India is one of the few countries in the world where women and men have nearly the same life expectancy at birth. The fact that the typical female advantage in life expectancy is not seen in India suggests there are systematic problems with women’s health. Indian women have high mortality rates, particularly during childhood and in their reproductive years due to various social and economic issues related to health and society in general. Well Mini Review takes a look at this important aspect and delves into the details of the situation and the hygiene required which is a very crucial requisite in order to decrease maternal mortality.

Current Trends briefly describes the importance of Antimicrobial Preservative Testing Efficacy which is designed to provide a laboratory test that gauges the level of biological activity possessed by the preservative system of a pharmaceutical product. It is not meant to be a simulation of a real – world situation, nor is it meant as a guarantor that a preservative system that meets its requirements will never allow a contaminant to grow in the product. The assay is one that a laboratory can effectively reproduce, and one that will yield comparable results among a variety of laboratories.

Malaria which still is a disease common in the tropics was once a disease that was almost always fatal, however Sir Ronald Ross was the person who correctly discovered the precise means of transmission of the disease and thus the spread of the disease could be considerably contained, which earned him the Nobel prize in physiology or medicine amongst many other honors. Sir Ross is In Profile for the issue.

Bug of the Month Legionella is common in many environments, it is a Gram negative bacterium and, includes species which cause legionellosis or Legionnaires' disease, most notably *L. pneumophila*. which may be readily visualized with a silver stain.

Did You Know that Chromogenic agar which has been a trend setter in the area of microbiology?, it is because it has made testing potential samples, a rapid and a less cumbersome process by making the test microbes distinctly visible from the rest of the plate inhabiting microbes of the same or different genera.

Essential to the survival of all organisms, water has always been an important and life – sustaining drink to humans. This water which is directly consumed in just a drink, and in food, has to be of a good quality. Water which can get easily contaminated with pathogens, metals and radioactive particles, has to be strictly checked in order to ensure good health. Best Practices lays emphasis on the Quality of Potable Water.

In life one always has to move on, why not move on with a smile! well Relax Your Mood and let your mind tick again while tracking the answers to the Quiz.

While you go ahead and enjoy yet another informative issue of the Journal, we invite you to give us your constructive suggestions and comments which would make the Journal a better and a more interactive interface.
Maternal Mortality: Underlying Factors

The extent of maternal mortality is an indicator of disparity and inequity in access to appropriate health care and nutrition services throughout a lifetime, and particularly during pregnancy and childbirth. Every day, 1500 women die from pregnancy- or childbirth-related complications. In 2005, there were an estimated 536,000 maternal deaths worldwide. Most of these deaths occurred in developing countries, and most were avoidable. Extensive efforts have been made since 1987 to describe the extent and etiology of maternal mortality. Maternal deaths take place for two reasons: a direct obstetric death which is caused by complication that develops directly as a result of pregnancy, delivery or the postpartum period; an indirect obstetric death which is due to existing medical conditions that are made worse by delivery or pregnancy.

India is one of the few countries in the world where women and men have nearly the same life expectancy at birth. The fact that the typical female advantage in life expectancy is not seen in India suggests there are systematic problems with women’s health. Indian women have high mortality rates, particularly during childhood and in their reproductive years due to various social and economic issues related to health and society in general. The health of Indian women is intrinsically linked to their status in society. Research on women’s status has found that the contributions Indian women make to families often are overlooked, and instead they are viewed as economic burdens. There is a strong son preference in India, as sons are expected to care for parents as they age. This son preference, along with high dowry costs for daughters, sometimes results in the mistreatment of daughters.

Further, Indian women have low levels of both education and formal labor force participation. They typically have little autonomy, living under the control of first their fathers, then their husbands, and finally their sons. All of these factors exert a negative impact on the health status of Indian women.

Poor health has repercussions not only for women but also their families. Women in poor health are more likely to give birth to low weight infants. They also are less likely to be able to provide food and adequate care for their children. Finally, a woman’s health affects the household economic well-being, as a woman in poor health will be less productive in the labor force.

Many social traditions like child marriage have lead to women becoming more and more prone to health related problems. Marriage and child bearing at a very early age results in the already weak female child to becoming more weak and stressed with the burden of carrying not only for her infant but also her extended family. Pregnancy and abortions are related to many complications that may not seem so obvious in the beginning but eventually show effect.

Causes of maternal mortality
Women die from a wide range of complications in pregnancy, childbirth or the postpartum period. Most of these complications develop because of their pregnant status and some because pregnancy aggravated an existing disease. The four major killers are: severe bleeding (mostly bleeding postpartum), infections (also mostly soon after delivery), hypertensive disorders in pregnancy (eclampsia) and obstructed labour. Globally, about 80% of maternal deaths are due to these causes. Among the indirect causes (20%) of maternal death are diseases that complicate pregnancy or are aggravated by pregnancy, such as malaria, anaemia and HIV. Women also die because of poor health at conception and a lack of adequate care needed for the healthy outcome of the pregnancy for themselves and their babies. As stated by the 2005 WHO report following are the main reasons of maternal mortality:

- Severe bleeding/hemorrhage (25%)
- Infections (15%)
- Unsafe abortions (13%)
- Eclampsia (12%)
- Obstructed labour (8%)
- Unsafe abortions (13%)
- Other direct causes (8%)
- Indirect causes (20%)

Infection is estimated to be the second highest cause of under-reported maternal death. Obstetric infection accounts for more than 12% of maternal deaths. Despite the widespread application of standard aseptic techniques during vaginal birth, cesarean birth, and/or termination of pregnancy, post-pregnancy infections remain a significant source of maternal morbidity and mortality. Infection occurs most frequently in women who have cesarean births, and following spontaneous or elective termination of pregnancy.

Maternal mortality is a small but persistent aspect of induced abortion. Causes of maternal death that arise specifically from abortions include hemorrhage (ante partum or post partum), eclampsia, pre-eclampsia, obstructed and prolonged labor, infection, complications of abortion, disorders related to high blood pressure, anemia, ectopic pregnancy, and cardiomyopathy. The identification of medical and demographic risk factors may have significant implications creating initiatives aimed at decreasing the public health burden associated with maternal mortality.

Pregnancy related causes of maternal deaths
Maternal mortality and morbidity are two health concerns that are related to high levels of fertility. The high levels of maternal mortality are especially distressing because the majority of these deaths could be prevented if women had adequate health services. There are several underlying factors that need to be looked at intrinsically in order to understand the existing problems.

Few pregnant women receive prenatal care
Prenatal care and health care is very important for the health of the woman as well as her fetus. Essential and differential testing can indicate if there are any deficiencies that the woman is suffering from and thus after a differential diagnosis adequate treatment and health care can be provided in order to cause minimal or no birth and congenital defects that may be a direct or indirect cause of deficiency. Thus it is necessary that women should be adequately educated to ensure healthy pregnancies, sound knowledge of using beneficial health care and thus ensure safe childbirths.
Majority of births in India take place at home
Place of birth and type of assistance during birth have an impact on maternal health and mortality. Births that take place in non-hygienic conditions or births that are not attended by trained medical personnel are more likely to have negative outcomes for both the mother and the child. While health care is important, there are several other factors that influence maternal mortality and health. Medical research shows that early age at first birth and high numbers of total pregnancies take their toll on a woman’s health.

Anemia
Anemia (referred to as low levels of red blood cells or alternatively low levels of hemoglobin, which is the molecule that carries oxygen, or may also be low levels of iron in blood) accounts for one in five maternal deaths, is related to an easily treated problem. Anemia is one of the major causes of maternal mortality in India. It is noted painfully that after years of independence India leads iron deficiency anemia cases in the world and more than 90% of Indian women, adolescent girls and children are anemic. Everyone is aware of the fact that anemia results in physical weakness, mental shortcomings, low intelligence and increased vulnerability to a number of diseases and causes adverse pregnancy outcomes and even death of expectant mother. Anemic mothers also bear anemic children. In none of the states were services for anemia included as a component of antenatal care. Data from Rapid Household Survey indicated that even iron folic acid consumption is still very low. Only 22.3% of pregnant women consume Iron and Folic Acid supplementation for 90 days and the percentage is less than 10% among the non-educated women compared to 50% among the well-educated. Also the disparity between rural and urban areas is significant (18% and 34.5% respectively).

