MCB 410 E-NOTE TEMPLATE

COURSE CODE: MCB 410

COURSE TITLE: Principles of Epidemiology and Public Health

NUMBER OF UNITS: 2 Units

COURSE DURATION: Two hours per week

COURSE DETAILS:

Course Coordinator: Prof. D.A. Ojo *B.Sc, M.Sc, Ph.D*

E-mail: daojo3@yahoo.com

Office Location: Room A103, COLNAS

Other Lecturers: Arowosegbe A. and Dr. Shittu, O.B.

COURSE CONTENT:

Statistical application to epidemiology. Nature of epidemics. Epidemiological investigations. Spectrum of infections. Herd immunity. Latent of multifactorial systems in epidemics. Zonoses. Antigenic drift. Biological products for immunization and recommendation schedules.

COURSE REQUIREMENTS:

Departmental course for B.Sc Microbiology

READING LIST:

1. Banor, J.D., Akermann, P.G., Toro, G.: Clinical Laboratory Methods. 8th Edition. The C.V. Mosby Company. Saint Louis, U.S.A. 1974
2. Brooks, G., Balel, J., Morge, S.: In: Jaetz, Melnick and Adelberg (Editor). Medical Microbiology. 21st Ed. Aphenton and Lange. Stanford, California. 1998
3. Davidson, I. and Henry, J.B.: Clinical Diagnosis. 15th Ed. W.B. Saunder Company. 1974

LECTURE NOTES:

INTRODUCTION

A number of infective diseases are emerging that are important public health problems, even in developed countries. In developing countries, which include about 75% of the world's population, infectious diseases are still a major problem. Worldwide, infectious diseases account

for nearly one-third of total of 52 million annual estimated death. For many of these diseases (for example, measles, whooping cough and even tuberculosis), effective vaccines are manufactured but are sometimes not available in developing countries. The infectious diseases remain an important, but approachable, public health problem throughout the world. The current acquired immunodeficiency syndrome (AIDS) epidemic, which has spread worldwide in 20 years or less, is only one example of the devastating consequences of a new infectious disease in a global theater. Eradication or even effective control of infectious diseases must involve scientific, economic, political and educational solutions and ultimately, global cooperation.

The study of the occurrence, distribution and control of disease in populations is the field of EPIDEMIOLOGY i.e. Epidemiology is the study of disease in population. The epidemiologist traces the spread of a disease to identify its origin and mode of transmission. Epidemiologic data are obtained from clinical studies, disease-reporting survey, insurance questionnaires, and interview with patients to define common factors that constitute a disease. For infectious disease, the epidemiologist develops methods for the control of infectious disease by finding the interactions of the pathogen in the host population.

EPIDEMIOLOGICAL TERMINOLOGY

A number of terms having specific meanings are used by the epidemiologist to describe pattern of disease.

1. **PREVALENCE:** The prevalence of a disease in a population is defined as the proportion (or percentage) of diseased individuals in a population at any one time.

   i.e. Prevalence = $\frac{\text{Event}}{\text{population}} \times 100$

   e.g. In 2004 = 350 cases of 1200 population

$\therefore$ prevalence = $\frac{350}{1200}$ x 100%

In observational studies, it is called cross-sectional studies.

2. **INCIDENCE:** The incidence of a disease is the number of diseased individuals in a population. That is, the attribute of interest are measured initially in this group of persons, then these persons are followed up over a period of time for the development of the disease being studied. It is also known as cohort studies in observational studies. That is, the proportion of new cases of infection per year e.g.

In 2004 = 300 cases
In 2005 = 400 cases } as a population of 1,200

$\therefore$ Incidence rate = $\frac{400 - 350}{1200}$ x 100%

***Q*:** *Give account of two types of observational studies*.

3. **PREVENTION:** Inhibition of the development of a disease before it occurs and if it occurs interruption or slowing the progression of the disease.

4. **CONTROL:** This is to exercise restrain or regulation to correct or restore to normal operation aimed at reducing the prevalence of the disease to a level where it is not a major public health problem.

