

## Editorial

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Ushering the month of November with yet another issue of the Journal of Hygiene Sciences is as much pleasure for us here as we hope it will be for you to read. Being the last issue of the year, as the saying goes, 'save the best for the last' we have strived to make this issue as interesting as possible so that our esteemed customers and readers would enjoy the same.

The section on 'Mini review' takes a look into the vast, elaborate world of 'the ducks of the microbial world' – Mycobacteria. The genus comprises of a diverse variety of microbes ranging from parasites to saprophytes, and their significance to man and the environment as a whole. However we have touched upon the medically significant species of the genus, which are of particular concern to humans.

Science and Technology is improving and so are the related standards, there are many chemical molecules that are prepared and have been researched upon, one such substance is an intelligent; safe and effective biocide namely Surfacine. Current trends delves into this new genre of biocidal agents which harnesses silver's long established safe and broad-spectrum biocidal properties and delivers them via a non-eluting, efficient delivery system that preferentially and actively transfers silver into microorganisms. This certain chemical delivery system differs from other silver antimicrobial technologies that deliver silver compounds non-preferentially to the microorganism's environment rather than the microorganism itself.

Blood transfusions have come a long way, from the time wrong transfusions would claim lives to now when even the most minor blood components are checked before a transfusion is carried out. Distinguished research done on blood groups, and the basic existence of the different types of antigens on the surface of the RBC which were responsible for transfusion related deaths carried out by Karl Landsteiner is outstanding. Today as we know of the different factors that dominate medical principles of blood grouping and transfusion is attributed to this man who is 'In Profile' for this issue.

*Neisseria gonorrhoeae* is a Gram negative kidney bean shaped diplococci bacteria responsible for the sexually transmitted disease, gonorrhoea. The organism is responsible also for neonatal bacterial conjunctivitis. A trivial fact of the 'Bug of the Month' is that its able to pull 100,000 times its own weight and it has been claimed that the pili used to do so are the strongest biological motor known to date, exerting one nanonewton.

'Did you Know' Bacterial Enumeration is a complex area and there are various methods that are employed for enumerating bacterial cells. These methods may comprise of either the classical plate method or the use of cytometry which is although a comparatively new and fast technique of estimating bacterial cells however also has its own demerits. Enumeration techniques will vary depending of the location and the existing infrastructure of the area that is readily available.

Our section on 'Best Practices' gives different aspects that one should consider while choosing a disinfectant or a sanitizer that is best suited for the purpose for which it is employed. Also the article mentions the merits, demerits and the applications of the different cleaning agents. In addition also gives the 'Dos' and the 'Don'ts' for the use of disinfectants in general.

In addition to the above there is another informative section that is the Encyclopedia. To cheer you, make you smile and twitch your mind a little we treat you to, 'Relax Your Mood'.

We love to hear from you, we appreciate the feedback that we receive and will be glad to incorporate constructive ideas forwarded by you to us.

# Mycobacteria and their significance

Mycobacteria are a large group of microorganisms that inhabit a diverse range of natural environments: some species of which are capable of infecting humans and animals.

Mycobacteria are slender rods that sometimes show branching filamentous forms resembling fungal mycelium. Hence the name 'mycobacteria', meaning fungus – like bacteria. They do not stain readily, but once stained, resist decolorization with dilute mineral acids. Mycobacteria are therefore called 'acid fast bacilli' or 'AFB'. They are aerobic, nonmotile, noncapsulated and nonsporing. Growth is generally slow. The genus includes obligate parasites, opportunist pathogens and saprophytes.

The first member of this genus to be identified was the lepra bacillus discovered by Hansen in 1868. Koch (1882) isolated the mammalian tubercle bacilli and proved its causative role in tuberculosis by satisfying Koch's postulates. Tuberculosis in man was subsequently shown to be caused by two types of the bacillus – the human and bovine types, designated *Mycobacterium tuberculosis* and *M. bovis*, respectively.

Saprophytic mycobacteria were isolated from a number of sources. These included *M. butyricum* from butter, *M. phlei* from grass, *M. stercois* from dung and *M. smegmatis* from smegma.

Though there are a wide variety of Mycobacterial species that dwell in different environs, the need for understanding the medically significant mycobacteria is essential.

## *Mycobacterium tuberculosis*

*M. tuberculosis* is a straight or slightly curved rod, 1 – 4 $\mu$  X 0.2 – 0.8 $\mu$  occurring singly, in pairs or in small clumps. The size depends on conditions of growth, and long, filamentous, club shaped and branching forms may sometimes be seen. It is noteworthy to mention that *M. bovis* is usually straighter and shorter. *M. tuberculosis* is non motile, non sporing and non capsulated.

Tubercle bacilli have been described as Gram positive, though strictly speaking they cannot be so described, as after staining with basic dyes, they resist decolorization by alcohol, even without the mordanting effect of iodine. The Gram positivity is independent of the mordanting effect of iodine and appears to be determined by the same factors as are responsible for acid fastness. When stained with carbol fuchsin by the Ziehl – Neelsen method or by the fluorescent dyes (auramine O, rhodamine), they resist decolorization by 25% sulphuric acid and absolute alcohol for ten minutes (acid and alcohol fast). Acid fastness has been variously ascribed to the presence in the bacillus of an unsaponifiable wax (mycolic acid) or to a semipermeable membrane around the cell. It is related to the integrity of the cell and appears to be a property of the cell wall. Staining may be uniform or granular. Beaded or barred forms are frequently seen in *M. tuberculosis* while *M. bovis* stains more uniformly.

Non acid fast rods and granules have been reported in young cultures. Much (1907) demonstrated Gram positive granules in cold abscess pus in which acid fast bacilli could not be found, but which could produce tuberculosis when injected into susceptible animals. Much suggested that these granules (Much's granules) were non acid fast forms of tubercle bacilli.

## Cultural Characteristics

The bacilli grow slowly, the generation time in vitro being 14 – 15

hours. Colonies appear only in about two weeks and sometimes may be delayed up to 6 – 8 weeks. Optimum temperature is 37°C and growth does not occur below 25°C or above 40°C. Optimum pH is 6.4 to 7.0. *M. tuberculosis* is an obligate aerobe while *M. bovis* is microaerophilic on primary isolation, becoming aerobic on subculture. Growth is stimulated by 5 – 10 percent CO<sub>2</sub>. *M. tuberculosis* grows luxuriantly in culture as compared to *M. bovis* which grows sparsely. They are therefore, termed 'eugonic' and 'dysgonic' respectively. The addition of glycerol (0.5 per cent) improves the growth of human strains, while it is without effect or may even inhibit bovine strains. Sodium pyruvate improves the growth of both types of bacilli. *M. tuberculosis* does not grow in media containing 500 mg of P – nitrobenzoic acid /l unlike other slow growing nonchromogens.

Tubercle bacilli do not have exacting growth requirements, but are highly susceptible to even traces of toxic substances like fatty acids in culture media. The toxicity is neutralized by serum, albumin or charcoal. Several media, both solid and liquid, have been described for the cultivation of tubercle bacilli. The solid media contain egg (Lowenstein – Jensen, Petragnini or Dorset), blood (Tarshis medium), serum (Loeffler's serum slope) or potato (Pawlowsky's), however solid medium most widely employed for routine culture is the Lowenstein – Jensen (LJ) medium without starch, as recommended by the International Union Against Tuberculosis (IUAT). This consists of coagulated hen's egg, mineral salt solution, asparagine and malachite green, the last acting as a selective agent inhibiting other bacteria. A simple medium containing only eggs, malachite green, and coconut water has been reported to be a useful and a cheap alternative to the Lowenstein Jensen medium. Among the several liquid media described, Dubos', Middlebrook's, Proskauer and Beck's, Sula's and Sauton's media are the more common. Liquid media are not generally employed for routine cultivation, but are used for sensitivity tests, chemical tests and preparation of antigens and vaccines.

On solid media *M. tuberculosis* forms dry, rough, raised, irregular colonies with a wrinkled surface. They are creamy white initially, becoming yellowish or buff colored later. They are tenacious and easily emulsified. The colonies of *M. bovis* are in comparison, flat, smooth, moist and white breaking up easily when touched.

In liquid media without dispersing agents, the growth begins at the bottom, creeps up the sides and forms a prominent surface pellicle that may extend along the sides above the medium. Diffuse growth is obtained in Dubos' medium containing a detergent Tween – 80 (sorbitan monooleate). Virulent strains tend to form long serpentine cords in liquid media, while avirulent strains grow in a more dispersed fashion. The cord factor by itself is not responsible for virulence. The cord factor consists of two mycolic acids linked to a molecule of trehalose. It is present in some nonpathogenic species of mycobacteria as well. Colonial morphology may be modified by the presence of bacteriophage in the strain.

Tubercle bacilli may also be grown in chick embryos and in tissue culture.

## Resistance

Mycobacteria are not specially heat resistant, being killed at 60°C in 15 – 20 minutes. Survival is influenced by the material in which

the bacilli are present. Cultures may be killed by exposure to direct sunlight for two hours, but bacilli in sputum may remain alive for 20 – 30 hours. Bacilli may remain viable in droplet nuclei for 8 – 10 days. Cultures remain viable for 6 – 8 months at room temperature and may be stored for two years in the deep freeze cabinet at  $-20^{\circ}\text{C}$ .

Mycobacteria are relatively resistant to chemical disinfectants, surviving exposure to 5% phenol, 15% sulphuric acid, 3% nitric acid, 5% oxalic acid and 4% sodium hydroxide. It is destroyed by tincture of iodine in five minutes and by 80% ethanol in 2 – 20 minutes, 80% ethanol has been recommended as a disinfectant for skin, rubber gloves and clinical thermometers. It sterilizes pieces of cloth in ten minutes or less.

