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Editorial

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Lord Chesterfield once quoted, "Whatever is worth doing at all is worth doing well".

Our attempt of bringing forth to you; what is worth knowing and practicing well, still continues and this issue of the Journal is packed with many more interesting topics to look forward to.

The world that we are living in today is more and more demanding and it is not just an essential but is a requisite that we have to rely on materials that are synthesized from cheap and easily available sources, and the products so produced are also cheap, affordable and readily available. In Our Mini Review section we have described what are called as 'Single Cell Proteins', a brief description of their preparation and their significance is mentioned.

Microbiology is very important, in the field of industry and medicine and thus the media which is used to cultivate micro organisms plays a vital role in the isolation, identification and characterization of microbes. One of the most recent and upcoming areas is the harmonization of microbiological media by the European, United States and Japanese Pharmacopoeias. The section on Current Trends will give our readers a brief insight of this aspect.

Hygiene and Cleanliness, is said to be second only to Godliness, but it took a lot more than just endurance for one man to prove a point; which simply had its basis as hygiene. Even today when basic hygiene may be taken for granted; the achievement and the dedication that this man showed to Hygiene was outstanding. Our In Profile section covers the contribution of Ignaz Philipp Semmelweis to medical hygiene and how the work done by him has influenced medicine in general and obstetrics in particular.

The Shigella species, best known for its etiological role in bacillary dysentery is the organism that we have covered in the Bug of the Month section this time. Our section, Did You Know gives a brief account of the usefulness of the chemical compound Chlorxylenol, which has an antiseptic action and is safe to be used on skin. It also possesses good residual activity.

Good knowledge is not, the only requisite to become a good microbiologist, it is also important that the individual possesses good techniques and has good observation skills, the section on Best Practices deals with Microscopic techniques, which the observer needs to know to visualize the specimen in the most appropriate manner. Our page dedicated specially to Relax Your Mood will definitely cheer you up.

As you read on!!! we hope that you enjoy the information and we look forward to your valuable suggestions.

Single Cell Proteins

Single cell proteins may be defined as feed supplements that are rich in protein content and are produced from cultured algae, yeast and bacteria. These are commonly used in animal feed. Single cell proteins develop when microbes ferment their waste products. Certain microorganisms are capable of growing on agro based waste and these organisms accumulate high concentrations of protein in their cell bodies , these cells are referred to as Single Cell Protein (SCP).

Biomass and Single cell proteins

Microbial biomass has been eaten by man since time immemorial either directly as food; like mushrooms (fruiting bodies of certain basidiomycetes fungi; some of these fruiting bodies are edible while others are poisonous and non edible), or as a part of fermented foods.

In contrast biomass produced by unicellular and multicellular organisms like bacteria, yeast, filamentous fungi and algae is used and processed as human food or animal feed supplement. This biomass is also referred as Single cell protein.

Microbial cells are produced for two main applications

- As a source of protein for human or animal food, (SCP).
- For use as a commercial inoculum in food fermentations, for agriculture and waste treatment.

Single cell proteins have the potential to be developed into very large source of supplemental proteins that could be used in livestock feeding. In certain regions single cell proteins could become the principal protein source that could be used for domestic livestock, depending upon the population growth and the availability of plant feed protein sources. This could develop because microbes can be used to ferment some of the vast amounts of food materials, such as straws; wood and wood processing wastes; food, cannery and food processing wastes; and residues from alcohol production or from human and animal excreta.

Producing and harvesting microbial proteins is not without costs, unfortunately. In nearly all instances where a high rate of production will be achieved, the single cell protein will be found in rather dilute solutions, usually less than 5 % solids.

General process for the preparation of Single Cell Proteins

Regardless of the organism that is employed, process involved or the substrate that is used as the raw material, the steps of preparation can be summarized into:

1. Preparation of a suitable medium with a suitable carbon source; the medium for SCP production varies according to the micro organism. Among other things, the medium must contain a carbon source for cultivating the heterotrophic micro organisms, although green algae (Chlorella, Scenedesmus, Spirulina etc.) can be cultivated without the presence of a carbon source.

   The carbon sources for heterotrophic bacteria can be divided into two groups, namely fossil and renewable.
   - Fossil carbon sources include n - alkanes, gaseous hydrocarbons, methanol, ethanol etc. and the
   - Renewable sources include carbon dioxide, molasses, whey, polysaccharides, hydrolysates, effluents (of breweries, distilleries, confectioneries, industries, potato and canning industries and wood pulp industries) or other solid substrates.

     However n - alkanes seem to be a preferred source of carbon for SCP production.

     In addition to the alkane fraction, salts of pol - , K+, Mn2+, Zn2+, Fe2+ and gaseous ammonia (as source of nitrogen) are also added for cultivating many micro organisms including *Saccharomycopsis lipolytica.*

     The main carbon source may require physical or chemical pre- treatment prior to use.

2. In addition to the carbon source, nutrient sources which can provide phosphorus, nitrogen and other necessary elements must be provided in order to support optimal growth of the selected organism. Polymeric substrates are often hydrolyzed before being incorporated with sources of nitrogen, phosphorus and other essential nutrients.

3. Prevention of contamination of the medium and the plant can be done simply; by maintaining sterile and hygienic conditions.
   - The medium components may be heated or sterilized by filtration.
   - The circulating air and the gaseous components of the medium (NH3, CO2) are sterilized by passing through specialized filters.
   - It is a common practice to sterilize other components by passing through steam.
   - In addition to the components required for the fermentation and medium components the fermentation equipment is also sterilized.

4. Production of the desired microorganism; the desired micro organism has to be produced in a sufficient quantity in medium best suited for its growth, then inoculated into a medium similar to the fermentation medium and finally introduced into the main fermentation broth.

   At each step the purity of the culture has to be checked and can

be done either by Gram staining or plating of the inoculated medium on to microbiological media.

5. Recovery of the microbial biomass from the spent medium.
Methods available for concentrating Single cell proteins include filtration, precipitation, coagulation, centrifugation and the use of semi permeable membranes. These de - watering methods require equipment that is quite expensive and would not be suitable for most small scale operations. Single cell protein must be dried to about 10 % moisture, or condensed and has to be acidified to prevent spoilage from occurring or can alternatively be fed shortly after being produced.

The microbial biomass can be harvested by a variety of methods.
Centrifugation : Single celled organisms like yeasts and bacteria are normally recovered by centrifugation. Centrifugation will result in the settling of cells at the bottom while the medium will be distinctly atop the cell mass.

Filtration: Filamentous organisms are recovered by filtration, which will require the spent medium along with the filamentous mass to be passed through a mesh of desired pore size, which will retain the biomass and allow the medium to flow through.

However the recovery process for fungi is much more easier compared to that of yeast and bacteria like sedimentation and centrifugation.

6. Processing of the biomass for enhancing its usefulness and or storability.
In addition to acidification for preservation purposes, it is also necessary to decrease the concentration of RNA. Some of the methods employed for reducing nucleic acid content in microbial biomass are, alkane hydrolysis, chemical extraction, and activation of endogenous nucleases during final stage of microbial biomass production.

7. Aeration is necessary for most SCP production; except may not be required for algal SCP production. However bacterial and yeast fermentations are easier to aerate

Fermentation process, type, aeration and all the necessary factors are designed in such a way so as to obtain an optimum concentration and quality of the desired SCP, and the SCP type is largely dependent on the organism employed and hence it is important to know the following **Characteristics of the organism and the fermentation product**

1. It should be non pathogenic to plants, animals and to humans
2. It should have good nutritional value
3. It should be usable as food or feed
4. It should not contain any toxic compounds
5. The production cost for it should be low
6. the organism must exhibit high specific growth rates, good productivity and show optimum yields on a given substrate.
7. pH and temperature tolerance of the organism also plays a vital role in designing the process and have to be optimized before large scale production of SCP is commenced.
8. Growth morphology in the reactor.
9. Ease of recovery
10. Protein, RNA and nutritional composition of the product must be estimated.
11. Structural properties of the finished product are necessary.
12. Organisms should be stable genetically so that the strain with optimal biochemical and physiological characteristics may be maintained in the process through many hundreds of generations.