Anemia can be treated relatively simply and inexpensively with iron tablets. Severe anemia accounts for 20 percent of all maternal deaths in India and can also increase the chance of dying from a hemorrhage during labor.

Unhygienic conditions or practices
Unhygienic conditions and several practices that are carried out during gestation and consecutively during deliveries can endanger the life of the mother. Unhygienic conditions which is often a consequence of poor monitoring during delivery or of untreated sexually transmitted diseases (STDs), accounts for some 15% of maternal deaths. Infections can be effectively prevented by careful attention to clean delivery and by detection and management of STDs during pregnancy. Systematic postpartum care will ensure rapid detection of infection and its management by appropriate antibiotics.

Sepsis
Sepsis, a very severe infection – is one of the most frequent cause of maternal death. It can be eliminated if aseptic techniques are respected and if early signs of infection are recognized and treated in a timely manner. Another major cause of maternal deaths, due to infections may arise from unsafe abortions, anaemia and improper care during pregnancy. Women who do not eat nutritious food during pregnancies are susceptible to infection. In rural India this is one of the commonest causes of maternal deaths.

Toxemia
Toxemia another cause of maternal mortality, also may be a result of unhygienic conditions and malpractices, however, refers to the presence of toxin in the human system, which can not only be life threatening, but in certain instances be fatal and hence even claim a woman’s life.

Other causes include
Eclampsia:
There are various other causes of maternal mortality. Eclampsia is one of them, which is a fallout of pregnancy-induced hypertension. This usually happens due to improper antenatal care. Hypertension during the course of pregnancy can ultimately culminate in convulsions. Eclampsia if not treated with care in time may lead to the death of the mother.

Hemorrhage:
Another reason of maternal death is Hemorrhage. This may once again be caused by poor antenatal care, anemia during pregnancies or during operative deliveries.

Obstructed or prolonged labor
This occurs when the fetus does not deliver in the anticipated time. This may be due to the wrong position of the fetus, if it is a too large a baby or if the pelvis of the mother is narrow. In urban India, obstructed labor is generally not among the primary causes of maternal deaths anymore but in rural India, due to lack of interest in institutional delivery it is still a cause of maternal deaths.

Intermediate causes
They include the low social status of women, lack of awareness and knowledge at the household level, inadequate resources to seek care, and poor access to quality health care. Other causes are untimely diagnosis and treatment, poor skills and training of care providers, and prolonged waiting time at the facility due to lack of trained personnel, equipment and blood. The other prominent dark chapters of our society are the early age of marriage and child bearing, child spacing, family size and fertility patterns, literacy, socio-economic status and social customs and beliefs.

Reproductive factors
The risk of a woman dying in pregnancy and childbirth depends on the number of pregnancies she has in her lifetime. The higher the number of pregnancies the greater the lifetime risk of pregnancy related death. Maternal mortality rates are also higher among very young women, those aged 35 years and older and those with four or more children.

Socio-economic and cultural factors
The ability of women to command resources and make independent decisions about their fertility, their health and health care also has an impact on maternal mortality. Where women are afforded a low status in society their health needs are often neglected, and existing health facilities may not be accessed by the women in need. Additionally lack of education and understanding around health related issues can contribute to delays in seeking care when it is needed or to the inappropriate management of life threatening pregnancy complications.

• Woman's age: The optimal child bearing age is from 20 to 30 years. There is a gradual increase in the risk of maternal mortality < 20 years and >30 years. Pregnancies outside the age mentioned can be complicated and may have deleterious effects on the child as well as the mother.

• Parity: Parity means the number of children. The higher the
parity, the higher will be the chances of maternal mortality during or after partum.

- Birth interval: There is an increased risk of maternal mortality with short birth intervals. When the interval between pregnancies and child birth is not adequate, there are also chances that the child may be weak and more prone to infections.
- Poor socioeconomic status: This would mean that there is no proper maternal nutrition, this too can be dangerous for the mother as well as her unborn child.
- Bad cultural practices and beliefs.
- Nutritional status, for instance malnutrition.
- Environmental factors like poor sanitary conditions: May make the mother sick and hence the fetus may also get affected in certain cases.
- Lack of maternity services.
- Shortage of manpower in the health sector.
- Poor communication and transport facilities: In cases of emergencies, communication and transportation facilities are indispensable.

### Time to death for most common obstetric emergencies:

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Time to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postpartum hemorrhage</td>
<td>2 hrs</td>
</tr>
<tr>
<td>Antepartum hemorrhage</td>
<td>12 hrs</td>
</tr>
<tr>
<td>Ruptured uterus</td>
<td>1 day</td>
</tr>
<tr>
<td>Eclampsia (severe hypertensive disorder of pregnancy)</td>
<td>2 days</td>
</tr>
<tr>
<td>Obstructed labor</td>
<td>3 days</td>
</tr>
<tr>
<td>Infection</td>
<td>6 days</td>
</tr>
</tbody>
</table>

### Antenatal and intra partum care must contain the following features:

- Early registration of pregnancy (12 - 16 weeks);
- Minimum three Ante-Natal Check-ups;
- Screening all pregnant women for major health, nutritional and obstetric problems;
- Identification of women with health problems/complications, providing prompt and effective treatment including referral wherever required;
- Universal coverage of all pregnant women with tetanus toxoid (TT) immunization and malaria prophylaxis;
- Delivery in a very clean environment;
- Institutional delivery for women with bad obstetric history and high risk factors;
- Training of traditional birth attendants and female health care workers;
- Promotion of family planning;
- Prevention of complications such as eclampsia, malpresentations and ruptured uterus;
- Screening for anemia and providing Iron-Folic Acid (IFA) tablets to prevent anemia;
- Advice on food, nutrition and rest;
- Promotion of institutional delivery / Safe deliveries by trained personnel etc.

### The main problem areas of antenatal check ups lie herewith:

- Inadequate coverage; lack of trained health personnel in antenatal screening, risk identification and referral services;
- Over crowding in PHCs/hospitals;
- Lack of Emergency Obstetric services etc.

One of the major goals of Government of India's Department of Health and Family Welfare is to reduce maternal mortality and morbidity. The focus has shifted from individualized interventions to attention to the reproductive health care, which includes skilled attendance at birth, operationalizing Referral Units and 24 hours delivery services at Primary Health Centers.

### Role of the Government

The challenge for the government however is to help direct and improve privately provided services through appropriate regulatory arrangements and by encouraging an expansion of their scope to include promotion and prevention, in addition to curative care.

The link between pregnancy-related care and maternal mortality is well established. National programmes and plans have already stressed on the need for universal screening of pregnant women and operationalising essential and emergency obstetric care. Focused antenatal care, birth preparedness and complication readiness, skilled attendance at birth, and access to emergency obstetric care are factors that can help reduce maternal mortality.

### The mind boggling high maternal mortality rate in India can be reduced by following the strategies enumerated below:

- Effective initiative from the government is required in terms of proper allocation of resources to all the health institutions specially Primary Health Centers. Even more important is to ensure that the funds actually reach the users whenever it is needed.
- Early registration of antenatal cases and effective health education of couples to make them understand the importance of antenatal check ups, hospital deliveries and small family norms.
- Local dais / birth attendants and female health workers should be imparted periodic training to update themselves with improved techniques and be incorporated as an integral part of health care system. The importance of observing proper aseptic measures while conducting deliveries should be emphasized to them.
- Prevention and early treatment of infection, ante partum and postpartum hemorrhage.
- Wide spread availability / supply of Iron – Folic acid tablets and nutritious food to the poor and remotest corners of the country.
- Treatment of illnesses like diabetes, tuberculosis and malaria during pregnancy should be ensured.
- Construction of better roads and transport facilities is required especially in the rural areas and urban slums to make the health care facilities more available and accessible to people in need.
- Providing facilities for hospital deliveries for high risk cases like severe anaemia, hypertension, diabetes and heart disease.

Women lying in the high risk groups should be given adequate care and proper treatment to prevent fatalities. Treatment must be immediate and sustained with oxytocic drugs and plasma expanders; the means of referral to an equipped facility must be available to women with hemorrhage. Risk factors for obstructed labor include very young age, height below 145 cms, previous prolonged labor or stillbirth, and previous...
cesarean, abnormal presentation, or labor progression. Delivery for these women must be in a facility offering trained doctors and well-equipped operating rooms. Prevention of infection is possible with pre-sterilized delivery kits, antibiotics in kits or within facilities, cleanliness of hands and delivery areas, and maternal tetanus immunization. Identification of edema in pregnancy would prevent eclampsia. Abortion complications could be prevented with safe and early practices and women’s control over fertility.