***Q*:** *What is (i) prevention (ii) control*

5. **EPIDEMIC:** A disease is said to be epidemic when it occurs in an unusually high number of individuals in a population at the same time.

6. **PANDEMIC:** A pandemic is a widely distributed epidemic.

7. **ENDEMIC:** An endemic disease is one that is constantly present, usually at low incidence, in a population. In an endemic disease, the pathogen may not be highly virulent or the majority of the individuals may be immune, resulting in low disease incidence. However, as long as an endemic situation lasts, a few infected individuals remain, who serve as reservoirs of infection.

8. **SPORADIC:** A sporadic cases of a disease occur when individual cases are recorded in geographically separated areas, implying that the incidents are not related.

9. **OUTBREAK:** A disease outbreak occurs when a number of cases are observed, usually in a relatively short period of time, in an area previously experiencing only sporadic cases of the disease.

10. **MORTALITY:** Mortality is expressed as incidence of death in the population. Infectious diseases were the major causes of death in 1900 in developed countries, whereas currently they are of much less significance. Noninfectious diseases such as heart disease and cancer are of greater importance. In developing countries, infectious diseases are still the major killers.

MORBIDITY: Morbidity refers to the incidence of disease in populations and include both fatal and unfatal diseases. The major causes of illness are quite different from the major causes of death. Major illness include acute respiratory diseases (common cold) and acute digestive system condition, which are generally due to infectious agents and seldom cause death.

***Q*:** *Distinguish between an endemic disease, an epidemic disease and a pandemic disease*.

***Q*:** *Distinguish between mortality and morbidity*

DISEASE RESERVOIRS

Reservoirs are sites in which viable infectious agents remain alive and from which infection of individuals may occur. Reservoirs may be either animate or inanimate. Some pathogens are primarily saprophytic (living on dead matter and only incidentally infect and cause disease). For example, *Clostridium tetani* (the actual agent of tetanus) normally inhabits the soil. Infection of animals by this organism is an accidental event.

Many pathogens use other living organisms as their only reservoirs. In these cases, the reservoir is an essential component of the natural life cycle of the infectious agent. Some infections occur only in human, and main animate of the cycle involves person-to-person transmission. This type of pathogen cycle is common for viral and bacterial respiratory diseases, sexually transmitted diseases, staphylococcal and streptococcal infection, diphtheria, typhoid fever and mumps.

ZOONOSIS

A number of infectious diseases that occur in human also occur in animals. A disease that occurs primarily in animals but is occasionally transmitted to humans is called ZOONOSIS. Control of a zoonosis in the human population is in no way eliminates it as a public health problem. Control of the human disease can generally be achieved only through elimination of the disease in the animal reservoir. Considerable success has been achieved in the control of two diseases that were often transferred to humans from domestic animals, bovine tuberculosis and brucellosis. Control was achieved primarily by identifying and destroying infected animals. Pasteurization of milk was also of considerable importance in the prevention of the spread of bovine tuberculosis to humans because milk was the main vehicle of transmission.

CARRIERS

A carrier is an infected individual with no obvious signs of clinical disease. Carriers are potential sources of infection for others and are critically important for the spread of disease. Carriers may be individuals in the incubation period of the disease, in which case the carrier state precedes the development of actual symptoms. These individuals are prime sources of respiratory infections because they are not yet aware of their infection and so are not taking any precautions against infecting others. Such person are ACUTE CARRIERS because the carrier state lasts for only a short time. On the other hand CHRONIC CARRIERS may remain infected and carry disease for extended periods of time. Chronic carriers usually appear perfectly healthy.

Carriers can be identified in populations by using a variety of diagnostic techniques such as mass culture surveys or mass serological (antibody) surveys.

Diseases in which carriers are important for the spread of infection include hepatitis, tuberculosis and typhoid fever.