#### Biochemical reactions

Several biochemical tests have been described for the identification of mycobacterial species. The more important of them are the following:

- Niacin test: Human tubercle bacilli form niacin when grown on an egg medium. When 10% cyanogen bromide and 4% aniline in 96% ethanol are added to a suspension of the culture, a canary yellow color shows a positive reaction. The human bacilli give a positive reaction, while the bovine type is negative. The production of niacin was originally thought to differentiate human strains of *M. tuberculosis* from all other mycobacteria, but it also occurs in *M. simiae* and a few strains of *M. chelonii*.
- Aryl Sulphatase test: The enzyme aryl sulphatase is formed by atypical mycobacteria only. The organisms are grown in a medium containing 0.001M tripotassium phenolphthalien disulphate. 2N NaOH is added dropwise to the culture. A pink color indicates a positive reaction.
- Neutral Red test: Virulent strains of tubercle bacilli are able to bind neutral red in alkaline buffer solution, while avirulent strains are unable to do so.
- Catalase peroxidase tests help in differentiating tubercle bacilli from atypical mycobacteria and provide an indication of the sensitivity of the strain to isonicotinic acid hydrazide (INH). Most atypical mycobacteria, are peroxidase positive. Catalase negative strains of tubercle bacilli are avirulent for guinea pigs.  
A mixture of equal volumes of 30 vol. H<sub>2</sub>O<sub>2</sub> and 0.2% catechol in distilled water is added to 5.0 ml of a test culture and allowed to stand a few minutes. Effervescence indicates catalase production and browning of colonies indicates peroxidase activity.
- Amidase tests: The ability to split amides has been used to differentiate atypical mycobacteria. A useful pattern is provided by using five amides viz. acetamide, benzamide, carbamide, nicotinamide and pyrazinamide. A 0.00164M solution of the amide is incubated with the bacillary suspension at  $37^{\circ}\text{C}$  and 0.1 ml of MnSO<sub>4</sub>·4H<sub>2</sub>O. 1.0 ml of phenol solution and 0.5 ml of hypochlorite solution are added. The tubes are placed in boiling water for 20 minutes. A blue color developing indicates a positive test.
- Nitrate reduction test: This is positive with *M. tuberculosis* and negative with *M. bovis*.

#### Antigenic properties

Many antigens have been identified in mycobacteria. Group specificity to protein antigens. Following infection by tubercle bacilli, delayed hypersensitivity is developed to the protein of the bacillus (tuberculin). Tuberculin from *M. tuberculosis*, *M. bovis*

and *M. microti* appear to be indistinguishable. Some degree of antigenic relationship exists between the protein antigens of the tubercle bacilli and some atypical mycobacteria, as shown by weak cross reactions in skin testing with different tuberculin. Mycobacteria secrete a class of chelating agents termed exochelins. There is also some antigenic relationship between lepra and tubercle bacilli. A ribonucleoprotein from *M. tuberculosis* reacts with sera from patients with lepromatous leprosy.

#### Pathogenesis

The fate of tubercle bacilli entering the body is influenced by a variety of factors such as the inoculum dose, virulence and mode of entry of the bacillus, and host factors would include the age, resistance and hypersensitivity of the host. Tubercle bacilli enter the body commonly by inhalation, less often by ingestion and rarely by inoculation into the skin. When they are inhaled, the bacilli lodge in the pulmonary alveoli, where they are promptly phagocytosed by alveolar macrophages. But instead of being killed, the bacilli multiply intracellularly and eventually disrupt the phagocyte. Phagocytes with ingested bacilli may even act as vehicles transporting the infection to different parts of the body. Intracellular multiplication of the bacillus is interrupted only with the development of specific cellular immunity which sets in about 6 – 8 weeks after infection.

In children, primary infection leads to the 'primary complex'. This consists of a subpleural focus of tuberculosis pneumonia in the lung parenchyma (Ghon focus) usually found in the lower lobe or the lower part of the upper lobe, together with the enlarged draining lymph nodes. The primary complex is usually an asymptomatic lesion undergoing spontaneous healing, resulting in hypersensitivity to tuberculin (tuberculin allergy) and some degree of specific acquired resistance (immunity). Rarely, the primary infection may lead to hematogenous spread and the development of miliary tuberculosis, meningitis and lesions in different organs such as the spleen, liver and kidneys.

The essential pathology of tuberculosis consists of the production, in infected tissues, of a characteristic lesion, the tubercle. This is an avascular granuloma composed of a central zone containing giant cells, with or without caseation necrosis, surrounded by epithelioid cells and a peripheral zone of lymphocytes and fibroblasts. The tubercle bacillus does not appear to contain or produce a toxin. The basis of the virulence components of the bacillus have been shown to possess different biological activities which may influence the pathogenesis, allergy and immunity in the disease.

The cell wall of the bacillus induces resistance to infection, causes delayed hypersensitivity, increases reactivity to endotoxin and can replace the whole bacillus in Freund's adjuvant. Tuberculin can elicit the tuberculin reaction and, when bound to a lipid, can induce delayed hypersensitivity. In tissues it induces the formation of monocytes, macrophages, epithelioid cells and giant cells. The bacterial polysaccharide induces immediate hypersensitivity and causes exudation of neutrophils from blood vessels into tissues. Lipids cause the accumulation of macrophage and neutrophils. Phosphatides induce the formation of tubercles consisting of epithelioid cells and giant cells, with even rare caseation.

Tuberculosis lesions are primarily of two types – exudative and productive. The exudative type is an acute inflammation reaction with accumulation of edema fluid, polymorphonuclear

leucocytes, and later of monocytes around the bacilli. The lesions may heal by resolution, lead to necrosis of the tissue or develop into productive type. The productive type of lesion is predominantly cellular, composed of a number of tubercles, which may enlarge, coalesce, liquefy and undergo caseation.

The adult type of tuberculosis is generally due to reactivation of the primary infection (post primary progression, endogenous reinfection), or exogenous reinfection. The adult type of pulmonary lesion may heal by resorption, fibrosis and occasionally calcification, or progress to chronic fibrocaceous tuberculosis with tubercle formation, caseation, cavitation and shedding of tubercle bacilli in sputum (open tuberculosis). Rarely an acute, rapidly fatal infection may occur in adults.

#### Epidemiology

Tuberculosis is an ancient disease, evidence of spinal tuberculosis having been discovered in Egyptian mummies. It has been for many centuries the most important of human infections, in its global prevalence, with devastating morbidity and mortality. It has been called 'the captain of all men of death'. Its prevalence increased greatly following the Industrial Revolution, with rapid urbanisation and overcrowding. With improvements in the standards of living, its incidence has come down in the affluent countries. Therefore it has been aptly called 'a barometer of social welfare'.

There is high prevalence of both infection and active disease in the developing countries. Practically everyone is infected by the age of 20 and the infected rates are as high as 10 – 15 per cent in the first grade of school. Mortality is high in infants and in children below five years.

Low socioeconomic status and malnutrition are important predisposing factors. Dusty occupations, especially exposure to silica dust, favor tuberculosis. Doctors, nurses and laboratory workers who have contact with patients and infectious materials are prone to develop the disease.

The major source of infection is the 'open' human case shedding the bacillus in the sputum. The bacilli remain viable for weeks in dust. Inhalation of such dust is the principal mode of infection. The bovine tubercle bacillus used to be responsible for the bulk of intestinal, grandular, bone and joint tuberculosis in the West, infection having been acquired by consumption of milk from infected cattle. Strict control of dairy cattle and of milk has eliminated this danger. In India, human infection with bovine strains is extremely rare.

#### Laboratory diagnosis

Laboratory diagnosis of tuberculosis may be established by demonstration of the bacillus in the lesions by microscopy, isolating it in culture or transmitting the infection to experimental animals. Demonstration of hypersensitivity to tuberculo-protein is of some diagnostic value, but serological tests are not useful.

#### Pulmonary tuberculosis:

The specimen tested is the sputum. Bacillary shedding is abundant in lesions with caseation and relatively scanty in miliary tuberculosis. The sputum is best collected early in the morning before any meal. If the sputum is scanty, a 24 – hour collected sample may be examined. In early or convalescent cases, bacillary shedding may be intermittent and three consecutive samples should be examined for better results. The sputum

should be collected directly into a sterile wide mouthed container free from antiseptics. Disposable waxed cardboard containers are ideal. Where sputum is not available, laryngeal swabs may be examined. In children who tend to swallow the sputum stomach washing may be tested.

#### Extrapulmonary tuberculosis:

In tuberculosis meningitis, the CSF is examined by smear, culture and animal inoculation. On standing, a spider web clot may be formed in the CSF, which contains enmeshed tubercle bacilli. Pleural effusion and other exudates are collected with citrate to prevent coagulation. If free from other bacteria, they are centrifuged and the sediment cultured. If contaminant bacteria are present, the deposit is concentrated before culture.

#### Prophylaxis

In the prevention of tuberculosis general measures such as adequate nutrition, good housing and health education are as important as specific antibacterial measures. The latter consist early detection and treatment of cases, chemoprophylaxis and immunoprophylaxis.

#### Treatment

Chemotherapy has revolutionized the management of tuberculosis. It has been established that sanatorium regimens, bed rest, fresh air and good food, as well as operative interventions, such as artificial pneumothorax and thoracoplasty, are not essential for cure and that domiciliary treatment with appropriate antibacterial drugs is all that is required. The anti tuberculosis drugs employed are rifampicin, isoniazid, pyrazinamide, streptomycin, ethambutol, ethionamide, thiacetazone, para-amino salicylic acid and cycloserine. The first four of these are bactericidal and the others bacteriostatic. Antituberculosis treatment has to be always with multiple drugs – generally, three simultaneously. Prolonged treatment, usually two years or more, used to be the rule. But with the advent of actively bactericidal drugs like rifampicin, short course regimens of about six months have been found adequate in many cases.

