

Editorial

Contents

■ Editorial	1
■ Mini review	2
■ Current Trends	4
■ In Profile	9
■ Relax Mood	10
■ Bug of the Month	11
■ Did you Know	13
■ Best Practices	14
■ In Focus	16

This issue of the Journal of Hygiene Sciences contains loads of information that will keep you occupied with the subject of Microbiology & Disinfection.

Hygiene refers to conditions and practices that help to maintain health and prevent the spread of diseases. promotion is a general term used to cover a range of strategies aimed to improve one's hygiene behavior so as to prevent the spread of disease.

Hygiene Promotion is a planned, systematic attempt to enable people to take action prevent or mitigate water, sanitation, and hygiene related diseases and provides a practical way to facilitate community participation and accountability in emergencies. Our Mini Review focuses on why Hygiene Promotion is important in emergencies.

We all have heard about Probiotic Foods. Our Current Trends section sheds light on some of the facts about Probiotics & the WHO guidelines for evaluation of Probiotics in Foods. Probiotics represent one of the fastest growing and most reputable segments in nutritional supplements. Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'

Our In Profile Scientist of the month is Dr. Sidney Altman, The molecular biologist who discovered that ribonucleic acid (RNA) can initiate some biological reactions, acting as a biocatalyst and seemingly playing the role of a protein enzyme. While at Yale, Altman's Nobel Prize work came with the analysis of the catalytic properties of the ribozyme RNase P. In recent years Dr. Altman and his research team at Yale University have applied their knowledge of RNA molecular biology to develop a method to inhibit the expression of any gene in any organism.

Gastric & peptic ulcers were attributed to different factors like stress, pungent food & acidity, but the actual etiological agent for these conditions is Helicobacter pylori, which is the Bug of the Month for this time.

Urinary tract infections are one of the most common causes of hospital admissions and clinic visits globally, making urine, the most frequent sample received for culture. In most of the clinical laboratories of the developing world, a combination of Blood agar and MacConkey's Agar is traditionally used for urine culture. Chromogenic UTI Medium is a differential agar which provides presumptive identification of the main pathogens which cause infection of the urinary tract.

Microbiologically contaminated surfaces can serve as reservoirs of potential pathogens. The transferral of microorganisms from environmental surfaces to patients is largely via hand contact with the surface. Although hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections. Our best Practices section focuses on the cleaning strategies in the Patient care areas.

Have a well deserved laugh & tickle your mind a little with our Relax Mood section.

Your feedback /suggestions will be taken as a productive input in making this Journal more informative & interesting.

So go on, turn the page & explore the information.....

Hygiene Promotion in Emergencies

Water and Sanitation related diseases cause significant deaths and sickness in emergencies. Even without the disruption of an emergency, diarrhea kills over 30,000 children per week worldwide. During protracted war and conflict in particular, simple diarrheal diseases can often kill more people than the fighting itself.

What is Hygiene Promotion?

Hygiene Promotion is the planned, systematic attempt to enable people to take action to prevent or mitigate water, sanitation, and hygiene related diseases and provides a practical way to facilitate community participation and accountability in emergencies.

Effective hygiene promotion is widely accepted to be one of the most valuable tools to reduce the burden of diarrheal diseases after a disaster. Hygiene promotion is, nevertheless, given significantly less emphasis than other water supply and sanitation initiatives.

Hygiene promotion is a general term used to cover a range of strategies aimed to improve people's hygiene behavior and so prevent the spread of disease. This note focuses on behavior related to water supply and sanitation.

Components of Hygiene Promotion

Below represents the different components of Hygiene Promotion in an emergency situation and examples of the specific activities related to each component are then provided.

Community Participation e.g.:

- Consult with affected men, women, and children on design of facilities, hygiene kits, and outreach system
- Identify and respond to vulnerability e.g. the elderly or those with disabilities
- Support and collaborate with existing community organizations, organizers, and communicators

Use and Maintenance of facilities e.g.:

- Feedback to engineers on design and acceptability of facilities
- Establish a voluntary system of cleaning and maintenance
- Encourage a sense of ownership and responsibility
- Lay the foundations for longer term maintenance by identification, organisation and training of water and sanitation committees

Selection and distribution of hygiene items e.g.:

- Decide on content and acceptability of items for hygiene kits
- Ensure the optimal use of hygiene items (including insecticide-treated bed nets where used)

Community and Individual Action e.g.: Apply principles of Behavior Change Communication and Social Mobilization

- Train outreach system of hygiene promoters to conduct home visits
- Organize community dramas and group activities with adults and children
- Use available mass media e.g. radio to provide information on hygiene

Communication with WASH stakeholders e.g.:

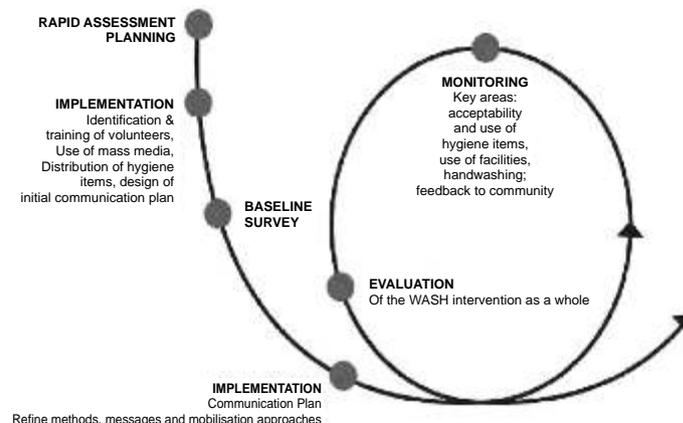
- Collaborate with and/or orientate government workers
- Train women's groups/co-operatives and national NGOs

Monitoring: Collect, analyze and use data on:

- Appropriate use of hygiene items
- Optimal use of facilities
- Community satisfaction with facilities

How do you do Hygiene Promotion in an emergency?

A simplified model of the Project Cycle



In any emergency intervention, be it chronic or acute, the hygiene promotion aspect of the programme should follow the project cycle and include assessment, planning, implementation and monitoring as shown in the diagram above.

Hygiene Promotion in different phases and contexts of an emergency

Emergency contexts are very varied and the specific approach to Hygiene Promotion will depend on the existing situation and what is feasible in terms of population customs, culture, and resources. The key difference between Hygiene Promotion interventions in different phases

of the emergency or different contexts will usually relate to the intensity and scale of the intervention, which is dependent on the level of public health risk. In general, the early stages of the emergency will be characterized by the need to at least provide information to the affected population but as soon as possible a more interactive approach should be used. At all times the emphasis should be on mobilizing people to take action.

Hygiene Promotion approaches and methods

The most commonly used approach to access the population in emergencies is that of identifying and training community outreach workers (volunteers/mobilisers/animators).

A cascade system, where outreach workers (at least 1:500 per population or more if intensive work is required or if populations are spread out), are supervised by trained hygiene promoters who are supported by skilled professionals, is the most common model used, but others are possible. A network of peer educators might also be established e.g. teenagers or young mothers. Hygiene clubs could also be established in each affected area. A key aspect of the initial Hygiene Promotion assessment is to identify existing local capacity and skills.

Cascade Outreach System



It is recommended that both the available mass media (e.g. radio or leaflets) AND other more interactive methods are employed (see orientation workshop). Even in an acute emergency some initial discussions with individuals and community groups can

take place and as the emergency evolves more widespread use of methods that foster discussion should be encouraged.

Among the most useful participatory methods are 'community mapping' exercises, focus group discussions, exercises using visual aids to stimulate discussion and mobilization activities such as three pile sorting, chain of contamination, and pocket chart voting. An assessment of the existing resources available for hygiene promotion is important as this will help to ensure that

culturally appropriate methods and tools are employed.

It is important to note that health benefits are not always the main motivating factor for changes in behavior. The need for privacy and safety, convenience, social status, and esteem may sometimes be stronger driving forces than health arguments.

HYGIENE PROMOTION STEPS

Step	Key issues/activities
Step 1 Assessment Conduct rapid assessment to identify risk practices and get an initial idea of what the community knows, does, and understands about water, sanitation, and hygiene.	Which specific practices allow diarrheal microbes/other diseases to be transmitted? Which practices are the most harmful?
Step 2 Consult women, men, and children on contents of hygiene kit	What specific hygiene needs do men, women, and children have e.g. sanitary towels, razors, potties?
Step 3 Planning Select practice(s) and hardware for intervention (define objectives and indicators)	Which risk practices are most widespread? Which will have the biggest impact on public health? Which risk practices are alterable? What can be done to enable change of risky practice?
Step 4 Define target audiences (this may be all the affected community with priority focus on those who care for young children) and stakeholders	Who employs these practices? Who influences the people who employ these practices? E.g. teachers, community leaders, Traditional Birth Attendants etc.
Step 5 Define initial mode of intervention Determine initial key messages and channels of communication Determine advocacy and training needs for stakeholders	What mass media methods are available? E.g. 60% of people have radios but they are often used only by men What methods do the target audiences trust? E.g. traditional healer, discussions at women's group meetings Where/how can men & women be accessed? E.g. distribution queue, water point
Step 6 Recruit/identify and start to train fieldworkers and outreach system	What capacity (systems, skills, and approaches) already exists in government/national NGOs?
Step 7 Implementation Begin implementation and continue assessing situation	Distribute hygiene kits Emphasis initially on providing information and use of mass media e.g. radio spots, campaigns, and home visits by volunteers Organise group meetings/interviews and discussions with key informants and stakeholders to initiate a more interactive approach.
Step 8 Ongoing assessment Develop baseline Understand motivational factors/ refine key messages	Obtain quantitative data where feasible. Carry out systematic collection of qualitative data using participatory methods (co-ordinate with others and be careful not to overwhelm communities with over questioning) What motivates those who currently use safe practices? What are the advantages of the safe practices?
Step 9 Monitor	Are hygiene kits being used/are people satisfied with them? Are toilets being used/are people satisfied with them? Do men and women feel safe when accessing facilities? Are people washing their hands? Is drinking water in the home free from contamination?
Step 10 Implementation Refine communication plan Rapidly adapt intervention according to outcome of monitoring Continue training Continue monitoring	Emphasis more on interactive methods e.g. group discussions using mapping, three pile sorting etc. Identify and train (with engineers) longer term structures e.g. committees.