Q. *What is a disease reservoir*?

Q. *Distinguish between acute and chronic carriers*.

INFECTIOUS DISEASE TRANSMISSION

Epidemiologists follow the transmission of a disease by correlating geographical, seasonal and age group incidence of a disease with possible modes of transmission. A disease limited to a restricted geographical location may suggest a particular vector, malaria, for example, is transmitted by a mosquito found mainly in tropical regions. A marked seasonality to a disease is often indicative of certain modes of transmission, such as in the case of chicken pox, where the number of cases jump sharply when children enter school and come in close contact.

A pathogen can be transmitted directly from one host to another or indirectly by means of living agent called a vector. Pathogen can also be transmitted by inanimate object (formites) and common vehicles such as food and water.

***Direct Host-to-Host Transmission*:** Host-to-host transmission occurs whenever an infected host transmits the disease to a susceptible host. Transmission by the respiratory route and by direct contact is very common. Transmission by infectious droplets is the most frequent means by which upper respiratory infections such as the common cold and influenza are propagated. However, some pathogens are so sensitive to environmental influences that they are unable to survive for significant period of time away from the host and must be transmitted from host to host by direct contact. The best examples of pathogens transmitted in this ways are those responsible for sexually transmitted diseases, such as *Treponema pallidum* (syphilis) and *Neisseria gonorrhoeae* (gonorrhea).

***Indirect Host-to-Host Transmission*:** Indirect transmission can occur by either living or inanimate means. Living agents transmitting pathogens are called VECTORS. They are generally arthropods (for example, insects, mites, ticks or fleas) or vertebrates (for example, dog, cats, rodents).

Inanimate agents such as bedding, toys, books and surgical instruments can also transmit disease. These inanimate objects are collectively referred to as fomites. Food and water are referred to as disease vehicles. Fomites can also be disease vehicles, but major epidemics originating from a single source are usually traced to food or water because these are actively consumed in large amount by a number of individuals in a population.

EPIDEMICS

Two major types of epidemics can be distinguished: (1) common-source and (2) Host-to-host.

1. **Common-Source Epidemic:** A common-source epidemic arise as the result of infection (or intoxication) of a large number of people from a contaminated common source, such as food or water. Usually such contamination occurs because of a malfunction in the sanitation of a central distribution system. Foodborne and waterborne diseases are primarily intestinal diseases. The pathogen leaves the body in fecal material, contaminates food or water via improper sanitation procedures and then enters the intestinal tract of the recipient during ingestion. The disease incidence for a common-source outbreak is characterized by a rapid rise to a peak because a large number of individuals ingest contaminated food or water and become ile within a relatively brief period of time.

2. **Host-to-Host Epidemic:** In a host-to-host epidemic, the disease incidence shows a relatively slow, progressive rise and gradual decline. A host-to-host epidemic can be initiated by the introduction of a single infected individual into a susceptible population, with this individual infecting one or more people in the population. The pathogen replicates in susceptible individuals, reaches a communicable stage, and is transferred to other susceptible individuals, where it again replicates and becomes communicable.

Q. *Distinguish between a common-source epidemic and a host-to-host epidemic*.

HERD IMMUNITY

Herd immunity is the resistant of a group to infection and spread of a pathogen resulting from immunity of a high proportion of the members of the group. If the proportion of immune individuals is sufficiently great, then the whole population will be protected. From

epidemiological studies of the incidence of polio in large populations, it appears that if a population is 70% immunized, polio will be essentially absent in the population. The immunized individuals protect the rest of the population because they cannot acquire and pass on the pathogen, thus breaking the cycle of infection. For a highly infectious disease such as influenza, the proportion of immune individuals necessary to confer herd immunity is higher, about 90-95%. A value of about 70% has also been estimated for diphtheria.

Generally the pathogens do not change their nature because they are continuously transferred from one individual to the other and perpetrate in susceptible individuals. In addition, there are some pathogens that go on continuous changes and cause new epidemics, for example AIDS, and influenza virus. This feature of pathogens is called ANTIGENIC SHIFT which is genetically determined by major character of the pathogens. Due to antigenic shift they are not recognized by the immune system of the host. For example, antigenic shift in influenza virus occurs due to hybridization between two antigenic type, or two different influenza viruses (animal virus and human virus). Sometimes smaller antigenic changes also occur in pathogen just to escape from immune system of the host. These smaller changes occurring in pathogen from time to time are called ANTIGENIC DRIFT. When resistance in a given population is so high (herd immunity), the pathogen cannot infect humans. In such situation they infect animals.