Single cell protein basically comprises protein, fats, carbohydrates, ash ingredients, water and other elements such as phosphorus and potassium. The composition and quality of the SCP depends upon the organism and the substrate which it utilizes for its growth.

Potential substrates for SCP production

Sulfite waste liquor
Candida utilis has been produced as a protein supplement by fermentation of sulfite waste liquor in Germany.

Cellulose
Cellulose from natural sources and waste wood is an attractive starting material for SCP production because of its abundance. The association of cellulose with lignin in wood makes it somewhat intractable to microbial degradation. However thermal or chemical pretreatment, used in combination with enzymatic hydrolysis, is usually required.

Whey (used in Bel process)
Whole milk whey or deproteinized whey is a carbohydrate source, which creates disposal problems (high BOD). Problems associated with whey for SCP production are usually insufficient substrate, seasonal supply variations and its high water content (>90 %) which makes transport prohibitively expensive. *Kluyveromyces marxianus* is an organism that is capable of utilizing lactose as a carbon source, and can thus utilize whey.

Starch (used in Symba process)
The Symba process was developed in Sweden to produce SCP from potato starch using two yeast strains. *Saccharomyces fibuligera* produces the enzyme necessary for starch degradation enabling co - growth of *Candida utilis*. This process is referred to as simultaneous saccharification and fermentation.

Glucose
Food grade glucose was the substrate chosen for the production of fungal SCP using *Fusarium graminearum*. The resultant SCP was very similar to meat.

Higher Alkanes
Substrate costs are low, however due to their crude nature, exhaustive processing was required to recover the yeast free of a gas - oil flavor taint.

Methane / methanol
Methane was initially considered as a SCP raw material because, as a gas product, purification problems after fermentation would be minimal. Methane however has many disadvantages, including that a mixture of methane and oxygen can be explosive. Methane is however easily converted to methanol; which requires less oxygen, less fermenter cooling, is highly water soluble and has minimal explosion risks.

Fermenter vessel

The choice of fermenter (cultivation vessel) largely depends upon the requirements of the micro organisms employed in the process. Aeration is an important operation in the cultivation, which is either carried out manually, in case of certain small scale production or commonly done mechanically. Heat is however also generated during the process which may be lethal to the desired organism or its product and is thus necessary to keep a check on. For this purpose, the fermenter is also required to have a provision for a temperature sensing and for cooling.

The production is usually continued for an indefinite period of time, because in this case the process tends to be more economical. The fermentation may be aseptic or run as a 'clean' operation depending upon the particular objectives.

Various SCP production Processes

Bel process
Whey, which is the byproduct of the dairy industry is used in SCP production. This has a high pollution load, and a high Chemical Oxygen Demand (COD) of 60 g oxygen per liter of whey. Whey contains approximately 45 g/L lactose and 10 g/L protein. It is particularly suitable for the production of SCP using lactose - utilizing yeast. The Bel process was designed by the Bel Industries in France, and was developed with the aim of reducing the pollution load of dairy industry waste, while simultaneously producing a marketable protein product. A number of proteins are operated using *Kluyveromyces lactis* or *K. marxianus* (formerly *K. fragalis*) to produce a protein; Protibel, which is used for both human and animal consumption.

This process initially involves whey pasteurization, during which 75 % of the whey proteins are precipitated. The lactose concentration is adjusted to 34 g/L and mineral salts are also added. This supplemented whey is introduced into a 22m3 continuous fermenter, maintained at 38^{0}C, and pH of 3.5, with an aeration rate of 1700 m3/h. The yeasts utilize the lactose and attain a biomass concentration of 25 g/L, with a biomass yield of 0.45 - 055 g / g of lactose. Yeast cells are recovered by centrifugation then resuspended in water, recentrifuged and finally roller - dried to 95 % solids. Levels of residual sugar remaining in the spent medium are less than 1g/L.

It is important to recover as much water as possible prior to the final drying. The whole operation is to be done under clean and hygienic conditions to keep the product and the broth, that leaves the plant free of bacterial contamination. The final dried products are bacteriologically stable.

If handled properly. In some cases, an after treatment of the biomass is desirable to reduce the unwanted compounds in the product or to isolate the protein. One of the important task is to reduce nucleic acid content, which is high in micro organisms (4 - 6 % in algae, 10 - 16 % in bacteria, 6 - 10 % in yeasts and 2.5 - 6 % in fungi) and can be hazardous to health.

Symba Process
The Symba process was developed in Sweden to produce SCP for animal feed from potato processing wastes. It is not economically attractive as a stand alone operation. However alternative routes of the purification of these waste waters are difficult and expensive, as they contain up to 3 % solids and have COD values of over 20 g oxygen per liter. A high proportion of the available substrate is starch, which many microbes cannot directly utilize. To overcome this problem two micro organisms were selected that grow in a symbiotic association. They are the yeasts *Saccharomycopsis fibuligera*, which produces the hydrolytic enzymes necessary for starch degradation and *Candida utilis.* The process is operated in two stages. In the first stage *S. fibuligera*, is grown in a small reactor on the sterilized waste, supplemented with a nitrogen source and phosphate. At this point the starch is hydrolyzed. The resulting broth is then pumped into a second larger fermenter of 300 m3 capacity where both the organisms are present. However *C. utilis* comes to dominate the second stage and constitutes up to 90 % of the final product. The Symba process operates continuously and after 10 days the pollution rate is reduced by 90 %. Resultant protein rich biomass (45% protein) is concentrated by centrifugation and is finally spray or drum dried.

Pekilo process
This process began operating in 1975 and was the first commercial continuously operating process for the production of a filamentous fungus. The process was developed in Finland for the utilization of spent sulfite liquor, derived from food processing, that contains monosaccharides and acetic acid. Supplements of other carbon sources, usually molasses, whey and hydrolyzed plant wastes were added. The organism of interest in this process is *Paecilomyces variotii.* This continuous process is operated aseptically and produces over 10000 tonnes of SCP a year from two 360m3 fermenters. Resulting dried Pekiloprotein containing up to 59 % crude protein, is used in the preparation of compounded animal feed.

Bioprotein process
There has been a considerable amount of research into the production of SCP using alkanes as carbon sources, notably methane and liquid straight chain hydrocarbons from C12 to C20. However these compounds present certain technical problems.

They are not miscible in water, methane in particular, is explosive when mixed with oxygen. Some of these substrates also require purification, or the protein product derived from that needs to be treated to remove adsorbed toxic compounds. In addition these compounds present cooling and aeration problems, as these highly exothermic processes require substantially more oxygen than when carbohydrates are used.

The Bioprotein process, developed by 1990s by Norferm , uses methane rich natural gas as a sole carbon and energy source for the growth of *Methylococcus capsulatus*. A mixture of heterotrophic bacteria is also present, which helps to stabilize the process. The fermentation is carried out in the loop fermenter. Biomass is continuously harvested by centrifugation and ultra filtration, prior to heat inactivation and spray drying. The final product contains 70 % protein and is marketed as Pronin

Pruteen Process

Methanol has several advantages over methane and many other carbon sources particularly it is miscible with water and is available in a very pure form. Consequently the resultant protein does not have to undergo purification. This process uses a methylotrophic bacterium, *Methylophilus methylotrophus*, to produce a feed protein for chickens, pigs and veal calves, marketed as Pruteen.