PROBIOTIC FOODS

Probiotics - Definition

Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2002).

Advantages Of Probiotics

Although evidence is still being gathered, researchers say there are enough data to rate the effectiveness of probiotics for several specific illnesses. In 2008, a panel of experts at Yale University reviewed the latest findings. They concluded that probiotics are most effective for:

- Acute childhood diarrhea
- Preventing antibiotic-associated diarrhea
- Preventing pouchitis, an intestinal inflammation that can follow serious intestinal surgery
- Regulating immune response
- Treating and preventing eczema associated with cow's milk allergy

Many different individuals take Probiotics for a variety of different reasons.

Better Digestion: There are numerous forms of good bacteria in the body such as lactobacilli that works to keep virus and other pathogens from causing strain and stomach related illnesses. This good bacterium also helps with the digestion process by making it easier for the body to break down and absorb food.

Irritable Bowel Syndrome: Many of the problems associated with Irritable Bowel Syndrome (IBS) such as Diarrhea can be caused by an infection or irritation in the digestive tract of the body. By adding microflora, yeast, and other good bacteria that is contained in Probiotics to the digestive tract, it can help calm the stomach and prevent Diarrhea and other problems that are brought on by IBS.

Candida: One of the most popular ways to help relieve the symptoms from the fungus Candida is by taking Probiotics. While having a certain amount of Candida in our body is a good thing and healthy, having too much of it can be detrimental to our immune system and can lead to health related problems. Taking Probiotic supplements can help aid the growth of excess Candida and help regulate the amount.

Vaginal Bacteria Infections: For women the vagina can be very susceptible to bacterial infections and issues. Taking antibiotics is one of the leading causes of bacteria issues and even taking daily birth control can be problematic. Probiotics can help restore the normal balance of bacteria within the vagina preventing yeast infections and other health issues.

Urinary tract infections: Many women who have recurring and frequent urinary tract infections (UTI) have found Probiotics to help reduce the frequency of them occurring. According to a study published by the Clinical Infectious Diseases magazine, a study was completed where women were given a Probiotic that contained Lactobacillus those who received the Probiotic had a less frequent percentage of having a reoccurring UTI.

Disadvantages

Potential Side Effects

Probiotics are usually sought out by people with digestive problems in search for a cure for indigestion, diarrhea or heartburn. While probiotics may alleviate some of these problems they can also cause similar complications. The National Center for Complementary and Alternative Medicine describes the potential gastrointestinal side effects of probiotics. The most common side effects of probiotics are gas and bloating. In more serious and more rare instances, probiotics can cause infections, especially in immunocompromised people. If you have a weak immune system, or, if you are taking medicine that may weaken your immune system, avoid probiotics.

Infections:

One concern with the use of probiotics is that in certain patient populations, there may be an increased risk of infections, according to MedlinePlus. Specifically, patients with weak or absent immune systems - for instance, patients undergoing chemotherapy, or patients with HIV/AIDS -- may be unable to fight off an overgrowth of the bacteria in probiotic supplements. This abnormal growth of bacteria could then lead to potentially dangerous infections. Medline Plus notes that these are rare situations, but patients who are immunocompromised should be careful when making the decision to take a probiotic containing supplement, and discuss the risks and benefits with their health care providers.

Price

One of the biggest drawbacks of probiotics is that they are not cheap. In a discussion about probiotics, Dr. Otis Brawley points out that probiotics are expensive and not likely to be covered by insurance any time soon. Most probiotic supplements are sold in specialty stores or on nutrition websites for high prices. Some probiotic supplements are designed specifically for infants with colic and charge parents a premium for a potential colic cure. If you do not have extra money in your budget to afford a probiotics habit, you may want to avoid probiotics unless they are specifically recommended by your physician.

Factors affecting viability of Probiotics in foods

Some factors, both intrinsic and extrinsic, may influence the survival of probiotics in food, and so have to be considered in all stages of probiotic food manufacturing.

- physiological state of the added probiotic in the food
- physicochemical conditions of food processing
- physical conditions of product storage, like temperature
- chemical composition of the product, such as content of nutrients, oxygen or pH
- interactions with other product components, that can be inhibitory or protective

Physiological State:

The physiological state of bacteria when prepared and remaining in a product itself are important factors for survival of the

probiotics. Dryness in a food product keeps the bacteria in a relatively quiescent state during storage, while a wet product establishes potentially active metabolism.

Temperature:

The temperature at which probiotic organisms grow is an important factor in food applications where fermentation is required, is also a critical factor influencing probiotic survival during manufacture and storage. The lower the temperature the more stable probiotic viability in the food product will be. During processing, temperatures over 45–50°C will be detrimental to probiotic survival, this means that the higher the temperature, the shorter the time period of exposure required to severely decrease the numbers of viable bacteria, ranging from hours or minutes at 45–55°C to seconds at higher temperatures. Therefore it is obvious that probiotics should be added downstream of heating/cooking/pasteurization processes in food manufacture to avoid the high temperatures. Elevated temperature also has a detrimental effect on stability during the product process of shipping and storage. Again, the cooler a product can be maintained, the better probiotic survival will be, like in vegetative probiotic cells in liquid products, where refrigerated storage is usually essential. If the product is dried, the bacteria will be in a quiescent state, so acceptable probiotic viability can be maintained in dry products stored at ambient temperatures for 12 months or more.

pH:

Some bacteria like Lactobacilli and bifidobacteria can tolerate lower pH levels because they produce organic acid and products from carbohydrate metabolism. Indeed, numerous in vitro and in vivo studies have demonstrated that in gastric transit where the cells are exposed to low pH values and with a time of exposure relatively short, some probiotic organisms can survive. In fermented milks and yogurts with pH values between 3.7 and 4.3 lactobacilli are able to grow and survive, while Bifidobacteria tend to be less acid tolerant, with most species surviving poorly in fermented products at pH levels below 4.6. *B. animalis* subsp. *lactis* is most commonly used in acidic foods because is more acid tolerant than human intestinal species, and *B. thermoacidophilum*, is even more tolerant to low pH (and heat), but has not yet been characterized thoroughly for probiotic traits and is not used commercially.

Regarding to fruit juices (pH 3.5–4.5) commercially successful products have been produced, such as Gefilus (Valio Ltd, Finland), which contains *Lactobacillus rhamnosus* GG. The viability at low pH can be improved with carriers such as dietary fibers. Survival of lactobacilli in low pH has also been enhanced in the presence of metabolizable sugars, that allow cell membrane proton pumps to operate and prevent lowering of intracellular pH. This can improve survival during gastric transit, but may not be applicable to improving probiotic survival over the time stages of shelf-storage.

Water quality:

For quiescent probiotic bacteria, water activity is a crucial determinant of survival in food products during storage. The higher the moisture levels and water activity, the lower survival of probiotics. There is a substantial interaction between water activity and temperature with respect to their impact on the

survival of quiescent probiotics. As the storage temperature is increased, the detrimental impact of moisture is magnified. Here, the osmotic stresses appear to play a role, with the presence of smaller molecules resulting in poorer bacterial survival, although the exact cell death mechanisms have not been elucidated yet.

Oxygen:

Both bifidobacteria and lactobacilli are considered strict anaerobes and oxygen can be detrimental to its growth and survival. However, the degree of oxygen sensitivity varies considerably between different species and strains, for example, lactobacilli, which are mostly microaerophilic, are more tolerant of oxygen than bifidobacteria, to the point where oxygen levels are not an important consideration in maintaining the survival of lactobacilli. Most probiotic bifidobacteria do not grow well in the presence of oxygen, although, many bifidobacteria have enzymatic mechanisms to limit the oxygen toxicity. For oxygen sensitive strains, some strategies can be used to prevent oxygen toxicity in food products. Antioxidant ingredients have been shown to improve probiotic survival, as well as the use of oxygen barrier or modified-atmosphere packaging. Therefore, it is advisable to minimize processes that are highly aerating, particularly when using bifidobacteria.

