JOURNAL OF
HYGIENE SCIENCES
Committed to the advancement of Clinical & Industrial Disinfection & Microbiology
VOLUME - VI ISSUE - VI FEB-MAR 2014

Editorial

Contents

"Hope Smiles from the threshold of the year to come, Whispering 'it will be happier'..." — Alfred Tennyson.

With these words ringing in our ears, we all look forward to a Bright and Joyous New Year 2014.

It is rightly said Good health is not something we can buy. However, it can be an extremely valuable savings account. Resolve to take better care of yourself this year.

Coming to this issue, in the Mini review section we will be briefly discussing on the role of microbiology laboratory in controlling the emerging Noscomial infections. A nosocomial, or hospital-acquired, infection as you must be aware is a new infection that develops in a patient during hospitalization. The microbiology laboratory's rapid and consistent identification of nosocomial pathogens is a keystone in the surveillance and control of hospital-acquired infections.

Stains have been used for diagnosing infectious diseases since the late 1800s. Many infectious agents grow slowly on culture media or may not grow at all; stains may be the only method to detect these organisms in clinical specimens. In the hands of experienced clinical microscopists, stains provide rapid and cost-effective information for preliminary diagnosis of infectious diseases. A review of the most common staining methods used in the clinical microbiology laboratory is presented in our Current Trends section.

Joseph Lister is the surgeon who introduced new principles of cleanliness which transformed surgical practice in the late 1800s. He chose dressings soaked with carbolic acid (phenol) to cover the wound and the rate of infection was vastly reduced. Lister then experimented with hand-washing, sterilising instruments and spraying carbolic in the theatre while operating, in order to limit infection. His lowered infection rate was very good and Listerian principles were adopted throughout many countries by a number of surgeons. Read more about the 'father of antiseptic surgery' in our In Profile Section.

Caulobacters are the most prevalent organisms adapted solely for survival in nutrient-poor aquatic and marine environments. In our Bug of the Month we cover *Caulobacter crescentus* which plays an important part in biogeochemical cycling of organic nutrients, thus exhibiting a significant role in the carbon cycle. In addition, due to its distinctive ability to survive in nutrient-limiting conditions, *C. crescentus* has been selected for extensive studies in broad environmental habitats where contamination may be present.

Indicator bacteria are not themselves dangerous to the health but are used to indicate the presence of a health risk. Indicator bacteria are useful for estimation of fecal contamination of water. Our Did You Know section this time deals different types of Indicator Bacteria and their sources.

Disc diffusion has been the mainstay for antimicrobial susceptibility testing (AST) in most clinical microbiological laboratories. In our Best Practices we deal with Trouble shooting for this particular test method.

Have fun in working your brains with the Brainteasers and laugh the way to a wonderful health with our Enjoy the Humour section. We would love to hear your views on JHS so do write to us.

Nosocomial Infections - The Role of Microbiology Laboratory

Millions of surgical procedures and even more invasive medical procedures, including gastrointestinal endoscopic procedures performed each year. Each procedure involves contact by a medical device or surgical instrument with a patient's sterile tissue or mucous membranes. A major risk of all such procedures is the introduction of pathogens that can lead to infection. Failure to properly disinfect or sterilize equipment carries not only risk associated with breach of host barriers but also risk for person-to-person transmission (e.g., Hepatitis B virus) and transmission of environmental pathogens (e.g., *Pseudomonas aeruginosa*).

Sterilization is essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients.

Multiple studies in many countries have documented lack of compliance with established guidelines for disinfection and sterilization. Failure to comply with scientifically based guidelines has led to numerous outbreaks. This document presents a pragmatic approach to the judicious selection and proper use of disinfection and sterilization processes.

Here is the data which gives the picture, how this ignored area of medical practices paying for health of patients around the world.

Health care-associated infection in DEVELOPED countries

In Europe patients affected by Hospital Acquired Infections (HAI) is 4.6% to 9.3%, i.e. approximately 5 million cases annually. HAI contributes to death in at least 2.7% (i.e. 135 000) of cases.

In the USA, it is 4.5%, corresponding to 1.7 million affected patients and attributing to deaths of approximately 99 000 patients.

Health care-associated infection in DEVELOPING countries

Neonatal infections were reported to be 3–20 times higher among hospital-born babies in developing than in developed countries.

In India about 10 – 30 % of patients admitted to hospitals and nursing homes acquire a nosocomial infection.

11-83 % hospital-acquired infections reported in India. (Indian express)

The risk for patients to develop SSI in developing countries is significantly higher.

The most frequent type of infection hospital-wide:

- Urinary tract infection (UTI) (36%)
- Surgical site infection (SSI) (20%)
- Bloodstream infection (BSI) (11%)
- Pneumonia (11%)

Nosocomial or Hospital Acquired Infections (HAI)

Any infection which was:

Absent at the time of admission

Acquired from the health care worker during any health care procedure

Patient should develop symptoms 48 hours after the admission or up to 15 days after discharge to be regarded as HAI.

Modern healthcare employs many types of invasive devices and procedures to treat patients and to help them recover. But infections can be associated with the devices used in medical procedures, such as catheters, ventilators, etc. or patients can be exposed to this bacterium through contaminated surfaces or the spores can be transferred on contaminated hands of healthcare professionals.