Filter sterilized compressed air was used for both agitation and aeration. All streams into the fermenter were sterilized. The fermentation was performed at pH 6.5 to 6.9 and at a temperature of 34 - 37 ^{0}C with entirely inorganic commercial grade nutrients. Bacterial cells were recovered by a novel separation technique, involving initial concentration from 3 % (w/w) to 12 % (w/w) by flocculation, which was promoted by acid and heat shock. This was followed by centrifugal dewatering, with recycle of water, and air drying. The dried unprocessed product contained 16 % nucleic acids and over 70 % crude protein.

Quorn Production

The process utilizes only food grade material and is strictly aseptic. The product Quorn has a reduced RNA content. The fermenter for the process is operated continuously at 30 ^{0}C at pH 6.0. Food grade glucose syrup, derived from maize, potato or wheat starch is used as a carbon source with supplements of biotin and mineral salts. Ammonia is used to control the pH and also serves as a nitrogen source. Oxygen is supplied as sterile compressed air and must be controlled with strict limits. If the oxygen levels fall too low, ensuing anaerobic metabolism results in the formation of byproducts that give unacceptable flavor and aroma. However very high dissolved oxygen results in reduced productivity. During the fermentation the biomass doubles every 4 - 5 hours and achieves concentrations of 15 - 20 g /L. The filaments of the harvested organism is a critically important factor related to eating quality. Mycoprotein is different from other novel bacterial and yeast proteins because of its micro filamentous structure. This structure is similar to the meat fibers and thus makes it an ideal substitute for meat.

The fungal biomass generated contains 10 % RNA, which is too high for human consumption. RNA levels are subsequently reduced by a thermal shock, at 64^{0}C for 30 minutes, which renders the organism non viable and activates the organisms RNases. The resultant degraded RNA leaks out of the cell into the surrounding medium. RNA level is reduced to 2% (w/w) or less. Now the mycelium is continuously harvested by vacuum filtration. The filter cake formed is a mat of interwoven fungal hyphae; which can be frozen as sheets, formed into various shapes, granulated or powdered.

A brief comparison between bacterial and yeast SCPs

Though Bacteria have a high protein content the SCPs so produced have certain disadvantages

- Bacterial cells have small size and low density, which makes harvesting from fermented medium difficult and costly.
- Bacterial cells have a high nucleic acid content relative to yeast and fungi, this can be detrimental to human beings, tending to increase the uric acid content in blood. This may cause uric acid poisoning or gout. To decrease the nucleic acid levels additional processing step has to be introduced, and this increases the cost.
- The general public thinking is that all bacteria are harmful and produce disease. An extensive education program is required to remove this misconception and to make the public accept bacterial protein.

However yeast (eg. *Saccharomyces cerevisiae*) have many advantages that make it a more acceptable SCP

- They have a large size and are hence easier to harvest and can be obtained at low costs.
- Low nucleic acid content
- High lysine (amino acid) content
- Ability to grow at an acidic pH, but the most important advantage is familiarity and acceptability to the public in general because of the long history of use.

Disadvantages include lower growth rates, lower protein content (45 - 65 %), and lower methionine content than bacteria.

If SCPs are to be used successfully, there are seven main criteria to be satisfied;

1) The SCP must be safe to eat.
2) The overall cost for the production of the SCP must be low, affordable and profitable
3) The nutritional value dependent on the amino acid composition must be high.
4) It must be acceptable to the general public.
5) RNA content must be low
6) It must have the functionality, i.e.; characteristics that are found in common staple food.
7) The substrates on which the organisms grow should be safe, so that components that are toxic or allergenic to humans and animals should not be present in the processed SCP.

In recent years production of edible mushrooms, has also

acquired importance in many countries as a protein source. Not only is it a rich protein source, it also is considered a delicacy for human consumption. These mushrooms are cultured on solid substrates that range from straw to sawdust and are cultivated under controlled and well monitored conditions.

Lab scale production of oyster mushroom may be done as follows:

Requirements

1. Clean polythene bag
2. Antiseptic solution / 70 % alcohol
3. Straw
4. Scissor
5. Water
6. Oyster mushroom spawn (the mycelium or primary filamentous growth, of the mushroom; also, cakes of earth and manure containing this growth, which are used for the propagation of the mushroom).

Method

1. Cut the straw, approximately 2 inches in length and sterilize the bits.
2. Wipe the polythene bag with a cotton ball, soaked with antiseptic or 70 % alcohol, allow the bag to dry.
3. Wash the hands carefully, rinse with antiseptic, dry them.
4. Put the straw pieces in a layer, into the polythene bag, press the layer (1 inch in height of the straw layer).
5. Sprinkle a few spawn and water on the layer,
6. subsequently place another layer of straw, and sprinkle spawn and water in a similar fashion.
7. Make up to 3 - 4 layers
8. Then hold the mouth of the plastic bag tightly and tie it up with a string.
9. Pierce a few holes in the bag with a sterile pointer or a sharp tip, and place the bag in a cool dry place, and tie up the bag hanging from a suspension string in a vertical fashion.
10. Sprinkle water on the bag daily.
11. The first yield of mushroom will be visible in 7 - 10 days, and will look like white, or cream colored pin heads, tie the string in a cross fashion around the straw pack carefully. Now cut open the bag carefully with a pair of scissors, and you will observe a thick mass of straw (and hence it is important that one packs the straw tightly).
12. Subsequently the oyster mushrooms will be visible in 4 - 5 days.

Encyclopedia

Quarantine is voluntary or compulsory isolation, typically to contain the spread of something considered dangerous or contagious. Quarantine is designed to prevent the introduction, establishment, or spread of animal, plant or human pests and diseases.

Animal quarantine: The animals that are brought to a country from another country always need to be screened for the presence of diseases, pathogens and parasites. This type of quarantine is applicable even to insects, fish or birds.

Plant quarantine: All parts of plants such as fruits, seeds, cuttings, bulbs and corms, as well as things made from wood or bamboo, must be examined and if necessary treated. Living plants however have to be kept in special places called, 'quarantine stations' to eliminate the chances of possible pathogen or parasite presence on the plant body.

Human quarantine: In cases where there are immigrants, it is necessary to scan these individuals for the presence of pathogens, disease and parasite, in order to prevent such persons and pathogens from entering a country , which may lead to the spread of the disease or condition which may be difficult to treat and or eradicate. The term is very commonly employed in case when the disease or condition is highly contagious, for instance in case of smallpox.
Depending on the severity of the condition that on which quarantine is required; the duration of the quarantine may be of a brief period, in which case an individual has to take a decontamination shower, but there are other cases in which the quarantine period may range from a few days to several months and even years. The importance of quarantine is essential in order to stop or at least decrease the rate of the disease spread.

Quarantine is also necessary in certain cases where there are suspects also called 'carriers' (those that may be asymptomatic but yet are capable of carrying the disease), a classical example of which is the case of 'typhoid Mary' (original name Mary Mallon), who was responsible for spreading typhoid fever, infecting individuals and was also responsible for deaths due to typhoid.
Isolation of infected people, is useful to keep the individuals that are already infected away from the persons that are still healthy. Quarantine allows for the focused attention of specialized health care to people who are ill. People in isolation may be cared for in their homes, in hospitals, or in designated health care facilities. In cases where individuals are suffering from contagious respiratory diseases such as tuberculosis, it is necessary to isolate the infected individuals.

Quarantine is however not restricted to individuals who are showing signs and symptoms of a particular condition, it may be necessary to isolate even those individuals who are exposed to a particular infectious agents but are not yet ill, but may develop the infection after a certain period of time. In cases like these the individuals are isolated for a certain time period and medically examined. This type of quarantine is medically very effective in protecting the public.

The Centers for Disease Control and Prevention, through its division of Global Migration and Quarantine, also is empowered to detain, medically examine, or conditionally release persons suspected of carrying certain communicable diseases.
Quarantine may also be used to protect individuals suffering from certain disorders of the immune system, which may make them prone to infection. Such disorders include Severe Combined Immune Deficiency (SCID) and in conditions when an individual has converted from a HIV seropositive case to an AIDS condition.

