

## Editorial

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The immune system is one of the most active system in the human body and is also responsible for maintaining surveillance and keeping a check on the 'residents' of the human body. The term 'residents' refers to none other than the Normal microflora of the human body which resides on the surface as well as in the other internal organs such as the stomach, however there are areas in the body that normally are entirely sterile, such as the uterus. However when certain sites of the body tend to be neglected or there is no proper immune surveillance there is a possibility that the relatively non harmful microbiota of the body tend to take advantage and give rise to infections, which are referred to as 'opportunistic infections' for this the microflora is sometimes considered 'opportunistic'. The different facets of the host – microbe relationship is dealt with in brief in the 'Mini Review' titled 'Normal flora of the human body and opportunistic infections'.

Sterility testing is an important requisite of various health – care products so that they are free from any contamination. Such procedures would help to prevent the transfer of infectious particles and microbes in the body. Therefore it is essential that sterility testing is made mandatory and the guidelines for the same are followed sincerely. Current Trends delves into Sterility testing and its importance in different fields.

Diphtheria was considered a scourge to mankind and was a dreaded disease before the concept of serum therapy; which was put forth by Emil Adolf Von Behring, who came up with a vaccine for the prevention of diphtheria. This German scientist is 'In profile' of this issue of the Journal.

The 'Bug of the Month' for this issue is *Bordetella pertussis* which is the etiological agent of whooping cough. The bacterium is a Gram negative coccobacilli and is non – motile by nature.

With the increase in food and water borne disease, it would be essential to employ a method that can be used for the detection of pathogens and coliforms which are responsible for multiple diseases. One such method is the 'Hydrophobic grid membrane filter technique' which is described in the section on Did You Know.

With the rise in nosocomial infections and more and more people falling prey to these due to the increasing numbers of HIV cases across the globe, more specifically India, there is an urgent need for the proper maintenance of hygiene in domestic conditions, but more specifically in hospital environments from where infectious particles may get disseminated to other susceptible individuals. Our section on Best practices gives some quick tips on why 'Disinfection of Linen' is important.

Whether it is study, work or play there is always a need for relaxation and a urge to take a break.....Take a break, laugh, think and enjoy, 'Relax your Mood'!!!!!!!

We love to hear from you and every suggestion would draw our attention to some topics which our readers would appreciate and would like to know. So lets hear from you.....

The best thing about the future is that it comes one day at a time....hope each day is good and that you enjoy every bit of it.

# Normal Microflora of the human body and opportunistic infections

It has been estimated that there are more microbial cells inhabiting the human body than there are eukaryotic cells of which it is made up. This normal microflora usually co-exists relatively peacefully with the host and does not cause infection. The mechanism by which this co-existence is achieved are still not properly understood and the interaction between the normal flora and the host is far from simple. For a variety of reasons, however, this interaction can be disturbed and often results in the microflora becoming pathogens. The study of the diseases then caused is important both in terms of treatment and in terms of contributing to our understanding of the mechanisms by which the normal microflora usually interacts with the host.

## Human body and microbes (Human body Microflora)

Many microorganisms inhabit human body externally (on the surface) and indigenous microbes of the human body are commensals, i.e., they do not harm the host. They obtain their nourishment from secretions and excretory wastes of the human body. Some microbes however have mutualistic symbiosis.

They sometimes function as scavengers by ingesting excretory wastes and are beneficial to the host; for instance, certain intestinal bacteria synthesize vitamin B, E and K, whereas others protect the host from the pathogenic microbes by some of the following means:

### Primary colonization

The microbes colonize sites on the host organs and the surface of the skin, by this means the organism gains monopoly on that particular site, which enables it to have its establishment there, thus this colonization ensures that the other microbes, including pathogens do not gain easy access to colonizing the same site. The existing organisms also secrete substances like bacteriocins that are inhibitory to the growth and survival of bacteria from the same genus but a different species (from the one producing the bacteriocin). Thus these organisms guard the area which they inhabit.

### Competition

Amongst the bacterial species there also exists competition, by means of which the existing organism which may be in higher numbers easily outwit the invading organisms or those organisms which tend to threaten the existing population's survival and growth.

**Commensal microflora** consists of those microorganisms, which are present on the body surfaces covered by epithelial cells and are exposed to the external environment (gastrointestinal and respiratory tract, vagina, skin, etc.). Commensal bacteria co-evolved with their hosts, however, under specific conditions they are able to overcome protective host responses and exert pathological effects. Resident bacteria form complex ecosystems, whose diversity is enormous. The most abundant microflora is present in the distal parts of the gut, the majority of the intestinal

bacteria are Gram negative anaerobes. More than 50% of the intestinal bacteria cannot be cultured by conventional microbiological techniques. Molecular biological methods help in analyzing the structural and functional complexity of the microflora and in identifying its components. Resident microflora contains a number of components able to activate innate and adaptive immunity. Unlimited immune activation in response to signals from commensal bacteria could pose the risk of inflammation; immune responses to mucosal microbiota therefore requires a precise regulatory control. The mucosal immune system has developed specialized regulatory, anti-inflammatory mechanisms for eliminating or tolerating non-dangerous, food and air-borne antigens and commensal microorganisms (oral mucosal tolerance).

However at the same time the mucosal immune system must provide local defense mechanisms against environmental threats (eg. invading pathogens). This important requirement is fulfilled by several mechanisms of mucosal immunity: strongly developed innate defense mechanisms ensuring appropriate function of the mucosal barrier, existence of unique types of lymphocytes and their products, transport of polymeric immunoglobulins through epithelial cells into secretions (sIgA – secretory IgA) and migration and homing of cells originating from the mucosal organized tissues in mucosae and exocrine glands. The important role of commensal bacteria in development of optimally functioning mucosal immune system was demonstrated in germ-free animals (using gnotobiological techniques). Involvement of commensal microflora and its components with strong immunoactivating properties (eg. LPS, peptidoglycans, superantigens, bacterial DNA and HSP) in etiopathogenic mechanism of various complex, multifactorial and multigenic diseases, including inflammatory bowel diseases, periodontal disease, rheumatoid arthritis, atherosclerosis, allergy, multi-organ failure, colon cancer has been recently suggested.

## A) Microbes of the skin:

Though human skin secretes some anti – bacterial substances; many bacteria manage to grow on superficial squamous epithelium of the skin. Staphylococcus, Streptococcus. Diptheroids (aerobic corynebacteria), Propionibacterium, moulds, yeasts and some pathogenic bacteria live on the surface of the skin. The skin flora contains both aerobic bacteria, which account for about one half of the cultivable bacteria at most anatomical sites. The flora of the perineal area, however, contains organisms of intestinal origin. The skin flora receive their nutrition from the secretions of sebaceous and dead cells.

## B) Microbes of the mouth cavity:

The oral cavity harbors more than 300 different bacterial species. The concentrations of bacteria in saliva are  $10^8$  to  $10^9$  colony forming units/ml and anaerobic bacteria outnumber aerobic bacteria by 10:1. on the tooth surfaces, the

concentrations of bacteria are  $10^{10}$  to  $10^{11}$  cfu/ml with a predominance of anaerobes. Bacterial concentrations in gingival scrapings are  $10^{11}$  to  $10^{12}$  cfu/ml with anaerobic bacteria outnumbering aerobic bacteria by 1000:1. In the saliva and on the tongue surface, the predominant anaerobic bacteria are cocci, while in the gingival crevice large concentrations of Gram negative rods are recovered. Soluble nutrients and abundance of moisture continuously present in the mouth cavity provide a suitable environment for the growth of bacteria. Several microbes inhabit the buccal cavity; some common ones are: *Staphylococcus aureus*, *S. epidermidis*, *S. mitis*, Peptostreptococci, Lactobacilli, Actinomyces, *Haemophilus influenzae*, *Bacteroides oralis*, *Fusobacterium nucleatum*, *Candida albicans* and *Treponema denticola*.

C) Microbes of the gastro-intestinal tract:

The gastrointestinal tract is the main reservoir of bacteria, representing a surface of  $200\text{m}^2$  separating the  $10^{13}$  eukaryotic cells of the host from  $10^{14}$  bacterial cells. Marked variation in the concentrations and bacteriological patterns of the gastrointestinal flora are observed at different levels of the tract. Microorganisms, such as *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, -hemolytic streptococci, *Haemophilus influenzae* and Neisseria inhabit the pharynx.

A large number of microorganisms are found in the large intestines (colon). They include Gram negative bacilli (*Fusobacterium nucleatum*, *F. necrophorum*, *Bacteroides oralis*), Gram positive bacilli (Lactobacilli, *Eubacterium limosum*, Clostridium), Peptostreptococci, Enterococci, Enterobacter, *Escherichia coli*, Klebsiella, Proteus and *Candida albicans*. The number of ingested bacteria is reduced dramatically due to gastric acidity. A low concentration of organisms is maintained in the small bowel, the major mechanism of population control being intestinal motility, which ensures that non-adherent organisms simply pass through.

Microorganisms found in the upper intestinal tract are different from those in the lower intestinal tract. In the stomach and the proximal small bowel, the microorganisms found as normal flora are a reflection of the oral flora. Bacterial concentration in this region are  $10^2 - 10^5$  cfu/ml intestinal content. In the colon, bacterial concentrations of  $10^{11} - 10^{12}$  cfu/g feces are found. About 500 different bacterial species are recovered in the lower intestine. The most common anaerobic microorganisms are bifidobacteria, lactobacilli and bacteroides.

