hemicelluloses from barley or other crops.

Biocontrol agents:

As noted, these fungi are used, with or without legal registration, for the control of plant diseases. There are several reputable companies that manufacture government registered products. Some of these companies are listed at the end of this web page. This site will not knowingly list any nonregistered products or strains offered for sale in commercial agriculture even though these products are common and their sale is widely ignored by governmental regulatory agencies.

Plant growth promotion:

For many years, the ability of these fungi to increase the rate of plant growth and development, including, especially, their ability to cause the production of more robust roots has been known. The mechanisms for these abilities are only just now becoming known.

Some of these abilities are likely to be quite profound. Recently, we have found that one strain increases the numbers of even deep roots (at as much as a meter below the soil surface). These deep roots cause crops, such as corn, and ornamental plants, such as turfgrass, to become more resistant to drought.

Perhaps even more importantly, our recent research indicates that corn whose roots are colonized by Trichoderma strain T-22 require about 40% less nitrogen fertilizer than corn whose roots lack the fungus. Since nitrogen fertilizer use is likely to be curtailed by federal mandate to minimize damage to estuaries and other oceanic environment (there are a number of other sites on the web dealing with this topic, search for sites dealing with the 'dead zone') the use of this organism may provide a method for farmers to retain high agricultural productivity while still meeting new regulations likely to be imposed.

As a source of transgenes.

Biocontrol microbes, almost by definition, must contain a large number of genes that encode products that permit biocontrol to occur. Several genes have been cloned from *Trichoderma* spp. that offer great promise as transgenes to produce crops that are resistant to plant diseases. No such genes are yet commercially available, but a number are in development. These genes, which are contained in *Trichoderma* spp. and many other beneficial microbes, are the basis for much of "natural" organic crop protection and production.

Reference:

(1) Altomare, C., Norvell, W. A., Björkman, T., and Harman, G. E. 1999. Solubilization of phosphates and micronutrients by the plant-growth promoting and biocontrol fungus Trichoderma harzianum Rifai 1295-22. Appl. Env. Microbiol. 65:2926-2933. (2) Bolar, J. P. *et al.* 2000. Expression of endochitinase from *Trichoderma harzianum* in transgenic apple increases resistance to apple scab and reduces vigor. *Phytopathology* **90**, 72–77. (3) Brunner, K. *et al.* 2003. The Nag1 *N*-acetylglucosaminidase of *Trichoderma atroviride* is essential for chitinase induction by chitin and of major relevance to biocontrol. *Curr. Genet.* **43**, 289–295. (4) Chet, I., Harman, G. E. & Baker, R. 1981. *Trichoderma hamatum*: its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microb. Ecol.* **7**, 29–38. (5) Elad, Y., and Kapat, A. 1999. The role of Trichoderma harzianum protease in the biocontrol of Botrytis cinerea. Eur. J. Plant Pathol. 105:177-189. (6) Harman, G. E. & Kubicek, C. P. Trichoderma *and* Gliocladium Vol. 2 (Taylor and Francis, London, 1998). Together with reference 87, this reference presents much of what was known about *Trichoderma* in 1998. (7) Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., Lorito, M. 2004. Trichoderma species opportunistic, avirulent plant symbionts. Nat Review Microbiol. 2 (1): 43-56. (8) Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The History and evolution of current concepts. Plant Dis.: 87, 4-10. (9) Howell, C.R., and Stipanovic, R. D. 1995. Mechanism in the biocontrol of *Rhizoctonia solani* induced cotton seedling disease by *Gliocladium virens*: Antibiosis. Phytopathology 85:469-472. (10) Howell, C. R., Hanson, L. E., Stipanovic, R. D., and Puckhaber, L. S. 2000. Induction of terpenoid synthesis in cotton roots and control of Rhizoctonia solani by seed treatment with Trichoderma virens. Phytopathology 90:248-252. (11) Inbar, J., Menendez, A. & Chet, I. 1996. Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. *Soil Biol. Biochem.* **28**, 757–763. (12) Kubicek, C. P. & Harman, G. E. 1998. Trichoderma *and* Gliocladium Vol. 1. (Taylor and Francis, London. (13) Schirmböck, M. *et al.* 1994. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *Appl. Environ. Microbiol.* **60**, 4364–4370. (14) Viterbo, A. *et al.* 2002. Expression regulation of the endochitinase chit36 from *Trichoderma asperellum* (*T. harzianum* T-203). *Curr. Genet.* **42**, 114–122. (15) Yedidia, I., Benhamou, N., and Chet, I. 1999. Induction of defense responses in cucumber plants (Cucumis sativus L.) by the biocontrol agent Trichoderma harzianum. Appl. Environ. Microbiol. 65:1061-1070. (16) Zeilinger, S. *et al.* 1999. Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. *Fungal Genet. Biol.* **26**, 131–140.