A major problem in the chemotherapy tuberculosis is the development of drug resistance. The mechanism of resistance can be prevented by simultaneous treatment with two more drugs. Resistance may be 'primary' or 'secondary' developing during treatment. Inadequate or irresponsible chemotherapy of tuberculosis facilitates the development and dissemination of drug resistant strains endangering the health of both the patient and the community at large.

#### *Mycobacterium leprae*

*Mycobacterium leprae* is the etiological agent of leprosy which, is a chronic granulomatous disease of man involving primarily the skin, peripheral nerves and nasal mucosa, but capable of affecting any tissue or organ. The disease may be classified into four types – lepromatous, tuberculoid, dimorphous and indeterminate. The type of disease is a reflection of the immune status of the host. It is therefore not permanent and varies with chemotherapy and alterations in host resistance. Bacilli isolated from different types of leprosy do not differ in virulence of other properties.

The two extreme or 'polar' forms of the disease are the lepromatous and tuberculoid types. The lepromatous type is seen where the host resistance is low. The bacilli are seen in large numbers or as globi inside lepra cells or extracellularly.

Superficial nodular lesions (lepromata) develop which consist of granulation tissue containing a dense collection of vacuolated cells in different stages of development from mononuclear cells to lepra cells. The nodules ulcerate, become secondarily infected and cause distortion and mutilation. Bacilli invade the mucosa of the nose, mouth and upper respiratory tract and are shed in large numbers in nasal and oral secretions. The reticuloendothelial system, eyes, testes, kidneys and bones are also involved. Bacillaemia is common. The lepromatous type is more infective than the other types. The prognosis is poor. Cell leprosy and the lepromin test is negative. On the humoral immune response, antibodies in high titre are seen against mycobacterial as well as several other antigens.

#### Epidemiology

Leprosy is an exclusively human disease and the only source of infection is the patient. The exact mode of infection is not clear. The mode of entry may be either through the respiratory tract or through the skin. Leprosy is not highly communicable. The disease develops in only about five per cent of spouses living with leprosy patients. The incubation period is very long and averages 2 – 5 years. As both the time of exposure and the onset of disease are difficult to identify, estimates of incubation period are only approximations.

#### Lepromin test

The lepromin test was first described by Mitsuda in 1919. The original antigen (lepromin) was boiled, emulsified, lepromatous tissue rich in lepra bacilli. The response to the intradermal injection of lepromin is typically biphasic consisting of two separate events. The first is the early reaction of Fernandez, which consists of erythema and induration developing in 24 – 48 hours and usually remaining for 3 – 5 days. This is analogous to the tuberculin reaction. Histologically the lesion consists of serous exudate with lymphocytic infiltration. The second is the late reaction of Mitsuda, appearing in three weeks and gradually subsiding in the next few weeks. The reaction consists of an indurated skin nodule, which may ulcerate. Histologically, there is infiltration with lymphocytes, epithelioid cells and giant cells. Both the early and late reactions are usually correlated in the same individual, but most leprologists use the late reaction as evidence of lepromin positivity.

#### **Atypical Mycobacteria**

Several mycobacteria, distinct from human or bovine tubercle bacilli, which have been isolated on occasion from human pathological material, have been grouped together under the loose term 'atypical mycobacteria'. They are also known as 'anonymous' or 'unclassified' mycobacteria. Unlike tubercle bacilli which are strict parasites, atypical mycobacteria may occur in soil, water and other sources.

Atypical mycobacteria are gaining increasing importance as human pathogens in advanced countries where tuberculosis has been brought under control. Some of them cause pulmonary disease indistinguishable from tuberculosis, while others cause lymphadenitis, urinary infections, cutaneous and subcutaneous lesions. In the developing countries, where tuberculosis is still rampant, atypical mycobacteria are comparatively of minor importance as human pathogens.

Group I. Photochromogens: These strains form colonies that produce no pigment in the dark, but when the young culture is exposed to light for one hour in the presence of air, and re – incubated for 24 – 28 hours, a yellow orange pigment appears.

They are slow growing, though growth is faster than that of tubercle bacilli. The most important species in this group is *M. kansasii*.

*M. kansasii* causes chronic pulmonary disease resembling tuberculosis, particularly in old persons with preexisting lung diseases. Infections are more common in cities and in industrial areas. Man to man transmission does not seem to occur. The bacilli have been isolated from soil and milk.

Group II. Scotochromogens: These strains form pigmented colonies (yellow – orange – red) even in the dark. They are widely distributed in the environment and sometimes contaminate cultures of tubercle bacilli. They do not usually cause human disease except for *M. scrofulaceum* which may cause scrofula (cervical adenitis) in children.

Group III. Nonphotochromogens: These strains do not form pigment even on exposure to light. Colonies may resemble those of tubercle bacilli. The medically important species are *M. intracellulare*, *M. avium* and *M. xenopi*.

*M. intracellulare* causes chronic pulmonary disease indistinguishable from tuberculosis. It may also cause renal infection and lymphadenopathy. It is commonly known as the Battey bacillus.

Group IV. Rapid growers: This is a heterogeneous group of mycobacteria capable of rapid growth, colonies appearing within seven days of incubation at 37°C or 25°C. Within the group, photochromogenic, scotochromogenic, and nonchromogenic species occur. All the chromogenic rapid growers are saprophytes. The medically important species are *M. fortuitum* and *M. chelonae* both of which can cause chronic abscesses in man.

#### **Pathogenic Environmental Mycobacteria**

However there are another category of mycobacteria that are clinically significant and these are Environmental mycobacteria which constitute a frequent cause of infection, and there is a growing body of evidence to show that water is a significant vehicle for the transmission of these organisms. The importance of the Pathogenic Environmental Mycobacteria (PEM), and especially the Mycobacterium avium complex (MAC), was recognized with the discovery of disseminated infection in immunocompromised people, particularly people with HIV and AIDS. Yet there are many forms of the disease, both minor and serious, that are caused by PEM.

PEM have been identified as a group of microorganisms that are widespread in the environment and which appear to be an emerging cause of waterborne disease. These are an important cause of pulmonary disease and the incidence is increasing in areas around the globe. Although the clinical manifestation of pulmonary infection with PEM can resemble TB, the disease is very different because the host acquires infection by exposure to environment sources of these organisms (eg., water and soil).

The causative agents for PEM are numerous, clinically, PEM infections can range from asymptomatic, indolent disease with minimal clinical symptoms to rapidly destructive pneumonic disease with significant morbidity and mortality. Therapy for these infections is difficult and often associated with toxicity and expense. For reasons that are not understood, extrapulmonary dissemination is strikingly rare when the systemic immune system is intact, despite very advanced pulmonary disease. Because PEM are ubiquitous in the environment, actual pulmonary disease needs to be differentiated from colonization and contamination in the laboratory. Finding environmental mycobacteria in respiratory secretion requires supportive clinical (symptoms, signs and radiographic evidence of disease) and

microbiological evidence of infection before treatment is commenced. Practically the decision to treat is sometimes very difficult and requires a mutual understanding between the patient and the responsible physician.

Occasionally, when complex situations arise due to diagnostic or therapeutic uncertainties, the patient can be referred to an institution with experience in treating these infections.

Clinical Aspects

Acquisition of pulmonary infection most likely occurs by the aerosol route. Given the prevalence of PEM in the environment, exposure must be fairly universal. After disease develops, the symptoms can include cough, sputum production, fatigue, weight loss, sweats, hemoptysis, pleuritic and non pleuritic chest pain. Occasionally symptoms are out of proportion to the amount of disease seen radiographically. For instance, patients with significant disease noted on chest radiograph can be surprisingly asymptomatic or those with minimal changes can have debilitating symptoms. Fever is unusual unless bacterial superinfection occurs; however, the presence of another pathogenic organism in the sputum does not always predict the presence of fever. Often it is difficult to sort out symptoms due to

the underlying lung diseases (eg., dyspnea associated with emphysema or sputum production from underlying bronchiectasis) from those due to the underlying mycobacterial infection.

Confirmation of PEM in respiratory secretions is essential before committing the patient to therapy. If sputum specimens are negative, many clinicians will go to bronchoscopy for diagnosis. Failure to recognize infection and disease and thus withholding therapy may result in unnecessary lung damage; nevertheless, pulmonary disease needs to be differentiated from colonization, which does not require immediate therapy.

The natural history of PEM lung disease is quite variable. There are two prototypical descriptions of disease: and indolent (Primary) form tends to occur in older non – smoking females and a more traditional form, which is usually secondary to underlying structural lung diseases. Interestingly, there seems to be a shift in disease presentation from the secondary to the primary form.

Depending on the patient response and the susceptibility of the infectious agent, therapy, prophylaxis and prognosis in each of the above cases can be deduced.

**Reference:**

**Encyclopedia**

**Hypothyroidism** is the disease state in humans and in animals caused by insufficient production of thyroid hormone by the thyroid gland. 'Cretinism' describes the form of the same in infants.

**Causes of Hypothyroidism**

There are two fairly common causes of hypothyroidism. ● The first is a result of previous (or currently ongoing) inflammation of the thyroid gland, which leaves a large percentage of the cells of the thyroid damaged (or dead) and incapable of producing sufficient hormone. The most common cause of thyroid gland failure is called autoimmune thyroiditis (also called Hashimoto's thyroiditis), a form of thyroid inflammation caused by the patient's own immune system.

● The second major cause is the broad category of 'medical treatments'. The treatment of many thyroid conditions warrants surgical removal of a portion or all of the thyroid gland. If the total mass of thyroid producing cells left within the body are not enough to meet the needs of the body, the patient will develop hypothyroidism.