D) Microbes in the Respiratory tract:

During respiration we inhale a large number of adsorbed microorganisms along with dust particles. Most of them are trapped in the nasal cavity which has a mechanism for trapping the dust and the microbes, however in certain cases the organisms may reach the upper respiratory tract. Some staphylococci, aerobic corynebacteria (diphtheroids), Gram negative cocci (*Branhamella* sp.) and Gram positive rods (*Haemophilus influenzae*) inhabit the nasal cavity. The upper airways, including the oral cavity, the nasal passages, the

nasopharynx and the oropharynx, present various aspects of microbial colonization. Bacteria of the saliva number  $10^8/\text{ml}$ , among which approximately one-half are anaerobic bacteria, while in the gingival crevice the concentration exceed  $10^{11}/\text{ml}$  and anaerobes account for over 99% of viable organisms. Despite anatomical continuity and juxtaposition, the sinus and eustachian tubes like the lower air passages, are generally sterile in the normal state.

E) Microbes of the Urinogenital organs:

Microbes normally present in the Urinary tract, which are relatively harmless but protect the urinary tract from infections by producing acid and other metabolites which inhibit the growth of microbes including pathogens. *Mycobacterium smegmatis* and mycoplasmas are usually found on the external parts of the genitals. Peptostreptococci, Enterobacteriaceae, Clostridium, Staphylococcus, *Candida albicans* and *Trichomonas vaginalis* are some common microorganisms associated with urinogenital organs.

F) Microbes of the mucous membrane of the eye:

Due to the lacramal glands the eye is continuously flushed with tears which wash the eye and excrete the microbes and dust particles present; therefore the eye is apparently free from microbes or at least has only a few micro-organisms present such as *Staphylococcus albus*, *Corynebacterium xerosis* and mycoplasmas and are usually associated with the mucous membrane of the eye.

The human host and its microbial flora constitute a complex ecosystem whose equilibrium serves as a remarkable example of reciprocal adaptation. Intestinal bacteria play an important role in the development of the immune system in neonates. The normal intestinal flora is responsible for resistance to colonization by exogenous pathogenic microorganisms. Nevertheless, it also constitutes a reservoir of potentially pathogenic bacteria in close contact with the host. These bacteria are responsible for opportunistic infections in immuno-compromised hosts. The equilibrium of the flora can be upset by antibiotics, leading to infections as a result of proliferation of antibiotic resistant pathogenic bacteria.

The human body is separated from a myriad of endogenous microorganisms by a simple epithelial layer. Most of these organisms are anaerobic bacteria that must eschew oxygen to survive, multiply and propagate. Paradoxically, they enjoy a commensal existence with a host needing atmospheric oxygen for its own survival. This is made possible by the existence of microclimates with prevailing conditions of low oxidation-reduction potential. The surfaces of the human body harbor a complex indigenous microflora including aerobic and anaerobic bacteria. Qualitative and quantitative aspects of the human microflora vary, depending on the different anatomical sites, which are described as ecological niches.

Anaerobic bacteria are prevalent among the bacterial populations of the human body, particularly on mucus membrane surfaces. The major sites with a rich anaerobic normal flora are the mouth, the gastrointestinal tract and the female genital tract.

**Opportunistic Infections**

An opportunistic infection is an infection caused by microorganisms that usually do not cause disease in a healthy immune system. A compromised immune system, however, presents an "opportunity" for the pathogen to infect. Thus, by taking advantage of a lowered immunity, harmless or rather 'silently' present bacteria tend to cause infections and disease which is a direct result of immunodeficiency.

**Causes**

Immunodeficiency or immunosuppression which result in opportunistic infections can be caused by:

**Malnutrition:**

Adequate nutrition is a basic need for a healthy growth and survival. In cases when the body is deprived of necessary nutrients it may be unable to produce essential proteins (including antibodies) that are responsible for guarding the body against invading pathogens and organisms that may be the normal flora of the human body. The normal flora of the body is kept under constant surveillance by the immune system and thus these do not produce infection, however when the immune system is weakened in the absence of nutrients these microbes tend to invade the tissues causing infection. In cases when these infections are not treated they may result in adverse complications like septicemia and in certain cases even death may occur.

**Recurrent infections:**

Refer to those infections which are recurrent and are responsible for putting the immune system under stress, thus in presence of such an infection other pathogens may tend to increase in number and an otherwise non-harmful microbe may be responsible for a consecutive infection.

Infections acquired earlier in life that becomes active again (as the immune system becomes less effective, the germ flares up and causes the disease).

**Immunosuppressants for organ transplant recipients:**

In order to prevent organ rejection in organ transplant patients, immunosuppressants are prescribed. These drugs are responsible for masking the adverse effects of the immune system on the transplanted organ, but during this time the immune system may also be suppressed from acting against invading pathogens. Therefore during this therapy the patient has to be housed in hygienic conditions. The transplant patient has to take all the required preventive measures to abate infection.

**Chemotherapy for cancer:**

Chemotherapy which is meant to destroy carcinogenic cells in the body also affects healthy cells and makes the individual more prone to infections from bacteria and fungi. Also this therapy puts the body under stress, since the body has to repair the damage that the treatment may have caused.

**HIV or AIDS infection:**

Acquired Immuno-Deficiency Syndrome which is caused by the Human Immunodeficiency Virus creates a condition in the body that weakens the immune system and in an advanced stage of the

disease, fully paralyzes the sophisticated immunity from recognizing and destroying pathogens that can cause potential disease. Therefore it may be noted that an HIV positive person when advances to AIDS can get infected with any microbe even with a low virulence and pathogenicity and the infection can even result in death. At this stage the body may not even react to treatment and may finally succumb to the syndrome (and/or related diseases).

**Genetic predisposition:**

Some individuals are born with a genetic predisposition of a weak immune system which may be due to the several reasons including the fact that antibody producing cells may be immature. There are various types of immunodeficiency disorders which include Severe Combined Immunodeficiency (SCID) in which case there is a defect in the Cell mediated immunity (CMI) as well as Humoral mediated immunity (HMI). In this case the afflicted individual has to be treated with special care, since such individuals are highly prone to infections which can be life threatening.

**Skin damage:**

Skin forms an excellent protective barrier against the entry of pathogens and prevents externally present microbes from coming in contact with the underlying tissues. But in case of a break in the skin, for instance, in cases when there are cuts, bruises, or even in cases of accidents the break in the skin results in the entry of the externally present microbes into the circulatory system which can give rise to opportunistic infections such as a carbuncle which are caused by *Staphylococcus aureus* which is actually included in the normal flora of the skin. Alternatively staphylococcal species may cause Staphylococcus scaly skin syndrome (SSSS).

**Antibiotic treatment:**

Antibiotics are the primary line of treatment for a wide variety of bacterial and fungal infections, but amongst the different side effects, antibiotics may be responsible for the alteration of the gut flora. Antibiotic therapy may also result in the initial flora of the body getting altered, which, can in turn result in opportunistic infections.

**Medical procedures:**

Any medical procedures when done have to be conducted with immense caution and hygiene, devices that are used in surgeries or in any invasive procedures, including injections needles should never be re-used. All the instruments and devices have to be maintained under sterile conditions especially immediately prior to use since these can act as fomites and can carry infectious agents, thus resulting in 'iatrogenic' (physician induced) infections. Negligence in handling such procedures can also result in septicemia (presence of bacteria in the blood stream, which can cause blood poisoning).

**Pregnancy:**

During gestation there are various changes in the physiology of the body and it is these changes that may cause the normal flora of the body to get altered, hence making the body more prone to microbial invasions.

**Potential Opportunists*****Pneumocystis jirovecii:***

Was previously referred to as *Pneumocystis carinii* f. hominis. is a fungus that has been known to be causing a rare form of pneumonia which if left untreated could result in death, however the situation is not so adverse with the advent of better health care. Though the situation has changed the microbe still continues to be of significance in individuals suffering from HIV / AIDS. In fact the disease is a common feature that has been seen in HIV infected persons.

***Candida albicans:***

This fungal infection can be treated with antifungal drugs, depending on the site which is infected. The infection was earlier referred to as Moniliasis, and can cause infection in the mouth, vagina and can even infect the eyes and respiratory tract.

***Staphylococcus aureus:***

Infections that are caused by this bacterium are attributed to minor punctures and breaks in the skin through which this relatively harmless bacterium gains entry into the system and may result in minor localized infections to major diseases that can become systemic. The bacterium may also gain entry via respiratory organs. These bacteria if not properly managed can even result in cardiac arrests which can also be fatal if not immediately acted upon. To avoid this conditions adequate measures have to be taken to either prevent or treat the infection at an early stage. Linezolid is the drug of choice for the treatment of resistant strains.