Moreover, due to antigenic shift or drift against the population of susceptible individual increases. In this situation the Public Health Officials have to make sure that about 70% of individuals must be immunized so that the herd immunity could be maintained.

CYCLE OF DISEASE

The concepts of epidemics and herd immunity can also explain why certain diseases occur in cycle. A good example of a clinical disease is chickenpox, which occurs in a high

proportion of school children. Because the chickenpox is transmitted by the respiratory route, its infectivity is high in crowded situation such as school. On entry into school at age 5, most children are susceptible, so that on the introduction of virus into the school, an explosive propagated epidemic results. Virtually every individual becomes infected and develops immunity, and as the immune population built up, the epidemic dies down. A single infected child can initiate an epidemic causing disease in nearly all previously unexposed children.

HOSPITAL-ACQUIRED (NOSOCOMIAL) INFECTION

A hospital may not only be a place where sick people get well but may also be a place where sick people get sicker. Cross-infection from patient to patient or from hospital personnel to patients presents a constant hazard. Hospital infection are called nosocomial infections (Nosocomium is the Latin word for hospital) and occur in about 5% of all patients admitted. In certain clinical services, such as intensive care units, up to 10% of the patients acquire a nosocomial infection. Hospital infections are partly due to the prevalence of diseased patients but are more often due to the presence of pathogenic microorganisms that are selected for and maintained within the hospital environment. Even multiple-drug-resistant organisms are often spread from host to host as part of the normal flora. Therefore, virtually all nosocomial pathogens are normal flora in either patients or hospital staff.

THE HOSPITAL ENVIRONMENT

Infectious diseases are spread easily and rapidly in hospital environments for several reasons:

1. Many patients have weakened resistance to infectious disease because of their illness (compromised hosts).
2. Hospitals treat patients suffering from infectious disease, and these patients may be reservoirs of highly virulent pathogens.
3. The housing of multiple patients in rooms and wards increases the chance of cross-infection.
4. Hospital personnel move from patient to patient, increasing the probability of transfer of pathogens.
5. Many hospital procedures, such as catheterization, hypodermic injection, spinal puncture and removal of tissue sample (biopsy) or fluids, breach the skin barrier and carry with them the risk of introducing pathogens to the patient.
6. In maternity wards of hospitals, newborn infants are usually susceptible to certain kinds of infection because they lack well-developed defense mechanisms.
7. Surgical procedures are a major hazard because internal organs are exposed to sources of contamination and the stress of surgery often diminishes the resistance of the patient to infection.
8. Certain therapeutic drugs, such as those use for immunosupression in transplant patients, increase susceptibility to infection.
9. Use of antibiotics to control infection selects for antibiotic-resistant organisms, which may not be easily controlled if they cause further infection.

HOSPITAL PATHOGENS

Hospital pathogens preferentially infect several sites notably the urinary tract, blood, wounds and the respiratory tract. A relatively small number of pathogens cause majority of nosocomial infections at these sites.

One of the most important and widespread hospital pathogen is *Staphylococcus aureus*. It is the most common cause of surgical wound infections and the second most common cause of blood infections. *S. aureus* is also particularly problematic in nurseries. Many strains are unusually virulent and are also resistant to common antibiotics, making their treatment very difficult.

Excherichia coli is the most common cause of urinary tract infections in hospitals, but *Pseudomonas nemaginosa*, Candida *albican* and *Klebsiella pneumonia* infections are also very common. Candida and Pseudomonas are good examples of opportunistic pathogens. *Pseudomonas aeruginosa* from hospital isolates is often resistant to multiple antibiotics, complicating treatment. *E. coli* is generally sensitive to at least common antibiotics.