● There are several other rare causes of hypothyroidism, one of them being a completely 'normal' thyroid gland that is not making enough hormone because of a problem in the pituitary gland. If the pituitary does not produce enough thyroid stimulating hormone (TSH) then the thyroid simply does not have the 'signal' to make hormone. So it doesn't.

Type	Origin	Description
Primary	Thyroid gland	The most common forms include Hashimoto's thyroiditis (an autoimmune disease) and radioiodine therapy for hyperthyroidism.
Secondary	Pituitary gland	Occurs if the pituitary gland does not create enough thyroid stimulating hormone (TSH) to induce the thyroid gland to produce enough thyroxine and triiodothyronine. Although not every case of secondary hypothyroidism has a clear – cut cause, it is usually caused by damage to the pituitary gland, as by a tumor, radiation, or surgery.
Tertiary	Hypothalamus	Results when the hypothalamus fails to produce sufficient thyrotropin – releasing hormone (TRH). TRH prompts the pituitary gland to produce thyrotropin (TSH). Hence may also be termed 'Hypothalamic-pituitary-axis hypothyroidism'.

Certain factors can increase a person's chances of developing thyroid disorders. Individuals may need more regular testing if they  
 (1) Have had a thyroid problem before, such as goiter or thyroid surgery. (2) Have a family history of thyroid disease. (3) Have other autoimmune diseases including Sjogren's syndrome, pernicious anemia, type 1 diabetes, rheumatoid arthritis and lupus. (4) Have Turner syndrome, a genetic disorder that affects girls and women. (5) Are older than 60. (6) Have been pregnant or delivered a baby within the past 6 months. (7) Have received radiation to the thyroid, neck or to the chest. (8) Getting tested routinely helps uncover thyroid problems – especially subclinical problems. Subclinical means a person has no apparent symptoms.

**Symptoms**

Since the main purpose of thyroid hormone is to 'run the body's

metabolism' it is understandable that people with this condition will have symptoms associated with a slow metabolism.

(1) Fatigue (2) Weakness (3) Weight gain or increased difficulty losing weight (4) Coarse, dry hair (5) Dry, rough pale skin (6) Hair loss (7) Cold intolerance (can't tolerate cold temperatures like those around you) (8) Muscle cramps and frequent muscle aches (9) Constipation (10) Depression (11) Irritability (12) Memory loss (13) Abnormal menstrual cycles (14) Decreased libido (15) Slow heart rate

Each individual patient may have any number of these symptoms, and they will vary with the severity of the thyroid hormone deficiency and the length of time the body has been deprived of proper amount of hormone.

# Surfacine: A safe and effective biocide

To maintain a clean environment, where we have the assurance that the surfaces that we are touching around us at least where we dwell, are free from microbes especially pathogens which can cause illness is a requisite. With a lot more importance that is given to hygiene, there is a continuous need to provide a technology which can provide us antimicrobial agents with the following features:

- The antimicrobial agent that is used should be effective in low concentrations.
- The lethal dose for the organism should be much less than the level that cause toxicity to mammalian cells, or alternatively the agent should be such that there is no toxicity to mammalian cells and is selectively effective only against microbes.
- The agent should be cost effective.
- Should be such that the microbes are unable to develop resistance to it, so that there are no resistant strains of microorganisms for the same agent.
- The agent should not be inactivated by light (photoreduced), so that even a surface that is constantly exposed to light is also protected.
- The agent should not be inactivated when used together with other antimicrobial solvents such as alcohols or water.
- The agent should also have a wide range of pH stability.
- The agent should have a good residual activity.

However with modern technology, research and development, there are alternatives and even solutions to the problems that health care personnel were unable to handle in the past, may be due to lack of knowledge or lack of other alternative measures instead.

Surfacine is one such agent which hails from a new genre of disinfecting products which challenges to disinfect and sterilize while being safe for use in cases when it comes in contact with mammalian tissue and or cells.

Surfacine is unique because it continues to disinfect and kill microorganisms for sustained periods of time without elution of its active components into contacting liquids. Surfacine can be safely applied to both skin and surfaces and works by laying down a transparent, yet highly active antimicrobial shield on surfaces to which it is applied. Further, Surfacine is non toxic, proven safe for humans and does not release any harmful components into the environment.

Surfacine is a new persistent antimicrobial agent that may be used on animate or inanimate surfaces. It incorporates a water insoluble antimicrobial compound (silver iodide) in a polymeric biguanidine matrix. It provides sustained antimicrobial action (even after rinsing / washing), has an excellent safety profile, provides broad spectrum antimicrobial efficacy and is cost effective. Surfacine kills microorganisms on contact with a

surface and does not elute or leach into solution.

Contact biocides are a relatively new form of infection resistant materials. Surfacine is a silver based antimicrobial coating that can be immobilized on the surface of most alloplastic materials used to fabricate devices. It exhibits broad spectrum antimicrobial activity exclusively at the surface without elution and does not induce antimicrobial activity in contacting fluids such as urine regardless of volume. Because it is permanently immobilized on the material surface, the coating is non toxic to cells and is not anticipated to exhibit immunogenicity, teratogenicity, or carcinogenicity.

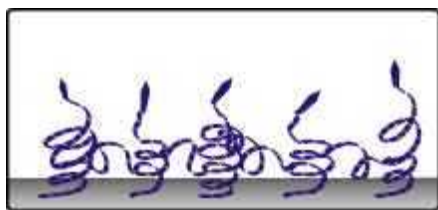
## Gross contamination

Contaminated environmental surfaces have been associated with transmission of certain nosocomial pathogens, principally vancomycin resistant *Enterococcus* spp. (VRE), methicillin resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*. The incidence of nosocomial infections caused by VRE in particular has dramatically increased in the past decade. Cross contamination is thought to result from transient hand carriage by hospital personnel, who may potentially be colonized directly from contact with colonized or infected patients or indirectly by contact with a contaminated environmental surface. Cultures of surfaces in rooms of patients colonized or infected with VRE have yielded positive cultures in 7% to 37% of samples.

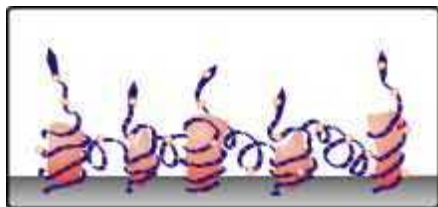
## Mode of action

Surfacine is a new, persistent antimicrobial agent that may be used on animate or inanimate surfaces. It incorporates a water-insoluble antimicrobial compound (silver iodide) in a surface-immobilized coating (a modified polyhexamethylenebiguanide) that is capable of chemical recognition and interaction with the lipid bilayer of the bacterial outer cell membrane by electrostatic attraction. The intimate microbial contact with the surface results in transfer of the antimicrobial component (silver) directly from the coating to the organism. Microorganisms contacting the coating accumulate silver until the toxicity threshold is exceeded; dead microorganisms eventually lyse and detach from the surface. The amount of silver present and the number of microorganisms in contact with the treated surface determine how long the coating is effective. Antimicrobial activity is retained when the surface is subjected to repeated dry wiping or wiping with a quaternary ammonium compound, however inactivation times for the organisms vary.

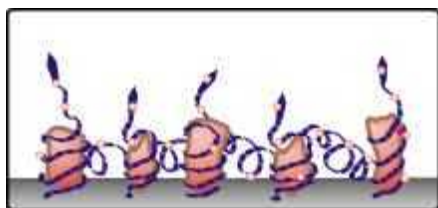
The persistent antimicrobial agent transfers the active biocide (silver) 'on demand' directly to the organism without elution of silver ions into solution. The coating therefore functions in a chemically intelligent way, i.e., antimicrobial response is triggered only upon microbial contact. The mechanism of silver release differs from that of conventional, topically applied silver compounds (eg., silver nitrate and silver sulfadiazine), which work by generating a bactericidal level of silver ions. (the ions are released into aqueous solution either by silver oxide or dissolution of the silver salt).



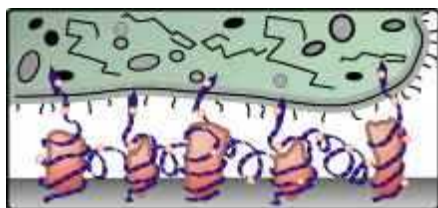
A 3-D polymeric network is immobilized onto the substrate surface.



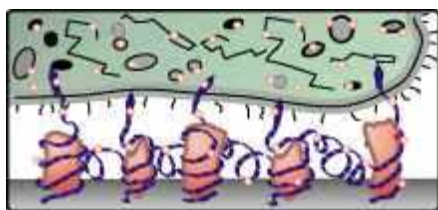
The immobilized surface network is impregnated with small (sub-micron) particles of silver halide that result in the formation of silver halide/polymer complex.



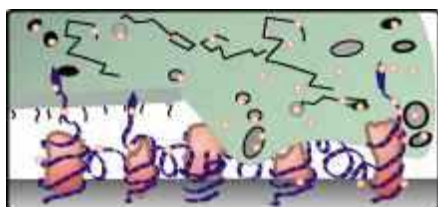
Solubility characteristics of the silver halide/polymeric complex prevent ionic silver from leaching into solutions contacting the surface coating.



Silver is available, however, to react with bacterial cells contacting the coating.



Silver is preferentially transferred directly to the microorganism causing a toxic accumulation that results in cell death.



Silver accumulated within dead microorganisms is not toxic to neighboring cells because it remains effectively complexed by the proteins of the dead microorganism. The silver halide reservoir within the polymeric network replenishes the coating surface with silver, allowing the coating to maintain high surface anti microbial activity to microorganisms that contact it for further challenges.

**Characteristics**

This new antimicrobial agent can be applied to animate and inanimate surfaces by dipping, brushing or spraying without prior surface treatment. The coating does not undergo photoreduction, degradation or color change when exposed to intense UV irradiation. This new antimicrobial agent has excellent adhesion to virtually all substrates, is optically clear, and does not delaminate, flake or crack. Treated surfaces subjected to a wipe test retained their antimicrobial efficacy.