Harmonized Microbiological Culture Media

Microbiology forms the basis of many field, including medicine and industry, and micro organisms are important in the production of certain compounds like antibiotics. Microbes also play a vital role in biodegradation, and are also significant as etiological agents in many diseases. 'Harmonization of Microbiological Culture media' is a concept that is put forth by three different pharmacopoeias and is restricted only to non sterile products like products used for environmental monitoring and is not applicable to sterile products such as injectables.

The composition of the media components is dictated by Pharmacopoeias, and media that are produced with descriptions provided by different pharmacopoeias do not differ in their composition, however may differ in the concentration / amount of the different components that is present in the given media and or the means by which the same component is named.

The revolutionary concept of harmonized culture media has come forth as a result of several years of discussion between microbiologists in the US, Europe and Japan that there is now for the first time ever a harmonized testing procedure available for microbiological quality control. This concept will bring about the use of certain similar protocols in different aspects. For instance in the future, these Pharmacopoeial methods will use identical media, tests organisms and performance test parameters in the US, in Europe and Japan, thus bringing about uniformity in the testing procedures.

Stakeholders : authorities responsible for the change

At about the same time as the formation of the Pharmacopoeial Discussion Group (PDG) and, the recognition for the need for a joint regulatory – industry forum, the International Conference on Harmonization of Technical Requirements for registration of pharmaceuticals for Human Use (ICH) was formed in April 1990. Members of the ICH are as follows:

1. European Medicines Agency (EMEA)
2. European Federation of Pharmaceutical Industries and Associations (EFPIA)
3. Ministry of Health, Labor and Welfare, Japan (MHLW)
4. Japan Pharmaceutical Manufacturers Association (JPMA)
5. US Food and Drug Administration (FDA)
6. Pharmaceutical Research and Manufacturers of America (PhRMA).

The aim of global pharmacopoeia harmonization is to promote consistency of microbiological methods used by companies throughout the world and was an attempt to harmonize the differing standards and specifications contained within the regional compendia. It is to such specifications that the true test of harmonization is applied, i.e. for a given set of analytical procedures and acceptance criteria (APAC) the same accept / reject decision should be made regardless of geographical location.

The 6th supplement to the Ph. Eur. 5th edition contains detailed descriptions of the methods for microbial enumeration tests and tests for specified micro organisms, harmonized with regulations in the USA and Japan. Among other modifications, the harmonized methods provide much more detail in terms of demonstrating method suitability (method validation) and media growth promotion.

Before harmonization comes into full effect, pharmaceutical laboratories must present evidence for registered products that the new methods are suitable to replace the current methods. At least for Europe and the US, after the deadlines specified, only the new harmonized methods will be official EP / US Pharmacopoeia methods.

The methods that are proposed by the harmonization procedure will not only be applicable to the products that are already registered pharmaceutical products but, also to the other products that are not yet to be released, all these have to be tested according to the new methods. As a consequence the harmonization procedure requires that pharmaceutical companies must re validate each manufactured pharmaceutical product.

Harmonized chapters

In terms of microbiology, the harmonized chapters closest to implementation are those associated with the testing of non sterile products. Formerly the Microbial limits, the harmonized chapters now refer to Microbial Enumeration Tests (USP <61>, EP 2.6.12); tests for specified micro organisms (USP <62>, EP 2.6.13); and Microbiological Quality of Non sterile Pharmaceutical Products (USP <1111>, EP 5.1.4).

Implementation dates have been pushed – out from 2007 to 2009 or 2010 depending upon the region but allowance is made for the early adoption of harmonized chapters in the development of new products.

The pharmacopoeias have been granted a certain time span, which is referred as 'Transition time' so that the new methods become official, eg. The European pharmacopoeias have

proposed that the (old) Ph. Eur. Method will remain the official valid method until 31st December 2008 while the methods in US Pharmacopoeia stay valid until 30th April 2009.

The Changes

- Changes of existing culture media to be compliant with the pharmacopoeia requirements and introduction of new media to the pharma market.
- Also quality control testing methods and specifications will have to be done according to the new harmonized methods.
- The microbial enumeration tests are those designed to report a count for bacteria, yeast and filamentous fungi. Methodology can be pour plate, surface spread plate or, something new to the harmonized chapter, such as membrane filtration. Incubation times and temperatures will change from some current regional chapters as well as those in detail which cover the preparation of inoculum and the growth promotion requirements which are better defined.
- The tests specified micro organisms will result in changes to incubation times and temperatures, the choice of species to be used and media quality control. Requirements and the formulation of growth media used. Some regions will have the option of re testing removed in the harmonized chapters.
- All the potential changes will result in re validation of methodologies and media used. This will be a huge challenge to laboratories with implementation deadlines either over or a couple of months away.
- The impact will also be felt by suppliers to the industry in terms of reformulation of media and changes to quality control procedures and also those contract laboratories providing service to the pharmaceutical industry.

Test of suitability

The European Pharmacopoeia specified that the ability of the test to detect micro organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing procedure or a change in the test within the product, which may affect the outcome of the test, is introduced.

Frequency of Suitability testing

It is not regulated in the pharmacopoeia but industrial practice and is recommended by the FDA that the entire test should be performed three different times followed by an evaluation for consistency and reproducibility of results.

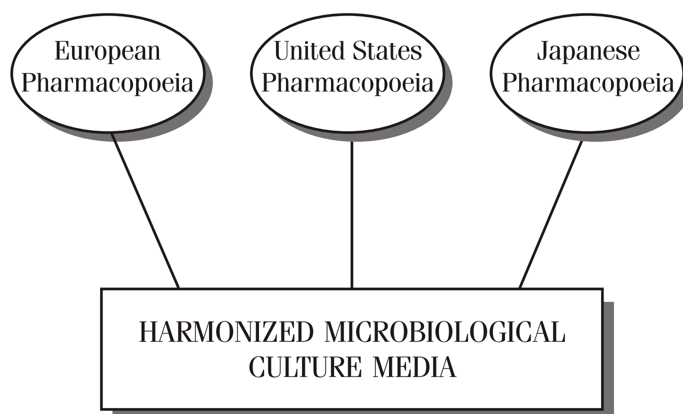
Usually three batches of each of the pharmaceutical products and not three batches of the culture media are used for this procedure.

For validation, the test results should be acceptable for three consecutive analyses. The three tests should also be performed from three different lots to demonstrate the test is not affected by within specification variations of different lots of the formulation.

Significance of Harmonized Microbiological media in Brief

Consistency of microbiological methods used by companies globally.

Harmonized method provides more detail than any of the pharmacopoeial methods in terms of demonstration of method suitability and in terms of media growth promotion.



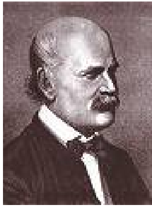
Future Prospects of Harmonization in general

As harmonization continues and implementation begins, the process of licensing of pharmaceuticals should become independent of geographical location i.e. interchangeability of licenses within a certain region.

A more streamlined development process, linked to international co - operation in licensing should dramatically lower the cost of bringing medicines to the market. It should also reduce the time taken to bring new and current medicines to areas of the world where they are badly needed.

In terms of patient welfare this is a significant progression, as the extra work (and cost) involved in the current licensing of pharmaceuticals between jurisdictional boundaries results in inevitable delays in the availability of medicines.

If pharmacopoeial harmonization delivers the above benefits then, through the efforts of the PDG and ICH, the achievement of what they have begun as an unrealistic goal will transform the reality of human health care in the 21st century.



Ignaz Philipp Semmelweis

Birth : July 1, 1818
Death : August 13, 1865
Nationality : Hungarian
Known for : Prophylaxis of puerperal fever

Puerperal fever had afflicted many women during and immediately after childbirth, and thus also called 'childbed fever'. It was the meticulously planned work carried out by Ignaz Philipp Semmelweis that in long term helped to devise a prophylactic measure that proved instrumental in decreasing the infection rate and the deaths that resulted due to the infection.