Though there are a number of factors that play a key role in maternal mortality, in developing countries infections also attribute to deaths, these infections can be prevented by using basic measures such as the following:

- Deliveries should be handled at proper health care facilities under the guidance of professional obstetrics and trained medical staff and also all the emergency equipment that may be required in cases of emergencies.
- All the devices that are used during deliveries or abortions have to be sterile, so that there is no transmission of infectious particles to the mother and or the fetus.
- The hands of all the attendants and the place where the delivery is undertaken have to be essentially monitored and maintained under stringent aseptic conditions, again, in order to minimize infections both for the mother and the child.
- Also vaccines that are to be administered during gestation have to be taken, to prevent infections like tetanus at bay which can lead to death of mothers pre and post-partum.

Disinfection
Disinfection and sterilization are essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients. Several antiseptic agents are available for hand hygiene, skin antisepsis, surface and instrument disinfection. The antiseptic used should be able to perform adequate antisepsis & disinfection. Traditional antiseptics score low in this point due to resistance development, low bioburden tolerance and cytotoxicity. They also should be non-irritating and non-staining. It should also not be malodourous. The traditional alcohol-containing products and iodophors (povidone-iodine) are the most commonly used agents. None of these antiseptics are completely safe and effective.

Alcohols
- Lacks a sustained residual effect on the skin.
- Irritant and toxic to tissue cells, therefore, are unsuitable for application on mucous membranes.

Iodophors (povidone-iodine)
- Have a minimal residual effect.
- Get deactivated in the presence of organic matter and body fluids.
- Not recommended in neonates, particularly pre-term infants.
- Not recommended for thyroid patients.
- May be toxic to tissues.
- Allergy/hypersensitivity is possible.

Modern disinfectants such as Polyhexamethylene biguanidine (PHMB) is an excellent choice. PHMB a polymeric biguanidine, is a broad spectrum cationic surface active antimicrobial agent. It is also one of the multipurpose antimicrobial agents that can be used for skin, surface and instrument disinfection.

Benefits of PHMB:
Chemically stable & non volatile
- PHMB has very low surface activity, having a surface tension essentially identical to water, & consequently can be readily water rinsed from surfaces & do not have residual streaks or tackiness.
- Odorless, non foaming, clear & colorless.
- Easily handled & applied.
- Effective & stable over a wide pH range (4-10).

Unique biguanide chemistry
- Novel non specific mode of action.
- No known evidence of development of organism resistance.

Broad spectrum of activity
- High activity against Pseudomonas, MRSA, VRE, food borne pathogenic organism, viruses & so on.
- Retains activity in presence of organic matter.

Safe antiseptic
- Not cytotoxic to human cells.
- No skin sensitization/irritation.
- Can be used for neonates and thyroid patients.

Mode of action of PHMB
1. Rapid action towards the bacterial surface.
2. Binding to a receptive site on the surface.
3. Overcoming bacterial defense mechanism.
4. Attraction towards the cytoplasmic membrane.
5. Leakage of low molecular weight cytoplasmic components and inhibition of membrane bound enzymes.
6. Extensive disruption of cytoplasmic membranes and leakage of macromolecular components.
7. Precipitation of cell contents and cell death.

Advantages of PHMB over the classically used disinfectants such as povidone iodine
1. Unlike povidone iodine, is not inactivated in the presence of organic matter.
2. Unlike povidone iodine, PHMB is not cytotoxic.
3. Unlike povidone iodine, PHMB can be used for thyroid patients.
4. PHMB is resistance free.
5. PHMB is not affected by sunlight, water, temperature and pH fluctuations. This stability allows PHMB as a better antimicrobial agent.
6. PHMB has Low acute toxicity via dermal & oral route.
7. Low skin & eye irritation potential at in-use concentration.
8. Low toxicity following long term exposure.
9. Not teratogenic & shows no reproductive effects when studied over two generations.
10. Non genotoxic in range of studies.
11. Not considered carcinogenic in humans.

Interventions and Solutions to reduce Maternal Mortality
The persistence of a high maternal mortality rate (MMR) despite half a century of efforts to bring it down indicates that somehow India has not been able to establish appropriate maternal health services especially in the rural areas. An improved, accountable health care system at primary level is essential for decreasing maternal mortality to the desired level. For the same, the
following has to be implemented:
1) Make the antenatal, intranatal and postnatal services accessible to women;
2) Ensure delivery by skilled attendant nurses or doctors;
3) Improve hygienic conditions;
4) Provide better parental care.

In conclusion it can be said that, a maternal death is often not only the result of technical incompetence or negligence, but is also caused by ineffective health system and limited knowledge, social attitudes, poor health and midwife practices by the family and community itself. The health of mother is directly related to her child's health; and without due attention to the causes behind high maternal mortality ratios, we are simply ignoring an important determinant of the health of our nation. In doing so, maybe we are running the risk of damaging our chances for an all-encompassing prosperity in future.

### Psoriasis

Psoriasis is a chronic, non-contagious disease that affects mainly the skin. It is currently suspected to be autoimmune in origin. It commonly causes red, scaly patches to appear on the skin, although some patients have no dermatological symptoms. The scaly patches caused by psoriasis, called 'psoriatic plaques', are areas of inflammation and excessive skin production. Skin rapidly accumulates at these sites and takes on a silvery-white appearance. Plaques frequently occur on the skin of the elbows and knees, but can affect any area including the scalp, palms of hands, soles of feet, and genitals. In contrast to eczema, psoriasis is more likely to be found on the extensor aspect of the joint.

The disorder is a chronic recurring condition that varies in severity from minor localized patches to complete body coverage. Fingernails and toenails are frequently affected (psoriatic nail dystrophy). Psoriasis can also cause inflammation of the joints, which is known as psoriatic arthritis. Ten to fifteen percent of people with psoriasis have psoriatic arthritis. Factors that may aggravate psoriasis include stress, withdrawal of systemic corticosteroid, excessive alcohol consumption, and smoking. There are many treatments available, but because of its chronic recurrent nature psoriasis is a challenge to treat.

**Causes**

The cause of psoriasis is not fully understood. There are two main hypotheses about the process that occurs in the development of the disease. The first considers psoriasis as primarily a disorder of excessive growth and reproduction of skin cells. The problem is simply seen as a fault of the epidermis and its keratinocytes. The second hypothesis sees the disease as being an immune-mediated disorder in which the excessive reproduction of skin cells is secondary to factors produced by the immune system. T cells (which normally help protect the body against infection) become active, migrate to the dermis and trigger the release of cytokines (tumor necrosis factor-alpha TNFα, in particular) which cause inflammation and the rapid production of skin cells. It is not known what initiates the activation of the T cells.

Compromised skin barrier function has a role in psoriasis susceptibility. Psoriasis is a fairly idiosyncratic disease. The majority of people's experience of psoriasis is one in which it may worsen or improve for no apparent reason. Studies of the factors associated with psoriasis tend to be based on small (usually hospital based) samples of individuals. Conflicting findings are often reported. Nevertheless, the first outbreak is sometimes reported following stress (physical or mental), skin injury, and streptococcal infection. Conditions that have been reported as accompanying a worsening of the disease include infections, stress, and changes in season and climate. Certain medicines, including lithium salt and beta blockers, have been reported to trigger or aggravate the disease. Excessive alcohol consumption, smoking and obesity may exacerbate psoriasis or make the management of the condition difficult. Hairspray, some face creams and hand lotions, can also cause an outbreak of psoriasis. Psoriasis occurs more likely in dry skin than oily or well-moisturized skin, and specifically after an external skin injury such as a scratch or cut. This is believed to be caused by an infection, in which the infecting organism thrives under dry skin conditions with minimal skin oil, which otherwise protects skin from infections. The case for psoriasis is opposite to the case of athlete's foot, which occurs because of a fungus infection under wet conditions as opposed to dry in psoriasis. This infection induces inflammation, which causes the symptoms commonly associated with psoriasis, such as itching and rapid skin turnover, and leads to drier skin as the infecting organism absorbs the moisture that would otherwise go to the skin. To prevent dry skin and reduce psoriasis symptoms, it is advised to not use shower scrubs, as they not only damage skin by leaving tiny scratches, they also scrape off the naturally occurring skin oil. It is recommended to use talc powder after washing as that helps absorb excess moisture which would otherwise go to the infecting agent. Additionally, moisturizers can be applied to moisturize the skin, and lotions used to promote skin oil gland functions.