Toxicity Of Ingredients

Interactions between probiotics and other ingredients could happen and those interactions can be protective, neutral, or detrimental to probiotic stability. Obviously, the inclusion of antimicrobial preservatives can inhibit probiotic survival and elevated levels of ingredients such as salt, organic acids, and nitrates can inhibit probiotics during storage, while starter cultures can sometimes inhibit the growth of probiotics during fermentation through the production of specific bacteriocins.

Freeze - Thawing

The damages made to cell membranes by freezing, probiotics are detrimental to survival, and also can make the cells more vulnerable to environmental stresses. To prevent or at least mitigate cell injury, protectants are usually added to cultures to be frozen or dried. Once frozen, probiotics can survive well over long shelf lives in products such as frozen yogurts and ice-cream. Using alternative methods of freezing, such as slow-cooling rates or pre-freezing stress, can significantly improve cell survival. Repeated freeze–thawing cycles are highly detrimental to cell survival and should be avoided.

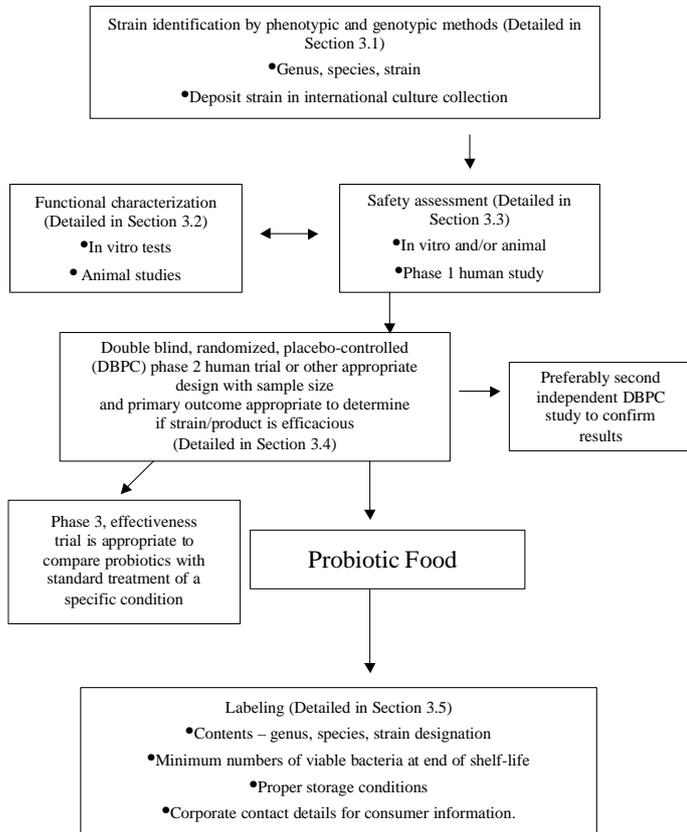
WHO/FAO Guidelines For The Evaluation Of Probiotics In Food In order to claim that a food has a probiotic effect, the guidelines set forth by a joint report of WHO/FAO (World Health Organization/Food and Agriculture Organization of the United Nations) should be followed. A scheme outlining these guidelines for the evaluation of probiotics food use is shown in Fig. 1 on page 6.

3.1. Genus/species/strain identification

It was recognized that it is necessary to know the genus and species of the probiotic strain. The current state of evidence suggests that probiotic effects are strain specific. Strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. A

Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food
London, Ontario, Canada, April 30 and May 1, 2002

Figure 1. Guidelines for the Evaluation of Probiotics for Food Use



possible exception is the ability in general of *S. thermophilus* and *L. delbrueckii* spp. *bulgaricus* enhance lactose digestion in lactose intolerant individuals. In this case, or in other cases where there is suitable scientific substantiation of health benefits that are not strain specific, individual strain identity is not critical. It is recommended that a combination of phenotypic and genetic tests be used.

DNA-DNA hybridization is the reference method to specify that a strain belongs to a species; however, as it is time consuming and beyond the resources of many laboratories, requiring a large collection of reference strains, the use of DNA sequences encoding 16S rRNA is suggested as a suitable substitute. In this case, it is recommended that this genotypic technique be combined with phenotypic tests for confirmation. Patterns generated from the fermentation of a range of sugars and final fermentation products obtained from glucose utilization are key phenotypes that should be investigated for identification purposes.

Strain typing has to be performed with a reproducible genetic method or using a unique phenotypic trait. Pulsed Field Gel Electrophoresis (PFGE) is the gold standard. Randomly Amplified Polymorphic DNA (RAPD) can also be used, but is less reproducible. Determination of the presence of extra chromosomal genetic elements, such as plasmids can contribute to strain typing and characterization. It is recommended that all strains be deposited in an internationally recognized culture collection.

3.2. In vitro tests to screen potential probiotics.

In addition, in vitro are useful to gain knowledge of strains and the mechanism of the probiotic effect. However, it was noted that the currently available tests are not fully adequate to predict the functionality of probiotic microorganisms in the human body. It was also noted that in vitro available for particular strains are not sufficient for describing them as probiotic. Probiotics for human use will require substantiation of efficacy with human trials. Appropriate target-specific in vitro that correlate with in vivo are recommended.

Currently used in vitro tests for the study of probiotic strains

- Resistance to gastric acidity
- Bile acid resistance
- Adherence to mucus and/or human epithelial cells and cell lines
- Antimicrobial activity against potentially pathogenic bacteria
- Ability to reduce pathogen adhesion to surfaces
- Bile salt hydrolase activity
- Resistance to spermicides (applicable to probiotics for vaginal use)

3.3. Safety considerations: Requirements for proof that a probiotic strain is safe and without contamination in its delivery form

Historically, lactobacilli and bifidobacteria associated with food have been considered to be safe (Adams & Marteau, 1995). Their occurrence as normal commensals of the mammalian flora and their established safe use in a diversity of foods and supplement products worldwide supports this conclusion. However, probiotics may theoretically be responsible for four types of side effects (Marteau, 2002)

1. Systemic infections
2. Deleterious metabolic activities
3. Excessive immune stimulation in susceptible individuals
4. Gene transfer

In recognition of the importance of assuring safety, even among a group of bacteria that is Generally Recognized as Safe (GRAS), the Working Group recommends that probiotic strains be characterized at a minimum with the following tests:

1. Determination of antibiotic resistance patterns
2. Assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation)
3. Assessment of side-effects during human studies
4. Epidemiological surveillance of adverse incidents in consumers (post-market)
5. If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition (SCAN, 2000)
6. If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required

Assessment of lack of infectivity by a probiotic strain in immune compromised animals would add a measure of confidence in the safety of the probiotic.

3.4. In vivo studies using animals and humans

In some cases, animal models exist to provide substantiation of in vitro and determination of probiotic mechanism. Where appropriate, the Working Group encourages use of these prior to human trials. The principal outcome of efficacy studies on probiotics should be proven benefits in human trials, such as statistically and biologically significant improvement in condition, symptoms, signs, well-being or quality of life; reduced risk of disease or longer time to next occurrence; or faster recovery from illness. Each should have a proven correlation with the probiotic tested.

Probiotics have been tested for an impact on a variety of clinical conditions. Standard methods for clinical evaluations are comprised of

Phase 1 (safety)

Phase 2 (efficacy)

Phase 3 (effectiveness) and

Phase 4 (surveillance)

Phase 1 studies focused on safety are discussed in Section 3.3 above.

Phase 2 studies, generally in the form of randomized, double blind, placebo-controlled (DBPC) design, measure efficacy compared with placebo. In addition, phase 2 studies measure adverse effects. A general recommendation for the testing of probiotic foods is that the placebo would be comprised of the food carrier devoid of the test probiotic. Sample size needs to be calculated for specific endpoints. Statistically significant differences must apply to biologically relevant outcomes.

Probiotics delivered in food generally are not tested in Phase 3 studies, which are concerned with comparison with a standard therapy. When a claim is made for a probiotic altering a disease state, the claim should be made based on sound scientific evidence in human subjects.

In Phase 2 and 3 studies, the Working Group recognizes the value of validated quality of life assessment tools. It is recommended that human trials be repeated by more than one Center for confirmation of results. It is recommended that information accumulated to show that a strain(s) is a probiotic, including clinical trial evidence be published in peer-reviewed scientific or medical journals. Furthermore, publication of negative results is encouraged as these contribute to the totality of the evidence to support probiotic efficacy.

3.5. Health claims and labeling

Currently in most countries, only general health claims are allowed on foods containing probiotics. The Working Group recommends that specific health claims on foods be allowed relating to the use of probiotics, where sufficient scientific evidence is available, as per the guidelines set forth in this report. Such specific health claims should be permitted on the label and promotional material. For example, a specific claim that states that a probiotic 'reduces the incidence and severity of rotavirus diarrhea in infants' would be more informative to the consumer than a general claim that states 'improves gut health'. This would better comply with Codex General Guidelines on Claims (CAC/GL 1-1979) to avoid misleading information.