PUBLIC HEALTH MEASURES FOR THE CONTROL OF DISEASE

An understanding of the epidemiology of an infectious disease makes it possible to develop methods for control of the disease. Public health refers to the health of the general population and to the activities of public health authorities in the control of disease. The following are very essential measures for the control of disease:

1. **Control Directed against Reservoir:** If the disease occurs primarily in domestic animals, then infection of human can be prevented if the disease is eliminated from the infected animal population. Immunization procedures or destruction of infected animals

may be used to eliminate the disease in animals. These procedures have been quite effective in eliminating brucellosis and bovine tuberculosis from humans.

When this reservoir is a wild animal, then eradication is much more difficult. Rabies is a disease that occurs in both wild and domestic animals but is transmitted to domestic animals primarily by wild animals. The control of rabies can be achieved by immunization of domestic animals.

If the reservoir is an insect (such as a mosquito in case of malaria), effective control of the disease can be accomplished by elimination of the reservoir with chemical insecticides or other lethal agents.

When humans are the reservoir (for example, AIDS) then the control and eradication can be much more difficult, especially if there are assymptomatic carriers.

2. **Control Directed against Transmission of the Pathogen:** If the organism is transmitted via food or water, then public health procedures can be instituted either to prevent contamination of these vehicles or to destroy the pathogen in the vehicle. Water purification methods have been responsible for dramatic reductions in the incidence of typhoid fever and the pasteurization of milk has helped in the control of bovine, tuberculosis in humans. Transmission of respiratory pathogen is much more difficult to prevent. Attempts at chemical disinfection of air have been unsuccessful. However, air filtration is a viable method, but is limited to small enclosed areas.

3. **Immunization:** Smallpox, diphtheria, tetanus, pertussis (whooping cough), and poliomyelitis have been controlled primarily by means of immunization. When childhood diseases occur in adults, they can have severe effects. If a woman contacts rubella (a viral disease) during pregnancy, the unborn child can be affected by serious

developmental and neurological disorders. Measles and polio are also much more serious disease in adult than in children.

All adults are advised to review their immunization status, Tetanus immunizations, for example, must be renewed at least every 10 years to provide effective immunity. Reimmunization for polio is not recommended for adults unless they are travelling to countries in Africa and Asia where polio may still occur.

4. **Quarantine:** Quarantine involves restricting the movement of individual with active infections to prevent spread of disease to other members of the population. The time limit of quarantine is the longest period of communicability of the given disease.

By international agreement, six diseases are considered quarantinable: smallpox, cholera, plaque, yellow fever, typhoid fever and relapsing fever. Spread of certain other higher contagious diseases such as Ebola hemorrhagic fever and meningitis, may be controlled using quarantine methods.

5. **Surveillance:** Surveillance is the observation, recognition and reporting of diseases as they occur.

6. **Pathogen Eradication:** Disease eradication can be accomplished in some specific cases and has been successful in the case of smallpox. Although smallpox, a viral disease, cannot be treated, immunization practices were very effective.

Vaccination with a related viral strain conferred virtually complete immunity to lethal smallpox infection.

Polio, another viral disease with very effective immunization program, is also targeted for eradication (polio is already eradicated from the Western hemisphere). Leprosy, another disease with a human reservoir is also targeted for eradication, but this

is because active cases can now be effectively treated with a multidrug therapy that cures the patient and also prevents spread of *Mycobacternin leprae*. Other diseases that are being targeted for eradication are Chagas' disease (treat active cases and destroy the insect vector of this parasitic worm) and dracunculiaisis (treat drinking water to prevent transmission of the Guinea worm parasite). Other diseases that are thought to be reasonable candidates for eradication include syphilis and rabies.

Q. *Outline public health methods can be used to halt the spread of an epidemic disease once it has begun.*

EMERGING AND REEMERGING INFECTIOUS DISEASES

Diseases that suddenly become prevalent are referred to as emerging diseases. Emerging infectious are not limited to new diseases but also include reemergence of diseases thought to be controlled, especially when antibiotics become less effective and public health system fail.