Sakazakii: an Opportunistic Food Borne Pathogen

(Enterobacter) sakazakii is a gram-negative rod that causes severe illness in human infants, including necrotizing enterocolitis, septicemia, and meningitis. In 2008 the genus Cronobacter was proposed to accommodate (E). sakazakii, which is referred to here as (E). sakazakii gen. nov. until formal acceptance of the new genus (Iversen et. al., 2008). Advances in supportive care have decreased the mortality rates of infections caused by (E). sakazakii, but survivors of meningitis face severe neurological sequelae. Most cases occur in infants less than 28 days old, and premature or lowbirth-weight infants are especially susceptible, probably due to impaired immune response compared to full-term infants and adults. Adults have been infected with (E). sakazakii, but no cases of adult meningitis have been reported, and nearly all adults infected had underlying disease such as cancer. Antibiotic therapy against (E). sakazakii is effective, although recent evidence suggests that antibiotic resistance may be increasing.

(E). sakazakii largely an opportunistic pathogen, infecting primarily infants and, occasionally, immunocompromised or elderly patients. Infants, defined as children less than 1 year of age, and especially infants less than 28 days old, are the primary victims of (E). sakazakii (FAO/WHO, 2004). The well-documented outbreaks have occurred in hospital settings, especially neonatal intensive care units. However, the Centers for Disease Control and Prevention (CDC) has documented cases of (E). sakazakii in infants at home. In infants, there are three main classes of illness associated with (E). sakazakii: meningitis, an inflammation of the membranes surrounding the brain, bacteremia or the more serious sepsis, and necrotizing enterocolitis (NEC).

Contaminated commercial infant formula powders have been implicated in several outbreaks and are suspected to be the main vehicle for (E). sakazakii . In a study of powdered infant formula around the world, 14% of samples were positive for (E). sakazakii, although none of the samples had more than 1 CFU/g. Controversy exists regarding the potential for (E). sakazakii survive infant formula (IF) powder processing, but the ability of stationary-phase cells of (E). sakazakii survive osmotic stress and drying for extended periods of time has been documented. Although (E). sakazakii been isolated from a wide variety of sources, including foodmanufacturing plants, indicating that it is widespread, the natural reservoir for (E). sakazakii unknown. The ability to adhere to surfaces, including rubber, silicon, polycarbonate, and stainless steel, may explain the persistence of (E). sakazakii infant formula preparation equipment and in food-manufacturing environments. Traditionally, identification of (E). sakazakii several steps, taking up to 7 days for positive identification. Recently, selective differential media have been developed, shortening the process by several days. Conventional and real-time PCR (polymerase chain reaction) methods have also been developed but do not yet have official approval. Isolation and detection techniques will continue to improve as more information becomes available. The full genome has been sequenced (4.36 Mb).

Among infants infected with (E). sakazakii, most were less than 28 days old, about half weighed less than 2000 g, and two-thirds were born at less than 37 weeks' gestation, making them premature (FAO/WHO, 2004). Several factors probably contribute to the high susceptibility of such infants to (E). sakazakii. First, the immune system of preterm infants is deficient in several respects compared to full-term infants and adults. Polymorphonuclear leukocytes of preterm infants show impaired antibacterial response to lipopolysaccharide from *E. coli* (Henneke et al., 2003). Expression of interleukin-8 is also lower in preterm infant monocytes than in full-term infant and adult monocytes, which reduces the chemotactic response of neutrophils to the site of infection (Schibler et al., 1993). Finally, low levels of IgG antibodies, low levels of complement, proteins that signal antibody-mediated destruction of bacterial pathogens, and exhaustion of neutrophil storage pools, which phagocytize bacterial pathogens, also increase the susceptibility of preterm infants (Haeney, 1994).

(E). sakazakii adheres to latex, silicon, polycarbonate, and to a lesser extent, stainless steel when grown in IF (Iversen et al., 2004b). One study demonstrated that an encapsulated strain of (E). sakazakii produced biofilms of a higher cell density than those produced by a nonencapsulated strain (Iversen et al., 2004b).