**Diagnosis**

A diagnosis of psoriasis is usually based on the appearance of the skin. There are no special blood tests or diagnostic procedures for psoriasis. Sometimes a skin biopsy, or scraping, may be needed to rule out other disorders and to confirm the diagnosis. Skin from a biopsy will show clubbed Rete pegs if positive for psoriasis. Another sign of psoriasis is that when the plaques are scraped, one can see pinpoint bleeding from the skin below (Auspitz's sign).

**Treatment**

Medications with the least potential for adverse reactions are preferentially employed. If the treatment goal is not achieved then therapies with greater potential toxicity may be used. Medications with significant toxicity are reserved for severe unresponsive psoriasis. This is called the psoriasis treatment ladder. As a first step, medicated ointments or creams, called topical treatments, are applied to the skin. If topical treatment fails to achieve the desired goal then the next step would be to expose the skin to ultraviolet (UV) radiation. This type of treatment is called phototherapy. The third step involves the use of medications which are taken internally by pill or injection. This approach is called systemic treatment. Over time, psoriasis can become resistant to a specific therapy. Treatments may be periodically changed to prevent resistance developing (tachyphylaxis) and to reduce the chance of adverse reactions occurring. This is called treatment rotation. Antibiotics are generally not indicated in routine treatment of psoriasis. However, antibiotics may be employed when an infection, such as that caused by the bacteria Streptococcus, triggers an outbreak of psoriasis, as in certain cases of guttate psoriasis.
The antimicrobial effectiveness test (AET) is designed to provide a laboratory test that gauges the level of biological activity possessed by the preservative system of a pharmaceutical product. It is not meant to be a simulation of a real-world situation, nor is it meant as a guarantor that a preservative system that meets its requirements will never allow a contaminant to grow in the product.

The assay is one that a laboratory can effectively reproduce, and one that will yield comparable results among a variety of laboratories.

The antimicrobial effectiveness test demonstrated the effectiveness of the preservative system in a product. A product is inoculated with a controlled quantity of specific microorganisms. The test then compares the level of microorganisms found on a control sample versus the test sample over a period of 28 days.

**What is an Antimicrobial Preservative?**
Antimicrobial preservatives are substances added to non-sterile dosage forms to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. In the case of sterile articles packed in multidose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced by repeatedly withdrawing individual doses. Examples of antimicrobial preservatives include formaldehydes and alcohol.

**Product Categories**
For testing purposes, the US Pharmacopoeia has divided the test articles into four separate categories:
- **Category 1**: Injections, other parenterals including emulsions, otic, sterile nasal products made with aqueous bases or vehicles.
- **Category 2**: Topically used products made with aqueous bases or vehicles, non sterile nasal products, and emulsions, including those applied to nasal membranes.
- **Category 3**: Oral products other than antacids made with aqueous bases or vehicles.
- **Category 4**: Antacids made with an aqueous base.

**Selection of Preservative**
The preservative must be largely undissociated at the pH of the formulation. Product storage temperature must not harm, as well as other active substances should not interact with the preservatives, this may alter the effective concentration required for its function.

**Importance of antimicrobial efficacy testing during dosage form stability studies**
Pharmaceutical products are subjected to long term as well as short term (stress testing) stability studies to forecast the time for which product remains within specifications. The short term stability studies are used for rapid assessment of stability and as support to long term studies. International Conference for Harmonization advocates short term stability testing of new drug products at 40°C and 75°C relative humidity. Since the preservative, an antimicrobial substance, is aiding in the keeping quality of the product it is necessary to ensure the activity of the same is maintained till the expiry of the product. This necessitates analyzing the concentration of the preservative during stability studies. Relying on chemical assays to provide the necessary assurance of preservative protection would not be sufficient. Since the chemical assay value may be unchanged but the biological activity is altered. This may arise due to:
- Modification in the antimicrobial activity in presence of the excipients in the formulation.
- The preservative may lose its activity due to adsorption onto suspended solids or partition into the non-aqueous phase of an emulsion.
- Leaching of the ingredients from the container may interact with the preservative and modify its activity.
- The level of bioburden associated with the excipients is one common source of contamination in nonsterile pharmaceuticals if it is derived from animal, botanical or mineral sources. Apart from the concentration of the antimicrobial preservative the activity may be influenced due to pH and redox potential which may change during storage.
- The preservative may degrade resulting in an enhanced activity due to its degradation.
- Presence of two or more type of preservative system increases the complexity of the analytical assays.

These factors clearly point to the requirement of the assessment of the preservative efficacy biologically as well as analytically during stability studies.

**Test Organisms**
The test organisms specified were to be tested separately. This method differed from the method supported by some other labs, which used a test with a mixed population of 21 different organisms and assayed for survivors over a 10 week period. The USP used the five species individually which was subsequently shown to be a better indicator of preservative effectiveness than challenging with a mixed culture. Now, when AET is performed in accordance with the United States Pharmacopoeia (USP), five indicator organisms are utilized for the purpose of challenging the preservative system in a product. Three of the five USP indicator organisms, *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538), addresses the growth of bacteria. *Candida albicans* (ATCC 10231) is the representative yeast, while *Aspergillus niger* (ATCC 16404) is a mold. These are specific strains.

The above listed microorganisms are ATCC cultures and must be harvested under current USP guidelines to assure validity.
However, other test microorganisms may be incorporated into the testing as per the requirements of the customer and the specific product needs.

A product is inoculated (contaminated) with a number of microorganisms between $1 \times 10^3$ (100,000) to $1 \times 10^7$ (1,000,000) colony forming units (CFU) per milliliter (mL) of product. At various intervals, depending on the category, the product is tested to determine its ability to control reproduction or destroy the microorganisms.

**Product Criteria**

A logarithmic reduction is evaluated at each test interval required for the category. By test definition, any growth over the allotted amount for any of the indicated microorganisms renders the preservative in the product not effective.

When does a product need an antimicrobial effectiveness test?

As part of the stability study, it is beneficial to determine if a preservative system will stand up to the product's shelf life. It may also be beneficial to determine if the preservative system chosen for a product is compatible with the formulation of the product. The USP procedure is intended for a self-contained finished product. However, it is also necessary to retest the effectiveness of the preservative system any time the formulation is changed or when significant product or packaging changes occur.

Factors influencing reproducibility of the test

The preservative efficacy tests may be influenced by many parameters as:

- Test organisms used are intended to be examples of those that might realistically represent contaminants of the product from the user.
- An infection hazard posed by the products intended use.
- The raw materials used in the manufacturing and its contaminants.
- Presence of contaminants which survive under unfavorable conditions.

Major difference in approach of EP and USP is that EP recommends efficacy testing as requirement in product development documentation while USP recommends the same as mandatory product performance testing.

Preservative efficacy assessment by rapid methods

Since the tests for preservative efficacy are time consuming, they require a 28 day sampling period (in addition to incubation time) and lot of operator input. They are not quantitative, cannot be extrapolated to statistical evaluation, and utilize large volumes of materials; it has become the need of the hour to develop a testing procedure that would help to diminish shortcomings. These tests are not replacements but prelude to pharmacopoeial testing procedures. The term rapid assumes two different meanings:

1. One uses traditional microbiological technique but short sampling period which are extrapolated to predict efficacy over 28 days. The fact that microbial inactivation follows first order kinetics makes it possible to use linear plot of log viable cell count vs. exposure time and D (decimal reduction values) to predict surviving fraction after longer exposure. However, such procedures have limitations such as small error in calculation of D value or slope is magnified in large error in calculated percentage of survivors projected over 28 days. Test organisms may grow back after initial decline to higher than initial value. Many organisms may not follow first order kinetics of inactivation or pseudo first order in case of gross excess of preservative. Most Probable Number (MPN) technique using microtitre plates have also been used for this.

2. Procedures where sampling period is not shortened but measure of viability is completed within shorter time than that required for plate count. These employ alternatives to traditional counting procedures based on biomedical indexes. These are the substances which are present in a living cell which can be used as a marker for detection of these organisms. The methods used to detect these markers include bioluminescence, viability staining using dye reduction and conduction impedance methods that detect electrical changes in culture media resulting from microbial growth.