It is recommended that it be the responsibility of the product manufacturer that an independent third party review by scientific experts in the field be conducted to establish that health claims are truthful and not misleading. The Working Group recommends that the following information be described on the label:

- Genus, species and strain designation. Strain designation should not mislead consumers about the functionality of the strain
- Minimum viable numbers of each probiotic strain at the end of the shelf-life
- The suggested serving size must deliver the effective dose of probiotics related to the health claim
- Health claim(s)
- Proper storage conditions
- Corporate contact details for consumer information

Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery

The ability of probiotic microorganisms to survive and multiply in the host strongly influences their probiotic benefits. The bacteria should be metabolically stable and active in the product, survive passage through the upper digestive tract in large numbers and have beneficial effects when in the intestine of the host. The standard for any food sold with health claims from the addition of probiotics is that it must contain per gram at least 10 - 10 cfu of viable probiotic bacteria (FAO/WHO, 2001). However, there are still several problems with respect to the low viability of probiotic bacteria in dairy foods. Several factors have been reported to affect the viability of probiotics in fermented dairy products, including titratable acidity, pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains of associative fermented dairy product organisms, concentration of lactic and acetic acids and even whey protein concentration. Here we focus on the current knowledge and techniques used in the microencapsulation of probiotic microorganisms to enhance the performance of these organisms during fermentation, downstream processing and utilization in commercial products.

Survival is, of course, essential for organisms targeted to populate the human gut, one of the most important issues in health benefit provision by probiotic bacteria. Different approaches that increase the resistance of these sensitive microorganisms against adverse conditions have been proposed, including appropriate selection of acid- and bile-resistant strains, use of oxygen impermeable containers, two-step fermentation, stress adaptation, incorporation of micronutrients such as peptides and amino acids, and microencapsulation.

Microencapsulation technology

Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific conditions. A microcapsule consists of a semi permeable, spherical, thin, and strong membrane surrounding solid/liquid core, with a diameter varying from a few microns to 1 mm.

Microcapsules and microspheres can be engineered to gradually release active ingredients. A microcapsule may be opened by

many different means, including fracture by heat, solvation, diffusion, and pressure. A coating may also be designed to open in the specific areas of the body.

Conclusions and future directions

Sophisticated shell materials and technologies have been developed and an extremely wide variety of functionalities can now be achieved through microencapsulation. Any type of triggers can be used to prompt the release of the encapsulated ingredients, such as pH changes, mechanical stress, temperature, enzymatic activity, time, osmotic force, etc.

Encapsulated probiotic bacteria can be used in many fermented dairy products, such as yoghurt, cheese, cultured cream and frozen dairy desserts, and for biomass production. In the encapsulated form, the probiotics are protected from bacteriophage and harsh environments, such as freezing and gastric solutions. Thus, encapsulation facilitates the manufacture of fermented dairy products in which the bacteria have consistent characteristics and higher stability during storage and higher productivity than non encapsulated bacteria. With the encapsulated products, the residence time, acidity and continuous inoculation of milk with a constant bacilli/cocci ratio can be controlled at a desired pH.

The use of microencapsulated probiotics for controlled release applications is a promising alternative to solving the major problems of these organisms that are faced by food industries. Even so, the challenges are to select the appropriate microencapsulation technique and encapsulating materials. To date, the research on the encapsulation of probiotics has focused mainly on maintaining the viability of the probiotic bacterial cells at low pH and high bile concentrations. One important challenge for cell encapsulation is the large size of microbial cells (typically $1\text{e}4\text{ mm}$) or particles of freeze-dried culture (more than 100 mm). This characteristic limits cell loading for small capsules or, when large size capsules are produced, can negatively affect the textural and sensorial properties of food products in which they are added. In almost all cases, gel entrapment using natural biopolymers, such as calcium alginate, carrageenan, gellan gum, and chitosan are favored by researchers. However, although promising on a laboratory scale, the developed technologies for producing gel beads still present serious difficulties for large-scale production of food-grade microencapsulated microorganisms.

Another major challenge is to improve the viability of probiotics during the manufacturing processes, particularly heat processing. Consequently, there appears to be no commercial probiotic products available that are stable at high temperatures. Keeping in view the importance of producing thermo resistant probiotic microorganisms, as well as the interests of food and pharmaceutical companies, new approaches are needed in further research.

There are at least two options: (1) discovering new strains of probiotic bacteria that are naturally heat stable or that have been genetically modified and (2) developing an encapsulation system that effectively acts like an "insulation material". We currently need to exploring approaches to tackle this challenging area and is focusing on developing novel encapsulation systems. This is

based on an understanding of the thermal conductivity properties of several food-grade biopolymers and lipids that are used as encapsulating shell materials, individually and in combination.

References:

1. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada: 2002. April, May. Guidelines for evaluation of probiotics in food. US Food and Drug Administration. Generally Recognized As Safe (GRAS)
2. Adams MR, Marteau P (1995): On the safety of lactic acid bacteria. *Int J Food Micro*, 27: 263-264.
3. Conway PL, Gorbach SL, Goldin BR (1987): Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J Dairy Sci*, 70: 1-12.
4. Hennequin C, Kauffmann-Lacroix C, Jobert A, Viard JP, Ricour C, Jacquemin JL, Berche P (2000): Possible role of catheters in *Saccharomyces boulardii* fungemia. *Eur J Clin Microbiol Infect Dis*, 19: 16-20.
5. Mackay AD, Taylor MB, Kibbler CC, Hamilton-Miller JMT (1999): *Lactobacillus endocarditis* caused by a probiotic organism. *Clin Microbiol Infect*, 5: 290-292.
6. Marteau P (2002): Safety aspects of probiotic products. *J Nutr*, (In Press). Oggioni MR, Pozzi G, Balensin PE, Galieni P, Bigazzi C (1998): Recurrent septicemia in an immunocompromised patient due to probiotic strains of *Bacillus subtilis*. *J Clin Microbiol*, 36: 325-326.
7. Rautio M, Jousimies-Somer H, Kauma H, Pietarinen I, Saxelin M, Tynkkynen S, Koskela M (1999): Liver abscess due to a *Lactobacillus rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG. *Clin Infect Dis*, 28: 1159-60.
8. Richard V, Auwera P, Snoeck R, Daneau D, Meunier F (1988): Nosocomial bacteremia caused by *Bacillus* species. *Eur J Clin Microbiol Infect Dis*, 7: 783-785.
9. Report of the Scientific Committee on Animal Nutrition on the Safety of Use of *Bacillus* Species in Animal Nutrition. European Commission Health & Consumer Protection Directorate-General. <http://europa.eu.int/comm/food/fs/sc/scan/out41.pdf>
10. Spinosa MR, Wallet F, Courcol RJ, Oggioni MR (2000): The trouble in tracing opportunistic pathogens: cholangitis due to *Bacillus* in a French hospital caused by a strain related to an Italian probiotic? *Microb Ecol Health Dis*, 12: 99-101.



Sidney Altman

Born : 7 May 1939 (age 73)
(Montreal, Quebec, Canada)
Nationality : Canada & American
(since 1984)
Fields : Molecular biology
Known for : Ribozymes
Notable awards : Nobel Prize in
Chemistry (1989)

INTRODUCTION:

Molecular biologist Sidney Altman discovered in 1983 that ribonucleic acid (RNA) can initiate some biological reactions, acting as a biocatalyst and seemingly playing the role of a protein enzyme. This overturned the established scientific understanding that RNA acts only as a carrier of genetic information, and challenged basic biological tenets on the origins of life. He won the Nobel Prize for Chemistry in 1989, sharing the award with Thomas R. Cech, who reached very similar conclusions in his own independent work.

Early Life: Altman was born on May 7, 1939 in Montreal, Quebec, Canada. His parents were immigrants to Canada, each coming from Eastern Europe as a young adult, in the 1920s. Altman's mother was from Bialystok in Poland, and had come to Canada with her sister at the age of eighteen, learning English and working in a textile factory to earn money to bring the rest of their family to Quebec. Altman's father, born in Ukraine, had been a worker on a collective farm in the Soviet Union. He was originally sponsored to come to Canada as a farm worker, but later, as a husband and a father of two sons, he supported the family by running a small grocery store in Montreal.

As Altman reached adulthood, the family's financial situation had become secure enough that he was able to pursue a college education. He went to the United States to study physics at the Massachusetts Institute of Technology. While at MIT, he was a member of the ice hockey team. After achieving his bachelor's degree from MIT in 1960, Altman spent 18 months as a graduate student in physics at Columbia University. Some months later, he enrolled as a graduate student in biophysics at the University of Colorado Medical Center. His project was a study of the effects of acridines on the replication of bacteriophage T4 DNA. He received his Ph.D. in biophysics from the University of Colorado in 1967.

Personal Life: Altman was married to Ann Korner in 1972. They are the parents of two children, Daniel and Leah. lived primarily in the United States since departing Montreal to attend MIT in 1958, Altman became a U.S. citizen in 1984, maintaining dual citizenship as a Canadian citizen as well.

Career: After receiving his Ph.D., Altman embarked upon the first of two research fellowships. He joined Matthew Meselson's laboratory at Harvard University to study a DNA endonuclease involved in the replication and recombination of T4 DNA. Later, at the MRC Laboratory of Molecular Biology in Cambridge, England, Altman started the work that led to the discovery of RNase P and the enzymatic properties of the RNA subunit of that enzyme. "The discovery of the first radio chemically pure precursor to a tRNA molecule enabled me to get a job as an assistant professor at Yale University in 1971, a difficult time to get any job at all.