Some of the diseases that suddenly emerged into prominence in the past were syphilis (caused by *Treponema pallidum*) and plague (caused by *Yersinia pestis*).

Some factors responsible for the emergence of new pathogens are: (1) human population shifts (demographics) and behavior, (2) technology and industry, (3) economic development and land use, (4) international travel and commerce, (5) microbial adaptation and change, (6) breakdown of public health measures and (7) abnormal natural occurrences that upset the usual host-pathogen balance.

INVESTIGATION AND FOLLOW-UP OF OUTBREAKS OF DISEASE

When an outbreak of disease occurs within any area of the hospital, extensive investigation is indicated. The microbiologist and epidemiologist play major role in this

investigation and should be consulted at the earliest possible moment, since as time passes it becomes more and more difficult to determine sources of infection. The course the investigation takes and the measures taken to prevent further spread will depend on the type of infection and the situation that exists within the area or institution involved.

One must attempt to determine whether carriers provide a continuous reservoir, whether there is a single source, such as food at a given meal, and whether there are breaks in technique in the day-to-day operation of the area involved. Information may be obtained in a number of ways, such as a careful study of the nature and occurrence of the infections and the isolation of a particular microorganism from a significant number of cases. The complete identification of the microorganism is important and can be definite.

To take a specific example, this is perhaps the only situation in which phage typing of *Staph. aureus* can be justified at the present time. If, in outbreaks of staphylococcal disease, all or most cases are caused by a single phage type, the search for a source of the outbreak can be arrowed. Once the type of infection is known and its source is located, the procedure for preventing recurrences should become obvious.

The occurrence of such diseases as diphtheria and meningitis in patients occupying beds in an open ward or in other areas where other patients or hospital personnel may have been exposed also presents a problem. Here again the measures taken to prevent exposed or possibly exposed individuals from developing disease will depend on circumstances.

The prevention of serum hepatitis is important not only in the hospital but also in doctors' offices and other places where parenteral inoculations are done. Transmission of this disease occurs when the serum of a person carrying the virus is introduced into another person. Thus when doing finger punctures, a new sterilized needle must be used for each patient. Disposable

syringes and needles are available and should be used. Transfusion is another potent source of transmission and all possible precautions must be taken in the blood bank. No person with a history of jaundice should ever be used as a donor.

NOTE-UNDER EPIDEMIOLOGY

I. **TERMINOLOGIES:**

Epidemiology (opi = upon, demos = population, logy = study) is the science that deals with occurrence, determination, distribution and control of a disease. An individual who studies the epidemiology is called epidemiologist. When a disease occurs occasionally at irregular intervals in a human population, it is known as sporadic disease e.g. typhoid. A disease maintaining a steady low level frequency at a regular interval is called ENDEMIC disease e.g. common cold. However, a sudden increase in occurrence of a disease beyond a limit is called epidemic (upon the people). If the occurrence of a disease increases within a large population over a wide region, it is called PANDEMIC (pan = all).

II. **FREQUENCY OF A DISEASE**

Frequency of a disease refers to its repeated occurrence as fractions in a given population. To measure the frequency the epidemiologists use statistics and find out the ratio of increase over the pre-existing cases. It is measured as an increase over per 100 or per 1000 individuals. By measuring frequency one can speculate how severe a disease is? However it is also related to morbidity or mortality. Morbidity is the number of individuals becoming ill by a specific disease within a susceptible population during a defined period. It is measured as:

Morbidity rate = Number of new cases of a disease during a specific period in a population

Total number of individual in the population

Similarly, mortality rate refers to death of individuals due to a specific disease with respect to size of population of sufferers with the same disease.

Mortality rate = Number of death due to a given disease

Size of total population of sufferers with same disease

BIOCHEMICAL TESTS AND REACTIONS

Detection of bacter, a species is based primarily on the determination of the presence or absence of different enzymes coded by the genetic material of the bacterial chromosome. These enzymes direct the metabolism of bacteria along one of several pathways that can be detected by special media used in culture techniques. Substitutes upon which these enzmes can react are incorporated into the culture medium, together with an indicator system that can detect either the decay of the substrate or the presence of specific metabolic products. By selecting a series of media that measure different metabolic characteristics of the microorganism to be tested, a biochemical fingerprint can be determined for making a species identification.