Validation

The first time a product is tested for antimicrobial effectiveness, a validation is necessary to show the microorganisms are able to withstand the formulation. A full validation is performed in three independent studies with each of the studies recovering not less than 70 percent of the growth inoculum versus the control.

It is necessary to re-validate a product, whenever a formulation change has occurred, when the manufacturing process has been changed, or when changes in packaging occur.

How much product is required?

If a product is submitted in liquid form, a volume of not less than 20 mL is preferred. When submitting granular or powdered dosage forms a weight of 20 grams is preferred. The validation of this product requires an additional 100 mL or grams. At the time of product submittal, it is necessary to note the category to be tested for the product.

Testing time intervals; under normal conditions, the turnaround time to perform the Antimicrobial Effectiveness Test is seven weeks due to incubation requirements. However, if scheduling arrangements are made prior to product submittal, it is possible to reduce the time needed to prepare the necessary organisms used in testing.

Other Antimicrobial Effectiveness Tests

Simulated In-use Antimicrobial Effectiveness Testing

The concern on in-use stability is a prudent one in situations where a volatile component of the preparation can be affected by opening of the container. However, many preparations are very stable to exposure to oxygen, and are packaged in oxygen permeable containers that provide years of stability data during development of the product. These tests are primarily geared to demonstrate the chemical stability of the preparation, but there are also multiple recommendations to perform microbial monitoring of product dispensed as a patient would.

References:

Sir Ronald Ross

Birth: May 13, 1857
Death: September 16, 1932
Nationality: Scottish
Known for: Discovery of the method of transmission of malaria parasites

Ronald Ross was born on May 13, 1857, as the son of Sir C.C.G. Ross, a General in the English army. He commenced the study of medicine at St. Bartholomew's Hospital in London in 1875; entered the Indian Medical Service in 1881. He commenced the study of malaria in 1892. In 1894 he determined to make an experimental investigation in India of the hypothesis of Laveran and Manson that mosquitoes are connected with the propagation of the disease. After two and a half years' failure, Ross succeeded in demonstrating the life-cycle of the parasites of malaria in mosquitoes, thus establishing the hypothesis of Laveran and Manson.

In 1899 he joined the Liverpool School of Tropical Medicine under the direction of Sir Alfred Jones. He was immediately sent to West Africa to continue his investigations, and there he found the species of mosquitoes which convey the deadly African fever. Since then the School has been unremitting in its efforts to improve health, and especially to reduce malaria in West Africa. Ross' researches have been confirmed and assisted by many distinguished authorities, especially by Koch, Daniels, Bignami, Celli, Christophers, Stephens, Annett, Austen, Ruge, Ziemann, and many others.

In 1901 Ross was elected a Fellow of the Royal College of Surgeons of England and also a Fellow of the Royal Society, of which he became Vice-President from 1911 to 1913. In 1902 he was appointed a Companion of the Most Honorable Order of Bath by His Majesty the King of Great Britain. In 1911 he was elevated to the rank of Knight Commander of the same Order. In Belgium, he was made an Officer in the Order of Leopold II.

In 1902 a movement was set on foot to commemorate the valuable services rendered to the School of Tropical Medicine by its originator and Chairman, Sir Alfred Jones, by founding a Chair of Tropical Medicine in University College to be connected with the School. The movement was met with enthusiastic support, and an amount of money was quickly collected sufficient to found «Sir Alfred Jones’ Chair of Tropical Medicine».

Ross was appointed to the Professorship in 1902 and retained the Chair until 1912, when he left Liverpool, and was appointed Physician for Tropical Diseases at Kings College Hospital, London, a post which he held together with the Chair of Tropical Sanitation in Liverpool. He remained in these posts until 1917, when he was appointed Consultant in Malaria to the War Office, his service in this capacity, and in special connection with epidemic malaria then occurring on combatant troops, being recognized by his elevation to the rank of Knight Commander, St. Michael and St. George, in 1918.

He was later appointed Consultant in Malaria to the Ministry of Pensions. In 1926 he assumed the post of Director in Chief of the Ross Institute and Hospital of Tropical Diseases and Hygiene, which had been created by admirers of his work, and he remained in this position until his death. He was also a President of the Society of Tropical Medicine. His Memoirs (London, 1923) were «inscribed to the people of Sweden and the memory of Alfred Nobel».

During this active career, Ross' interest lay mainly in the initiation of measures for the prevention of malaria in different countries of the world. He carried out surveys and initiated schemes in many places, including West Africa, the Suez Canal zone, Greece, Mauritius, Cyprus, and in the areas affected by the 1914-1918 war. He also initiated organizations, which have proved to be well established, for the prevention of malaria within the planting industries of India and Ceylon.

He made many contributions to the epidemiology of malaria and to methods of its survey and assessment, but perhaps his greatest was the development of mathematical models for the study of its epidemiology, initiated in his report on Mauritius in 1908, elaborated in his Prevention of Malaria in 1911 and further elaborated in a more generalized form in scientific papers published by the Royal Society in 1915 and 1916. These papers represented a profound mathematical interest which was not confined to epidemiology, but led him to make material contributions to both pure and applied mathematics. Those related to «pathometry» are best known and, 40 years later, constitute the basis of much of the epidemiological understanding of insect-borne diseases.

Through these works Ross continued his great contribution in the form of the discovery of the transmission of malaria by the mosquito, but he also found time and mental energy for many other pursuits, being poet, playwright, writer and painter. Particularly, his poetic works gained him wide acclamation which was independent of his medical and mathematical standing.

He received many honours in addition to the Nobel Prize, and was given Honorary Membership of learned societies of most countries of Europe, and of many other continents. He got an honorary M.D. degree in Stockholm in 1910 at the centenary celebration of the Caroline Institute. Whilst his vivacity and single-minded search for truth caused friction with some people, he enjoyed a vast circle of friends in Europe, Asia and America who respected him for his personality as well as for his genius.

Ross married Rosa Bessie Bloxam in 1889. They had two sons, Ronald and Charles, and two daughters, Dorothy and Sylvia. His wife died in 1931, Ross survived her until a year later, when he died after a long illness, at the Ross Institute, London, on September 16, 1932.

Reference:
http://nobelprize.org/nobel_prizes/medicine/laureates/1902/ross-bio.htm
Prosecutor: “Now tell the jury the truth, madam. Why did you shoot your husband with a bow and arrow?”
Defendant: “I didn't want to wake the children.”

Surgeon enters the operation theater with a garland in his hand
Patient asks: “What are the flowers for?”
Surgeon says if the operation is successful, its for me, if not its for you.

A ship was sinking.
CAPTAIN – Does anyone know to pray?
PRIEST – Yes I can pray.
CAPTAIN – ok, you pray, everyone else will wear a life jacket as we are one jacket short.

Track your brain

Use the hints provided to complete the crossword.

**Across**
1. ______ (10) & infections are two of the major causes of maternal mortality.
7. Sir Ronald Ross is constantly associated with the discovery of the transmission of ______ (7).
9. _____ (11) media has revolutionized microbiological testing.
10. _____ (13) of drinking water poses a major threat to human health.
12. _____ (4) is sometimes a metal contaminant in drinking water.

**Down**
2. Hypertensive disorders in pregnancy are referred to as ______ (9).
3. ______ (7) refers to the presence of toxin in the human system.
4. ______ (9) is a chronic, non contagious disease that affects mainly the skin.
5. Development of resistance to a certain drug therapy is referred to as ______ (13).
6. Category 1 of the test articles that requires Antimicrobial Effectiveness Testing include _____ (11).
8. Transmission of Legionella pathogen occurs by means of _____ (14).
11. Gross alpha test is used to determine any dissolved ______ (13) in water.
Legionella species

Legionella is a Gram negative bacterium, including species that cause legionellosis or Legionnaires' disease, most notably L. pneumophila. It may be readily visualized with a silver stain. Legionella is common in many environments, with at least 50 species and 70 serogroups identified. The side chains of the cell wall carry the bases responsible for the somatic antigen specificity of these organisms. The chemical composition of these side chains both with respect to components as well as arrangement of the different sugars determines the nature of the somatic or O antigen determinants, which are essential means of serologically classifying many Gram-negative bacteria.