Altman's career at Yale followed a standard academic pattern with promotion through the ranks until he became Professor in 1980. He was Chairman of his department from 1983–1985 and in 1985 became the Dean of Yale College for four years. On July 1, 1989 he returned to the post of Professor on a full-time basis.

While at Yale, Altman's Nobel Prize work came with the analysis

of the catalytic properties of the ribozyme RNase P. RNase P is a ribonucleoprotein particle consisting of both a structural RNA molecule and one (in prokaryotes) or more (in eukaryotes) proteins. Originally, it was believed that, in the bacterial RNase P complex, the protein subunit was responsible for the catalytic activity of the complex, which is involved in the maturation of tRNAs. During experiments in which the complex was reconstituted in test tubes, Altman and his group discovered that the RNA component, in isolation, was sufficient for the observed catalytic activity of the enzyme, indicating that the RNA itself had catalytic properties, which was the discovery that earned him the Nobel Prize. Although the RNase P complex also exists in eukaryotic organisms, his later work revealed that in those organisms, the protein subunits of the complex are essential to the catalytic activity, in contrast to the bacterial RNase P.

Recent Advances by Altman: In recent years Dr. Altman and his research team at Yale University have applied their knowledge of RNA molecular biology to develop a method to inhibit the expression of any gene in any organism. They have patented a technique that stops the expression of virus genes in human tissue culture cells. Some day they expect it will work in vivo — in people.

HONOURS:

- Dean's List - MIT, 3 semesters
- University Fellow, U. of Colorado 1964-67
- Damon Runyon Fund Fellow 1967-69
- Anna Fuller Fund Fellow 1969-70
- Fellow - American Association for the Advancement of Science 1987
- Member - American Academy of Arts and Sciences 1988
- Rosenstein Award for Basic Biomedical Research 1989
- Member - Connecticut Academy of Arts and Sciences 1989
- Fairchild Fellow, California Institute of Technology 1989-90
- National Institutes of Health Merit Award 1989
- Nobel Prize in Chemistry 1989
- Sterling Professor of Biology, Yale University 1990
- Yale Science and Engineering Association Award 1990
- Member - American Philosophical Society 1990
- Member - National Academy of Sciences (USA) 1990
- Distinguished Service Medal, Teachers College, Columbia University 1990
- William Clyde DeVane Professor - Yale College 1991
- Fellow, Whitney Humanities Center, Yale University 1991-1994
- Innovators of Science Award, Medical College of Virginia 1992
- Montgomery Fellow, Dartmouth College 1992
- Feodor Lynen Award, University of Miami/Miami Winter Symposia 1995
- Julius K. Schultz Visiting Professor, University of Miami Medical School 1998
- Visiting Professor, Guy's Medical School, King's College, University of London (UK) 1999-2001
- Novartis-Drew Award in Biomedical Research 1999
- Burroughs-Wellcome Visiting Professor, Ohio State University 2000

REFERENCES:

- (1) www.ncbi.nlm.nih.gov
- (2) www.nobelprize.org
- (3) Nobel Laureates in Chemistry, 1901–1992. American Chemical Society and Chemical Heritage Foundation. 1994. p. 737.
- (4) Altman, Sidney; Karl Grandin, ed. (1989). "Sidney Altman Autobiography". Les Prix Nobel. The Nobel Foundation.
- (5) Newton, Carolyn D. (1990). "Altman, Sidney". Encyclopædia Britannica. **1990 Britannica Book of the Year**. Chicago: Encyclopædia Britannica. pp. 81. (6) "Book of Members, 1780–2010: Chapter A". American Academy of Arts and Sciences.

Enjoy the humour

Three old men went to see God.

The first old man, an American, asked God when my country will come out of recession. "100 years," God said. The American started weeping profusely. "I will not live to see that day".

Second man, a Russian asked God "When will my country become prosperous and a real Super Power again?" "Fifty years," came the reply. The Russian too started weeping profusely. "I will not live to see that day" Finally the Indian asked God, "When will my country become corruption-free?"

God started weeping profusely. "I will not live to see that day".

Two Ladies Talking in Heaven

1st woman: Hi! My name is Wanda.

2nd woman: Hi! I'm Sylvia. How'd you die?

1st woman: I froze to death.

2nd woman: How horrible!

1st woman: It wasn't so bad. After I quit shaking from the cold, I Began to get warm & sleepy, and finally died a peaceful death. What about you?

2nd woman: I died of a massive heart attack. I suspected that my Husband was cheating, so I came home early to catch him in the act. But instead, I found him all by himself in the den watching TV.

1st woman: So, what happened?

2nd woman: I was so sure there was another woman there somewhere that I started running all over the house looking.

I ran up into the attic and searched, and down into the basement. Then I went through every Closet and checked under all the beds.

I kept this up until I had looked everywhere, and finally I became so exhausted that I just Keeled over with a heart attack and died.

1st woman: Too bad you didn't look in the freezer ---we'd both still be Alive.

A man was sleeping in his house. Suddenly Yam raj appeared & said, "Go out & enjoy. Nothing will happen to you for the next 10 years." He did so & met with an accident & died.

On the way to heaven is hell... saw Yam raj whistling & relaxing. He asked Yam raj, why did you lie to me?

"..... Sorry Son, Appraisal time, had to achieve Target..."

Back when Bill Clinton and Hillary got married Bill told her, there's one thing I want you to know. There's a box under my bed and I don't want you to look in it until I die.

Hillary agreed to this but, over the years, the curiosity got the better of her and she finally looked in it. She found three beer cans and 1.5 million dollars in cash.

When she asked Bill what the beer cans were for, he replied, well, those are for all the times I've cheated on you.

Hillary said, well, that's not bad after all these years and you being a politician and traveling and all.

She was about to leave, but then she said, Hey, Bill, what about the 1.5 million dollars?

Bill replied that's for all the times the box got full and I had to cash the cans in.

THOUGHTS BY GREAT PEOPLE

"You can do anything, but not everything."

(David Allen)

"Perfection is achieved, not when there is nothing more to add, but when there is nothing left to take away."

(Antoine de Saint)

"Courage is not the absence of fear, but rather the judgment that something else is more important than fear."

(Ambrose Redmoon)

"To the man who only has a hammer, everything he encounters begins to look like a nail."

(Abraham Maslow)

"A wise man gets more use from his enemies than a fool from his friends."

(Baltasar Gracian)

"The real voyage of discovery consists not in seeking new lands but seeing with new eyes."

(Marcel Proust)

"Try a thing you haven't done three times. Once, to get over the fear of doing it. Twice, to learn how to do it. And a third time, to figure out whether you like it or not."

(Virgil Garnett Thomson)

"People often say that motivation doesn't last. Well, neither does bathing – that's why we recommend it daily."

(Zig Ziglar)

"I am thankful to all those who said NO to me, it's because of them I did it myself..."

(Einstein)

Helicobacter pylori



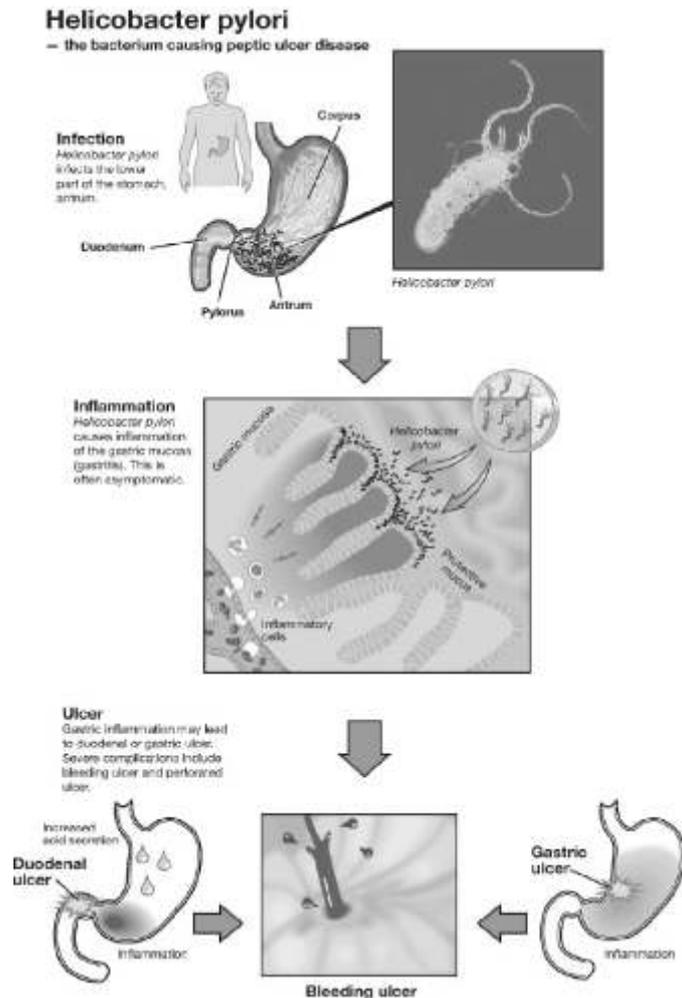
CLASSIFICATION:

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Epsilon Proteobacteria
Order: Campylobacteriales
Family: Helicobacteraceae
Genus: Helicobacter
Species: H. pylori
Binomial Name: Helicobacter pylori

Helicobacter pylori previously named as Campylobacter pyloridis, is a Gram-negative bacterium found in the stomach. It was identified in 1982 by Barry Marshall and Robin Warren, who found that it was present in patients with chronic gastritis and gastric ulcers, conditions that were not previously believed to have a microbial cause.