(HAIs) include central line-associated bloodstream infections, catheter-associated urinary tract infections, and ventilator-associated pneumonia. Infections may also occur at surgery sites-surgical site infections or contaminated hand or surfaces-associated gastrointestinal infections, etc.

There are a number of factors that can increase the risk of acquiring an infection during healthcare interventions, but high standards of infection control practices like instrument sterilization minimize the risk of occurrence.

Role of The Laboratory In Infection Control

The success of the hospital's infection control efforts hinges to a large extent on the active involvement of the laboratory in all aspects of the infection control program. Laboratory personnel should understand why infection control is necessary, the approaches being taken by the hospital's infection control program to meet its objective to reduce nosocomial infections, and how the laboratory can support and cooperate with the program.

Surveillance of Nosocomial Infections

Surveillance is defined as "the ongoing, systematic collection, analysis, and interpretation of health data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know"

Surveillance, which is an essential element of an infection control program, provides the data to identify infected patients and determine the site of infection and the factors that contributed to the infection. When infection problems are recognized, the hospital is able to institute appropriate intervention measures and evaluate their efficacy. Surveillance data are also used to assess the quality of care in the hospital. If the data collected are to be most useful for decision making, the hospital should focus on their most important and predominant problems and use surveillance methods that adhere to sound epidemiologic principles.

The nosocomial infection surveillance system may be sentinel event based or population based or both. A sentinel infection (or sentinel group of infections) is one that clearly indicates a failure in the hospital's efforts to prevent infections and, in theory, requires individual investigation. Denominator data are usually not collected in sentinel event based surveillance. Sentinel event-based surveillance will identify only the most serious problems and should not be the only surveillance system in the hospital. Population based surveillance, that is, surveillance that is done on patients with similar risks, requires both a numerator (the infection) and denominator (number of patients or days of

exposure to the risk). If the infection rates are to be used for inter hospital comparisons, the rates must be adjusted for patients' intrinsic and extrinsic risks of infection. To calculate risk-adjusted rates from population-based surveillance data, corresponding risk factors in both the numerator and denominator must be collected. The risk factors may be patient characteristics such as underlying disease conditions, or they may be procedures or devices used to diagnose or treat the patient.

The system employs a population-based surveillance system that provides risk-adjusted rates that can be used for inter hospital comparisons. Data are collected for four surveillance components that target different populations of inpatients: (i) all patients in the hospital (called hospital-wide), (ii) patients in the ICU, (iii) patients in the high-risk nursery, and (iv) patients who undergo an operative procedure. Except for the hospital-wide component, important and specific risk factors are collected for the population of patients monitored. For example, in the ICU surveillance component, data are collected on the type of ICU and the total number of days that patients are exposed to a urinary catheter, central vascular line, or ventilator; these are called device-days.

Requirements for a surveillance system

A hospital should have clear goals for doing surveillance. Furthermore, these goals must be reviewed and updated frequently to meet new infection risks in changing patient populations, the introduction of new high-risk medical interventions, and changing pathogens and their resistance to antibiotics.

A surveillance system should include the following elements-

Trained personnel

A typical ICP will spend about half of her or his time performing surveillance. The ICP should have, at minimum, knowledge about clinical patient care, epidemiology, and microbiology. Unfortunately, some hospitals appoint individuals to the infection control position but do not provide them with training to adequately perform infection control functions. Courses in infection control are available through the Association for Practitioners in Infection.

Control and Epidemiology and its local chapters

Individuals who meet certain time and practice qualifications and successfully pass a written examination can be certified in infection control. Criteria for all data collected in the surveillance system must be defined and must be used uniformly by all who perform surveillance. They should be reviewed and approved by the hospital's infection control committee.

Readily available sources of data for identifying infections

The infection control program must have access to all patient and hospital records and should have the full cooperation of all hospital personnel and departments to obtain the necessary data to conduct routine surveillance or investigate an outbreak. For routine surveillance, the infection control program uses laboratory and clinical data for two reasons: case finding, i.e., screening for patients with possible infections; and determining the site of infection, associated risk factors, and outcomes. The surveillance system should not rely solely on other hospital personnel, such as coders, to collect data to identify infected patients because the application of some infection criteria is complex and patient medical records often are not complete. The diagnostic practices of the physicians practicing in the hospital are an important factor in the ability of the infection control program to detect infections, since most infections are identified through microbiologic cultures and other laboratory tests. If most of the patients in the hospital are treated empirically, without cultures being done, the infection control program cannot use culture results as its primary source for detecting infections and must instead adopt clinically based infection criteria. The infection control program staff, through various hospital committees, may be able to influence physicians' diagnostic practices to encourage appropriate culturing and other testing.

Accurate and complete denominator data

Where to obtain denominator data and how to collect them vary among hospitals, depending on the sources available in the hospital and the resourcefulness of the infection control program in gaining the cooperation of the patient care staff and other hospital departments.