Legionella acquired its name after a July, 1976 outbreak of a then-unknown "mystery disease" sickened 221 persons, causing 34 deaths. The outbreak was first noticed among people attending a convention of the American Legion – a congressionally chartered association of U.S. military veterans. On January 18, 1977 the causative agent was identified as a previously unknown bacterium, subsequently named Legionella.

Epidemiology
Although 64 Legionella serogroups have been identified among 42 species, L. pneumophila causes most legionellosis. L. pneumophila serogroup 1 alone is responsible for 70-90% of cases in adults. In a pediatric series, L. pneumophila serogroup 1 accounted for only 48% of cases, serogroup 6 accounted for 33%, and the remaining cases involved other serotypes and species. Legionella micdadei and L. dumoffii are the second and third most common species to cause Legionnaires disease in children, respectively.

Transmission
Transmission occurs by means of aerosolization or aspiration of water or mist droplets contaminated with Legionella organisms. Wounds may become infected after contact with contaminated water. The following systems are linked to transmission of Legionella organisms: Cooling towers, Humidifiers, Respiratory therapy equipment, Evaporative condensers, Potable water distribution systems (eg. showers, faucets).

Legionella organisms are aerobic, motile, and nutritionally fastidious pleomorphic Gram-negative rods. The growth of the organism depends on the presence of L-cysteine and iron in special media. The organism has been isolated in natural aquatic habitats (freshwater streams and lakes, water reservoirs) and artificial sources (cooling towers, potable water distribution systems). Freshwater amoebae appear to be the natural reservoir for the organisms. Optimal growth temperature is 28-40°C; organisms are dormant below 20°C and are killed at temperatures above 60°C.

Most nosocomial infections and hospital outbreaks have been linked to contaminated hot water supply. However, contamination of cold-water supply has also been reported. Nosocomial Legionnaires disease associated with water birth is reported in a few neonates. Person-to-person transmission has not been demonstrated.

Pathophysiology
Once inside a host, incubation may take up to two weeks. Initial symptoms are flu-like, including fever, chills, and dry cough. Advanced stages of the disease cause problems with the gastrointestinal tract and the nervous system and lead to diarrhea and nausea. Other advanced symptoms of pneumonia may also present. However, the disease is generally not a threat to most healthy individuals, and tends to lead to harmful symptoms only in those with a compromised immune system and the elderly. Consequently, it should be actively checked for in the water systems of hospitals and nursing homes.

Mucociliary action clears Legionella organisms from the upper respiratory tract. Any process that compromises mucociliary clearance (eg. smoking tobacco) increases risk of infection. Virulence varies between strains of L. pneumophila. For example, some strains can adhere to the respiratory epithelial cells via pili, whereas strains with a mutated gene that encodes for the pili show reduced adherence in vitro.

Organisms that reach the alveoli undergo phagocytosis by the alveolar macrophages but are not actively killed. Macrophages may actually support the growth of Legionella organisms. The bacteria multiply intracellularly until the cell ruptures. Liberated bacteria then infect other macrophages. Additional virulence factors include genes that potentiate infection of macrophages and inhibit phagosomal fusion, allowing intracellular growth.

Cell-mediated immunity appears to be the primary host defense mechanism against Legionella infection. Activation of macrophages produces cytokines that regulate antimicrobial activity against Legionella organisms. Individuals with certain deficiencies in cell-mediated immunity are at increased risk for legionellosis.

The role of neutrophils in host defense against Legionella infection is unclear; neutropenia does not appear to predispose patients to legionellosis. Humoral immunity may play a secondary role.

Once infection is established, Legionella organisms cause an acute fibrinopurulent pneumonia with alveolitis and bronchiolitis. In addition to the lungs, Legionella organisms may infect the lymph nodes, brain, kidney, liver, spleen, bone marrow, and myocardium.

Detection
Legionella is traditionally detected by culture on buffered charcoal yeast extract (BCYE) agar. Legionella requires the presence of cysteine to grow and therefore does not grow on common blood agar media used for laboratory based total viable counts. Common laboratory procedures for the detection of Legionella in water concentrate the bacteria (by centrifugation and/or filtration through 0.2 micrometre filters) before inoculation onto a charcoal yeast extract agar containing antibiotics (e.g. glycine vancomycin, polymixin, cyclohexamide, GVPC) to suppress other flora in the sample. Heat or acid treatment are also used to reduce interference from other microbes in the sample.

After incubation for up to 10 days, suspect colonies are confirmed as Legionella if they grow on BCYE containing cysteine, but not on agar without cysteine added. Immunological techniques are then commonly used to establish the species and/or serogroups of bacteria present in the sample.

Many hospitals use the Legionella Urinary Antigen test for initial detection when Legionella pneumonia is suspected. Some of the advantages offered by this test is that the results can be obtained in a matter of hours rather than the five days required for culture, and that a urine specimen is generally more easily obtained than a
sputum specimen. One disadvantage is that the urine antigen test only detects anti-bodies towards *Legionella pneumophila*; only a culture will detect infection by the other *Legionella* species. New techniques for the rapid detection of *Legionella* in water samples are emerging including the use of Polymerase Chain Reaction (PCR) and rapid immunological assays. These technologies can typically provide much faster results.

**Mortality and Morbidity**
The mortality rate in patients with Legionnaires disease is 5 – 80 %, depending on certain risk factors. The factors associated with high mortality rates include the following:
- **Age;** middle-aged and older adults have a higher risk of developing Legionnaires disease than do young adults and children. Among children, more than one third of reported cases have occurred in infants younger than 1 year.
- **Predisposing underlying conditions,** such as chronic lung disease, immunodeficiency, malignancies, end-stage renal disease, and diabetes mellitus.
- **Nosocomial acquisition.**
- **Delayed initiation of specific antimicrobial therapy.**
- **Sex,** since males are more than twice as likely as females to develop Legionnaires disease.

**Clinical History**
Pneumonia is the predominant clinical manifestation of Legionnaires disease (LD). After an incubation period of 2-10 days, patients typically develop the following nonspecific symptoms:
- **Fever**
- **Weakness**
- **Fatigue**
- **Malaise**
- **Myalgia**
- **Chills**
- **Respiratory symptoms may not be present initially but develop as the disease progresses.** Almost all patients develop a cough, which is initially dry and nonproductive, but may become productive, with purulent sputum and, (in rare cases) hemoptysis. Patients may experience chest pain.
- **Neurologic and GI symptoms are usually prominent.**
- **Neurologic complaints may include the following:**
  - Headache
  - Lethargy
  - Confusion
  - Cerebellar ataxia
  - Agitation
  - Stupor
- **Common GI symptoms include diarrhea (watery and non-bloody), nausea, vomiting, and abdominal pain.**
  - In neonates, Legionnaires disease can manifest as sepsis and/or pneumonia with a fulminant course, often diagnosed at autopsy.
  - **Extrapulmonary legionellosis is rare;** the most common site of extrapulmonary infection in adults is the heart. In children, extrapulmonary sites may include the liver, spleen, brain, and lymph nodes. Manifestations of extrapulmonary legionellosis may include the following:
    - **Sinusitis**
    - **Cellulitis**
    - **Peritonitis**
    - **Pyelonephritis**
    - **Pancreatitis**
    - **Wound infection**

**Risk Factors**
In adults, recognized risk factors for legionellosis include the following:
- **Cigarette smoking**
- **Chronic lung disease**
- **Immunosuppression (eg, malignancies, immunosuppressive therapy such as corticosteroids, human immunodeficiency virus [HIV], acquired immunodeficiency syndrome [AIDS])**
- **End-stage renal disease**
- **Diabetes mellitus**
- **Advanced age**
- **Surgery,** especially for head and neck malignancies and for solid organ transplantations, predisposes patients to nosocomial infections.
- **Risk factors for children are less well defined than they are in adults.** Apparent predisposing factors, from reported cases, include the following:
  - **Immunodeficiency (primary or secondary) -** Malignancies, severe combined immunodeficiency, chronic granulomatous disease, organ transplantation, and treatment with corticosteroids.
  - **Preexisting respiratory disease -** Acute or chronic lung disease, asthma, tracheal stenosis, and tracheobronchomalacia.
  - **Young age (especially neonates)**
  - **Rare cases of legionellosis are reported in children who are immunocompetent and who lack predisposing conditions.**

**Treatment**
Pontiac fever requires no specific treatment. Legionnaires' disease is treated with antibiotics. Treatment is started as soon as Legionnaires' disease is suspected, without waiting for test results. Erythromycin or a related antibiotic are the drugs of choice. In severe cases a second drug, rifampin, can be added to the prescription.
Chromogenic media revolutionized microbiological testing while still maintaining traditional agar testing techniques. This assures easy differentiation of microorganisms without complex and costly traditional detection procedures.