Ignaz Semmelweis was the fifth child out of ten of a prosperous German grocer and was born on July 1, 1818 to Jozsef and Terezia Muller Semmelweis in Taban, Buda, Hungary, Austria Hungary. He received his elementary education at the Catholic Gymnasium of Buda, then completed his schooling at the University of Pest between 1835 to 1837. Later in 1837 he traveled to Vienna, to enroll in its law school to comply to his father's wish that he became a military advocate in the service of the Austrian bureaucracy. Soon after his arrival to Vienna he was attracted to medicine and seemingly without any parental opposition he matriculated in the medical school. Though Semmelweis returned to Buda , he came back to Vienna where he met Karl Von Rokitansky, Josef Skoda and Ferdinand Von Hebra.

After graduation Semmelweis remained in Vienna, repeating a two month course in practical midwifery. And received a Master's– Magister – degree in the subject. He completed his surgical training and spent 15 (Oct 1844 to Feb 1846) months with Skoda learning diagnosis and statistical methods. Later Semmelweis applied for the position of assistant in the first obstetrics clinics of the University's teaching institution, the Vienna General Hospital. It was here that Semmelweis concerned himself with the problem of Puerperal fever which had afflicted the European child birth clinic in the 19th century. The spread of the infection was attributed to many factors including crowdedness, poor ventilation and commencement of lactation. These factors failed to convince him of the etiology of the disease, and determined, to identify the precise cause of the disease he carried out systematic work in this direction. Finally he hypothesized that the medical interns who came in contact with human cadavers transferred some material from the cadavers to laying – in women in labor and thus Semmelweis also suggested a prophylaxis. By the end of May 1847 he asserted that if hands of examining physicians were washed with chloride of lime after the dissection of cadavers during post - mortem examination, then the transfer of particles would be prevented and so also the transfer of infection. He also soon formed the opinion that not only infection from septic viruses caused puerperal fever but it also came from other sources of putridity. The younger physicians understood the importance of his discovery and supported him. His superior however, was critical, probably because he did not understand Semmelweis. Despite the indifference that Semmelweis had to go through in 1848 he widened his prophylaxis to include all instruments coming in contact with patients in labor.

However Semmelweis disliked public speaking or writing, and this was supposedly the reason that Semmelweis did not get the recognition he deserved for his work and his opinions were misunderstood. Many scholars and doctors of his time regarded him unfavorably.

The first account of his discovery was published by Professor Ferdinand Hebra in December, 1847, in the Journal of the Imperial and Royal Society of Physicians of Vienna, followed by a supplementary statement from the same physician four months later in April,1848. Professor Josef Skoda delivered an address upon the same subject in the Imperial and Royal Academy of Sciences in October, 1849. Never the less the mistakes that these friends of Semmelweis had committed, gave the impression that Puerperal fever was caused due to septic virus. It was only in May, 1850 that he gave a lecture upon his discovery before the society of Physicians; this address was followed by a second one on June 18, 1850.

In 1857 Semmelweis married Maria Weidenhoeffer, who was 19 years junior to him and fathered five children with her.

In 1861 Semmelweis finally published his momentous discovery in book from "Die Atiologie, des Begriff und die Prophylaxis des Kindbettfiebers" (The Etiology, Concept and Prophylaxis of Childbed fever). The work was written in German and discussed, at length, the historical circumstances that surrounded his discovery of the cause and prevention of Puerperal fever.

In 1861 Semmelweis' increasing bitterness and frustration at the lack of acceptance of his method finally broke his indomitable spirit. He became alternatively apathetic and pathologically enraged about his mission as a savior of mothers.

In July 1865 Semmelweis suffered what appeared to be a form of mental illness; and after a journey that was imposed by family and friends was committed to a private asylum in Vienna. There he became violent and was beaten by asylum personnel; from the injuries received he died within a fortnight on August 13, 1865 at the age of 47.

Semmelweis' achievements must be considered against the medical milieu of his time. The ontological concept of the disease insisted on specific disease entities that could be distinctly correlated both clinically and pathologically. Puerperal fever exhibited multiple and varying anatomical localizations and a baffling symptomatology closely related to the evolution of generalized sepsis. The apparent connection between this fever and erysipelas further clouded the issue. Moreover, the idea of a specific contagion causing the disease was not borne out of clinical experience. In the face of such theoretical uncertainties and the profusion of causes attributable to the disease, Semmelweis displayed a brilliant methodology borrowed from his teachers at Vienna.

However it was not until after his death that Semmelweis found full recognition as the predecessor of Lister and pioneer in antiseptic treatment.

Semmelweis wrote in his Atiologie foreward
"When I look back upon the past, I can only dispel the sadness which befalls upon me by gazing into that happy future when the infection will be banished......The conviction that such a time must inevitably sooner or later arrive will cheer my dying hour".

Enjoy the humour

- Yesterday scientists in the United States revealed that beer contains small traces of female hormones.

  'To prove this theory, they fed one hundred men twelve pints of beer and observed that 100 % of them started talking nonsense and could not drive.'

- A blind guy on a bar stool shouts to the bartender, " Wanna hear a blonde joke?"

  In a hushed voice, the guy next to him says, "before you tell that joke, you should know something".

  Our bartender is blonde, the bouncer is blonde. I'm a 6' tall, 200 lb black belt. The guy sitting next to me is 6'2", weighs 225, and he's a rugby player. The fella to your right is 6'5" pushing 300 and he's a wrestler. Each one of US is blonde. Think about it, Mister. Do you still wanna tell that joke?"

  The blind guy says, " Nah, not if I'm gonna have to explain it five times."

Thoughts to live by

- A man who dares to waste one hour of time has not discovered the value of life. (Charles Darwin)
- Believe that life is worth living and your belief will help create the fact. (William James)
- I rate enthusiasm even above professional skill. (Edward Appleton)
- Patience is the companion of wisdom. (Saint Augustine)
- In order to succeed, your desire for success should be greater than your fear of failure. (Bill Cosby)

Track your brain

Rearrange the letters to make familiar words to match the phrase.

SERIES I

(a) MSHOMORU is a single cell protein.

(b) ERPULPERA infection is also referred to as 'childbed fever' .

(c) LROYXEOLHCNL has a preservative action.

_ _ _ GE _ _ _ species are responsible for acute dysentery.

SERIES II

(a) OCEPMCROIS is used to magnify objects.

(b) AIMED is food for microbes.

(c) OYIBID is a bacterial species named after Boyd.

_ _ _ _ AN_ _ _ _ is essential for curbing the spread of a contagious disease

Check your Answers on Page 16

Shigella species

Shigella species are a group of Gram negative, short rods, non spore forming and non capsulated facultative intracellular pathogens. The genus is named after a Japanese microbiologist; Shiga who discovered the bacterial genus. Their etiological role in bacterial dysentery is known since 1890. This genus belongs to the family Enterobacteriaceae and tribe Escherichieae; they are grouped into four species, *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*. These species are also known as groups A, B, C and D respectively. Group A has 13 serotypes, Group B has 6 serotypes, Group C has 23 serotypes and Group D has a single serotype. Group A is most pathogenic, Group B is recently associated with outbreaks among homosexual males, Group C is endemic in India and Group D is most prevalent in the United States and has the mildest course of disease.

Morphological and cultural characteristics

Shigella are very similar to salmonellae, they are 2 – 4 µm X 0.6 µm, but are unlike salmonellae in that Shigella are non - flagellate and non motile organisms. Fimbriae are found only in *Shigella flexneri*, with the exception of serotype 6 and some strains in other serotypes.

The culture is aerobic and facultatively anaerobic. Shigella grows optimally at 37°C, but *Shigella sonnei* grows well even at 10°C and 45°C. Though Shigella shows good growth on conventional media, but none can grow on a simple medium without supplementation with nicotininc acid; although some strains require other growth factors.