H. pylori is a helix-shaped (classified as a curved rod, not spirochaete), microaerophilic; that is, it requires oxygen, but at lower concentration than is found in the atmosphere. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (H₂) produced by intestinal bacteria. Like other typical Gram-negative bacteria, the outer membrane of H. pylori consists of phospholipids and lipopolysaccharide (LPS).

Pathogenic action of H. pylori



Attachment:

The Helicobacter pylori enter the stomach and attach to the protective mucus lining of the stomach wall. The bacteria are able to survive in the strongly acid environment of the stomach because they excrete the enzyme urease which neutralized the acidic environment of the stomach by converting urea into the basic ammonia and buffer bicarbonate. Inside the mucus lining of the stomach wall, the bacteria cannot be killed by the body's immune system.

Toxin production:

The Helicobacter pylori produce toxins such as vacuating cytotoxin A (VAC A) that cause the cells in the lining of the stomach to die. This allows the bacteria to better access of nutrients as it decreases the competition from stomach lining cells.

Cell Invasion:

The bacteria invade the protective inner lining of the stomach so that they can be protected from immune system. The bacteria then kill the cells that they invade which creates holes in the mucus lining of the stomach, causing the formation of ulcers. Additionally, the substances released by the bacteria during the invasion, hurt the stomach cells ability to absorb calories from food in the stomach.

How is H. pylori spread?

Researchers are not certain how H. pylori is transmitted, although they think it may be spread through contaminated food or water. People may pick up the bacterium from food that has not been washed well or cooked properly or from drinking water that has come from an unclean source.

Other research is exploring how infection spreads from an infected person to an uninfected person. Studies suggest that having contact with the stool or vomit of an infected person can spread H. pylori infection and H. pylori have been found in the saliva of some infected people, which means infection could be spread through direct contact with saliva.

Signs & Symptoms:

Over 80% of people infected with H. pylori show no symptoms. Acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea. Where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia : stomach pains, nausea, burping, poor appetite, bloating, belching, weight loss and sometimes vomiting, black stool.

Individuals infected with H. pylori have a 10 to 20% lifetime risk of developing peptic ulcers and a 1 to 2% risk of acquiring stomach cancer. Inflammation of the pyloric antrum is more likely to lead to duodenal ulcers, while inflammation of the corpus (body of the stomach) is more likely to lead to gastric ulcers and gastric carcinoma.

Why testing is important in case of H. pylori infection?

Testing, whether the above symptoms are caused by H. pylori is important because treatment for a duodenal ulcer caused by H. pylori is different from that for an ulcer caused by non steroidal

anti-inflammatory agents (NSAIDs), like aspirin and ibuprofen.

How is an *H. pylori*-induced ulcer diagnosed?

Doctors use one of three simple, non invasive tests to detect *H. pylori* in a patient's blood, breath, or stool. Because the breath test and stool test more accurately detect *H. pylori* than the blood test, some doctors prefer to use one of these two tests. Invasive tests include Endoscopy.

Blood test - A blood sample is taken from the patient's vein and tested for *H. pylori* antibodies. Antibodies are substances the body produces to fight invading harmful substances-called antigens-such as the *H. pylori* bacterium.

Urea breath test - The patient swallows a capsule, liquid, or pudding that contains urea "labeled" with a special carbon atom. After a few minutes, the patient breathes into a container, exhaling carbon dioxide. If the carbon atom is found in the exhaled breath, *H. pylori* is present, as this bacterium contains large amounts of urease, a chemical that breaks urea down into carbon dioxide and ammonia.

Stool antigen test - The patient provides a stool sample, which is tested for *H. pylori* antigens.

Endoscopy - A procedure that involves snaking a thin, flexible tube with a camera down the esophagus, through the stomach, and into the small intestine to view the upper GI tract.

Recent advances in *H. pylori* Treatment

Helicobacter pylori plays a key role in dyspepsia, peptic ulcer disease, and gastric neoplasia and eradication of the infection has become an important treatment goal in clinical practice. Seven-day proton-pump inhibitor-amoxicillin-clarithromycin triple therapy is the current first-line therapy for *H. pylori* but eradication rates are compromised by poor compliance and antibiotic resistance. Ten-day sequential treatment may emerge as an alternative first-line therapy. Bismuth-based quadruple therapy is the second-line regimen of choice. Novel triple-therapy regimens containing rifabutin, levofloxacin, or furazolidone may be useful alternatives as second- or third-line therapy.

Anti microbial Therapy:

Medicines that reduce stomach acid include proton pump inhibitors (PPIs) and histamine receptor blockers (H2 blockers). Both acid-reducing medicines help relieve peptic ulcer pain after a few weeks and promote ulcer healing. PPIs and H2 blockers work in different ways:

- PPIs suppress acid production by halting the mechanism that pumps acid into the stomach.
- H2 blockers work by blocking histamine, which stimulates acid secretion.

Bismuth subsalicylate (Pepto-Bismol) coats ulcers, protecting them from stomach acid. Although bismuth subsalicylate may kill *H. pylori*, it is used with-not in place of-antibiotics in some treatment regimens.

In the United States, clarithromycin-based triple therapy-is the standard treatment for an ulcer caused by *H. pylori*. The doctor prescribes the antibiotic clarithromycin, a PPI, and the antibiotics amoxicillin or metronidazole for 10 to 14 days. Because research shows higher cure rates with 14 days of treatment, some doctors

now prescribe triple therapy for this longer period.

Bismuth quadruple therapy is another treatment strategy used in the United States. The patient takes a PPI, bismuth subsalicylate, and the antibiotics tetracycline and metronidazole for 10 to 14 days. Bismuth quadruple therapy is used to treat patients in one of several situations, including if the patient (1) cannot take amoxicillin-a penicillin-like antibiotic-because of a penicillin allergy (2) has been treated before with a macrolide antibiotic, such as clarithromycin (3) is still infected with *H. pylori* because triple therapy failed to kill the bacteria.

Table 2: Antibiotics Used for *Helicobacter pylori* Eradication

	DRUG	DOSE
Standard antibiotics	Amoxicillin	1g BID
	Metronidazole	500mg BID
	Clarithromycin	250-500mg BID
	Tetracylin	500mg QID
Salvage antibiotics	Levofloxacin	300mg BID/500mg OD
	Rifabutin	150mg BID
	Furazolidone	100-200mg BID

Non-antimicrobial therapy, in particular with probiotics, may reduce the side effects of triple therapy and simultaneously increase the efficacy of treatment, either by a direct effect on *H. pylori* or by improvement of therapy adherence due to reduction of side effects.

Non-antimicrobial Co-therapy

Various non-antimicrobial products have been studied for their effect on *H. pylori* when taken either alone or as co-therapy with triple therapy. These products include normal foods or food components, such as cranberry juice, ginger, oregano and broccoli sprouts, 23 food additives such as lactoferrin and various probiotics. The purpose of their use was either to reduce the side effects of eradication therapy or to improve the efficacy of this therapy, or both.

Various research groups have studied the effect of probiotic strains on *H. pylori*. In vitro experiments showed that *Lactobacillus* strains, in particular *L. casei*, *Shirota*, *L. brevis* and *L. gasseri*, can suppress *H. pylori* growth. This effect requires viable *Lactobacilli*. Additional in vivo experiments suggested that these *Lactobacillus* strains when given for three to four weeks may decrease *H. pylori* colonization density measured by urea breath testing, but do not lead to eradication. Combination of probiotics with triple therapy may decrease side effects of the triple combination, in particular diarrhea and nausea, but has no consistently reported effect on eradication rates.

References:

1. www.medicinenet.net
2. www.emedicinehealth.com
3. www.ncbi.nlm.nih.gov/in
4. *Helicobacter pylori*: Molecular Genetics and Cellular Biology. Caister Academic.
5. Kusters JG, van Vliet AH, Kuipers EJ (July 2006).
6. "Pathogenesis of *Helicobacter pylori* Infection". Clin Microbiol Rev 19 (3): 449-90.

Chromogenic UTI Agar

Accurate identification of pathogens in short time is the primary responsibility of a clinical microbiology laboratory. Various chromogenic media that could identify pathogens in reduced time with precision and ease have been developed to fulfill this task.

Urinary tract infections are one of the most common causes of hospital admissions and clinic visits globally, making urine, the most frequent sample received for culture. In most of the clinical laboratories of the developing world, a combination of Blood agar and MacConkey's Agar is traditionally used for urine culture.

The bacteria that cause urinary tract infections typically enter the bladder via the urethra. However, infection may also occur via the blood or lymph. It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy. After gaining entry to the bladder, *E. coli* are able to attach to the bladder wall and form a biofilm that resists the body's immune response. *E. coli* is the cause of 80–85% of urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5–10%. Rarely may they be due to viral or fungal infections. Other bacterial causes include: *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterobacter*. These are uncommon and typically related to abnormalities of the urinary system or urinary catheterization. Urinary tract infections due to *Staphylococcus aureus* typically occurs secondary to blood born infections.