Permanently treated surfaces remained chemically inert and retained their biocidal activity after exposure to various physical and chemical stresses such as; temperature (tested –20 to 130°C), solvents (alcohol), solutions with a pH of 4 to 10, solutions of high ionic strength, and sterilization by conventional methods (eg., steam, ethylene oxide, gamma irradiation). The coating contains low levels of silver iodide, and coated surfaces are resistant to biofilm formation. Surfaccine does not cause mammalian cell toxicity and passes the acute systemic toxicity tests recommended by the US pharmacopoeia.

**Applications**

If novel surface treatments such as this product prove to be effective in significantly reducing microbial contamination, are cost effective, and have long term residual activity, they may be extremely useful in limiting transmission of pathogens. The antimicrobial activity of this makes it potentially suitable for a wide range of applications, including disinfection of surfaces, microporous filters, and medical devices and use as a topical ointment or hand antiseptic.

Purpose	Features	Benefits
Disinfectants (similar to phenolics, quarter ammonium)	<ul style="list-style-type: none"> <li>● Antimicrobial persistence (&gt; 13 days).</li> <li>● May be used on animate and inanimate surfaces.</li> </ul>	<ul style="list-style-type: none"> <li>● Assess microbicidal activity against broad spectrum of pathogens.</li> </ul>
Antiseptics (similar to alcohol, iodophor, chlorhexidine gluconate)	<ul style="list-style-type: none"> <li>● Broad antimicrobial spectrum.</li> <li>● Transfers active agent (silver) to microbes on demand without elution.</li> <li>● Resistant to forming biofilm.</li> <li>● No toxicity to mammalian cells.</li> </ul>	<ul style="list-style-type: none"> <li>● Demonstration of efficacy to reduce nosocomial infections.</li> <li>● Human safety and toxicity data for use as an antiseptic.</li> <li>● Demonstrate antimicrobial activity in presence of organic matter.</li> </ul>

Reference: <http://www.surfaccine.com/>





## Karl Landsteiner

Birth: June 14, 1868

Death: June 24, 1943

Nationality: Austrian

Known for: Modern system of classification of blood groups

Karl Landsteiner was born in Vienna on June 14, 1868. His father, Leopold Landsteiner, a doctor of law, was a well known journalist and newspaper publisher, who died when Karl was six years old. Karl was brought up by his mother, Fanny Hess, to who he was highly devoted, until his own hour of death.

Landsteiner studied medicine at the University of Vienna, graduating in 1891. Even while he was a student he had, begun to do biochemical research and in 1891 he published a paper on the influence of diet on the composition of blood ash. To gain further knowledge of chemistry he spent the next five years in the laboratories of Hantzsch at Zurich, Emil Fischer at Wurzburg and E. Bamberger at Munich.

Returning to Vienna, Landsteiner resumed his medical studies at the Vienna General Hospital. In 1896 he became an assistant under Max von Gruber in the Hygiene Institute at Vienna. Even at this time he was interested in the mechanisms of immunity and in the nature of antibodies. From 1898 till 1908 he held the post of assistant in the University Department of Pathological Anatomy in Vienna, the head of which was Professor A. Weichselbaum, who had discovered the bacterial cause of meningitis, and with Fraenckel had discovered the pneumococcus. Here Landsteiner worked on morbid physiology rather than on morbid anatomy. In this he was encouraged by Weichselbaum, in spite of the criticism of others in this institute. In 1908 Weichselbaum secured his appointment as Prosector in the Wilhelminaspital in Vienna, where he remained until 1919. In 1911 he became Professor of Pathological Anatomy in the University of Vienna, but without the corresponding salary.

Up to the year 1919, after twenty years of work on pathological anatomy, Landsteiner with a number of collaborators had published many papers on his findings in morbid anatomy and on immunology. He discovered new facts about the immunology of syphilis, added to the knowledge of the Wasserman reaction, and discovered the immunological factors which he named haptens (it then became clear that the active substances in the extracts of normal organs used in this reaction were, in fact, haptens). He made fundamental contributions to the existing knowledge of paroxysmal haemoglobinuria.

He also showed that the cause of poliomyelitis could be transmitted to monkeys by injecting into them material prepared by grinding up the spinal cords of children who had died from this disease, and, lacking in Vienna; monkeys for further experiments, he went to the Pasteur Institute in Paris, where monkeys were available. His work there, together with that independently done by Flexner and Lewis, laid the foundation for further knowledge of the cause and immunology of poliomyelitis.

Landsteiner made numerous contributions to pathological anatomy, histology and immunology, all of which showed, not only his meticulous care in observation and description, but also his biological understanding. But his name no doubt will always be honored for his discovery in 1901 of, and outstanding work on, the blood groups, for which he was given the Nobel Prize for Physiology or Medicine in 1930.

In 1875 Landois had reported that, when man is given transfusions of the blood of other animals, these foreign blood corpuscles are clumped and broken up in the blood vessels of man with the liberation of hemoglobin. In 1901 – 1903, Landsteiner pointed out that a similar reaction may occur when the blood of one human individual is transfused, not with the blood of another animal, but with that of another human being, and that this might be the cause of shock, jaundice, and haemoglobinuria that had followed some earlier attempts at blood transfusions.

His suggestions however received little attention until, in 1909, he classified the bloods of human beings into the now well – known A, B, AB and O groups and showed that transfusions between individuals of groups A or B do not result in the destruction of new blood cells and that this catastrophe occurs only when the person is transfused with the blood of the person belonging to a different group. Earlier, in 1901 – 1903, Landsteiner had suggested that, because the characteristics that determine the blood groups are inherited, the blood groups may be used to decide instances of doubtful paternity. Much of the subsequent work that Landsteiner and his pupils did on blood groups and the immunological uses they made of them was done, not in Vienna, but in New York. For in 1919 the conditions in Vienna were such that laboratory work was very difficult and, seeing no future for Austria, Landsteiner obtained the appointment of Prosector to a small Roman Catholic Hospital at The Hague. Here he published, from 1919 – 1922, twelve papers on new haptens that he had discovered, on conjugates with proteins which were capable of inducing anaphylaxis and on related problems, and also on the serological specificity of the hemoglobins of the different species of animals. His work in Holland came to an end when he was offered a post in the Rockefeller Institute for Medical Research in New York and he moved there together with his family. It was here that he did, in collaboration with Levine and Weiner, the further work on the blood groups which greatly extended the number of these groups, and here in collaboration with Weiner studied bleeding in the new-born, leading to the discovery of the Rh – factor in blood, which relates the human blood to the blood of the rhesus monkey.

To the end of his life, Landsteiner continued to investigate blood groups and the chemistry of blood groups and the chemistry of antigens, antibodies and other immunological factors that occur in the blood. It was one of his great merits that he introduced chemistry into the service of serology.

Rigorously exacting the demands he made upon himself, Landsteiner possessed untiring energy. Throughout his life he was always making observations in many fields other than those in which his main work was done (he was, for instance, responsible for having introduced dark field illumination in the study of spirochaetes). By nature somewhat pessimistic, he preferred to live away from people.

Landsteiner married Helen Wlasto in 1916. Dr. E. Landsteiner is a son by this marriage.

In 1939 he became Emeritus Professor at the Rockefeller Institute, but continued to work as energetically as before, keeping eagerly in touch with the progress of science. It is characteristic of him that he died pipette in hand. On June 24, 1943, he had a heart attack in his laboratory and died two days later in the hospital of the Institute in which he had done such distinguished work.

**References:** [http://nobelprize.org/nobel\\_prizes/medicine/laureates/1930/landsteiner-bio.html](http://nobelprize.org/nobel_prizes/medicine/laureates/1930/landsteiner-bio.html)

# Enjoy the humour

A lion came across a pig and said, "I roar and the jungle fears"

Pig replies, "nowadays I sneeze and the world fears",

## HOW THINGS CHANGE WITH TIME

Man to doctor, "is there any way to a long life"?

Doctor: Get married

Man asks: Will it help?

Doctor: No, but the thought of long life will never come to your head.

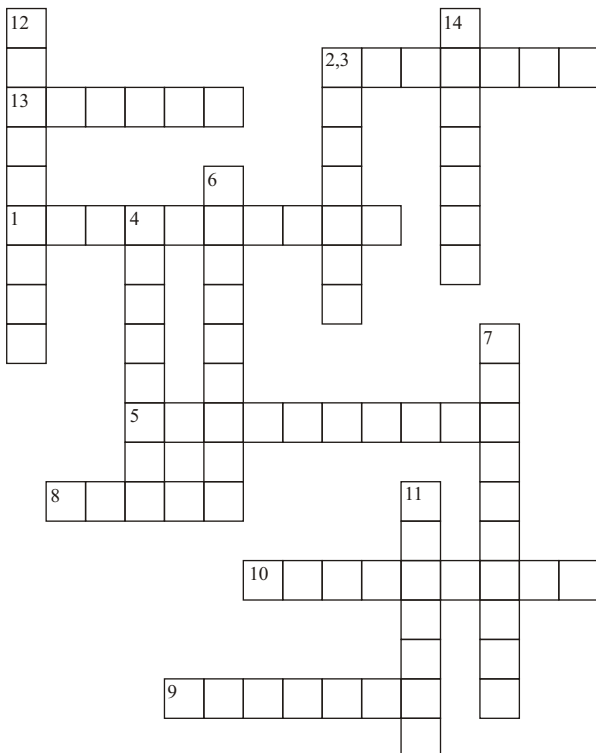
## NEWTON'S LAW OF ROMANCE

Love can neither be created nor destroyed.

It can only be changed from one girl to another.

## Track your brain

Use the hints provided to complete the crossword.