A Brief description of Shigella species

Shigella dysenteriae (subgroup A): type 1 of this species is the bacillus originally described by Shiga. It is indole negative and is the only member of the family that is always catalase negative, while the others are invariably catalase positive. *Shigella dysenteriae* type 1 forms a toxin (Shiga toxin), the earliest example of an exotoxin produced by a Gram negative bacillus. Three types of toxic activity have been demostrated in shigella culture filtrates:

1. Neurotoxicity, the toxin however seems not to affect the nervous system directly, but the primary site of action are the blood vessels, mainly of the central nervous system, with the neurological effects being secondary.
2. Enterotoxicity, which results in dysentery.
3. Cytotoxicity; the toxin appears to interfere with the host cellular machinery responsible for protein synthesis.

Shigella flexneri (subgroup B): this group is named after Flexner, who described the first of the mannitol fermenting shigellae. This group is biochemically heterogenous and antigenically the most complex among antigens.

Shigella boydii (subgroup C): this group consists of dysentery bacilli that resemble *Shigella flexneri* biochemically but not antigenically. The group is named after Boyd, who first described these strains from India (1931)

Shigella sonnei (subgroup D): this bacillus, first described by Sonne (1915) in Denmark, ferments lactose and sucrose late. It is indole negative. It is antigenically homogeneous but may occur in two forms – phase I and phase II- the latter forming colonies that are larger, flatter and more irregular. In many cases the disease may only be a mild diarrhea.

Antigenic Structure

Shigellae are differentiated by their somatic (O) antigens into serotypes identified by agglutination tests with absorbed specific antisera. Some serotypes may be identified by agglutination with unabsorbed sera, but absorbed sera must be used for other serotypes between which minor antigens are shared. Also some strains possess K antigens, though these are not relevant in typing but may sometimes interfere with agglutination by O antisera. Common fimbrial antigens may also occur, particularly in *Shigella flexneri*. It is, therefore important that the identification of shigellae should be made by a combination of antigenic and biochemical properties and not by slide agglutination alone.

Pathogenesis

Shigella infection occurs mainly via the fecal - oral route. Most Shigella infections are the result of the bacterium passing from stools or toiled fingers of one person to the mouth of another person, contaminated food and water also play a vital role in the spread of infection and may result in epidemics during natural calamities like floods. Shigella are present in the diarrheal stools of infected persons while they are ill and continue to shed bacteria for a week or two later. The spread of the infection is favored in areas which are heavily crowded and have poor sanitation conditions. Fomites (inanimate objects that aid in the transfer of micro organisms) are also significant in the spread of Shigellosis (infection caused by *Shigella* species). These bacteria are highly virulent and even a small population of the bacteria (10-200 bacteria) can cause an infection depending on the host immune response and virulence of the strain.

Epidemics may either be food borne of water borne. The infection may be acquired from eating food that has become contaminated by infected food handlers. Vegetables can become contaminated if they are harvested from a field with contaminated sewage or wherein infected field workers defecate. Shigella can also be transmitted by flies. Flies can breed in infected feces and then contaminate food. Shigella infections can be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or even if someone with shigellosis swims or bathes or, worse, defecates, in it.

Shigella species cause dysentery that results in the destruction of epithelial cells of the intestinal mucosa in the cecum and rectum.

Symptoms

Signs and symptoms indicating shigellosis may include

dysentery or even diarrhea resulting in bloody stools containing mucus, abdominal pain, nausea, vomiting, loss of appetite, high fever, painful bowel movement, dehydration and rectal pain. In severe cases shigellosis may lead to seizures, headache, stiff neck, tiredness and confusion.

Epidemiology

Epidemics of bacillary dysentery have always accompanied wars, natural calamities, and often influenced their outcome. However epidemics in civilian communities are associated with poverty and lack or sanitation. The only source of infection are human beings – cases, or less often carriers. Chronic carriage is rare, the bacilli disappearing from feces within a few weeks, except in some malnourished children or AIDS patients. Shigellae exhibit a high rate of secondary household transmission. In addition to the common modes of transmission mentioned , young male homosexuals may acquire shigellosis as part of the gay bowel syndrome. In developed countries the infection rate is lower compared to the developing countries, and may occur only sporadically, as outbreaks in mental asylums or day care centers.

Laboratory Diagnosis

Shigellae are rarely present in the body other than in the intestine and the laboratory diagnosis of bacillary dysentery can be made only by the isolation of a shigella from the feces.

It is necessary to determine the serotype of an isolate to confirm its identity as a shigella, which can further aid to identify the particular serotype which may be responsible for an epidemic.

However demonstration of antibodies in sera are not useful as indicators of infection.

Specimens : The specimen submitted to the laboratory are commonly feces or rectal swabs, rarely vomit. Feces is preferable to a rectal swab because a rectal swab is unlikely to give a positive culture unless it is moist and visibly soiled with feces. Fresh feces should be inoculated without delay or transported in a suitable medium such as Sach's buffered glycerol saline, pH 7.0 – 7.4. Highly alkaline transport media used for vibrios are inhibitory for shigellae.

For inoculation it is best to use mucus flakes if they are present in the sample. MacConkey and Dextrose Citrate agar plates are inoculated. After overnight incubation at 37^0C, the plates are inspected for nonlactose fermenting colonies, which are tested for motility and biochemical reactions. Any non motile bacillus that is urease, citrate, H_2S and KCN negative should be further investigated by biochemical test.

Biochemical tests : Tests for motility and biochemical reactions are used primarily to indicate that the isolate is a *Shigella* species and secondarily to suggest the species of shigella.

Microscopic examination : A wet film of a saline suspension of feces from severe bacillary dysentery is likely to show numerous erythrocytes (blood), pus and macrophages and therefore the slide has to be carefully examined for the specific bacilli.

Treatment

Persons with mild infection usually recover quickly without any antibiotic therapy. However appropriate antibiotic therapy kills shigella bacteria, and may also shorten the illness by a few days. The antibiotics commonly used for treatment are ampicillin, trimethoprim / sulfamethoxazole (also known as Bactrim or Septra), ceftriaxone, or among adults the use of ciprofloxacin is common.

Antidiarrheal agents such as loperamide or diphenoxylate with atropine, can make the illness worse and should be avoided.

The other therapy recommended to restore the fluids lost during dehydration include electrolyte solution and the WHO (World Health Organization) recommended ORS (Oral Rehydration solutions / Salts) is ideal, since it helps to rehydrate the body and also supplies essential salts lost during acute dysentery.

Prophylaxis

- There is no vaccine that is available for prevention of Shigellosis as yet. Infection with a particular strain of Shigella confers immunity in the body towards that strain for several years, and this immunity is attributed to the secretory IgA, whereas the individual is still susceptible to infection from other Shigella strains.

However the spread of infection can be prevented by:

- Frequent and careful handwashing with soap. Handwashing is important among all age groups. Handwashing among children should be frequent and supervised by an adult especially when the individual, may be a toddler, is not toilet trained, especially, in day care centers.
- In places such as public bathrooms and toilets, adequate handwashing facility is important. Daycare centers should not provide water play areas.
- If children with diapers acquire the infection , the person changing the diapers must wash their hands carefully, the child's hands must also be washed with soap and warm water, the area where the diaper is changed should also be washed clean with either household bleach or bacterial wipes and as far as possible infected children must not come in contact with uninfected children.
- Foods including vegetables must be washed thoroughly and cooked adequately in order to prevent the transfer of soil particles, and sewage if any.
- Basic food safety measures must be implemented to prevent the transmission of infection.
- People suffering from Shigellosis must not prepare food or drink at least up to complete cessation of diarrhea for 2 or more days.
- Only boiled water should be used for drinking purpose
- Eat only cooked hot foods or fruits you peel yourself.
- In case of an outbreak; the source of contamination / outbreak should be identified and appropriate measures must be taken to prevent further spread of infection.