For many years, the isolation of urinary pathogens has relied upon the use of cystine lactose electrolyte deficient (CLED) agar. This medium, first described by Sandys and later modified by Mackey and Sandys, has been widely used for diagnostic routine urinary bacteriology as a non-selective medium capable of supporting the growth of most urinary pathogens, and at the same time giving good colonial differentiation without surface spread of *Proteus* spp. Urine samples contribute greatly to the daily workload of a microbiology laboratory.

Several commercial companies have now developed **Chromogenic Agars**. These combine the basal cystine electrolyte depleted media with chromogenic substrates, rather than lactose and indicator, to enhance discrimination of the different species of microorganisms and permit rapid identification on the primary isolation medium by means of colony colour.

Chromogenic UTI Medium is a differential agar which provides presumptive identification of the main pathogens which cause infection of the urinary tract. It contains two specific chromogenic substrates which are cleaved by enzymes produced by *Enterococcus* spp., *Escherichia coli* and coliforms. In addition, it contains tryptophan which indicates tryptophan deaminase activity (TDA), indicating the presence of *Proteus* spp. It is based on Cystine Lactose Electrolyte Deficient (CLED) Medium which provides a valuable non-inhibitory diagnostic

agar for plate culture of other urinary organisms, whilst preventing the swarming of *Proteus* spp.

The chromogenic mixture, is targeted towards β -glucosidase enzyme activity, and allows the specific detection of enterococci through the formation of blue colonies. The other chromogen, Red-Galactoside, is cleaved by the enzyme β -galactosidase which is produced by *Escherichia coli*, resulting in pink colonies. Any uncertainty in identification may be resolved by removing suspect colonies from the plate and performing an indole test using. Cleavage of both the chromogens by members of the coliform group, results in purple colonies.

The medium also contains tryptophan which acts as an indicator of tryptophan deaminase activity (TDA), resulting in halos around the colonies of *Proteus*, *Morganella* and *Providencia* spp. It should be noted that organisms with a typical enzyme patterns may give anomalous reactions. For example, in a trial, over 45% of *Enterobacter cloacae* were shown to lack β -glucosidase, resulting in pink colonies which were indistinguishable from *Escherichia coli*. In such cases an indole test can be performed.

References:

1. Amdekar, S; Singh, V, Singh, DD "Probiotic therapy: immunomodulating approach toward urinary tract infection.". *Current microbiology* 2011; 63 (5): 484–90.
2. D Fallon et al., A comparison of the performance of commercially available chromogenic agars for the isolation and presumptive identification of organisms from urine. *Clin Pathol*, 2003; J 56(8): 608–612.
3. Edberg SC, Kontniqum CM. Comparison of β -glucuronidase based substrate systems for identification of *E. coli*. *J Clin Microbiol* 1986;24:368–71.
4. Fung JC, Lucia B, Clark E, Berman M, Goldstein J, D'Amato RF. Primary culture media for routine urine processing. *J Clin Microbiol* 1982; 16: 632-6.
5. Lane, DR; Takhar, SS "Diagnosis and management of urinary tract infection and pyelonephritis". *Emergency medicine clinics of North America* 2011; 29 (3): 539–52.
6. Mackey JP, Sandys GH. Diagnosis of urinary tract infections. *BMJ* 1966;1:1173.
7. Manafi M. New developments in chromogenic and fluorogenic media. *Int J Food Microbiol* 2000;60:205–18.
8. Nicolle LE "Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis". *Urol Clin North Am* 2008; 35 (1): 1–12.
9. Salvatore, S; Salvatore, S, Cattoni, E, Siesto, G, Serati, M, Sorice, P, Torella, M "Urinary tract infections in women." *European journal of obstetrics, gynecology, and reproductive biology* 2011; 156 (2): 131–6
10. Sandys GH. A new method for preventing swarming of *Proteus* spp with a description of a new medium suitable for use in routine laboratory practice. *J Med Lab Technol* 1960;17:224.

General Cleaning Strategies for Patient-Care Areas

INTRODUCTION

Microbiologically contaminated surfaces can serve as reservoirs of potential pathogens. The transferral of microorganisms from environmental surfaces to patients is largely via hand contact with the surface. Although hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections.

The principles of cleaning and disinfecting environmental surfaces take into account the intended use of the surface or item in patient care. CDC (U.S. Centers for Disease Control and Prevention) retains the Spaulding classification for medical and surgical instruments, which outlines three categories based on the potential for the instrument to transmit infection if the instrument is microbiologically contaminated before use. These categories are “critical,” “semicritical,” and “noncritical.” In 1991, CDC proposed an additional category designated “environmental surfaces” to Spaulding’s original classification to represent surfaces that generally do not come into direct contact with patients during care. Environmental surfaces can be further divided into medical equipment surfaces (e.g., knobs or handles on hemodialysis machines, x-ray machines, instrument carts, and dental units) and housekeeping surfaces (e.g., floors, walls, and tabletops).

The following factors influence the choice of disinfection procedure for environmental surfaces: a) the nature of the item to be disinfected, b) the number of microorganisms present, c) the innate resistance of those microorganisms to the inactivating effects of the germicide, d) the amount of organic soil present, e) the type and concentration of germicide used, f) duration and temperature of germicide contact, and g) if using a proprietary product, other specific indications and directions for use.

Spaulding proposed three levels of disinfection for the treatment of devices and surfaces that do not require sterility for safe use. These disinfection levels are “high-level,” “intermediate-level,” and “low level.” The basis for these levels is that microorganisms can usually be grouped according to their innate resistance to a spectrum of physical or chemical germicidal agents.

The process of high-level disinfection, an appropriate standard of treatment for heat-sensitive, semi critical medical instruments (e.g., flexible, fiber optic endoscopes), inactivates all vegetative bacteria, mycobacterium, viruses, fungi, but not all the spores. High-level disinfection is accomplished with powerful, sporicidal chemicals (e.g. glutaraldehyde, peracetic acid etc). These liquid chemicals/high-level disinfectants are highly toxic. Use of these chemicals for applications other than those indicated in their label instructions (i.e., as immersion chemicals for treating heat-sensitive medical instruments) is not appropriate.

Therefore the new generation sterilants have come to the market which are non-toxic & have a wide range of applications. These sterilants are highly effective within one hour of contact time. (E.g. Fourth generation Quaternary ammonium compounds (QAC) – Didecyl dimethyl ammonium chloride (DDAC), polymeric biguanides, Poly Hexa Methylene Biguanide (PHMB), Hydrogen peroxide etc)

Intermediate-level disinfection does not kill bacterial spores, but it does inactivate Mycobacterium tuberculosis var. bovis, which is substantially more resistant to chemical germicides than ordinary vegetative bacteria, fungi, and medium to small viruses (with or without lipid envelopes). Chemical germicides with sufficient potency to achieve intermediate-level disinfection include

chlorine-containing compounds (e.g., sodium hypochlorite), alcohols, some phenolics and some iodophors.

Low-level disinfection inactivates vegetative bacteria, fungi, enveloped viruses (e.g., human immunodeficiency virus [HIV], and influenza viruses), and some non-enveloped viruses (e.g., adenoviruses). Low-level disinfectants include first generation quaternary ammonium compounds like cetrimide, some phenolics like chloroxyleneol and some iodophors. Sanitizers are agents that reduce the numbers of bacterial contaminants to safe levels as judged by public health requirements, and are used in cleaning operations, particularly in food service and dairy applications. Germicidal chemicals that have been approved by FDA as skin antiseptics are not appropriate for use as environmental surface disinfectants.

The selection and use of chemical germicides are largely matters of judgment, guided by product label instructions, information, and regulations. Liquid sterilant chemicals and high-level disinfectants intended for use on critical and semi-critical medical/dental devices and instruments are regulated exclusively by the FDA as a result of recent memoranda of understanding between FDA and the EPA that delineates agency authority for chemical germicide regulation. Environmental surface germicides (i.e., primarily intermediate- and low-level disinfectants) are regulated by the EPA and labeled with EPA registration numbers.

Strategies for cleaning and disinfecting surfaces in patient-care areas take into account a) potential for direct patient contact, b) degree and frequency of hand contact, and c) potential contamination of the surface with body substances or environmental sources of microorganisms (e.g., soil, dust, and water).

a. Cleaning of Medical Equipment

Manufacturers of medical equipment should provide care and maintenance instructions specific to their equipment. These instructions should include information about a) the equipments’ compatibility with chemical germicides, b) whether the equipment is water-resistant or can be safely immersed for cleaning, and c) how the equipment should be decontaminated if servicing is required. In the absence of manufacturers’ instructions, non-critical medical equipment (e.g., stethoscopes, blood pressure cuffs, dialysis machines, and equipment knobs and controls) usually only require cleansing followed by low- to intermediate-level disinfection, depending on the nature and degree of contamination. Ethyl alcohol or isopropyl alcohol in concentrations of 60%–90% (v/v) is often used to disinfect small surfaces (e.g., rubber stoppers of multiple-dose medication vials, and thermometers) and occasionally external surfaces of equipment (e.g., stethoscopes and ventilators). However, alcohol evaporates rapidly, which makes extended contact times difficult to achieve unless items are immersed, a factor that precludes its practical use as a large-surface disinfectant. Alcohol may cause discoloration, swelling, hardening, and cracking of rubber and certain plastics after prolonged and repeated use and may damage the shellac mounting of lenses in medical equipment.

b. Cleaning Housekeeping Surfaces

Housekeeping surfaces require regular cleaning and removal of soil and dust. Dry conditions favor the persistence of gram-positive cocci (e.g. coagulase-negative Staphylococcus spp.) in dust and on surfaces, whereas moist, soiled environments favor the growth and persistence of gram-negative bacilli. Fungi are also present on dust and proliferate in moist, fibrous material.