***Streptococcus pyogenes:***

The mode by which this infection may be acquired is similar to the means by which Staphylococcal infections are contracted. Effective treatment for Streptococcal infections are antibiotics, however the infection will be resolved in 3 to 7 days with or without treatment. However treatment decreases the time the individual is infectious. Antibiotics commonly used are penicillin, amoxicillin, cephalexin and erythromycin are commonly used. But in cases when the infection is left untreated with antibiotics the individual may remain contagious for up to 2 to 3 weeks. Care has to be taken that the infection does not worsen in cases when the infection is left untreated. During this time it is advisable that the individual avoids crowded areas like institutions, and public meetings. HIV infected individuals may be afflicted with diseases attributed to this genus, which is actually a normal commensal on human skin.

***Pseudomonas aeruginosa:***

This bacterium is known to be a opportunist bacterium not only in humans, but also in plants and animals and takes full advantage of a compromised immune system. Infections caused by *Pseudomonas aeruginosa* can be treated with a wide variety of antibiotics including aminoglycosides, quinolones, cephalosporins, carbapenems, polymyxins and monobactams.

***Acinetobacter baumannii:***

Acinetobacter strains, which are responsible for opportunistic infections in humans are multiple drug resistant strains and are referred to as Multiple Drug Resistant Acinetobacter baumannii (MDRAB), however there are some drugs which can be used in

the treatment of the infection, and they include polymyxins, tigecycline and aminoglycosides.

***Toxoplasma gondii:***

Infection by *Toxoplasma gondii* can be acquired by multiple means. The most common and the one causing the most problems is the accidental ingestion of oocysts from infected house cats. Infection may also be acquired by ingesting sarcocysts in undercooked meat. This can be prevented by thoroughly cooking the meat before eating. In healthy individuals, infection with *Toxoplasma gondii*, leads to the production of sarcocysts in various tissues and immunity to additional infections. Thus, an infected but asymptomatic person in good health generally does not need treatment. In case of pregnant women and immunocompromised individuals the drugs of choice are Spiramycin or Pyrimethamine plus sulfadiazine.

***Cytomegalovirus:***

The virus infects people worldwide. The treatment for an established infection is controversial at present. The drugs ganciclovir and foscarnet have been licensed for use in life threatening CMV infection in immunocompromised patients but have not been subjected to clinical trials therefore any damage that these drugs could cause is not known and therefore the therapy has to be closely monitored. CMV lung infection in AIDS patients is not normally treated because they do not mount the CMI necessary for immunopathology; due to which the infection is aggravated.

***Aspergillus sp.:***

Infection may be acquired by inhaling spores and such conditions are visible in persons suffering from HIV / AIDS. Voriconazole is currently the first-line of treatment for invasive aspergillosis. There are other drugs that can be used to treat invasive aspergillosis in patients who cannot take voriconazole. These include itraconazole, lipid amphotericin formulations, caspofungin, micafungin, and posaconazole. Whenever possible, immunosuppressive medications should be discontinued or decreased. In certain cases there may be fungus balls that are formed in the lungs which can lead the patient to spit out blood, which can be life threatening, in such situations a surgery may be conducted to remove the fungus ball that may be formed.

***Human Herpes Virus:***

Kaposi's sarcoma which is said to be caused by Human Herpes Virus (HHV8), is not considered to be curable. Neither surgical removal of the first-detected lesion nor obtaining a complete remission of multiple sites with chemotherapy or other techniques results in cure. Long term survival does occur both with or without treatment, however, survival in classic kaposi's sarcoma is usually years and sometimes decades. Some patients with AIDS related kaposi's sarcoma are still alive 10 years, though most survive only a few years and treatment decisions are usually aimed at palliation. All forms of kaposi's sarcoma are sensitive to radiation therapy. Radiation therapy is especially useful for lesions that are cosmetically disturbing, painful, bleed or protrude from the skin.

Chemotherapy can be used in treatment but there is concern that aggressive treatment might further depress the immune system.

The disease does respond to chemotherapy, both with single agents and a combination therapy.

### Treatment of Opportunistic infections (OIs)

Depending on the infecting microbe; specific treatment may be prescribed. The situation of treating an opportunistic infection may also be a complicated task, especially in cases where the infected individual is an HIV infected person. In such instances there is a need for antiretroviral therapy to be commenced together with specific treatment for opportunistic including bacterial infections.

For HIV positive individuals the treatment given should be highly specific, since the treatment for the opportunistic infections should not be interfering with the antiretroviral therapy, which can result in life threatening situations.

But the complications of opportunistic infections are far more severe when the person suffering from opportunistic infection is particularly a HIV / AIDS patient, in whom the immune system is compromised, in addition he or she is also on anti – retroviral therapy. Though it may not always be possible, 'prevention is better than cure'. The Prevention of a opportunistic infection most specially in these individuals is better than the effort that may be required to cure them.

As the HIV progresses to AIDS, the patient condition may deteriorate and the chances of acquiring opportunistic infections may increase, hence putting the individual at higher risks.

In any case; For any opportunistic infection, the best remedy would be specific treatment; if possible with narrow spectrum antibiotics, proper hygiene and a healthy diet consisting of plenty fresh fruits, pulses, milk, nuts and vegetables.

## Encyclopedia

**Allergy** is a reaction / disorder of the immune system often also referred to as atopy. Allergic reactions occur to normally harmless environmental substances known as allergens; these reactions are acquired, predictable and rapid. Strictly allergy is one of the four forms of hypersensitivity and is called type 1 (immediate) hypersensitivity. It is characterized by excessive activation of certain white blood cells called mast cells and basophils by a type of antibody known as IgE, resulting in an extreme inflammatory response. Common allergic reactions include eczema, hives, hay fever, asthma, food allergies, and reactions to the venom of stinging insects such as wasps and bees.

Mild allergies such as hay fever are highly prevalent in the human population and causes symptoms such as allergic conjunctivitis, itchiness and runny nose. Allergies can play a major role in conditions such as asthma. In some people, severe allergies to environmental or dietary allergens or to medication may result in life-threatening anaphylactic reactions and potentially death.

Variety of tests now exists to diagnose allergic conditions; these include testing the skin for responses to known allergens or analyzing the blood for the presence and levels of allergen-specific IgE. Treatments for allergies include allergen avoidance, use of antihistamines, steroids or other oral medications, immunotherapy to desensitize the response to allergen and targeted therapy.

**Signs and Symptoms:** Many allergens such as dust or pollen are airborne particles. In these cases, symptoms arise in areas in contact with air, such as eyes, nose and lungs. For instance, allergic rhinitis, also known as hay fever, causes irritation of the nose, sneezing, and itching and redness of the eyes. Inhaled allergens can also lead to asthmatic symptoms, caused by narrowing of the airways (bronchoconstriction) and increased production of mucus in the lungs, shortness of breath (dyspnea), coughing and wheezing.

Aside from these ambient allergens, allergic reactions can result from food, insect stings, and reactions to medications like aspirin and antibiotics such as penicillin. Symptoms of food allergy include abdominal pain, bloating, vomiting, diarrhea, itchy skin, and swelling of the skin during hives. Food allergies rarely cause respiratory (asthmatic) reactions, or rhinitis. Insect stings, antibiotics and certain medicines produce a systemic allergic response that is also called anaphylaxis; multiple organ systems can be affected, including the digestive system, the respiratory system and the circulatory system. Depending on the rate of severity, it can cause cutaneous reactions, bronchoconstriction, edema, hypotension, coma and even death. This type of reaction can be triggered suddenly, or the onset can be delayed. The severity of this type of allergic response often requires injections of epinephrine.

The nature of anaphylaxis is such that the reaction can seem to be subsiding, but may reoccur throughout a prolonged period of time.

Substances that come in contact with the skin, such as latex, are also common causes of allergic reactions, known as contact dermatitis or eczema. Skin allergies frequently cause rashes, or swelling and inflammation within the skin, in what is known as a 'wheal and flare' reaction characteristic of hives and angioedema.

### Cause:

Risk factors for allergy can be placed in two general categories, namely host and environmental factors.

**Host factors:** include heredity, sex age and race, with heredity being by far the most significant. However, there have been recent increases in the incidence of allergic disorders that cannot be explained by genetic factors alone.

**Environmental factors:** Four major environmental candidates are alterations in the exposure to infectious diseases during early childhood, environmental pollution, allergen levels and dietary changes.

### Diagnosis:

**Skin testing:** For assessing the presence of allergen-specific IgE antibodies, allergy skin testing is preferred over blood allergy tests because it is more sensitive and specific, and simpler to use, and less expensive. Skin testing is also known as 'puncture testing' and 'prick testing' due to the series of tiny puncture or pricks made into the patient's skin. Small amounts of suspected allergens and/or their extracts (pollen, grass, mite proteins, peanut extract, etc.) are introduced to sites on the skin marked with pen or dye (the ink/dye) should be carefully selected, lest it should cause an allergic response itself. A small plastic or metal device is used to prick or puncture the skin. Sometimes, the allergens are injected 'intradermally' into the patient's skin with a needle and a syringe.

**Blood testing:** During this test the total IgE level of the serum is tested. For this testing now even machines are available. Two types of immunoassays that have been used thus far for the determination of IgE levels in the serum, these are radiometric and colorimetric.

**Treatment:** Frequently the primary line of treatment would commence with distancing the allergen from the sensitive host. In case the allergen is already ingested or inhaled then measures are taken to reduce the inflammation and the effects that are brought about by the allergy. Since histamine that is released by the mast cells is the culprit for the major effects, antihistamines are important in reducing and suppressing the symptoms and is to be taken till the inflammation decreases and as directed by the physician.