References

Cruickshank R., Duguid J.P. Medical microbiology; volume 2 Edition 12, 1975.

Ananthanarayan and Paniker. Textbook of microbiology, Edition 7, 2005.

Chlorxylenol

Phenolics have been used for a long time, these compounds are known to have antimicrobial properties, but often these also have posed health problems, like phenolic fumes may be responsible for respiratory ailment and thus may not be ideal for use as a domestic disinfectant. Related compounds such as Chlorxylenol, which also is a phenolic compound and is chemically a halophenol (halogen substituted phenolic compound) also known as Parachlorometaxylenol (PCMX) that has been widely used as a surface disinfectant and also serves as a preservative.

Chlorxylenol is an aromatic chemical compound with a chemical formula $C_8H_9C_{10}$ and finds application in antisepsis, disinfection, preservation and other medical and non medical uses. It is sometimes used together with other antimicrobials like triclosan and has an antimicrobial activity similar to antiseptic agents such as chlorhexidine gluconate.

Mode of action

The precise mode of action of chlorxylenol has been little studied despite its widespread use for many years. It however has antiseptic and disinfectant properties depending on the concentration of chlorxylenol and the formulation of the solution. However because of its phenolic nature, it is expected to have similar effects on surface proteins and microbial cell walls. Phenolics are known to be responsible for the disruption of cellular membranes, precipitation of proteins and inactivation of enzymes. Halogens, known to be oxidizing by nature may also bring about the further oxidation of enzymes.

The efficacy of chlorxylenol against the organism can be potentiated by the presence of EDTA or other chelating agents, due to their removal of metal ions from the bacterial cell walls; which results in destabilization of the cell wall, easier penetration of the chemical and death of the cell due to leakage of cellular components.

When used as an antiseptic / disinfectant it is commonly used in concentrations ranging from 0.3 to 3.75 %.

Applications

- It has been used for both low risk as well as high risk applications. However formulations of the solution containing chlorxylenol is important when it is used against some Gram negatives such as Psuedomonas.
- Chlorxylenol has been used in a variety of antimicrobial soaps including surgical scrubs, preoperative preparations and handwashes.
- Other applications have included shampoos and medicated powders. Chlorxylenol is also effective against a large number of fungi, yeasts and molds.
- It may be used at a lower bacteriostatic and fungistatic concentrations as an effective preservative especially in antiseptics and cosmetics.
- In addition it is also used in a variety of other products such as paints, textiles, adhesives, emulsions, wash tanks and polishes in order to control bacterial, algal and fungal growth.
- These formulations can also enhance the action of the biocide against mycobacteria and viruses. Due to the disruption of lipid membranes , chlorxylenol may also be effective against enveloped viruses, but little activity has been reported against nonenveloped viruses.
- Chlorxylenol is used to sanitize bathroom premises, diaper pails, laundry equipment, human bedding and pet living quarters in households, hospitals and other institutions.
- Chlorxylenol is a stable biocide; it shows good skin penetration and can remain persistent on the skin for a number of hours following application to provide a further bacteriostatic and fungistatic barrier.
- Chlorxylenol is nontoxic at the concentrations typically used. Though it has a skin penetrating action, it is seldom, known to cause skin irritation or sensitivity.

Toxicity

Chlorxylenol generally is of moderate to low acute toxicity, but causes severe eye irritation and has been placed in Toxicity Category I (indicating the greatest degree of acute toxicity) for eye irritation effects. Chlorxylenol is of low acute toxicity via the inhalation route and causes only slight skin irritation, so it is placed in Toxicity Category IV (the lowest of four categories) for these effects. It is slightly toxic by the oral and dermal routes, and has been placed in Toxicity Category III for acute oral and dermal effects.

In case of accidental overexposure or ingestion of chlorxylenol, the effects may be similar to the effects that are caused by phenol and phenolic compounds. However depending on the composition of the solution comprising chlorxylenol the toxic effects of chlorxylenol can also be adverse. It can result in hepatitis and renal failure. It has the potential to cause central nervous system (CNS) depression, corrosion of oral mucosa and gastrointestinal tract, laryngeal edema and upper respiratory tract obstruction.

The main risk however, may be in aspiration which may cause respiratory distress syndrome, pneumonia and cardiorespiratory arrest. CNS depression is a prominent feature. Cardiovascular side effects mainly include arrhythmias. Also hypotension and cardiac depression may occur. Hypotension is caused as a result of hypovolemia secondary either to sequestration of fluids in the gastrointestinal tract or due to upper gastrointestinal bleed. Nephrotoxicity manifesting as oliguria, hematuria and elevated urea and creatinine.

Storage

Chlorxylenol whether in pure form or as a solution mixture is usually used in diluted form. Diluted working solution of chlorxylenol in use has to be carefully used. The solution has to be stored in well closed container in a cool place, since, the working solution may get contaminated during use.

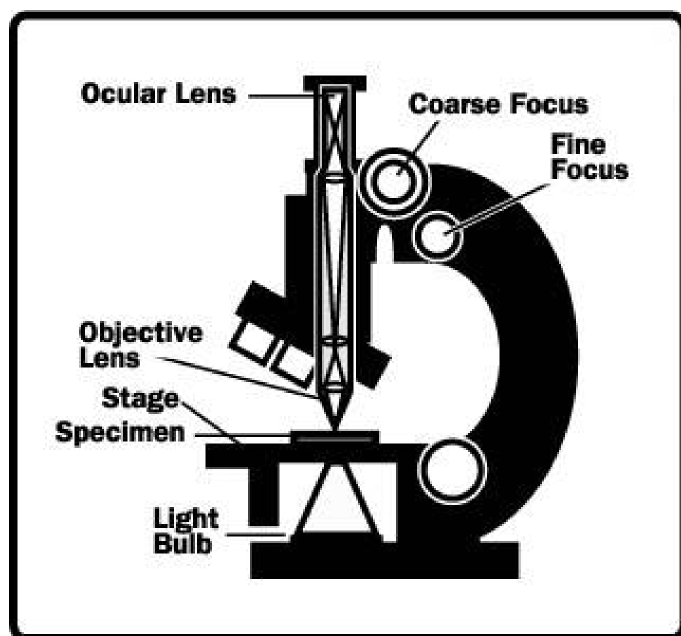
References

www.indmedica.com

Microscopic Techniques

A 'Microscope' the term which literally stands for 'Micro' means small and 'scope' means to 'look', was coined by Giovanni Faber in the year 1625 . The microscope is an optical instrument used to view objects that are too small to be visualized by the naked eye. It was designed to magnify objects so that their structure, morphology and their complexity could be studied.

To know the techniques that are used in microscopes it is also important to know, the essential components that make up a microscope.



All optical microscopes share the same basic components :

The eyepiece:

A cylinder containing two or more lenses to bring the image to focus for the eye. The eyepiece is inserted into the top end of the body tube. Eyepieces are interchangeable and many different eyepieces can be inserted with different degrees of magnification. Typical magnification values for eyepieces include 5x, 10x and 2x. In some high performance microscopes, the configuration of the objective lenses and the eyepiece are matched to give the best possible optical performance.

The Objective lens:

A cylinder containing one or more lenses, typically made of glass, to collect light from the sample. At the lower end of the microscope tube one or more objective lenses are screwed into a circular nose piece which may be rotated to select the required objective. Typical magnification values of objective lenses are 4x, 5x, 10x, 20x, 40x, 50x and 100x. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.

The Stage:

A platform below the objective which supports the specimen being viewed. In the center of the stage is a hole through which light passes to illuminate the specimen. The stage usually has arms to hold the slide.

The illumination source:

Below the stage, light is provided and controlled in a variety of ways. Usually daylight is directed via a mirror. Most microscopes, however, have their own controllable light source that is focused through an optical device called a condenser, with diaphragms and filters available to manage the quality and intensity of light.