Most, if not all, housekeeping surfaces need to be cleaned only

with soap and water or a detergent/disinfectant, depending on the nature of the surface and the type and degree of contamination. Cleaning and disinfection schedules and methods vary according to the area of the health-care facility, type of surface to be cleaned, and the amount and type of soil present.

If using a proprietary detergent/disinfectant, the manufacturers' instructions for appropriate use of the product should be followed. Consult the products' material safety data sheets (MSDS) to determine appropriate precautions to prevent hazardous conditions during product application. Personal protective equipment (PPE) used during cleaning and housekeeping procedures should be appropriate to the task.

Housekeeping surfaces can be divided into two groups – those with minimal hand-contact (e.g., floors, and ceilings) and those with frequent hand-contact (“high touch surfaces”). The methods, thoroughness, and frequency of cleaning and the products used are determined by health-care facility policy. However, high-touch housekeeping surfaces in patient-care areas (e.g., doorknobs, bedrails, light switches, wall areas around the toilet in the patient's room, and the edges of privacy curtains) should be cleaned and/or disinfected more frequently than surfaces with minimal hand contact. Infection-control practitioners typically use a risk-assessment approach to identify high-touch surfaces and then coordinate an appropriate cleaning and disinfecting strategy and schedule with the housekeeping staff.

Horizontal surfaces with infrequent hand contact (e.g., window sills and hard-surface flooring) in routine patient-care areas require cleaning on a regular basis, when soiling or spills occur, and when a patient is discharged from the facility. Regular cleaning of surfaces and decontamination, as needed, is also advocated to protect potentially exposed workers. Cleaning of walls, blinds, and window curtains is recommended when they are visibly soiled. Fogging with non toxic, environmental friendly disinfectants will be beneficial. Hydrogen peroxide leaves safe residue like water & oxygen & is effective within one hour. Para formaldehyde which was once used in this application is no longer registered by EPA for this purpose. Infection control, industrial hygienists, and environmental services supervisors should assess the cleaning procedures, chemicals used, and the safety issues to determine if a temporary relocation of the patient is needed when cleaning in the room.

Another reservoir for microorganisms in the cleaning process may be dilute solutions of the detergents or disinfectants, especially if the working solution is prepared in a dirty container, stored for long periods of time, or prepared incorrectly. Gram-negative bacilli (e.g., *Pseudomonas* spp. and *Serratia marcescens*) have been detected in solutions of some disinfectants (e.g. phenolics and first generation quaternary ammonium compounds). Contemporary EPA registration regulations have helped to minimize this problem by asking manufacturers to provide potency data to support label claims for detergent/disinfectant properties under real- use conditions (e.g., diluting the product with tap water instead of distilled water).

c. Cleaning Special Care Areas

Guidelines have been published regarding cleaning strategies for isolation areas and operating rooms. The basic strategies for areas housing immunosuppressed patients include a) Wet dusting horizontal surfaces daily with cleaning cloths pre-moistened with an EPA-registered hospital disinfectant or disinfectant wipes b) using care when wet dusting equipment and surfaces above the patient to avoid patient contact with the detergent/disinfectant; c) avoiding the use of cleaning equipment that produces mists or aerosols; d) equipping vacuums with HEPA filters, especially for the exhaust, when used in any patient-care area housing immunosuppressed patients and e) regular cleaning and

maintenance of equipment to ensure efficient particle removal. When preparing the cleaning cloths for wet-dusting, freshly prepared solutions of detergents or disinfectants should be used rather than cloths that have soaked in such solutions for long periods of time. Doors to immunosuppressed patients' rooms should be closed when nearby areas are being vacuumed. Bacterial and fungal contamination of filters in cleaning equipment is inevitable, and these filters should be cleaned regularly or replaced as per equipment manufacturer instructions.

Cleaning Strategies for Spills of Blood and Body Substances

Studies have demonstrated that HIV is inactivated rapidly after being exposed to commonly used chemical germicides at concentrations that are much lower than those used in practice. HBV is readily inactivated with a variety of germicides, OSHA has revised its regulation for disinfecting spills of blood or other potentially infectious material to include proprietary products whose label includes inactivation claims for HBV and HIV, provided that such surfaces have not become contaminated with agent(s) or volumes of or concentrations of agent(s) for which a higher level of disinfection is recommended. These registered products are listed in EPA's List D – Registered Antimicrobials Effective against Hepatitis B Virus and Human HIV. Additional lists of interest include EPA's List C –Registered Antimicrobials Effective against Human HIV-1 and EPA's List E – Registered Antimicrobials Effective Against *Mycobacterium* spp., Hepatitis B Virus, and Human HIV-1.

Sodium hypochlorite solutions are inexpensive and effective broad-spectrum germicidal solutions. Generic sources of sodium hypochlorite include household chlorine bleach or reagent grade chemical. Concentrations of sodium hypochlorite solutions with a range of 5,000–6,150 ppm to 500–615 ppm free chlorine are effective depending on the amount of organic material (e.g. blood, mucus, and urine) present on the surface to be cleaned and disinfected. EPA-registered chemical germicides may be more compatible with certain materials that could be corroded by repeated exposure to sodium hypochlorite.

Strategies for decontaminating spills of blood and other body fluids differ based on the setting in which they occur and the volume of the spill. In patient-care areas, workers can manage small spills with cleaning and then disinfecting using an intermediate-level germicide or an EPA-registered germicide from the EPA List D or E. For spills containing large amounts of blood or other body substances, workers should first remove visible organic matter with absorbent material (e.g., disposable paper towels discarded into leak-proof, properly labeled containment) and then clean and decontaminate the area. If the surface is nonporous and a generic form of a sodium hypochlorite solution (500ppm) is appropriate for decontamination assuming that a) the worker assigned to clean the spill is wearing gloves and other personal protective equipment appropriate to the task, b) most of the organic matter of the spill has been removed with absorbent material, and c) the surface has been cleaned to remove residual organic matter.

Protocols for cleaning spills should be developed and made available on record as part of good laboratory practice. Workers in laboratories and in patient-care areas of the facility should receive periodic training in environmental surface infection-control strategies and procedures as part of an overall infection-control and safety curriculum.

References:

- (1) www.cdc.gov/ncidod/hip/enviro/guide.htm
- (2) Spaulding EH. Chemical disinfection and antisepsis in the hospital. *J Hosp Res* 1972;9:5–31.
- (3) Favero MS, Bond WW. Chemical disinfection of medical and surgical materials. In: Block SS, ed. (4) Disinfection, sterilization, and preservation, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2001;881–917.

Microexpress

Introducing.....

Gamma Irradiated Sterile Disinfectants

Historically pharmaceutical, biotechnology and medical device manufacturers have been using disinfectants and filter through 0.22 micron filter for further usage. Lack of availability of good quality sterile disinfectants is a major hurdle facing GMP plants today, which is being overcome on an ad hoc basis by taking recourse to 0.22 micron filtration of available disinfectants, solutions and products. Such ad hoc methods give rise to the issue of credibility of the performance of such products and require ongoing validation of process that are used to aseptically filter the disinfectants through 0.22 micron filter. Thus gamma sterilized products take care of the aforesaid procedures and guarantee availability of sterile, effective and proven products for clean room usage.

Hand Disinfection

Cat No.	Products	Activity	Application
ANX0750	Alconox	Bactericidal, fungicidal and virucidal	Personal hand hygiene
PLG0750	Purellium Gel - C	Bactericidal, fungicidal and virucidal	Personal hand hygiene
TST0750	Triosept	Bactericidal, fungicidal and virucidal	Personal hand hygiene

Environment and Surface Disinfection

Cat No.	Products	Activity	Application
AST0750	Aerosept – C	Bactericidal, fungicidal and virucidal	For disinfecting laminar hoods, table tops, workstations, air and surface disinfection in critical areas.
MLE0750	Microlyse – C	Bactericidal, fungicidal and virucidal	For floor mopping and surface disinfection
NST0750	Nusept – C	Bactericidal, fungicidal and virucidal	For surface disinfection and general purpose disinfection
NVC0750	Novacide	Bactericidal, fungicidal and virucidal	For surface disinfection
ATR0750	Acitar	Bactericidal, fungicidal and virucidal	For environment (fumigation) and surface disinfection

Pack Size Available – 750 ml

BioShields Presents Nusept

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

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

ACTIVITY : Broad spectrum: Bactericidal, Fungicidal & Virucidal.

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

APPLICATIONS :

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

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

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