# Sterility Testing

Sterility testing is done in order to determine if a pharmacopeial article purporting to be sterile complies with the requirements of the test and is not by itself designed to ensure that a batch of product is sterile. This can only be accomplished by validation of the sterilization procedure or of the aseptic processing procedures. The working conditions in which the tests are performed should be monitored regularly by sampling the air and the surfaces of the working area and by carrying out control tests. The tests are based on the principle that if microorganisms are placed in a medium which provides nutritive material and water, and kept at a favorable temperature, the organisms will grow and their presence can be indicated by a turbidity in the original clear medium.

The test for sterility are designed to reveal the presence of microorganisms in the samples used in the test, interpretation of results is based on the assumption that the contents of every container in the batch have been tested, would also have compiled with the tests. Since every container cannot be tested, a sufficient number of containers should be examined to give a big degree of confidence in the results of the test.

## Different equipments that are useful in Sterility Testing

### Air Supply

Air supplied to the environment should be provided through terminal HEPA filters that should be fitted with audible and / or visual alarms to indicate pressure drop across the HEPA filters. There should be a pressure differential of 10 – 15 Pascals (guidance values) between each of the areas. ie, ambient / airlock and airlock / test rooms. A minimum of 20 air changes per hour is expected.

### Airlock

Entry to the clean room should be via an airlock where operators change into clean-room garments. The airlock should be designed to facilitate the operator from the unclean to the clean end of the room without compromising the aseptic gowning procedure. A step - over bench is a suitable division between these areas. The airlock should contain a full-length wall mirror, gowning instructions and hand washing / drying facilities.

### Clean-room fittings and surfaces

The clean-room should have a minimum of ledges and obstructions to flow of clean air. In general, fittings such as power outlets and light fittings should be flush with walls / ceiling surfaces and sealed to prevent the entrainment of unclean air. Surfaces should be smooth and impervious to the cleaning agents used. The joints between walls/ceiling/floors should be covered to facilitate cleaning. The intercom or communication system should be designed to allow hands-free use.

There should be no extraneous equipment within the clean-room environment.

Ultraviolet lights may be fitted only in pass-through hatches. If there is more than one parallel tube they should be shielded from each other. They should be checked at least annually or whenever new lamps are fitted.

## Other measures include:

### Cleaning, Sanitizing and Disinfection

There should be written instructions for daily, weekly and periodic cleaning and decontamination of the test suite. If an isolator is used, the method of disinfection/sterilization should be specified. Disinfectants should be free of microbiological contamination, which may be achieved by aseptic filtration or use of a product-compatible terminal sterilization method. All disinfectants and detergents should be monitored by testing for contamination.

All cleaning, sanitizing and disinfecting procedures should have been validated for minimum contact time and efficacy.

Surfaces and operators' gloved hands should be disinfected regularly during the test session.

### Environmental monitoring

Environmental monitoring should consist of air sampling, settle plates, surface monitoring and operators' gloved hand plates. Surfaces can be monitored by contact plates, films or swabs. The laminar flow area as well as background room area should be monitored.

Records should be maintained of the numbers and types of organisms isolated and results presented in a format that facilitates early detection of trends. Routine identification of environmental microorganisms to at least the genus level should assist in detecting trends. If the identity of organisms from the environment is to be used as the basis for invalidating a sterility test and performing a repeat sterility test, then a sensitive method of identification such as molecular typing techniques using RNA/DNA homology will be expected.

### Training

Sterility testing should be performed by personnel who have been trained, qualified and certified to perform the various tasks and procedures related to sterility testing. Personnel should undergo periodic re-certification, particularly when problems are detected during the course of routine environmental and negative control monitoring, or when operators perform the test infrequently.

### Clean-room garments

The sterility test operator should wear sterile clean-room garments that consists of a one-piece coverall suit, a head cover, a beard cover if applicable, overshoes, gloves and mask. Garments should be changed for each work session, or at least once daily if validated by personnel monitoring.

### Now.....Towards an improved sterility testing

The sterility test is generally recognized as a flawed test for its stated purpose. As early as 1956 Bryce published an article describing the two critical limitations of this test. He put forth that (1) the test was limited in that it can only recognize organisms able to grow under the conditions of the test, and that (2) the sample size is so restricted that it provides only a gross estimate of the state of 'sterility' of the product lot.

Other concerns about the sterility test include choice of sample size, choice of media, time and temperature of incubation.

Considering the fact that Sterility testing is a difficult task it must

be so designed that the false positives are eliminated, which may generally be due to laboratory contamination from the testing environment or technician error. The environment must meet requirements of the United States Pharmacopeial (USP) for levels viable microbial air and surface counts since USP is a compilation of validated methods and official monographs for pharmaceuticals and medical devices. Growth media used in sterility testing must be meticulously prepared and tested to ensure it can support microbial growth.

### Overview

Many products used in the laboratory need to be certified as sterile. Numerous applications and lab techniques require that the labware used be free of contaminating microbes. Only products that receive a strict and controlled schedule of sterility testing can meet these high demands. USP is considered an industry standard when it comes to sterility testing guidelines.

The validity of the sterility test also known as Bacteriostasis and Fungistasis Test (B&F) must be conducted for each pharmacopeial article. This test is necessary to demonstrate that the product is free from inhibiting factors, thus, eliminating the occurrence of false negative results.

The USP 71 Sterility Test begins with two qualifying assays. They are the Suitability Test (Growth Promotion Test) and the Validation Test (Bacteriostasis and Fungistasis Test).

The Suitability test confirms that each lot of growth media used in the sterility-test procedure will support the growth of less than 100 viable organisms. If the media cannot support the growth of the indicator organisms, then the test fails.

Secondly a portion of each media lot must be incubated and assessed for sterility according to the incubation parameters (time, temperature) established by the method. If the media is found to be non-sterile, then the test fails.

The Validation test determines if the test sample will inhibit the growth of microorganisms in the test media. Stasis, in terms of microbiology, is the inability of a microorganism to grow and proliferate in microbiological media. Bacteriostatic media does not necessarily kill bacteria, it simply may retard bacterial growth and proliferation. The Validation test must be performed on each product prior to and during sterility testing. This test determines if the media volumes are valid for the particular product.

### Processes in Sterility Testing

#### Membrane Filtration Method

This method can be used, in some cases, for solid products which are not readily soluble in the culture medium, a soluble powder or liquid that possesses instant bacteriostatic and fungistatic properties and or non bacteriostatic solid but is most often used for liquid products like oils and ointments. In the membrane filtration method, the test article is passed through a membrane filter, which is designed to retain microbial contaminants while permitting the passage of liquid test articles and inhibitors out of the test system. After the test article passes through the filter, the membrane is rinsed with an appropriate sterile rinse fluid. The membrane filters would capture the microorganism, if present.

The product samples are agitated for at least 15 seconds then filtered through a filter membrane. This membrane is then transferred to a bottle of Tryptic Soy Broth (TSB) media and a bottle of Fluid Thioglycollate media then incubated for 14 days. At day 3, day 7 and day 14 the filter and media are observed for growth. A passing USP sterility test shows no growth in either media over the entire incubation period.

All steps of this procedure are performed aseptically in a class

100 Laminar Flow Hood.

Advantages of the membrane filtration method include: Accommodation of large volume samples (up to 500 ml) and removal of inhibitory substances that inhibit the growth of microorganisms by rinsing the filter membrane with a suitable agent.

The disadvantage of this method: it can consume a large volume of final vial product during B&F testing, especially in the case of final container testing.

### Direct Transfer Method

Typically, the direct transfer method is used for solid dose forms, medical devices, ointments, and creams. For test articles produced by non aseptic manufacturing processes, a bioburden (or microbial limits) assay should be performed. Often, it is necessary to evaluate a non-sterile test article for the presence of objectionable organisms, depending on the intended use of the material. A microbial approach is recommended for the evaluation for objectionable organisms.

All steps of this procedure are performed aseptically in a Class 100 Laminar Flow Hood.

The product samples are aseptically transferred directly into the appropriate amount of Tryptic Soy Broth and Fluid Thioglycollate media. The samples and media are then incubated for 14 days. At day 3, day 7 and day 14 the filter and media are observed for growth. A passing USP sterility test shows no growth in either media over the entire incubation period.

### Direct Inoculation Method

In the direct Inoculation method, the test articles are inoculated directly into tubes or bottles that contain an appropriate medium and are incubated for a period of days.

This process provides a means of sterility testing for materials that cannot be easily filtered. And this process consumes less product volume during the conduct of the bacteriostasis and Fungistasis (B&F) testing.

The disadvantage of this method is volume constrain. Also, inherently bacteriostatic or fungistatic components cannot be removed or adequately neutralized.

Each sterility test should be qualified by a bacteriostasis and Fungistasis (B&F) test. The B&F test demonstrates that the test article itself is not inhibitory to the growth of microbial contaminants that can be present in the test sample. Therefore, it is important first to determine how the sterility test is intended to be conducted and then to design the B&F test to support the intended sterility test methodology. If bacteriostasis or fungistasis is observed during the conduct of the B&F test, adjustments to the testing methodology are made to eliminate, if possible, the cause of the stasis.