NOVEL PREVENTION STRATEGIES

A number of novel prevention strategies have been suggested for both specific anti-(E). sakazakii activity and for improving the safety of IF products. Naturally occurring fatty acids or their monoglycerides can inactivate a wide variety of bacterial pathogens, including Chlamydia trachomatis (Bergsson et al., 1998), Neisseria gonorrhoeae (Bergsson et al., 1999), Helicobacter pylori (Petschow et al., 1996), Haemophilus influenza and group B streptococci (Isaacs et al., 1995), Listeria monocytogenes and E. coli (Nair et al., 2004), and even some viral pathogens such as respiratory syncitial virus (Isaacs et al., 1995). Nair et al. (2004) found that (E). sakazakii in reconstituted IF can be reduced by greater than 5 log after 24 h of incubation at 4 or 8^{0}C in the presence of 25 and 50 mM monocaprylin. When held at higher temperatures, such as 37^{0}C, 25 and 50 mM monocaprylin reduce (E). sakazakii by 6 and 4 log, respectively. An alternative to addition of free fatty acids or monoglycerides to IF is the addition of lipase to IF. Isaacs et al. (1992) found that addition of lipase to IF releases antibacterial and antiviral fatty acids. However, the effect of addition of lipase to IF against (E). sakazakii has not been evaluated, and rancid flavors may lead infants to reject IF treated with lipases. In light of the severity of disease associated with (E). sakazakii, the use of such novel intervention strategies should be reconsidered.

References:

(1) Henneke P, Osmers I, Bauer K, Lamping N, Versmold HT, Schumann RR (2003): Impaired CD14-dependent and independent response of polymorphonuclear leukocytes in preterm infants. J Perinat Med. 31:176–183. (2) Schibler KR, Trautman MS, Liechty KW, White WL, Rothstein G, Christensen RD (1993): Diminished transcription of interleukin-8 by monocytes from preterm neonates. J Leukoc Biol. 53:399–403. (3) Haeney M (1994): Infection determinants at extremes of age. J Antimicrob Chemother. 34 (Suppl A): 1–9. (4) Iversen C, Forsythe SJ (2003): Risk profile of Enterobacter sakazakii, an emergent pathogen associated with infant milk formula. Trends Food Sci Technol. 11:443–454. (5) Nair MK, Joy J, Venkitanarayanan KS (2004): Inactivation of Enterobacter sakazakii in reconstituted infant formula by monocaprylin. J Food Prot. 67:2815–2189. (6) Isaacs CE, Litov RE, Marie P, Thormar H (1992): Addition of lipases to infant formulas produces antiviral and antibacterial activity. J Nutr Biochem. 3:304–308. (7) Bergsson G, Arnfinnsson J, Karlsson SM, Steingrimsson O, Thormar H (1998): In vitro inactivation of Chlamydia trachomatis by fatty acids and monoglycerides. Antimicrob Agents Chemother. 42:2290–2294.

Clean-Room Techniques

First cleanrooms were in hospitals to prevent disease transmission and infection in operating rooms (over 100 years ago). It is a valuable tool to prevent particulate and bio contamination.

A cleanroom is an environment used in manufacturing or scientific research. It has a low level of environmental pollutants such as dust, airborne microbes, aerosol particles and chemical vapors. More accurately, a cleanroom has a controlled level of contamination that is specified by the number of particles per cubic meter at a specified particle size. To give perspective, the ambient air outside in a typical urban environment contains 35,000,000 particles per cubic meter in the size range 0.5 µm and larger in diameter, corresponding to an ISO 9 cleanroom, while an ISO 1 cleanroom allows no particles in that size range and only 12 particles per cubic meter of 0.3 µm and smaller

Overview

Cleanrooms can be very large. Entire manufacturing facilities can be contained within a cleanroom with factory floors covering thousands of square meters. They are used extensively in semiconductor manufacturing, biotechnology, the life sciences and other fields that are very sensitive to environmental contamination.

The air entering a cleanroom from outside is filtered to exclude dust, and the air inside is constantly re-circulated through high efficiency particulate air (HEPA) and/or ultra low particulate air (ULPA) filters to remove internally generated contaminants.