## Thoughts to live by

- I am convinced that life is 10% what happens to me and 90% how I react to it. (Scipio Africanus).
- In every marriage more than a week old, there are grounds for divorce. The trick is to find, and continue to find, grounds for marriage. (Robert Anderson).
- A man's worth is no greater than his ambitions. (Marcus Aurelius).

### ACROSS:

**1.** Mycobacteria secrete a class of chelating agents referred to as (10). **3.** 80% (7) has been recommended as a disinfectant for skin, gloves and clinical thermometers. **5.** Iodine + solubilizing agents forms products termed as (10). **8.** *N. gonorrhoeae* characteristically releases outer membrane fragments called (5) during growth. **9.** (7) refers to painful urination. **10.** (9) is a highly effective biocide which can be safely used on inanimate surfaces as well as on human skin. **13.** (6) syndrome is a genetic disorder that affects girls and women.

### DOWN:

**2.** *M. tuberculosis* grows luxuriantly in culture and such growth is referred to as (7). **4.** (8) containing sanitizing agents can be highly corrosive when used on certain metals. **6.** (9) as sanitizers are commonly used in dairies, breweries and food production plants. **7.** Neisseria sp. are a (10) group of bacteria, they require media to be supplemented in order to grow. **11.** Paper used for sampling of surface bacteria is (7) filter paper. **12.** (9) is the technique used for direct enumeration of bacterial cells. **14.** Active substances in the extracts of normal organs used in Wasserman's reaction are referred to as (7).



## *Neisseria gonorrhoeae*

*Neisseria gonorrhoeae* got its name after it was described by Albert Neisser 1879. It is also known as Gonococci (plural), or Gonococcus (singular), is a species of Gram negative kidney bean shaped diplococci bacteria responsible for the sexually transmitted disease, gonorrhoea.

*Neisseria* are fastidious cocci, requiring nutrient supplementation to grow in laboratory cultures. Specifically, they grow on chocolate agar with carbon dioxide. These cocci are facultatively intracellular in polymorphonuclear leukocytes (neutrophils) of the gonorrhoea pustular exudate and typically appears in pairs (diplococci).

*Neisseria* is usually isolated on Thayer – Martin agar – an agar plate with three different antibiotics and nutrients which not only facilitate the growth of *Neisseria* species, but inhibit the growth of other Gram positive organisms and most bacilli and fungi. Further testing to differentiate the species includes testing for oxidase (all *Neisseria* show a positive reaction) and the carbohydrates; maltose, sucrose and glucose test in which *N. gonorrhoeae* will only oxidize (that is, utilize) glucose.

Cultures are grown at 35 – 36 degrees in an atmosphere of 3 – 10 % added CO<sub>2</sub>.

### Characteristics

*N. gonorrhoeae* possesses a typical Gram negative outer membrane composed of proteins, phospholipids and lipopolysaccharide (LPS). However, neisserial LPS is distinguished from enteric LPS by its highly – branched basal oligosaccharide structure and the absence of repeating O – antigen subunits. For these reasons, neisserial LPS is referred to as lipooligosaccharide (LOS). The bacterium characteristically releases outer membrane fragments called 'blebs' during growth. These blebs contain LOS and probably have a role in pathogenesis if they are disseminated during the course of an infection.

*N. gonorrhoeae* is a relatively fragile organism, susceptible to temperature changes, drying, UV light and other environmental conditions. *N. gonorrhoeae* are able to pull 100,000 times their own weight and it has been claimed that the pili used to do so are the strongest biological motor known to date, exerting one nanonewton.

### Disease

Symptoms of infection with *N. gonorrhoeae* differ depending on the site of infection. Infection of the genitals can result in purulent (or pus – like) discharge from the genitals which may be foul smelling, the other symptoms include inflammation, redness, swelling, dysuria and a burning sensation during micturition. *N. gonorrhoeae* can also cause conjunctivitis, pharyngitis, proctitis or urethritis, prostatitis and orchitis.

Conjunctivitis is common in neonates and silver nitrate or antibiotics are often applied to their eyes as a preventive measure against gonorrhoea. Neonatal gonorrhoeal conjunctivitis is caused when the infant is exposed to *N. gonorrhoeae* in the birth canal, and can result in corneal scarring and perforation.

Disseminated gonorrhoeal infections can occur, resulting in endocarditis, meningitis, or gonococcal dermatitis – arthritis syndrome presents with arthralgia, tenosynovitis and painless non – pruritic dermatitis.

Infection of the genitals in females with *N. gonorrhoeae* can result in Pelvic Inflammatory Disease (PID) if left untreated

which can result in infertility. PID results when *N. gonorrhoeae* travels into the pelvic peritoneum (via the cervix, endometrium and fallopian tubes).

### Infections caused by *N. gonorrhoeae*

The disease gonorrhoea is a specific type of urethritis that practically always involves mucus membranes of the urethra, resulting in a copious discharge of pus, more apparent in the male than the female. The first usage of the term 'gonorrhoea' by Galen in the second century, implied a "flow of seed". For centuries thereafter, gonorrhoea and syphilis were confused, resulting from the fact that the two diseases were often present together in infected individuals. The discovery of gonorrhoea having a different etiological agent came to light only after A. Neisser described it.

Gonorrhoeal infection is generally limited to superficial mucosal surfaces lined up with columnar epithelium. The areas most frequently involved are the urethra, cervix, rectum, pharynx, and conjunctiva. Squamous epithelium, which lines the adult vagina, is not susceptible to infection by the *N. gonorrhoeae*. However, the prepubescent vaginal epithelium, which has not been keratinized under the influence of estrogen, may be infected. Hence gonorrhoea in young girls may present as vulvovaginitis. Mucosal infections are usually characterized by a purulent discharge.

Uncomplicated gonorrhoea in an adult male is an inflammatory and pyogenic infection of the mucous membranes of the anterior urethra. The most common is a discharge that may range from a scanty, clear or cloudy fluid to one that is copious and purulent. Dysuria (difficulty in urination) is usually present. Asymptomatic infections occur in males, as well. Males with asymptomatic urethritis are an important reservoir for transmission and are at an increased risk for developing complications.

Endocervical infection is the most common form of uncomplicated gonorrhoea in women. Such infections are usually characterized by foul smelling vaginal discharge and sometimes by dysuria. About 50% of women with cervical infections are asymptomatic. Asymptomatic males and females are a major problem as unrecognized carriers of the disease, who may unknowingly transmit the infection to their partners.

The course that the organism takes in both the gender differ, in males, the organism may invade the prostate resulting in prostatitis, or extend to the testicles resulting in orchitis. In the female, cervical involvement may extend through the uterus to the fallopian tubes resulting in salpingitis, or to the ovaries resulting in oophoritis. The involvement of testicles, fallopian tubes or ovaries may result in sterility. Occasionally, disseminated infections occur.

### Virulence factors present in *N. gonorrhoeae*

Designation	Location	Contribution
PilE	Major fimbrial protein	Initial binding to epithelial cells
P.II (Opa)	Outer membrane protein	Contributes to invasion
P.I (Por)	Outer membrane protein	May prevent phagolysosome formation in neutrophils and / or reduce oxidative burst

<b>LOS</b>	Outer membrane Lipooligosaccharide (LOS)	Elicits inflammatory response, triggers release of TNF
<b>P.III (Rmp)</b>	Outer membrane protein	Elicits formation of ineffective antibodies that block bactericidal antibodies against PI and LOS
<b>Tbp1 and Tbp2</b>	Outer membrane receptors for transferrin	Iron acquisition for growth
<b>Lpb</b>	Outer membrane receptors for lactoferrin	Iron acquisition for growth

### Laboratory Diagnosis

In the acute stage of disease, diagnosis can be established readily, but chronic cases sometimes present great difficulties. In acute gonorrhoea, the urethral discharge contains gonococci in large numbers. The meatus is cleaned with a gauze, this gauze is soaked in saline. Also a sample of the discharge is collected with a platinum loop for culture, or directly on slides for smears. In females, besides the urethral discharge, cervical swabs should also be collected. This should be done carefully, using a speculum. High vaginal swab is not satisfactory.

In chronic infections there may not be any urethral discharge. The 'morning drop' of secretion may be examined or some exudate may be obtained after prostatic massage. It may also be possible to demonstrate gonococci in the centrifuged deposits of urine in cases where no urethral discharge is available.

The demonstration of intracellular, Gram negative diplococci in stained smears provides a presumptive evidence of gonorrhoea in the male. It has to be emphasized that diagnosis of gonorrhoea by smear examination is unreliable in females as some of the normal genital flora have an essentially similar morphology. The use of fluorescent antibody techniques for the identification of gonococci in smears has increased the sensitivity and specificity of diagnosis by microscopy.

For culture, specimens should be inoculated on pre-warmed plates immediately on collection. If this is not possible, specimens should be collected with charcoal impregnated swabs and sent to the laboratory in Stuart's transport medium. From acute gonorrhoea, cultures can be obtained readily on chocolate agar incubated at 35°C to 36°C under 5 to 10 per cent CO<sub>2</sub>. But in chronic cases, where mixed infection is usual and in the examination of lesions media such as Thayer – Martin's medium should be used. The growth is identified by morphology and biochemical reactions.

It may not be possible to obtain gonococci in culture from some chronic cases or from patients with metastatic lesions such as arthritis. Serological tests may be of value in such instances. The complement fixation test has been used with varying degrees of success. It becomes positive only some weeks after the infection is established and may remain positive for months or years after the disease has been cured. The test may be positive following meningococcal infections. It is necessary to use a polyvalent antigen because of the antigenic heterogeneity of gonococcal strains. The test is not suitable for routine use, but may be employed in special situations.