Colonies of specific microorganisms are recognizable at a glance by the color. This increases the efficiency of laboratory testing and also saves time and labor costs. The advantages of the media include: (1) fewer samples are positive and have to be checked and (2) there is no further need to investigate 10 different colonies per sample. Overall workload will be reduced and in a routine examination one can detect with higher frequency the samples containing the specific pathogen.

Different chromogenic media and their applications are as follows:

**Chromogenic agar for Salmonella species**
The conventional media for the detection of Salmonella has a very poor specificity creating an abundance of false positives (Citrobacter, Proteus, etc. as suspect colonies) among the rare real positive Salmonella.

In recent times chromogenic media have been developed for the rapid and more reliable identification of Salmonella. For instance a certain chromogenic product may combine two chromogens for the detection of Salmonella sp., 5-Bromo-6-Chloro-3-Indolyl caprylate (Magenta-caprylate) and 5-Bromo-4-Chloro-3-Indolyl b-D-galactopyranoside (X-gal). X-gal is a substrate for the enzyme b-D-galactosidase. Hydrolysis of the chromogen, Mag-caprylate, by lactose negative Salmonella species results in magenta colonies. The medium contains bile salts to inhibit the growth of Gram-positive organisms. Novobiocin is used to inhibit Proteus growth and ceftazidin is added to inhibit growth of Pseudomonads.

**Chromogenic agar for Urinary Tract Infections**
Urinary tract infections (UTI) continue to be a common problem. The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogen. Early information enables the selection of the appropriate antibiotic prior to the results of susceptibility tests and may thereby prevent outbreaks. For many years blood, cystine lactose electrolyte-deficient, and MacConkey agars have been used for the detection of urinary tract pathogens, as well as for the differentiation of a few of them. Chromogenic agar aids in the simultaneous presumptive identification of Gram-positive and Gram-negative bacteria and yeasts on a single medium by means of distinct colony colors produced by reactions of genus- or species-specific enzymes with a suitable chromogenic substrate.

**Chromogenic agar for Staphylococcus aureus**
*Staphylococcus aureus* is a major pathogenic bacterium found in clinical field and in food industry. Nosocomial infections due to *Staphylococcus aureus* create an increasing number of problems, so it is becoming more and more important to detect *Staphylococcus aureus* and in particular, methicillin resistant Staphylococcus aureus (MRSA).

New chromogenic agar have been specifically developed to screen clinical samples for the presence of MRSA, providing accurate, easy to read results in as little as 18 hours without the need to reincubate negative plates. The medium may be inoculated directly from swabs, isolates or culture suspensions.

**Chromogenic agar for Candida species**
Candida chromogenic agar is an alternative chromogenic formulation to the traditional media for the detection and isolation of Candida spp. In this chromogenic type medium, for example three different species of *Candida albicans*, *Candida tropicalis* and *Candida krusei* can be differentiated due to the chromogenic substrates present within the medium. Candida chromogenic agar allows the easy and rapid identification and differentiation of all 3 species by producing easy-to-read results in one plate, since they present different colored colonies. Colonies of *Candida albicans* are green, those of *Candida krusei* are purple-pink and those of *Candida tropicalis* are blue. In the medium Glucose is the fermentable carbohydrate providing carbon and energy. Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Chloramphenicol is an antibiotic which aids in isolating pathogenic fungi from heavily contaminated material, as it inhibits most contaminating bacteria. It is the recommended antibiotic for use with media due to its heat stability and wide bacterial spectrum. The chromogenic mixture allows the identification and differentiation of all 3 species by producing easy-to-read results in one plate, since they present different colored colonies.

**Chromogenic agar for Vancomycin-resistant Enterococcus**
Vancomycin-resistant Enterococcus (VRE) infections are especially aggressive and have been associated with high mortality rates. Also, the possibility of transfer of vancomycin resistance genes to other gram-positive organisms raises significant concerns. The detection and differentiation of the Enterococci strains carrying a transmissible resistance (E. faecalis and E. faecium) is a top priority in the epidemic control. Chromogenic agar gives a rapid detection and clear differentiation of the VRE.faecalis/VRE.faecium from other bacteria.

**Chromogenic agar for Group B Streptococci**
The Group B Streptococci (GBS), also known under the name of *Streptococcus agalactiae* are an important cause of serious neonatal infection. A higher risk exists for newborns whose mothers are colonized with GBS in the genital areas shortly before birth. Detecting vaginal colonization by GBS in pregnant women is the most effective strategy to prevent neonatal infections. This screening allows to determine the need of intrapartum antibiotic prophylaxis.

**Chromogenic agar for Listeria monocytogenes**
*Listeria monocytogenes* is a pathogenic bacteria which can cause serious food poisoning. For the detection of *Listeria monocytogenes*, conventional methods are long and they require heavy work load. On the contrary, chromogenic agar helps to easily differentiate *Listeria monocytogenes* from other *Listeria* directly at the isolation step.

**Chromogenic agar for Vibrio sp.**
*V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* are a pathogenic bacteria which can cause serious seafood poisoning. For the detection of these bacteria, conventional methods (TCBS) are long, require heavy workload and are not very sensitive.

On the contrary, specific chromogenic agar for *Vibrio* helps to easily differentiate *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* from other *Vibrio* directly at the isolation step by colony color with a sensitivity higher than conventional methods.

The result of this research and application can help the world to solve its chronic and constant difficulties, and help to check and identify of the emerging infectious diseases. The significance of chromogenic agar for the isolation of different microorganisms is vast.
Quality of Potable Water

Essential to the survival of all organisms, water has always been an important and life-sustaining drink to humans. Excluding fat, water composes approximately 70% of the human body by mass. It is a crucial component of metabolic processes and serves as a solvent for many bodily solutes. Drinking water or Potable water is water of sufficiently high quality that it can be consumed for drinking as well as cooking purposes without risk of immediate or long-term harm.

Water Contamination & Parameters For Drinking Water
Throughout most of the world, the most common contamination of raw water sources is from human sewage and in particular human fecal pathogens and parasites.
Parameters for drinking water quality typically fall under two categories:

- Chemical parameters: Include heavy metals, trace organic compounds, total suspended solids (TSS), and turbidity. These parameters tend to pose more of a chronic health risk through build-up of heavy metals although some components like nitrates/nitrites and arsenic may have a more immediate impact.
- Physical parameters: Affect the aesthetics and taste of the drinking water and may complicate the removal of microbial pathogens.
- Microbiological parameters: Include coliform bacteria, E. coli, and specific pathogenic species of bacteria (such as cholera – causing Vibrio cholerae), viruses and protozoan parasites, serves as an indication of contamination by sewage. Microbial pathogens are however of greater concern since these agents are highly infectious.

Indicators of safe drinking water
Access to safe drinking water is indicated by the number of people using proper sanitary sources. These improved drinking water sources include household connections, public standpipe, borehole condition, protected dug well, protected spring and rain water collection. Sources that don’t encourage improved drinking water include: unprotected well, unprotected spring, rivers or ponds, vendor-provided water, bottled water (consequent of water include: unprotected well, unprotected spring, rivers or ponds, vendor-provided water, bottled water (consequent of water collection. Sources that don't encourage improved drinking

water from deep wells or springs. The extent of treatment depends on the source of the water. Appropriate technology options in water treatment include both community – scale and household – scale point – on – use (POU) designs.

The most reliable way to kill microbial pathogenic agents is to heat water to a rolling boil but this requires abundant sources of fuel and is very onerous on the households, especially where it is difficult to store boiled water in sterile conditions. Other techniques, such as filtration, chemical disinfection, and exposure to ultraviolet radiation (including solar UV) have been demonstrated.

Solar water disinfection: Is a low-cost method of purifying water that can often be implemented with locally available materials. Unlike methods that rely on firewood, it has low impact on the environment.