For either methods; Upon completion of each sterility test performed, a report and Certificate of Analysis are issued and mailed to the specified contact.

### Certification

The test environment, which includes the laminar flow cabinet or isolator, should be certified at least annually by a competent person for compliance with the specified conditions.

Depending upon the nature of the material that is to be tested and the purpose for which its going to be used determine the protocol for Sterility testing.



## Emil Adolf Von Behring

Birth: March 15, 1854  
 Death: March 31, 1917  
 Nationality: German  
 Known For: Diphtheria vaccine

Emil Adolf Von Behring was born on March 15, 1854 at Hansdorf, Deutsch-Eylau. He is best known for Serum therapy and for his related contribution of diphtheria vaccine. Diphtheria was a disease that was a life-threatening infection of the time and was considered a scourge to mankind.

Behring was the eldest son of the second marriage of a school master with a total of 13 children. Since the family could not afford to keep Emil at a University, he entered, in 1874, the well known Army Medical College at Berlin. This made his education financially practicable, but according to obligation of the institute Behring carried the obligation to stay in military services for several years after he had taken his medical degree (1878) and passed his State Examination (1880). He was then sent to Wohrlau and Posen in Poland. In Posen, Behring carried out a lot of practical work, never the less he also found time during the day to study (at the Chemical Department of the Experimental Station) problems connected with septic diseases.

In the year 1881 – 1883 he carried out important investigations on the action of iodoform, he stated that it does not kill microbes but may neutralize the poisons given out by them, thus being antitoxic. His first publications on these questions appeared in 1882. The governing body concerned with military health, which was specially interested in the prevention and combating of epidemics, being aware of the ability of Behring, sent him to the pharmacologist C. Binz at Bonn for further training in experimental methods. In 1888 they ordered him back to Berlin, where he worked - undoubtedly in full agreement with his own wishes – as an assistant at the institute of Hygiene under Robert Koch. He remained there for several years after 1889, and followed Koch when the latter moved to the Institute for Infectious Diseases. This appointment brought him into close association, not only with Koch, but also with P. Ehrlich, who joined, in 1890, the brilliant team of workers Koch had gathered round him. In 1894 Behring became professor of Hygiene at Halle, and the following year he moved to the corresponding chair at Marburg.

Behring's most important researches were intimately bound up with the epoch-making work of Pasteur, Koch, Ehrlich, Loeffler, Roux, Yersin and others, which led the foundation of our modern knowledge of the immunology of bacterial diseases; but he is, himself, chiefly remembered for his work on diphtheria and on tuberculosis. During the years 1888 – 1890 E. Roux and A. Yersin, working at the Pasteur Institute in Paris, had shown that filtrate of diphtheria cultures which contained no bacilli, contained a substance which they called a toxin, that produced, when injected into animals, all the symptoms of diphtheria. In 1890, L. Brieger and C. Fraenkel prepared, from cultures of diphtheria bacilli, a toxic substance, which they called 'toxalbumin', which when injected in suitable doses into guinea-pigs, immunized these animals to diphtheria.

Starting from his observations on the action of iodoform, Behring tried to find whether a disinfection of the living organism might be obtained if animals were injected with material that had been

treated with various disinfectants. Above all the experiments were performed with diphtheria and with tetanus bacilli. They led to the well known development of a new kind of therapy for these two diseases. In 1890 Behring and S. Kitasato published their discovery that graduated doses of sterilized broth cultures of diphtheria or of tetanus bacilli caused the animals to produce, in their blood, substances which could neutralize the toxins which these bacilli produced (antitoxins). They also showed that the antitoxins thus produced by one animal could immunize another animal and that it could cure an animal actually showing symptoms of diphtheria. This great discovery was soon confirmed and successfully used by other workers.

Earlier in 1898, Behring and F. Wernicke had found that immunity to diphtheria could be produced by the injection into animals of diphtheria toxin neutralized by diphtheria antitoxin, and in 1907 Theobald Smith had suggested that such toxin-antitoxin mixtures might be used to immunize man against this disease. It was Behring, however, who announced, in 1913, his production of a mixture of this kind, and subsequent work which modified and refined the mixture originally produced by Behring resulted in the modern methods of immunization which have largely banished diphtheria from the scourges of mankind. Behring himself saw in his production of this toxin-antitoxin mixture the possibility of the final eradication of diphtheria; and he regarded this part of his effort as the crowning success of his life's work.

From 1901 onwards Behring's health prevented him from giving regular lectures and he devoted himself mainly to the study of tuberculosis. To facilitate his work a commercial firm in which he had a financial interest, built for him well-equipped laboratories at Marburg and in 1914 he himself founded, also in Marburg, the Behringwerke for the manufacture of sera and vaccines and for experimental work on these. His association with the production of sera and vaccines made him financially prosperous and he owned a large estate at Marburg, which was well stocked with cattle which he used for experimental purposes.

The great majority of Behring's numerous publications have been made easily available in the editions of his *Gesammelte Abhandlungen* (Collected papers) in 1893 and 1915.

Numerous distinctions were conferred upon Behring. Already in 1893 the title of Professor was conferred upon him, and two years later he became 'Geheimer Medizinalrat' and officer of the French Legion of Honor. In the ensuing years followed the honorary membership of Societies in Italy, Turkey and France; in 1901, the year of his Nobel Prize, he was raised to the nobility, and in 1903 he was elected to the privy Council with the title of Excellency. Later followed further honorary memberships in Hungary and Russia, as well as orders and medals from Germany, Turkey and Roumania. He also became an honorary freeman (Ehrenburger) of Marburg.

In 1896 Behring married the 18 years old Else Spinola, daughter of the Director of the Charite at Berlin. They had seven children. Behring died at Marburg, Hessen-Nassau on March 31, 1917.

The Nobel Prize medal won by Behring, is kept on display at the International Red Cross and Red Crescent Museum, in Geneva.

### Reference

[www.nobelprize.org/nobel\\_prizes/medicine/laureates/1901/beh-ring-bio.html](http://www.nobelprize.org/nobel_prizes/medicine/laureates/1901/beh-ring-bio.html)

# Enjoy the humour

This psychiatrist walks into his waiting room and sees two men.

One is hanging upside down from his ceiling. The other is sawing an imaginary piece of wood.

The doctor approaches the man who is sawing and asks him what he is doing. "I am sawing wood", the man replies.

And what is your friend doing? Asked the doctor again.

"Oh! He thinks he's a light bulb"

"Well don't you think you should tell him to get down?, the blood is gushing to his head".

"What! And work in the dark?"

Three sons went out on their own and prospered. Getting back together they discussed the gifts they were able to give their elderly mother.

The first said, "I built a big house for our mother". The second said, "I sent her a Mercedes with a driver". The third smiled and said, "I've got you both beat. You remember how mom enjoyed reading the Bible? And you know she can't see very well anymore. I sent her a remarkable parrot that recites the entire Bible. It took elders in the church 12 years to teach him. He's one of a kind. Mama just has to name the chapter and verse, and the parrot recites it."

Soon thereafter, Mom sent out her letters of thanks: "Milton," she wrote to one son, "the house you built is so huge. I live in only one room, but I have to clean the whole house." "Gerald," she wrote to another, "I am too old to travel any more. My eyesight isn't what it used to be. I stay most of the time at home, so I rarely use the Mercedes. And the driver is so rude!" "Dearest Donald," she wrote to her third son. "you have the good sense to know what your Mother likes. The chicken was delicious!"



## Thoughts to live by

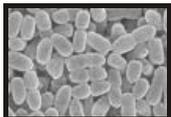
- We shall never know all the good that a simple smile can do. (Mother Teresa)
- Never be bullied into silence. Never allow yourself to be made a victim. Accept no one's definition of your life. Define yourself. (Harvey Fierstein)
- There is only one difference between a long life and a good dinner: that, in the dinner, the sweets come last. (Robert Louis Stevenson)
- Common looking people are the best in the world: that is the reason the Lord makes so many of them. (Abraham Lincoln)
- Better to be silent and to be thought a fool than to speak out and remove all doubt. (Abraham Lincoln)



### Track your brain

1. *Bordutella pertussis* is the causative agent of \_\_\_\_\_.
2. Disinfection of linen can prevent the spread of \_\_\_\_\_ infections
3. Emil Adolf Von Behring was responsible for the development of \_\_\_\_\_ vaccine.
4. \_\_\_\_\_ is an exaggerated immune response to usually harmless substances.
5. The classical treatment for bacterial opportunistic infections is \_\_\_\_\_.
6. The early development of the immune system is largely dependent on the \_\_\_\_\_.
7. Mild acidic solution which when used to treat alkalinity, is referred to as, '\_\_\_\_\_ '.
8. *B. pertussis* releases a protein that moiety ' \_\_\_\_\_ ' that helps the bacterium to bind to the cilia.
9. \_\_\_\_\_ is a device that is used to break food sample mass into smaller parts.
10. A systemic allergic response is also called \_\_\_\_\_.

Check your Answers on Page 16



## *Bordetella pertussis*

*Bordetella pertussis* is a Gram-negative, aerobic coccobacillus of the genus *Bordetella*, and the causative agent of pertussis or whooping cough. *B. pertussis* is a non motile bacterium. *B. pertussis* appear singly or in pairs. Its metabolism is respiratory, never fermentative.