HEPA filters are composed of a mat of randomly arranged fibres. The fibres are typically composed of fiberglass and possess diameters between 0.5 and 2.0 micrometer. Key factors affecting function are fibre diameter, filter thickness, and face velocity. The air space between HEPA filter fibres is much greater than 0.3 µm. The common assumption that a HEPA filter acts like a sieve where particles smaller than the largest opening can pass through is incorrect. Unlike membrane filters, where particles as wide as the largest opening or distance between fibres cannot pass in between them at all, HEPA filters are designed to target much smaller pollutants and particles. These particles are trapped (they stick to a fibre) through a combination of the following three mechanisms:

Interception, where particles following a line of flow in the air stream come within one radius of a fibre and adhere to it. Impaction, where larger particles are unable to avoid fibres by following the curving contours of the air stream and are forced to embed in one of them directly; this effect increases with diminishing fibre separation and higher air flow velocity. Diffusion, an enhancing mechanism is a result of the collision with gas molecules by the smallest particles, especially those below 0.1 µm in diameter, which are thereby impeded and delayed in their path through the filter; this behavior is similar to Brownian motion and raises the probability that a particle will be stopped by either of the two mechanisms above; it becomes dominant at lower air flow velocities.

Diffusion predominates below the 0.1 µm diameter particle size. Impaction and interception predominate above 0.4 µm. In between, near the Most Penetrating Particle Size (MPPS) 0.3 µm, both diffusion and interception are comparatively inefficient. Therefore, the HEPA specifications use the retention of these particles to define the filter.

Biomedical applications

Hospital staff modeling a HEPA filter, which can be used if a patient has active tuberculosis. HEPA filters are critical in the prevention of the spread of airborne bacterial and viral organisms and, therefore, infection. Typically, medical-use HEPA filtration systems also incorporate high-energy ultra-violet light units to kill off the live bacteria and viruses trapped by the filter media. Some of the best-rated HEPA units have an efficiency rating of 99.995%, which assures a very high level of protection against airborne disease transmission.

Staff enter and leave through airlocks (sometimes including an air shower stage), and wear protective clothing such as hats, face masks, gloves, boots and coveralls.

Equipment inside the cleanroom is designed to generate minimal air contamination. Even specialized mops and buckets exist. Cleanroom furniture is also designed to produce a minimum of particles and to be easy to clean.

Common materials such as paper, pencils, and fabrics made from natural fibers are often excluded; however, alternatives are available. Cleanrooms are not sterile (i.e., free of uncontrolled microbes) and more attention is given to airborne particles. Particle levels are usually tested using a particle counter.

Some cleanrooms are kept at a positive pressure so that if there are any leaks, air leaks out of the chamber instead of unfiltered air coming in.

Some cleanroom HVAC systems control the humidity to low levels, such that extra equipment ("ionizers") are necessary to prevent electrostatic discharge (ESD) problems.

Low-level cleanrooms may only require special shoes, ones with completely smooth soles that do not track in dust or dirt. However, shoe bottoms must not create slipping hazards (safety always takes precedence). Entering a cleanroom usually requires wearing a cleanroom suit.

In other cleanrooms, in which the standards of air contamination are less rigorous, the entrance to the cleanroom may not have an air shower. There is an anteroom (known as a "gray room"), in which the special suits must be put on, but then a person can walk in directly to the room.

Some manufacturing facilities do not use fully classified cleanrooms, but use some cleanroom practices together to maintain their cleanliness requirements.

Cleanrooms maintain particulate-free air through the use of either HEPA or ULPA filters employing laminar or turbulent air flow principles. Laminar, or unidirectional, air flow systems direct filtered air downward in a constant stream towards filters located on walls near the cleanroom floor or through raised perforated floor panels to be recirculated. Laminar air flow systems are typically employed across 80 percent of a cleanroom ceiling to maintain constant air processing. Stainless steel or other non-shed materials are used to construct laminar air flow filters and hoods to prevent excess particles entering the air. Turbulent, or non-unidirectional, air flow uses both laminar air flow hoods and non-specific velocity filters to keep air in a cleanroom in constant motion, although not all in the same direction. The rough air seeks to trap particles that may be in the air and drive them towards the floor, where they enter filters and leave the cleanroom environment.

Cleanroom classifications

Cleanrooms are classified according to the number and size of particles permitted per volume of air. Large numbers like "class 100" or "class 1000" refer to FED-STD-209E, and denote the number of particles of size 0.5 µm or larger permitted per cubic foot of air. (For e.g. , class 1000; i.e. 1000 particles of size 0.5 µm present in one cubic foot of air).

A discrete-particle-counting, light-scattering instrument is used to determine the concentration of airborne particles, equal to and larger than the specified sizes, at designated sampling locations.