CATALASE TEST: Add a drop of 3% H_2O_2 to a microscope slide. Touch a loopful of the organism to the drop of H_2O_2 foaming or bubbling indicates a positive test – Evolution of H_2O and O_2. The Reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

CITRATE UTILIZATION (CHRISTENSEN): The slant is inoculated with the organism and incubated at 37°C for 18-24 hours. Of the organism utilizes citrate as its source of energy in the presence of organic nitrogen, a deep red color will occur in the slanted portion of the tube. Strong reactions may be obtained even after 4-8 hours. This is a good test for separating *Shigella* species (Christensen positive from the Christensen citrate negative *E. coli*.

COAGULASE TEST (SLIDE): Using an inoculating loop make a heavy milky suspension of the staphylococcus organism in a drop of distilled water on a microscope slide. The suspension must be homogenous. Flame the loop and add a loopful of rabbit plasma to the suspension mix. Coagulase production is denoted by almost immediate chumping of the suspension. This test differentiates coagulase positive *Staphylococcus aures* from coagulase negative *Staphylococcus epidermidis*.

COAGULASE TEST (TUBE): Aseptically pipette 0.5mL of rabbit plasma into a sterile small tube and inoculate heavily with a 24-hour culture of the organism. Incubate at 37°C in a water bath. Complete or partial coagulation in 1 to 4 hours is interpreted as a positive test for free coagulase and identifies the organism as *Staphylococcus aureus*.

CELATIN LIQUIFICATION (TUBE): Heavily inoculate the organism into 15% gelatin tubes and incubate for 48 hours or longer at 37°C. To read the results place the tubes in a refrigerator for approximately 15 minutes. If gelatinase production has occurred the gelatin will be liquid after removal from the refrigerator. If the organism does not produce gelatinase the gelatin in the tubes will be completely solid after removal from the refrigerator.

H_2S Production: Blackening along the line of inoculation or some part of the medium indicates that hydrogen sulfide has been produced and has reacted with iron in the medium to give the black iron sulfide precipitate. Triple sugar iron (TSI) or SIM (sulfide, indol, motility) medium can be used. The utilization if lead acetate paper strips is a more sensitive method for detection of H_2S production. These strips can be placed into TSI or SIM medium just above the agar surface. The H_2S reacts with lead acetate to give lead sulfide and the strip turns black.

IMVIC Reaction: This strands for indol. methyl red, voges – proskaner and citrate.

This pattern of biochemical tests is most often used for the differentiation of *E. coli* from the Klebsiella – Enterobacter groups of organisms.

	I	M	Vi	C
E. coli	+	+	-	-
Klebsiella-Enterobacter	-	-	+	+

Indol Production: (Sulfide Indol, Motility), SIM medium is inoculated with an inoculating needle to the bottom of the tube and incubated at 37°C for 18-24 hours. Add a dropperful of chloroform to the tube to extract the indol if present. Next add equal amount of KOVAC;s reagent. The presence of a red color indicates the production of indol from the amino acid tryptophane. A yellow color indicates that the organism does not produce typtophane deaminase and there is no splitting off of indol from tryptophane.

LITMUS Milk Test: Litmus milk medium containing litmus blue indicator is inoculated and incubated at 37°C for 48 hours or longer. A number of reactions can be obtained:

(a) A pink color indicates an acid reaction due to the fermentation of lactose.

(b) A purple or blue color, which is an alkaline reaction, indicates no fermentation of lactose.

(c) Coagulation or Clot formation is caused by the precipitation of casein due to the acid produced from the lactose fermentation. *Clostroidium perfringes*, an etiological of gas gangrene, will produce tremendous amount of gas in the medium thus literally causing the clot to explode. This is referred to as "storming fermentation".