Pertussis or whooping cough is a respiratory tract infection characterized by a paroxysmal cough. It was first identified in the 16<sup>th</sup> century by Bordet, after whom the genus got its name. Another of *Bordetella* species has also been associated with whooping cough in humans, *B. parapertussis*. Before the advent of vaccinations, pertussis was the major cause of morbidity and mortality among infants and children. Reported cases decreased by more than 99% after the introduction of vaccine combined with diphtheria and tetanus toxoids in the 1940. Despite the introduction of vaccines, whooping cough still remains a disease of concern in the domain of public health.

### Habitat

*B. pertussis* colonize the cilia of the mammalian respiratory epithelium. The epithelium is a tissue composed of a layer of cells. The main purpose of the respiratory epithelium is to moisten and protect the airways. It was originally thought that *B. pertussis* did not invade the tissues. Though, recent research has shown that tissue invasion may occur. The bacterium is sometimes known to reside in alveolar macrophages.

These bacteria are nutritionally fastidious and are usually cultivated on rich media supplemented with blood. They can be grown in synthetic medium, however, which contains buffer, salts, an amino acid energy source, and growth factors such as nicotinamide (for which there is a strict requirement). Even on blood agar the organism grows slowly and requires 3 – 6 days to form pinpoint colonies.

### Pathogenesis

After *B. pertussis* has invaded the respiratory tract, the bacteria use several toxins to bind and destroy the epithelial cells. It begins by using hemagglutinin, a protein, which aids the bacteria in binding to the cilia surface. Next, the pertussis toxin, an endotoxin, enters the cells and activates the production of cAMP. This molecule is one of the messengers in cell protein synthesis regulation. Finally, *B. pertussis* releases the tracheal cytotoxin, which causes the destruction of the cilia in the epithelial cells.

The disease pertussis has two stages: The first stage, colonization, is an upper respiratory tract (Mouth, nose and throat) disease which is characterized by fever, malaise and coughing, which increases in intensity over about a 10-day period. During this stage the organism can be recovered in large numbers from pharyngeal cultures, and the severity and duration of the disease can be reduced by antimicrobial treatment. Adherence mechanisms of *B. pertussis* involve a 'filamentous hemagglutinin' (FHA), which is fimbrial-like structure on the bacterial surface, and cell-bound pertussis toxin (PTx). Short

range effects of soluble toxins also play a significant role in the invasion during the colonization stage.

The second or toxemic stage of pertussis follows relative nonspecific symptoms of the colonization stage. It begins gradually with prolonged and paroxysmal coughing that ends in a characteristic inspiratory gasp (whoop). During this second stage of pertussis infection the bacterium can rarely be recovered, and antimicrobial agents have no effect on the progress of the disease.

### Toxins produced by *B. pertussis*:

*B. pertussis* produces a variety of substances with toxic activity in the class of exotoxins and endotoxins.

It secretes its own invasive adenylate cyclase which enters mammalian cells. This toxin acts locally to reduce phagocytic activity and probably helps the organism to initiate infection. This toxin is a 45 Kda protein that may be cell-associated or released into the environment. Adenylate cyclase was originally identified as a hemolysin because it lyses red blood cells. Also it is responsible for hemolytic zones around colonies of *B. pertussis* growing on blood agar. Probably it inserts into the erythrocyte membrane which causes hemolysis.

*B. pertussis* also produces a 'lethal toxin' (formerly called dermonecrotic toxin) which causes inflammation and local necrosis adjacent to sites where *B. pertussis* is located. The precise role of the toxin in whooping cough is not known.

It also produces another substance called the tracheal cytotoxin which is toxic to the ciliated respiratory epithelium and which will stop the ciliated cells from beating. This substance in concentrations of 10<sup>-8</sup>M is also known to affect important neutrophil functions. Therefore it may also contribute to the survival of *B. pertussis* within the airways in vivo.

This substance is not a classical bacterial exotoxin since it is not composed of protein. The tracheal cytotoxin is a peptidoglycan fragment, which appears in the extracellular fluid where the bacteria are actively growing. The toxin kills ciliated cells and causes their extrusion from the mucosa. It also stimulates release of cytokine IL-1, and so causes fever.

Additionally *B. pertussis* produces the pertussis toxin (Ptx), a protein that mediates both the colonization and toxemic stages of the disease. PTx is a two component, A + B bacterial exotoxin. The A subunit (S1) is an ADP ribosyl transferase. The B component, composed of five polypeptide subunits (S2 through S5), binds to specific carbohydrates on the cell surfaces.

### Epidemiology

Pertussis occurs worldwide and is a human disease. No animal or insect source or vector is known to exist. Adolescents and adults are an important reservoir for *B. pertussis* and are often the source of infection for infants.

**High risk groups include:**

Children who are too young to be fully vaccinated and those who have not completed the primary vaccination series are at highest risk for severe illness. Like measles, pertussis is highly contagious with up to 90% of susceptible household contacts developing clinical disease following exposure to an index case. Adolescents and adults become susceptible when immunity wanes, but can receive a booster shot of the new combination vaccine.

**Transmission**

Pertussis is a classical example of 'droplet infection'. Direct contact with droplets from coughing or sneezing by an infected person can carry the infection droplets to another individual. The infected individual can continue to transmit the bacteria even up to three weeks after the coughing spell has stopped. However the infection can also be carried by individuals who are immunized to pertussis and through them the infection can be transmitted to those who are not immunized.

Transmission occurs less frequently by contact with freshly contaminated articles of an infected person. Therefore the possibility of fomites serving as infection reservoirs is declined as compared to other infectious agents.

**Symptoms**

Incubation period for the bacterium is five to ten days. The infected persons usually first shows cold symptoms (i.e., runny nose, sneezing fever and mild cough). The cough worsens and comes in bursts.

At the end of the cough, the infected person takes in air with a high pitched 'whoop' from which the infection gets its name. This sound is also the precise distinguishing characteristic of the infection.

**Complications**

Major complications are most common among infants and young children and include hypoxia, apnea and pneumonia, seizures, encephalopathy and malnutrition. Young children can die from pertussis. Most deaths occur among unvaccinated children or children too young to be vaccinated.

The other complications can include dehydration and inflammation of the middle ear. Infection can also result in loss of appetite.

**Laboratory Diagnosis**

The diagnosis of pertussis is based on a characteristic clinical history (cough for more than two weeks with whoop, paroxysms, or posttussive vomiting) as well as a variety of laboratory tests (culture, polymerase chain reaction [PCR], direct fluorescent antibody [DFA] and serology).

Culture is considered the gold standard laboratory test and is the most specific of the laboratory tests for pertussis. However fastidious growth requirements make *B. pertussis* difficult to culture. The yield of culture can be affected by specimen collection, transportation and isolation techniques. Specimens

from the posterior nasopharynx, not the throat, using calcium alginate swabs. Isolation rates are highest during the first 3 to 4 weeks of illness (catarrhal and early paroxysmal stages). Cultures are variably positive (30 – 50%) and may take as long as two weeks. So results may be too late for clinical usefulness. Cultures are less likely to be positive if performed later in the course of illness (more than 2 weeks after cough onset) or on specimens from persons who have received antibiotics or have been vaccinated. Since adolescents and adults have often been coughing for several weeks before they seek medical attention, it is often too late for culture to be useful.

DFA testing of nasopharyngeal specimens may be useful as a rapid screening test for pertussis. Use of the monoclonal DFA test has improved the specificity, but DFA still has a low sensitivity and should not be relied upon as a criterion for laboratory confirmation.

Serological testing could be useful for adults and adolescents who present late in the course of their illness, when both culture and PCR are likely to be negative.

**Treatment**

The medical management of pertussis cases is primarily supportive, although antibiotics are of some value. Erythromycin is the drug of choice. The therapy eradicates the organism from secretions, thereby decreasing communicability and, if initiated early, may modify the course of the illness.

An antibiotic effective against pertussis (such as azithromycin, erythromycin or trimethoprim-sulfamethoxazole) should be administered to all close contacts of persons with pertussis, regardless of age and vaccination status. All close contacts younger than 7 years of age who have not completed the four-dose primary series should complete the series with the minimal intervals.

Since there is loss of body fluids, a diet with increased fluids intake should be preferred. Also the patient feels extreme weakness therefore adequate rest should be taken as curative measure.

**Case classification:**

**Probable:** Meets the clinical case definition, but is not laboratory confirmed and is not epidemiologically linked to a laboratory – confirmed case.

**Confirmed:** A clinically compatible case that is laboratory confirmed or epidemiologically – linked to a laboratory confirmed case.

The clinical case definition above is appropriate for endemic or sporadic cases. In outbreak settings, including household exposures, a case can be defined as an acute cough illness lasting at least 2 weeks without other symptoms.

**Prevention**

Immunization with pertussis vaccine.

## Hydrophobic Grid-Membrane Filter Technique

With the increase in food borne diseases there is always a need to use better methods for the detection and the enumeration of microorganisms in food and thus techniques including hydrophobic grid membrane filter (HGMF) technique are widely employed.