Gross Alpha: The gross alpha test is used to determine if the water has any dissolved radionuclides that emit alpha particles. Generally, this will be an indication of whether or not the water has uranium or radium dissolved in it. This test does not indicate the presence of radon.

Test for organics and pesticides: Testing for organics or pesticides is usually only done if there is some suspicion of contamination. Close proximity to a waste site, fuel tanks or farm may be enough to warrant this type of test.

Lead Testing: Although there is much concern about lead in drinking water, the techniques and philosophies for testing and control are less than perfect. In almost all cases, lead found in drinking water is deposited there by the corrosion of lead in the distribution system. The source of the lead is usually lead street connections, old soldered joints (solder used today does not contain lead) or brass fixtures.

Testing Public Water Supplies
Generally public water supplies are not tested. Though there are agencies to test safety of drinking water, this does not, however, mean there is no complaint or there is no problem with the supply. It may need one of the secondary (aesthetic not health) standards of the supply treated, especially, if the supply is from a well. Other concerns such as those about lead, taste, sediment, staining, cyst etc... may motivate someone to have their water treated. Follow-up testing after the installation of equipment for health related parameters should always be done. Use two tests. One of the tests will be of treated water and one will be raw or untreated water. Two tests are needed to show if performance standards are met.

Microbiological testing of water
The most common and widespread health risk associated with drinking water is contamination; whether directly or indirectly, by human or animal excreta, particularly faeces. If such contamination is recent, and if those responsible for it include carriers of communicable enteric disease, some of the pathogenic microorganisms that cause these diseases may be present in the

Water Treatment & Testing
Most water requires some type of treatment before use. Even
water. Drinking the water, or using it in food preparation, may then result in new cases of infection. The pathogenic agents involved include bacteria, viruses, and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhea, dysentery, hepatitis, or typhoid fever, most of them are widely distributed throughout the world. Faecal contamination of drinking water is only one of several faeco oral mechanisms by which they can be transmitted from one person to another or, in some cases, from animals to people.

Other pathogens cause infection when water containing them is used for bathing of for recreation involving water contact, rather than by the oral route. Some may also cause infection by inhalation when they are present in large numbers in water droplets, such as those produced by showers and some air-conditioning systems or in the irrigation of agricultural land. Ideally, all samples taken from the distribution system including consumers’ premises should be free from coliform organisms. In practice, this is not always attainable. To control purity of water the following microbiological parameters for water collected in the distribution system is therefore recommended.

Indian standard IS 1622 : 1981

a. Throughout any year, 95 % of samples should not contain any coliform organisms in 100 ml.
b. No sample should contain E. coli in 100 ml.
c. No sample should contain more than 10 coliform organisms per 100 ml.
d. Coliform organisms should not be detectable in 100 ml of any two consecutive samples.

E. coli

E. coli is a Gram-negative, non-spore forming, rod-shaped bacterium which can be either motile or nonmotile (motile cells are peritrichous); growth is aerobic or facultatively anaerobic. Metabolism is both respiratory and fermentative; acid is produced by the fermentation of glucose and lactose.

1. E. coli is found in large numbers in the feces of humans and of nearly all warmblooded animals; as such it serves as a reliable index of recent fecal contamination of water.

2. E. coli is abundant in human and animal feces, in fresh feces it may attain concentrations of 109 per gram. It is found is sewage, treated effluents, and all natural waters and soils subject to recent fecal contamination; whether from humans, wild animals, or agricultural activity.

3. E. coli may be present or even multiply in tropical waters not subject to human fecal pollution. However, even in the remotest regions, fecal contamination by wild animals, including birds, can never be excluded, because animals can transmit pathogens that are infective in humans, the presence of E. coli must not be ignored.

Total Coliform

The term “coliform organisms (total coliforms)” refers to Gram negative, rod shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, and able to ferment lactose at 35 – 37°C with the production of acid, gas, and aldehyde within 24 – 48 hours. They are also oxidase-negative and non-spore-forming. These definitions have recently been extended by the development of rapid and direct enzymatic methods for enumerating and confirming members of the coliform group.

The existence both of non-fecal bacteria that fit the definitions of coliform bacteria and of lactose negative coliform bacteria limits the applicability of this group as an indicator of fecal pollution. The coliform test can therefore be used as an indicator both of treatment efficiency and of the integrity of the distribution system.

Microbiological water quality testing methodology:

Sample collection procedure for Bacteriological analysis of drinking water.

1. Remove any attachment from the tap.
2. Using a clean cloth outlet of the tap wipe to remove any dirt.
3. Turn on the tap for maximum flow and the water may run for two minutes.
4. Outlet of the tap is sterilized by means of flame from cigarette lighter.
5. Tap again opened to flow for 1 to 2 minutes at medium flow rate.
6. Sterile 250 ml plastic bottle is taken for sample collection.
7. Carefully unscrew the cap and immediately hold the bottle under the water jet and fill.
8. Water filled up to 200 ml and a small air space is left to make shaking before analysis.
9. Collected sample delivered to laboratory within 20 to 30 minutes and inoculated immediately.

Method for testing Total Coliform & E. coli

Name of the method: Multitube fermentation technique/MPN method

Presumption test:

1. Inoculated in 10 ml tubes containing Mac Conkey broth and Durham tubes.
2. Tubes are kept in the incubator 37°C for 24 to 48 hours.
3. Any presence of bacteria will show gas production or color change of the broth from violet to yellow.

Confirmation test for total coliform:

1. Inoculate from the positive tube from presumptive test in Brilliant green broth which contains Durham tubes.
2. The temperature is 37 ± 0.5°C.
3. Presence of gas production confirm the presence of bacteria.

Confirmation test for E. coli

1. Inoculate from the positive tube from presumptive test in EC broth which contains Durham tubes.
2. The temperature is 44 ± 0.5°C.
3. Presence of gas production confirm the presence of bacteria.

Completed test for E. coli

1. Inoculate from the positive tube from conformation test for E. coli in EMB agar.
2. Presence of metallic sheen confirm the presence of E. coli.

Considering the important aspects of the need to test the potability of water, it is essential that water quality is continuously monitored and adequate steps are taken to ensure the same.
### BioShields Presents Nusept

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

**Description:** NUSEPT™ is a new generation, powerful, non-stinging, safe, highly effective and resistance-free microbialic antiseptic solution. NUSEPT™ is an ideal antiseptic for use in medical settings. The main active ingredient of NUSEPT™ is poly (hexamethylenebiguanide) hydrochloride (PHMB). PHMB is a polymeric biguanide. There is no evidence that PHMB susceptibility towards this agent.

**ACTIVITY:** Broad spectrum: Bactericidal, Fungicidal & Viricidal.

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

**APPLICATIONS:**

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

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**Track your brain**

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**Microxpress offers Chromogenic Media and Water Quality Testing Kits**

**Chromogenic Coliform Agar**
A chromogenic medium with sodium lauryl sulphate recommended for simultaneous detection of *Escherichia coli* and total coliforms in water and food samples.

**Chromogenic *E.coli* agar**
A chromogenic medium for detection and enumeration of *Escherichia coli* in foods without further confirmation on membrane filter or by indole reagent.

**Chromogenic Enterococci Broth**
A chromogenic medium for identification and differentiation of *Enterococci* from water sample.

**Chromogenic Improved Salmonella Agar**
A chromogenic medium for identification and differentiation of *Salmonella* from water sample.

**Chromogenic UTI Agar**
A chromogenic differential medium for presumptive identification of microorganisms mainly causing urinary tract infections.

**PA Coliform Test Kit**
The kit is used for detection of presence or absence of coliform bacteria from water samples.

**Rapid Coliform Test Kit**
The kit is used for rapid detection of *E. coli* and coliforms from water samples on the basis of enzyme substrate reaction.

**Rapid Enterococci Test Kit**
The kit is used for rapid identification and differentiation of enterococci from water samples.

**Rapid H,S Test kit**
The kit is used for simultaneous detection of *Salmonella*, *Vibrio* species, *Citrobacter* and *E. coli* from water samples.

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**Highlights of the coming issue**

- **Mini Review**
  - Microbes and Fermentation.

- **Current Trends**
  - Radiation as a means of sterilization.

- **In Profile**
  - Jules Bordet.

- **Bug of the Month**
  - *Bacillus cereus*.

- **Did You Know**
  - Povidone - iodine as an Antiseptic? Think Again.

- **Best Practices**
  - Validation of Microbiological Test Methods.