Small numbers refer to ISO 14644-1 standards, which specify the decimal logarithm of the number of particles 0.1 µm or larger permitted per cubic metre of air. So, for example, an ISO class 5 cleanroom has at most 10^5 = 100,000 particles per m^3.

Both FS 209E and ISO 14644-1 assume log-log relationships between particle size and particle concentration. For that reason, there is no such thing as zero particle concentration. The table locations without entries are non-applicable combinations of particle sizes and cleanliness classes, and should not be read as zero.

Class	maximum particles/ft³					ISO equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥5 µm	
1	35	7	3	1		ISO 3
10	350	75	30	10		ISO 4
100		750	300	100		ISO 5
1,000				1,000	7	ISO 6
10,000				10,000	70	ISO 7
100,000				100,000	700	ISO 8

Class	maximum particles/m³						FED STD 209E equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥5 µm	
ISO 1	10	2					
ISO 2	100	24	10	4			
ISO 3	1,000	237	102	35	8		Class 1
ISO 4	10,000	2,370	1,020	352	83		Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293	Class 1000
ISO 7				352,000	83,200	2,930	Class 10,000
ISO 8				3,520,000	832,000	29,300	Class 100,000
ISO 9				35,200,000	8,320,000	293,000	Room air

Microbial Contamination: Outer layer of human skin can host up to 1 million microorganisms per square cm. Human saliva up to 1 billion per mL. Bacteria is usually primary concern, but foreign organic matter, viruses, fungi, algae are all included here. Cross contamination can be a big issue.

Contamination Measurement: Particulate contamination typically measured with laser particle counter. Microbial contamination can be measured in several ways-Centrifugal sampler, Settle plate method, Contact plate method, Swabbing.

How to Use Measurements: Can use measurements to isolate problem areas. Regular measurements can help to track changes, which can then be tied back to protocol, personnel, or material changes Don't depend upon room to maintain itself. Possible to isolate culture lines responsible.

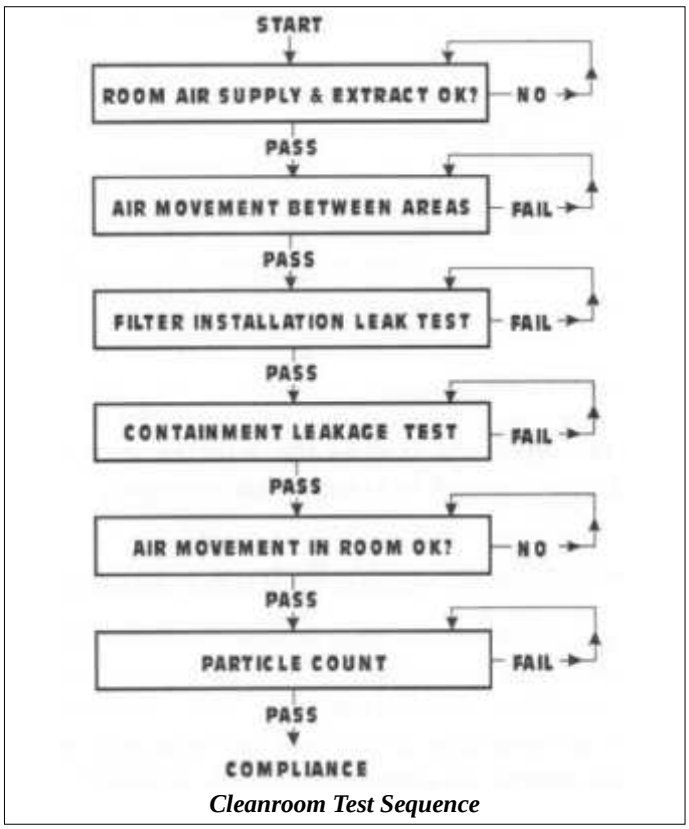
Cleaning: Critical to remove contaminants that cannot be removed by air handling. Important to follow procedures appropriate to your application. What is appropriate for one industry may not be appropriate for another. Most important thing is to develop standard procedures and FOLLOW THEM.

Dry and wet vacuuming: Dry has low (<25%) efficiency for particles smaller than 10 microns. wet uses liquids which result in greater force on the particles and hence better cleaning.

Wet wiping: Can be very efficient. Liquid breaks some bonds between surface and particles and allows particles to float off. Those adhering on surface can be rubbed off and retained in wiper. Must be careful not to redeposit particles.

Tacky rollers: Efficiency depends of tackiness/sticky ness of roller, cleanliness of tacky surface and softness of roller are also very important

Protocols to Improve Contamination Control



Cleanroom Test Sequence

Things to remember, always while working in a cleanroom area.