### Treatment and Prevention

If *N. gonorrhoeae* is resistant to the penicillin family of antibiotics, then ceftriaxone (a third generation cephalosporin) is often used. Sexual partners should also be notified and treated. Patients should also be treated for other sexually transmitted infections, especially Chlamydia infections, since co-infection

is frequent. Transmission can be reduced by the usage of condoms during intercourse, oral sex and by limiting sexual partners.

The recommended treatment for uncomplicated infections is a third generation cephalosporin or a fluoroquinolone plus an antibiotic (like doxycycline or erythromycin) effective against possible co-infection with *Chlamydia trachomatis*. The current CDC treatment guidelines recommend treatment of all gonococcal infections with antibiotic regimens effective against resistant strains, and the recommended antimicrobial agents are ceftriaxone, cefixime, ciprofloxacin or ofloxacin.

### Resistance

Gonococcus is a very delicate organism, readily killed by heat, drying and antiseptics. It is a strict parasite and dies in 1 – 2 hours in exudates outside the body. Formerly, it was highly susceptible to sulphonamides, penicillin and many other antibiotics. But gonococci have steadily developed resistance to one antibiotic after another.

In cultures, the coccus dies in 3 to 4 days, but survives in slant cultures at 35°C if kept under sterile paraffin oil. Cultures may be stored for years if frozen quickly and left at –70°C.

### Epidemiology

Gonorrhoea is an exclusive human disease, there being no natural infection in animals. Experimental disease may be produced in chimpanzees by urethral inoculation. A lethal infection can be produced in mice by intracerebral inoculation.

The only source of infection is a human patient or carrier. The existence of asymptomatic carriage in females makes them a reservoir serving to perpetuate infection among their male contacts. The mode of infection is almost exclusively venereal. Fomites do not play any significant role as the cocci die rapidly outside the human body. The only nonvenereal infection is ophthalmia neonatorum. Measures that are implemented include the practice of instilling silver nitrate solution into eyes of all newborn babies (Crede's method).

When sulphonamides and, later, penicillin were found very effective for treatment of gonorrhoea, it was hoped that the disease could be eradicated. But after a temporary decline, the incidence of the disease has been rising steeply. In some areas, gonorrhoeae has reached epidemic proportions, especially in adolescents and young adults. The reasons for the increase in gonorrhoeal infection are largely social and cultural. However a higher incidence of gonorrhoea has been observed in persons belonging to blood group B, the basis for which is not known.

### Control

There is no effective vaccine to prevent gonorrhoea. Candidate vaccines consisting of PilE proteins or Por are of little benefit. The development of an effective vaccine has been hampered by the lack of a suitable animal model and the fact that an effective immune response has never been demonstrated.

The evolution of antimicrobial resistance in *N. gonorrhoeae* may ultimately affect the control of gonorrhoea. Strains with multiple chromosomal resistance to penicillin, tetracycline, erythromycin, and cefoxitin have been identified in many parts of the world. Sporadic high-level resistance to spectinomycin and fluoroquinolones has been reported.

However safe sex, one or few sex partners, adequate screening of pregnant women and neonates born to infected women can serve as preventive measures for combating transmission of gonorrhoeal infection.

## Bacterial Enumeration

Microbes such as bacteria are ubiquitous in the environment and are present everywhere. Their presence in a particular condition can be either beneficial, harmful, or neither of both and may not be significant. However when there is a need for complete or at least a significant need for the absence of any sort of microbes, there is a need to substantially estimate the number of bacteria that are present, such as in consumables, per se food and milk.

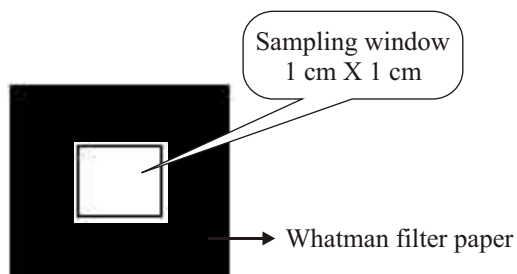
To ensure that bacteria are enumerated adequately there are different methods that can be employed to estimate the actual or at least the relative number of bacteria.

**Enumeration in water:** Will largely depend on the source from where the water sample is obtained. The turbidity of the water will also play a major role in determining the method that has to be used for the approximation of the microbial count. The sample of water is ideally collected in a sterile or clean container free from any debris and residue which may influence the bacterial count

**Enumeration in food:** Like water, food too has to be collected in clean, sterile containers before being tested. This food which may be collected in a solid form needs to be broken down into smaller parts and for this purpose gadgets like stomacher are used.

Food is firstly mixed with saline or any solvent that is recommended and then this food + solvent mixture is introduced into the stomacher which will macerate the food. This process will cause the bacteria in the food sample to come into the solution since maximum area of food will get exposed to the saline. Next this solution is taken and subjected to further analysis for purposes like enumeration.

**Enumeration on surfaces:** Bacteria that are present on the surface are collected in a very systematic fashion. For this purpose paper is cut in the form of a square within a square. Cut the inner square with the dimension 1 cm X 1 cm.



The paper used for sampling is Whatman filter paper. This paper is sterilized by being placed in a clean dry glass petriplate and is autoclaved for 30 minutes. Then with a sterile pair of forceps the dry whatman sampling paper is placed on the surface that has to be examined. Hold the paper firm in place and with a sterile non-absorbent cotton, wipe the area of the surface within the inner window. Now the cotton is introduced into sterile saline and rinsed, the cotton is removed and the solution is used for further analysis.

**Enumeration in air:** Microbes and bacteria are present even in air and are of significant importance. To enumerate the approximate amount of bacteria in the air, the most commonly used method is placing an agar plate in the area where the the air quality has to be tested. The preferable medium used is nutrient agar. Sterile nutrient agar plates are prepared, opened and placed in the

respective area for 10 to 20 minutes, the plates are closed and incubated at temperatures of choice; preferably at room temperature, alternatively also can be placed at 37 deg. C to estimate the approximate pathogenic load. The other medium that can be used may be Mac Conkey's plates.

### Techniques employed for enumeration

**Plating:** The solution that has been obtained from the prior steps is used in plating. Plating is either done as a spread plate technique or as a pour plate technique. The solution can be diluted depending on its turbidity.

- **Spread plate:** 0.1 ml of the bacteria containing solution is placed on the nutrient agar surface of the plate and with a sterile spreader the solution is uniformly spread on the surface of the medium, the plate is inverted and incubated at an optimum temperature for the period of 24 to 48 hours. Then the colonies are counted and the colony forming units (CFU) / ml calculated.
- **Pour plate:** 1ml of the bacteria containing solution is added to a molten agar which must be approximately 40 degrees in temperature, the solution is mixed uniformly with the molten agar and then this molten agar + solution is poured into a sterile petriplate and allowed to solidify. This plate is then inverted and incubated at the appropriate temperature for 24 to 48 hours.
- **Spiral plating:** For this plating technique a special device called spiral plater is used which rotates in a uniform fashion which in turn results in a systematic distribution of the bacteria thus, one can obtain a better and clearer count and one can obtain distinct CFU / ml.

**Turbidity measurement:** Turbidity is directly proportional to the bacteria present in the sample. According to the turbidity of the solution that has to be tested, dilutions can be made which will result in proper colony forming and less smothering of colonies thus a more appropriate count is obtained.

**Staining:** Bacteria can be stained using the appropriate staining techniques (Gram staining is most common) for the determination of Gram character of the bacteria and a gross estimation of the bacterial counts can be made, that are present in the sample obtained.

**Cytometry:** Is the direct enumeration of cells. By this method, viable as well as non viable cells are estimated, however this technique has the advantage of obtaining faster results, since there is no need for incubation of the sample, prior to enumeration of bacterial counts.

- **Hemocytometer:** Is a glass slide that is distinctively marked which facilitates the enumeration of cells on it. It has specialized squares which can also help to measure the size of the organism.
- **Flow cytometer:** Employs a technique to count cells as they are flowing. This system is complicated and requires the operator to have good skill and knowledge.

By far the above are a few techniques that are used in bacterial enumeration, which are employed by microbiologists on a common basis, however there are many other practices that are carried out which may serve the purpose and are used in various labs.

# Surface Disinfection

## Introduction

Surface disinfection covers a very broad spectrum of objects that surround us, whether they are while we travel, work, learn and touch to even objects which we use to sit, lay down or lean against. All these surfaces are what we are referring to and possibly others.

There is a need to maintain all dairies, abattoirs, breweries and food processing plants as clean and hygienic as possible. When dirty equipment is not in use, a rapid build – up of microorganisms occurs which can result in severe contamination of the foodstuff when the equipment is re – used. If proper attention is not given to the use of clean equipment and reduction of contamination, the foodstuff will spoil rapidly. Proper sanitization will reduce the number of bacteria in all work areas and on equipment in particular.

Sterilizing should not be confused with sanitizing or disinfection. To sterilize means to destroy all forms of life; applied specially to microorganisms, including bacterial and mold spores. There are no degrees of sterilization, a reference item so to speak is either sterile or it is not.

To disinfect is to literally free from infection. This term has come to imply chemical treatment of an inanimate surface or substance to rid it of harmful micro – organisms. Disinfectants are frequently expected to perform their function in the presence of significant quantities of dirt and / or organic matter. Though organic matter may increase bioburden for a particular disinfectant to work, it still has to have the capacity to function adequately.

Sanitizing is reducing the number of bacterial contaminants to levels judged safe by Public Health authorities. It implies a degree of physical cleanliness, i.e. the sanitizer is applied to a pre-cleaned surface. In commercial eateries, industries, farms etc, disinfection is carried out mainly using sanitizing solutions which suit the purpose and serve the benefit of the user as well as the consumer or the customer.