Course and Fine adjustment:

The course adjustment helps to focus the image and see the specimen, whereas in order to actually visualize the object in a precise fashion , and to adjust the focus, to one's vision, the fine adjustment of the microscope is a requisite.

On a typical compound optical microscope, there are three objective lenses : a scanning lens (4x), low power lens (10x) and high power lens (ranging from 20x to 100x). Some microscopes have a fourth objective lens, called an oil immersion lens. To use this lens a drop of immersion oil is placed on top of the cover slip, and the lens is very carefully lowered until the front objective immerses in the oil film. Such immersion lenses are designed so that the refractive index of the oil and of the cover slip are closely matched so that the light is transmitted from the specimen to the outer face of the objective lens with minimal refraction. An oil immersion lens usually has a magnification of 50x to 100x.

Though the functioning of the microscope has changed drastically over the years, the general principle of the working of the microscope is the same.

The objective lens is a very high powered magnifying glass i.e a lens with a very short focal length. This is brought very close to the specimen being examined so that the light from the specimen comes to a focus about 160 mm inside the microscope tube. This creates an enlarged image of the subject. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By carefully focusing a brightly lit specimen, a highly enlarged image can be seen. It is this real image that is viewed by the eyepiece lens that provides further enlargement.

In most microscopes the eyepiece is a compound lens, with one component less near the front and one near the back of the eyepiece tube. This forms an – air separated couplet, the virtual image comes to a focus and the second enabling the eye to focus

on the virtual image.

A major problem in observing specimens under a microscope is that their images do not have contrast. This is especially true of living things, although natural pigments such as the green in the leaves, can provide good contrast. One way to improve contrast is to treat the specimen with colored pigments or dyes that bind to specific structures within the specimen. The specializations are mainly in the illumination systems and the type of light passed through the specimen.

Various techniques in light microscopy

Brightfield : this is the basic microscope configuration. This technique has very little contrast; much of which is provided by staining the specimen.

Darkfield : this microscope uses a special condenser to block out most of the bright light and illuminate the specimen with oblique light. This optical setup provides a totally dark background and enhances the contrast of the image to bring out fine details.

Rheinberg illumination :

This set up is similar to darkfield, but uses a series of filters to produce an 'optical staining' of the specimen.

The following techniques use the same basic principle as Rheinfield illumination, achieving different results by using different optical components. The basic idea involves splitting the light beam into two pathways that illuminate the specimen.

Phase contrast :

This technique is best for looking at living specimens, such as cultured cells. In a phase contrast microscope, the annular rings in the objective lens and the condenser separate the light. The light that passes through the central part of the light path is recombined with the light that travels around the periphery of the specimen. The interference produced by these two paths produces images in which the dense structures appear darker than the background.

Differential interference contrast (DIC, or also called Nomarski) :

Uses polarizing filters and prisms to separate and recombine the light paths, giving a three dimensional appearance to the specimen.

Hoffman modulation contrast :

This technique is similar to DIC except that it uses plates with slits in both the axis and the off – axis of the light path to produce two sets of light waves passing through the specimen. In this case like DIC, a three dimensional image is produced.

Polarization :

The polarized light microscope uses two polarizers, one on either side of the specimen, positioned perpendicular to each other so that only light that passes through the specimen reaches the eyepiece. Light is polarized in one plane as it passes through the first filter and reaches the specimen. Regularly – spaced, patterned or crystallized portions of the specimen rotate the light that passes through. Some of this rotated light passes through the second polarizing filter, so these regularly spaced areas show up bright against a black background.

Fluorescence :

This type of microscope uses high energy, short wavelength light (usually ultraviolet) to excite electrons within certain molecules inside a specimen, causing those electrons to shift to higher orbits. When they fall back to their original energy levels, they emit lower energy, longer wavelength light (usually in visible spectrum), which forms the image.

Though microscopy and viewing a specimen using a microscope may seem very simple there are a lot of details that one has to bear in mind, in order to get the desired results.

Remember........

- Always clean the eyepiece, mirror, diaphragm and most importantly the lens of the microscope with a soft cloth or a paper napkin.
- In cases when the oil immersion is used, make sure that the oil is just enough to dip the lens and should not be in excess.
- Make sure that the slides used for microscopy are clean, grease – free, and have no scratches, the same however holds good for cover slips also.
- Be careful to place the cover slip in a manner that there are no air bubbles in between the slide and the cover slip, to obtain a clear view.
- Take care; to make the smear neither too thick, nor too thin.
- Tissue sections, must not be thick, because thick sections cause a hindrance to the proper viewing of the specimen.

Now try making a Simple Microscope

Requirements

1. 2 magnifying glasses
2. 1 sheet of printed paper

Method

1. Hold one magnifying glass a short distance above the paper. The image of the print will look a little bit large.
2. Place the second magnifying glass between your eye and the first magnifying glass.
3. Move the second magnifying glass up or down until the print comes into sharp focus. You will notice that the print appears larger than it does in the first magnifying glass.

References

www.science.howstuffworks.com.

In the section on Current Trends we have mentioned important aspects of Harmonized Microbiological Culture Media and significance of harmonization. Enlisted below is the range of harmonized culture media that are available with Accumix.

Buffer solution
- Buffered Sodium Chloride – Peptone solution pH 7.0

Microbial Enumeration tests
- Soybean Casein Digest Broth
- Soybean Casein Digest Agar
- Potato Dextrose Agar

Test for *Candida albicans*
- Sabouraud Dextrose Agar
- Sabouraud Dextrose Broth

Test for bile tolerant Gram negative bacteria
- Enterobacteria Enrichment Broth, Mossel
- Violet Red Bile Glucose Agar

Test for *Escherichia coli*
- MacConkey Broth
- MacConkey Agar

Test for *Salmonella* species
- Rappaport Vassiliadis Salmonella Enrichment Broth
- Xylose Lysine Deoxycholate Agar

Test for *Pseudomonas aeruginosa*
- Cetrimide Agar

Test for *Staphylococcus aureus*
- Mannitol Salt Agar

Test for Clostridia
- Reinforced Medium for Clostridia
- Columbia Agar

SERIES I	SERIES II
MUSHROOM	MICROSCOPE
PUERPERAL	MEDIA
CHLORXYLENOL	BOYDII
SHIGELLA	QUARANTINE

ACTALL™ - Two in One.

ACTALL is a safe and powerful Dual Action Antiseptic Solution for general purpose, for Disinfection and for use in first aid. It is a clear caramel colored solution with a Terpineol fragrance. Chlorxylenol and triclosan which are effective antimicrobials are active components present in the product. It is ideal for hospital, laboratory, food and Pharmaceutical industries.

COMPOSITION

2.4 % w / v Chlorxylenol	0.5 % w / v Triclosan
12 % v / v Isopropyl Alcohol (2-Propanol)	9 % v / v Terpineol
Caramel	

ACTALL
- Dual action antimicrobial activity
- Ideal for routine external antisepsis during midwifery
- Safe on scalp as an anti - dandruff solution
- Pleasant fragrance
- Ready to Use

USAGE DIRECTIONS

For Medical Use : Cuts, Bites, Abrasions, Insect Stings – Wash with 50 mL Actall to 1 Liter of water, cover with dry gauze. For urgent application undiluted Actall may be used, but not on sensitive skin types. Midwifery – 25 mL of Actall to 1 Liter of water, for routine external antisepsis.

For Personal Hygiene : Dandruff – Add 25 mL of Actall to 1 Liter of water and pour over scalp. Leave for 10 minutes before shampooing. Do not use undiluted. Bathing – 4 - 6 capfuls of Actall in the bath is hygienic and refreshing. Although a gentle antiseptic, do not use Actall for babies except on medical advice. Lavatories, Sinks Drain Etc. – Use undiluted Actall.

For epidemics : Add 25 mL of Actall to 1 Liter of water and use for disinfecting linen, floors.

Highlights of the coming issue

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