You as the User I: Very important to think about each and every action you take: How does this affect cleanliness? Why do we do this the way we do? Is there a better way to do it? What will happen if I do not follow proper protocols? You should know the answers to all of these questions!

You as the User II: Cleanroom environment is very fragile! Your actions have impact on other users. Important to follow procedures EVERY TIME. Make sure fellow workers follow procedures as well; nothing wrong with pointing out mistakes. Be an active participant: keep an eye out for areas that can be improved.

Minimalism is Good: Bring only required materials into cleanroom; if it is not necessary to perform the task, it should not be there. Personnel: only required personnel should be in the clean space. Reduce clutter—do not store materials in clean areas unless they have to be there!

Repetition is Good: Follow exact procedures every time. Wipe down surfaces with cleanroom wipes before and after every usage. Remember: this can seem tedious and unnecessary, but is essential to keeping the cleanroom maintained at its highest levels.

References: (1) Cleanroom Technology: Fundamentals of Design, Testing, & Operation, W. Whyte, Wiley & Sons, 2001, ISBN 0 471 86842 6. (2) Introduction to Contamination Control & Cleanroom Technology, Matts Ramstorp, Wiley-VCH, 2000, ISBN 3-527-30142-9. (3) Encyclopedia of Cleanrooms, Bio-Cleanrooms, and Aseptic Areas, Philip Austin, CRC Press, 2000, ISBN 0970113501. (4) Cleanroom Microbiology for the Non-microbiologist, David Carlberg, 1995, CRC Press, ISBN 0935184732 (2nd edition due Oct 2004). (5) Cleanroom Design, W. Whyte, Wiley & Sons, 1999, ISBN 0471942049. (6) Trade Publications:Cleanrooms Magazine, http://www.cleanrooms.com (7) A2C2 Magazine, http://www.a2c2.com

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2	**Rapid Automatic Elution** (The removal or separation of one material from another)	Instant and spontaneous release into liquid Media
3	**Improved Sample Collection**	Velvet brush-like texture rapidly and efficiently dislodges cells and collects liquid by capillarity.
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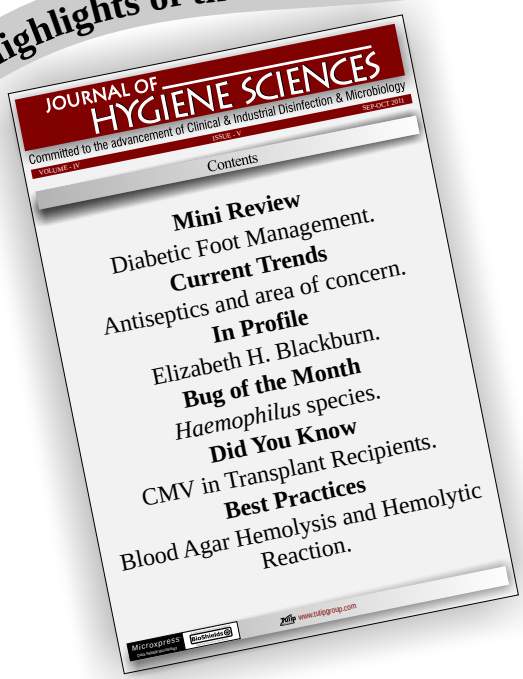
Medical: In Hospitals, Nursing homes, Medical colleges, Pathological laboratories for Inter-operative irrigation. Pre & post surgery skin and mucous membrane disinfection. Post-operative dressings. Surgical & non-surgical wound dressings. Surgical Bath/Sitz bath. Routine antisepsis during minor incisions, catheterization, scopy etc. First aid. Surface disinfection.
Industrial: In Pharmaceutical industry, Food & beverage industry, Hotel industry etc. General surface disinfection. Eliminating biofilms.

USAGE DIRECTIONS :

Surgical, postoperative, non surgical dressings	–	Use undiluted
Pre & post surgery, skin cleaning & disinfection	–	Use undiluted
Surgical/Sitz bath	–	Add 50 ml of NUSEPT™ in 1L of water & use
Antisepsis during minor incisions, catheterization,	–	Use undiluted scopy, first aid, bites, cuts stings etc
Midwifery, nursery & sickroom	–	Use undiluted
General surface disinfection	–	Add 100 ml of NUSEPT™ in 1L of water and gently mop the floor or surfaces

Highlights of the coming issue



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