Sanitizing of equipment and utensils is best carried out just prior to use. It is one of the most important step in the general sanitation operation for the following reasons:

- A variety of microorganisms remain on food processing equipment after it has been washed, even though it may appear clean. The organisms may be types which have been slowly accumulating on the equipment and or in the product during the processing operation. These can be removed, after cleaning the equipment, by thorough sanitizing.
- During the period that the processing equipment is unused, large numbers of bacteria may develop even though the equipment was cleaned and sanitized. This is especially true of surfaces which are difficult to dry. There are usually sufficient nutrients to support bacterial growth even on a clean surface and if it is moist, the increase in bacteria before the next usage may be tremendous.
- There may possibly be opportunity for insects or even rodents to contact idle equipment and this may result in appreciable

contamination and sometimes even spread of diseases.

- Water supplies occasionally become contaminated and even Municipal supplies are sometimes of questionable quality. When such water is employed for washing or rinsing equipment, spoilage organisms may contaminate the equipment. Thus the use of a sanitizing agent in the water used to rinse equipment helps prevent such contamination.
- A programme of effective sanitizing can make an appreciable and measurable contribution to the quality and shelf life of food products.

## Selection of Sanitizers

There are many types of chemical compounds used in the formation of disinfectants and sanitizers. However in the food industry the number of varieties which can be used is severely limited for a number of reasons.

- The compounds used for the purpose must not be toxic to humans in as much as their residues on food must not be harmful in any way to the quality of food and the consumer.
- They must not taint the product and must therefore be completely odorless.
- They must not color the product in any way.
- They must be relatively safe to use for hand cleaning situations.
- Most essential is high bactericidal activity.

When selecting a sanitizer, the six most popular types should be considered for their respective merits. Known by their primary ingredients they are chlorine compounds, iodophores or iodine compounds, quaternary ammonium compounds (QATS), acid anionic surfactant germicides, hydrogen peroxide and phenol.

## Chlorine based sanitizers

Are most commonly used. Proven in use and acceptance over the years, they have excellent germicidal power against a wide range of bacteria. In properly blended products, they are relatively non – toxic at use concentrations (200 ppm), colorless, non – staining, easy to prepare and apply. Generally they are also the most economical. Effective cleaning is essential when using these sanitizers as some of the available chlorine may be readily consumed by organic matter other than bacteria. Possible flavor problems associated with these products should be borne in mind in the brewing industry.

Chlorine is highly corrosive to a number of metals and its use is best confined to equipment fabricated in stainless steel. Temperature is another important parameter as the effectiveness of chlorine increases with increase in temperature. However above 50°C the liberated chlorine is rapidly lost to the atmosphere, reducing the effectiveness of the solution in situ.

Chlorine compounds should therefore not be used above 50°C neither should they be used where smoked products are being handled. This is because the phenolic compounds in the smoke

may react with the chlorine, producing chlorophenols which have a very strong odor, considered offensive in consumables.

### Iodophores

Are basically a combination of iodine and a solubilizing agent that releases free iodine when diluted with water. They possess quick microbial action against a wide variety of microorganisms. At use concentrations, they are non staining, relatively non toxic, non irritating and stable. No potable rinse is required if use concentration does not exceed 25 ppm available iodine.

Iodophores penetrate soil rapidly and are highly germicidal at virtually all concentrations. Many iodophores are approved for 'no rinse' sanitizing applications at 25 ppm. Iodophore-use solution temperatures should not exceed 48°C or they will begin to 'gas – off'. The germicidal performance of different iodophore formulations may differ greatly. Products yielding the same pH and iodine concentration may yield vastly different germicidal activities at equivalent dilutions. Iodophores can be used in very hard water.

### QATS

The quaternary ammonium compounds are types of cationic detergents possessing good antibacterial activity. Unfortunately their detergency properties are very poor, but they are good wetting agents. They are widely used throughout the food and meat industries and commonly formulated with detergents to form detergent / sanitizers, which clean and kill bacteria in one operation. They can also be used on their own. Although extremely effective for killing a wide spectrum of bacteria, some groups of bacteria are resistant to them. In use concentrations (200 ppm) QATS are odorless, colorless and non toxic. They are stable when heated and in the presence of organic soil. No potable water rinse is required if concentration is at or below 200 ppm active ingredient. They should not be used on processing equipment in a brewery because of possible adverse effects on head retention and flavor.

The bacteriostatic properties of the QATS plus their stability and the property of being absorbed onto surfaces results in such remaining sanitized for many hours after treatment.

QATS have generally been applied in preference to chlorine under conditions of heavy organic contamination where, to overcome the presence of the organic material, the strength of the chlorine would have to assume corrosive proportions. Generally they are combined with specific non ionic detergents for sanitizing dairy equipment.

QATS can be adversely affected by water hardness and may be incompatible with other compounds. They are completely inactivated by anionic compounds such as soaps. Acidity decreases the efficiency of many QATS to such an extent that at pH 3 their germicidal activities almost disappear while at pH 10, they show greatly improved activity. Temperature also affects their activity and an increase of about 20°C normally doubles it.

### Acid anionics

Acid anionic surfactant germicides are combinations of organic and inorganic acids with surface active agents. The acid is usually phosphoric. The germicidal effect is provided by the low pH as well as the activity of the surfactant. The acidity of this type of germicide is effective in removing or controlling the formation of

microbial films. Acid anionics are low foaming agents. They are effective in hard and soft water and eliminate the need for acid rinsing. They are also non corrosive to stainless steel.

### Peroxide

Hydrogen peroxide sanitizers can be used in dairies, breweries and food production plants. Using this sanitization method does away with many of the disadvantages held by other sanitization. Hydrogen peroxide containing sanitizers supersede conventional halogen sanitizers (Chlorine, Iodine, etc.) and cause the disinfection action to be rapid. They are not detrimental to the environment as when hydrogen peroxide decomposes, hydrogen and oxygen are formed. It is a broad spectrum, fast acting, sanitizer with extremely low toxicity.

### Phenols

Phenol based disinfectants should not be used inside a food processing plant, as they have a strong odor which will contaminate foods, they have good cleaning and disinfecting properties and should be used in stables, poultry growing houses, toilets, drains and compounds. They should be used diluted with warm or preferably hot water. They also have good deodorizing properties.

It is noteworthy to mention that all cleaning and disinfecting chemicals should be used in concentrations mentioned by the manufacturer. The temperature at which they are used should be checked for effective disinfection and hazard – free usage.

Certain chemicals, when mixed with other compounds with which they are not compatible may liberate dangerous toxic vapors and gases. For example acid compounds should never be mixed with strong alkaline or caustic compounds. Serious burns or even death may result.

All disinfectants / sanitizers have a recommended contact time. This is the time required for them to kill the majority of bacteria they come in contact with before manufacturing operations can begin again. As this time may vary from product to product, the manufacturers instruction must be followed strictly.

### Some do's and don'ts with sanitizers

#### DO's:

- Take the time to measure the sanitizer correctly.
- Add the sanitizer to the correct amount of water to make the correct solution for use.
- Use a clean, dry container or bucket for the solution.
- Wash away all the dirt before using the sanitizer.
- Discard the solution when the day's work is finished.

#### DON'Ts

- Use a sanitizer for sterilization.
- Store instruments or cleaning tools in a sanitizer solution.
- Top up sanitizer solution.
- Use yesterday's sanitizer solution.
- Mix sanitizers and detergents it may inactivate both.

Effective disinfection is a key ingredient which can help prevent or at least avoid the transmission of many microbes, more specifically pathogens.

**Microexpress recommends the use of the following kits for the detection and testing of Mycobacteria**

**Acid Fast Decolorizer:** Pre-diluted, ready to use 25% sulphuric acid solution for decolourization of acid fast smears for the screening of *M. tuberculosis* and *M. leprae*.

**ADA-MTB:** For the determination of adenosine deaminase activity in serum, plasma & biological fluids.

**Catalase Detection Kit:** For differentiation of isoniazide resistant strains of *M. tuberculosis* and *M. gastri* from genus mycobacterium based on catalase activity.

**Combicult:** Combipack of solid and liquid medium for *Mycobacterium tuberculosis* isolation.

**Lyfectol:** Mucolytic, disinfectant, specimen pretreatment and buffering system for AFB staining and culture.

**Mycocult:** Ready to use L J. solid medium for *Mycobacterium tuberculosis* isolation.

**Mycostain:** Acid fast stain set for screening of *M. tuberculosis* and *M. leprae*.

**Novachrom:** Rapid two step cold AFB stain.

**Sensicult Primary:** Primary drug containing L-J media panel for MTB sensitivity tests.

**Sensicult Secondary:** Secondary drug containing L-J media panel for MTB sensitivity tests.

**Sensivue Primary:** Drug susceptibility (primary anti-tubercular drugs) test for *M. tuberculosis* with a nitrate reductase assay using proportion method.

**Sensivue Secondary:** Drug susceptibility (secondary anti-tubercular drugs) test for *M. tuberculosis* with a nitrate reductase assay using proportion method.

**Bioshields offers SURFAX™ as a solution for surface disinfection**

Disinfectant Cleaner for Floor and Hard Surfaces

**COMPOSITION:** 0.6% w/v Sodium hypochlorite BP, Stabilizers, Perfume

**DESCRIPTION:** SURFAX is a colorless liquid with a characteristic mildly perfumed odor. It is an effective disinfectant cleaner based on a chlorine releasing action.

**BENEFITS:** Powerful synergistic action  
Enhanced Residual Effect  
Rapid cidal effect  
Disinfects + removes stains of blood and other body fluids  
Chlorine Release Formula  
Stabilized Formula  
Non-corrosive – all surface compatible

**ACTIVITY:** Bactericidal, Fungicidal, Virucidal

**APPLICATION: Medical**  
Disinfection of walls and floor of OT, maternity units, critical care areas, clinics, corridors, disposal areas, dressing rooms and toilets Industrial Pharmaceutical, Food and Dairy Industry.

**AVAILABILITY:** 500 ml, 5000 ml.