**Principle:** The HGMF technique is so designed that the enumeration of fecal coliforms depends on formation of blue growths inside HGMF grid cells, indicative of lactose fermentation, after incubation on m-FC (membrane filter coliform) agar at 44.5 deg. C for 18–24 hrs. The HGMF analysis takes approximately 24–26 hrs and yields counts that are as high, but with less random error than the tube Most Probable Number (MPN) method. A single dilution accommodates a wide range of contamination levels. Counting precision may be better than on conventional plates or membrane filters because the HGMF reduces the effect of individual visual acuity on the count. If a low count is expected, the detection levels can be lowered by filtering more of the suspension.

On the surface of conventional membrane filters with a pore size of 0.45µm a grid of hydrophobic material is printed on so that the filtration area is divided into growth compartments. The hydrophobic property of the grid lines determines the positions and limits of the lateral growth of microbial colonies and produces a miniaturized most probable number system equivalent to a large number of tubes inoculated from a single dilution. HGMF provides a counting range of more than 3 log 10 cycles on a single membrane filter.

The HGMF method is capable of detecting fecal coliforms that grow poorly or ferment lactose slowly in LST (Lauryl sulphate tryptose) or BGLB (Brilliant green lactose bile) media.

**Materials and Special equipment:** HGMF (1600 grid-cell, 0.45µm pore size), Membrane Filter forceps, Peptone water (PEP) or peptone water and tween 80 diluent (PT), Tryptic soy-magnesium sulfate agar (TSMS); if a resuscitation step is required, m-FC agar plates, Enzyme solutions as required for some food products which may require prior processing steps, Stomacher (device used for making smaller parts of large masses of sample), Spreadfilter with funnel. HGMF interpreter / reader, Water bath capable of maintaining 35–37 deg. C., Incubators capable of maintaining 25, 35 and 44.5 ± 0.5 deg. C.

### Procedure:

**Handling of sample units:** (1) In the laboratory prior to analysis, except for shelf stable foods, keep sample units refrigerated (0 to 5 deg. C.) or frozen, depending on the nature of the product. Thaw frozen samples in a refrigerator, or under time and temperature conditions which prevent microbial growth or death. (2) Analyze the sample units as soon as possible after their receipt in the laboratory.

**Preparation for analysis:** (1) Peptone water is adequate for many foods, but if foods contain appreciable quantities of fat (cheese and other dairy products, ground beef, etc.) the PT diluent should be used to improve filterability. Also it is preferable to use PT diluent if the spreadfilter is to be used. (2) Have ready sterile peptone water (PEP) or peptone / tween 80 (PT) diluent, tryptic soy magnesium sulfate agar (TSAM) plates, m-FC agar plates and enzyme solutions needed for food product category if required. (3) Clean the surface area of the working area with a suitable disinfectant. (4) Mark clearly the duplicate petri plates

identifying sample, sample unit (is defined as the portion of sample that is used for further analysis), dilution and date of inoculation.

**Preparation of Dilutions:** (1) To ensure a truly representative analytical unit agitate liquids or free flowing materials until the contents are homogenous. If the sample unit is a solid, obtain the analytical unit (from the quantity that is obtained for sampling there is a particular portion that is actually used for the test, which may require sample units from different parts of the same food mass) by taking a portion from several locations within the sample unit. (2) Prepare a 1:10 dilution of the food by aseptically adding 10g or mL (the analytical unit) into 90 mL of the PEP or PT diluent. If the spreadfilter is to be used to inoculate the HGMF, use PT diluent.

**Filtration:** Filtration through the HGMF will remove acids or other inhibitors. (1) Handle HGMF with sterile forceps. (2) Following the manufacturer's instructions for use of the filtration apparatus, aseptically pipette 1.0mL of the required dilution and inoculate the HGMF. Open the filter valve until all liquid has passed through and aseptically remove the HGMF. Do in duplicate. (3) Repeat with subsequent dilutions as required.

**Plating and incubation:** (1) If the organisms in the sample might have been stressed freezing or by other processing, then continue with step 2. (2) Transfer the HGMF to the surface of a TSAM or m-FC plate by rolling it onto the agar to avoid trapping air bubbles. Incubate plates in an inverted position in stacks of not more than two or three, at 25 deg. C for 4 hours for dry foods and 35 deg. C for 4 hours for all other foods OR at 44.5 ± 0.5 deg. C for 24 ± 2 hrs respectively.

**Counting and Scoring HGMF:** (1) The blue growth inside HGMF grid-cells is caused by lactose fermenting organisms, and are enumerated as fecal coliforms. (2) For automated counting, use an HGMF interpreter, following the manufacturer's instructions for its use. For manual counting, use a Linecounter. (3) Count 1 (one) for each grid-cell showing any shade of blue. (DO NOT count the individual colonies if a grid-cell contains more than one blue colony). If a rough estimate indicates fewer than 200 occupied grid-cells, count the whole HGMF. (4) For higher densities (up to 50 % occupied grid cells), rotate the HGMF so that the center indicator lies either to the left or right. Count positive (blue) grid-cells in the four rows immediately below the center and in the four rows immediately below the center (8 rows). Multiply the partial HGMF count by five to estimate the source of only one fifth of the HGMF was counted. (5) If the HGMF is so full that the counting negative (not-blue) grid-cells appears easier, then do so as in step 1 and 2. Subtract the HGMF negative count from 1600 or 320, as appropriate. (6) Multiply by 5 to obtain the score if only one-fifth of the HGMF was counted. (7) Record as too numerous to count (TNTC) any HGMF for which all grid cells are blue. (8) Record the scores of both of the duplicate HGMF. If there are no blue grid cells, record the score as zero.

The HGMF method can be used for the enumeration of fecal coliform organisms in food to determine compliance with the requirements of the Food and Drugs Act. The method has been shown to produce satisfactory results with fish, ground poultry meat, black pepper, cheese and nuts.

# Disinfection of Linen

The rise in the occurrence of nosocomial or hospital acquired infections (HAI) is becoming a huge problem in the hospital / health care sector. The incidence, type and magnitude of HAI varies from hospital to hospital. It is estimated to be around 10% of hospital admissions in South-East Asia. Given the prevailing conditions in the hospitals in developing countries, this is likely to increase.

In a developing country like India, communicable diseases especially infectious, parasitic, gastrointestinal, respiratory diseases and tuberculosis are among the leading causes of deaths. The number of patients with HIV/AIDS is also increasing. In order to fight such a grave situation, hospitals need to develop a programme for the implementation of good infection control measures to ensure the well being of both patients and staff by preventing and controlling HAI.

Three most important components which play an important role for an infection to occur in the hospital are - a susceptible host, a pathogenic micro-organism and an environment that is congenial for the multiplication of the pathogen. The inanimate hospital environment capable of spreading infections comprises of

- air, water, food and medicaments
- Used equipments and instruments
- **Hospital linen**
- Bio medical waste

Out of these used/dirty hospital linen (bed sheets, pillow covers, blankets, gowns, aprons, surgical drapes, scrub suits, towels, uniforms, lab coats etc) is a major potential source of infection as it is likely to be contaminated with a large number of pathogens and may contain sufficient moisture to allow these pathogens to continue to multiply in the warm hospital environment. Irrespective of patients diagnosis, all the used linen of a hospital/ health care center is potentially contaminated. Contaminated linen does not only pose a threat to the patients admitted in a health care facility and the health care workers, it is also dangerous for the laundry personnel responsible for processing hundreds of thousands of kilograms of contaminated reusable linens annually.

Careful management - handling and processing of linen, can therefore, play an important role in stopping or reducing actual pathogen transmission from linen to people or to the environment and cause diseases. Linen management is a complex procedure which requires

- Collection and transportation of contaminated linen
- Sorting of contaminated linen
- Washing and appropriate disinfection of contaminated linen
- Proper storage and distribution of clean linen

Linen management may vary on the basis of the climate, culture, systems and procedures of the individual organization. But it is important that the contaminated linen

- is handled with minimal agitation to avoid aerosolization of pathogenic micro-organisms.
- is placed in closed bags without sorting or washing at the location of use (CDC 1988; OSHA 1991).
- is rolled or folded to contain the heaviest soil in the center of the bundle.
- wet and foul linen/laundry is placed and transported in leak-proof closed bags or containers.
- from different areas, such as wards, surgical units, uniforms are separately collected and processed.
- is transported to laundry-processing area by trolleys / carts / containers with lids and never carried near the body.
- and clean linen is transported in separate and covered trolleys.

Trained and supervised hospital staff who understand the importance of safe handling of contaminated linen can minimize the spreading of pathogens. Measures like use of protective aprons, facial masks, gloves and implementation of effective hand hygiene for the staff handling and processing linen, reduces the risk of exposure to infectious materials and acquiring work-related infections. Collection of soiled linen is a tedious and long process. It may not be possible or practical for the linen handling staff to wash their hands properly with soap and water periodically. Effective hand hygiene can still be achieved by using a liquid quick drying antiseptic handrub which is capable of rapid and powerful cidal action and has residual activity. Thorough cleaning and disinfection of the bags / containers / trolleys / carts used to transport soiled linen after every use with a broad spectrum surface disinfectant can further minimize the chances of contamination.