Lysine Decarboxylase Test (Lysine Iron Agar Slant): Inoculate the slant and stab the butt to the bottom of the tube. After 24 hours incubation at 37^{o}C a lysine decarboxylase positive organism will give alkaline conditions (purple) in both the slant and butt. A lysine decarboxylase negative organism will give a yellow butt and alkaline slant. The formation of a black precipitate in the medium is indicative of H_2S production. So any intestinal gram negative rod is lysine decarboxylase positive and H_2S positive may be a *Salmonella sp*. Or Arizona and further biochemical and serological studies are warranted.

Note: The majority of Arizona and *Salmonella sp*. are H_2S positive. *Salmonella paratyphi A* is lysine decarboxylase negative and H_2S negative.

Methyl Red Test: Inoculate a tube of MRVP (methyl) red Voge-Proskaver) broth and incubate for 2-4 days at 37^{o}C. If upon the addition of 5 drops methyl red indicator the broth turns red, the test is considered MR positive and indicates a mixed acid type of fermentation from glucose. A negative methyl red test is denoted by a yellow color. *E. coli* is MR positive and *Klebsiella-Enterobacted* Group of organisms are MR negative.

ONPG-Test: A tablet of ortho-Nitrophenyl beta-galactopyranoside (ONPG) is dissolved is distilled water and inoculated with a heavy suspension of the organism to be tested followed by incubationa t 37^{o}C for 6 hours. Hydrolysis of ONPG is detected by the liberation of ortho-Nitrophenyl, with characteritistic yellow color, often within 30

minutes. Thus a positive ONPG test indicates that the organism contains lactose-fermenting enzymes and may be classified as lactose fermenter.

The ability of certain gram negative bacilli to ferment lactose is a useful criterion for the identification of certain members of the family Enterobacteriaceae.

Examples**:** *E. coli* is lactose positive while shigella sp. and salmonella sp. are lactose negative.

Oxidase Test (Plate): Place a drop of oxidase reagent (1% N, N-Dimethyl – P-phenylenediamine Monohydrochloride) on a group of colonies. A positive indophenols oxidase test is indicate by the development of a pinkish color in the colonies. Oxidase reagent is unstable and should not be used if it is black and contains a precipitate. The plate oxidase test is most useful for screening colonies of *Neisseria gonorrheal* from other colonies that constitute the normal, flora of vaginal cultures.

Oxidative-Fermentative Test (OF): Inoculate two tubes of OF Dextrose semisolid medium by stabbing the medium. One of the inoculated tubes is covered with 2mL of sterile mineral oil to provide anaerobic conditions and the other is left uncovered. The tubes are then incubated at 37^{o}C for 48 hours or longer. Fermentative organisms will produce and reaction (yellow) in both the covered and uncovered media. Oxidase organisms will reduce an acid reaction in the uncovered medium and yield slight to no growth without change (green) in the covered medium. A non-oxidative, non-fermentative organism will produce no change in the covered tube and no change or slightly alkaline reaction (blue) in the open tube.

Sugar fermentations: This test consists of a basal broth which contains a 1% concentration of the carbohydrate being-tested, i.e. glucose, maltose, lactose, etc. Broncresol purple is the

indicator employed. The medium is inoculated using an inoculating loop and incubated at 37^{o}C for 18-24 hours. If the sugar is not fermented the medium will remain neutral or turn slightly alkaline which is indicated by a purple color. If the sugar is fermented by the organism the acid end products will cause the indicator to turn yellow. Sugar fermentation patterns are useful in the differentiation and identification of many microorganisms especially the enteric bacteria.

Urea hydrolysis: Using an inoculating loop inoculate the organism onto the slant of the urea agar. Incubate at 37^{o}C for 24-28 hours. If the medium turns red, urea has been hydrolyzed by the bacterial enzyme urease resulting in the formation of ammonia. This test is most useful in differentiating the urea positive *proteus sp*. from the urea negative *providence group*.

$$NH_2 - CO - NH_2 + H_2O \longrightarrow 2NH_3 + CO_2$$