Collected hospital linen is transported to a sorting area where it is mainly segregated in two categories – foul linen (wet and contaminated with blood or body fluids) and soiled linen (not visibly dirty and not contaminated with blood or body fluids) before washing and is treated separately. Linen from patients suffering from resistant pathogens and linen to be hand washed are also segregated. Sorting of linen before washing is also important to ensure that there are no scalpels, sharp-tipped scissors, hypodermic and suture needles, sharp-tipped towel clips or soiled dressings. Storing of contaminated linen in the laundry area for long is not advisable.

Washing of contaminated linen is the most important step in linen processing. Proper washing using thermal and chemical measures (chemical agents/disinfectants capable of killing / eliminating the micro-organisms) can ensure disinfection of used

linen. The process of eliminating the micro-organisms is known as disinfection/sterilization. It is important to understand the difference between disinfection and sterilization. Disinfection in general refers to the elimination of only pathogenic micro-organisms and not more resistant spores while sterilization means complete elimination of pathogens as well as spores.

Many micro-organisms are physically removed from the linen, by the detergent and water, during the proper washing cycle. Washing linen at high temperature (71°C) in washing machines for at least 25 minutes with a preheating period for at least 5 minutes, is generally recommended as it kills most vegetative bacteria and viruses.

Recent studies have shown that a satisfactory reduction of microbial contamination can also be achieved at lower water temperatures (22-50°C) by monitoring and controlling the cycling of the washer, detergent and certain additives for adjusting the pH of the water and facilitating inactivation of pathogens present in the linen. Addition of a mild acidic "souring" agent neutralizes the alkalinity from the fabric, water and detergent. This shift in pH from approximately 12 to 5 may inactivate any remaining bacteria and reduce the potential for skin irritation.

Plain detergents (bar, liquid or powder) only lower surface tension and help in the removal of dirt/ debris but friction (scrubbing) is required to mechanically remove microorganisms. These detergents do not kill micro organisms. Addition of disinfectants for washing at higher temperatures may not be required. But if hand washing is the only option then contaminated linen needs to be pre-soaked in water, soap and disinfectant. This step helps in loosening and removing excess dirt/debris as well as decontaminating/disinfecting the linen.

Cleaning products having antimicrobial agents both clean and disinfect the linen. In most places along with plain detergents, chlorine releasing agents such as sodium hypochlorite and hydrogen peroxide are used as disinfectants. Use of washing soda and bleaching agents is not recommended as these chemical agents get rapidly neutralized in the presence of organic matter, blood, body fluids, soaps etc and become ineffective. Repeated use of chlorine releasing agents also causes the fabric to decolorize and deteriorate more quickly.

Hospital linen processing also needs to remove blood and body fluid stains from the linen. Therefore, use of cleaning agents is required which are not neutralized in the presence of organic matter, biodegradable, eco friendly, can clean, and disinfect linen without having any adverse effect on them. New quaternary ammonium compounds like Didecyl dimethyl ammonium chloride (DDAC) with broad spectrum disinfection power and additional surfactant action are good option as disinfectant and cleaner.

Spreading linen to dry in sunlight and open air (away from any source of contamination or pollution) allows exposure to ultraviolet sun rays, which produces natural disinfection. In places where drying the washed linen in sunlight is not possible electric dryers are used. Dryer heat is effective in ensuring disinfection. After drying linen is subjected to ironing which also has the same effect. This complete procedure cleans and disinfects the soiled and contaminated linen.

There are areas in a hospital, such as surgical units, which require sterilized linen (drapes, gowns etc). Sterilization of such linens is achieved with the help of steam sterilization/autoclaving following the normal washing and drying cycle to kill any residual spores. Re sterilization of previously sterilized linen requires laundering to rehydrate it.

Infection can be easily transferred between contaminated and uncontaminated items of clothing, laundry and the environment in which they are stored. It is important to store clean linen and supplies in a separate place away from the soiled linens. Shelves used for storing clean linen need to be cleaned periodically. Clean linen should be handled as little as possible.

While addressing one of the most important aspects of linen disinfection, that of preventing Nosocomial or Hospital Acquired Infections and the prevention of the dissemination of pathogens, does not mean that domestic linen should be neglected or only disinfected when visibly soiled.

Domestic linen too gathers dust, microbes and also has the presence of dead skin cells that are shed as a result of body friction with the linen. This linen too has to be cleansed properly as often as possible because the presence of dust particles and mites can cause sensitive individuals to get rashes and other allergic reaction, ranging from skin inflammations to respiratory tract disorders.

While considering linen disinfection, even linen that is soiled by infants and neonates has to be handled with precaution and care, especially in those cases when the infant is sick or shows symptoms of flu, dysentery, skin lesions including scabies, in such cases the linen has to be disinfected thoroughly before the reuse of such linen to avoid further disease progression.

Similar precautions have to be taken for sick, ailing adults. Especially those that are bedridden or having sores. Proper hygiene in such cases is indispensable since the accumulation of microbes on the linen can aggravate the disease and the sores.

Hygiene can not only ensure good health, it can also prevent infection and frequent infections in highly sensitive persons.

**Antibiotic Assay Media** are used for determining antibiotic potency by microbiological assay techniques. The activity or potency of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.

For this purpose, Microexpress offers a whole range of antibiotic assay media.

1. **Antibiotic Assay Medium A (No. 1) (Seed Agar)**  
Antibiotic Assay Medium A (No.1) (Seed Agar) is used for microbiological diffusion assay of  $\beta$ -lactam and other antibiotics.
2. **Antibiotic Assay Medium C (No. 3) (Assay Broth)**  
Antibiotic Assay Medium C (No.3) (Assay Broth) is used as the broth medium in turbidimetric or serial dilution assay of a wide variety of antibiotics.
3. **Antibiotic Assay Medium E (No. 5) (Streptomycin assay Agar with Yeast Extract)**  
Antibiotic Assay Medium E (No.5) (Streptomycin assay Agar with Yeast Extract) is used for microbiological assay of Dihydrostreptomycin, Framycetin & Kanamycin B using *Bacillus subtilis*.
4. **Antibiotic Assay Medium F (No. 8) (Base Agar with low pH)**  
Antibiotic Assay Medium F (No. 8) (Base Agar with low pH) is used for microbiological assay of Oxytetracycline, Tetracycline and Vancomycin.
5. **Antibiotic Assay Medium No. 11 (Neomycin, Erythromycin Assay Agar)**  
Antibiotic Assay Medium No.11 (Neomycin, Erythromycin Assay Agar) is used for microbiological assay of antibiotics.
6. **Antibiotic Assay Medium G (No. 19)**  
Antibiotic Assay Medium No. 19 is used for the microbiological assay of Amphotericin B, Candicidin and Nystatin using *Saccharomyces cerevisiae* (ATCC 9769, ATCC 2601) as the test organism.
7. **Antibiotic Assay Medium No. 32**  
Antibiotic Assay Medium No. 32 is used for preparing inoculum of *Bacillus subtilis* to be used as test organism for assaying Dihydrostreptomycin and Vancomycin by plate assay method.
8. **Antifungal Assay Agar**  
Antifungal Assay Agar is recommended for assaying the antifungal activity.
9. **Muller Hinton Agar**  
Muller Hinton Agar used for antimicrobial disc diffusion susceptibility testing of common, rapidly growing bacteria by the Bauer-Kirby method.

**Linen Disinfection as mentioned in the Best Practices is important in various industries including health care and hotels. Bioshields offers an effective and convenient alternative for linen disinfection.**

**LINOSAFE** is a Aldehyde Free Disinfectant Cleaner for Linen recommended for use in hospitals, hotels and industries with good cleansing power and a rapid bactericidal and fungicidal action. **LINOSAFE** removes all stains and odors, leaving behind a pleasant smell.

**COMPOSITION** : 5% w/v Didecyl dimethyl ammonium chloride.

**BENEFITS** : Aldehyde free  
Disinfectant + cleaner  
Fourth generation QAC  
Rapid Action  
Lint-free  
Hard water tolerant  
Fabric softener

**APPLICATION** : **Medical**  
Infected and non infected hospital linen  
**Industrial**  
Hotel and industrial linen  
**General**  
Home linen

**USAGE DIRECTIONS:**

**Non - Infected Hospital linen** - Pre-soak the Linen in a 0.5%v/v solution of **LINOSAFE** in water for 30 minutes (i.e., 50 ml in 10 Liters of water) and then wash off with plain water.

**Infected Hospital Linen** - Pre - soak the linen in a 0.75% v/v solution of **LINOSAFE** in water for 30 minutes (i.e., 75 ml in 10 Liters of water) and then wash off with plain water.

**Highly Infected Hospital Linen** - Pre - soak the linen in a 1% v/v solution of **LINOSAFE** in water for 30 minutes (i.e., 100 ml in 10 Liters of water) and then wash off with plain water. **Hotel and Industrial Linen** - Pre - soak the linen in a 0.5% v/v solution of **LINOSAFE** in water for 30 minutes (i.e., 50 ml in 10 Liters of water) and then wash off with plain water.

**Track your brain - Answer**

1. Whooping cough.
2. Nosocomial.
3. Diphtheria.
4. Allergy.
5. Antibiotics.
6. Intestinal bacteria.
7. Souring.
8. Hemagglutinin.
9. Stomacher.
10. Anaphylaxis.

**Highlights of the coming issue**