Analysis and dissemination of data to those who need the Information

Surveillance is incomplete until the data are analyzed, interpreted, and disseminated to those who need to have the information. The lack of risk-adjusted rates for most hospitals, which make the data difficult to interpret, may be an important reason why surveillance data are not useful.

Confidentiality of the data

The infection control program must be able to assure the hospital staff and physicians that the surveillance data will be used appropriately. Surveillance should be used to improve the quality of patient care and should not be used as a tool to punish or grade individuals, departments, or services without scrupulously protecting institutional and professional reputations. Several states have adopted laws protecting such data.

Selection of patients for monitoring

Traditionally, nosocomial infection surveillance systems have routinely monitored all patients in the hospital for infections at all sites and have used the overall infection rate to describe the magnitude of the infection problem. While an overall rate may provide an estimate of the infection problem, the value of such surveillance systems has recently been questioned. In order to monitor all patients for infections, a wide range of information sources must be reviewed in an ongoing fashion, and low-risk and high-risk patients are given equal time. Otherwise, the surveillance intensity will be uneven, resulting in an unacceptably low case-finding sensitivity. Furthermore, because most of the time is spent finding infections, there is little time left to collect data to adjust the rates by risk. A more efficient and effective alternative to hospital-wide surveillance is to focus on patients with the highest risk for infection. With the exception of the hospital-wide component, the NNIS system surveillance components are examples of surveillance protocols that target high-risk patients.

Strategies for identifying infected patients

Surveillance for nosocomial infections should be done prospectively, that is, patients should be actively and continuously monitored for infections while they are still in the

hospital. The case finding methods used to detect infected patients depend on the sources of information available in the hospital. In most hospitals, the microbiology laboratory reports are the most useful and efficient source for initial case finding. However, the microbiology laboratory should not be the sole source for case finding since cultures are not done for all patients with infections. Other sources of information to detect possible infections include the nursing care plan cards, antibiotic orders in the pharmacy, radiologic reports, autopsies, and verbal reports from patient care personnel. Like laboratory results, most of these require verification with other data, such as clinical findings recorded on the patient's medical record, to determine an infection site.

Use of surveillance data for continuous quality improvement

Over the last decade, the use of nosocomial infection rates as a basis for measuring quality of care has received considerable attention. To assist hospitals in using surveillance as a more effective tool to reduce nosocomial infection rates.

Continuous quality improvement is a general model for improving quality through continuous evaluation of performance in order to identify opportunities to improve the product or outcome. It is an approach that has been widely adopted by industry, including the health care industry, to provide high-quality products and services at a competitive and affordable price. Because the collection of reliable data is an essential element of this evaluation process, nosocomial infection surveillance can make an important contribution to continuous quality improvement in the hospital.

Hospitals use data to assess their quality of care by comparing their infection rates with external benchmark rates or by comparing changes in rates over time in their own hospitals. Many hospitals assume that any difference in the rates represents the success or failure of the patient care staff or institutional practices in preventing nosocomial infections.

While this may be true, there are other factors that could account for the differences in the rates. First, surveillance definitions or techniques may not be uniform among the hospitals or may be used inconsistently over time, causing variations to occur in sensitivity and specificity in infection case finding. Second, inaccurate or insufficient information about clinical and laboratory evidences of infections in the patient's medical record may seriously affect the validity and utility of the infection rate. The microbiology laboratory plays an essential role as a source of information on nosocomial infections and is discussed later. Third, the rates may not be adjusted for patients' intrinsic risks for infection. These risks are usually outside the control of the hospital and vary from hospital to hospital but are important factors in determining whether the patients will develop an infection. For example, a hospital with a large proportion of immune compromised patients would be expected to have a population at higher intrinsic risk for infection than a hospital without such a population of patients. The unsuccessful attempts to compare unadjusted mortality rates are reminders to those comparing infection rates that they must also pay attention to risk-adjusted infection rates.

Finally, the size of the population at risk (e.g., number of patients, admissions and discharges, patient-days, or operations) may not be large enough to calculate rates that adequately estimate the "true" rates for the hospital. Although it may not be possible to fully correct for these factors, hospitals should be aware of how they can affect the infection rate and take them into consideration when interpreting the data.

Specific Laboratory Support Functions

The microbiology laboratory should be actively involved in the infection control program. As the source of microbiologic culture information, the laboratory must provide easy access to high-quality and timely data and give guidance and support on how to use its resources for epidemiologic purposes. The services that the infection control program can offer to the laboratory include functioning as a liaison to the clinical services to improve the quality of specimens sent to the laboratory and promoting appropriate use of cultures and other laboratory tests. It can also assist the laboratory with its system for monitoring antimicrobial agent susceptibilities by identifying the pathogens that are of nosocomial origin.

Interaction of the laboratory with the infection control program

A current and thorough discussion of the role of the laboratory in infection control can be found in the text Hospital Infections. Other publications on this subject are also informative. In brief, the microbiology laboratory can support the infection control program in the following ways. Ensure high-quality performance in the laboratory. Because the surveillance system ordinarily uses the results of cultures and other tests ordered by physicians for the diagnosis and treatment of patients, the surveillance program benefits when the laboratory performs high-quality work on clinical specimens. Additional laboratory tests may be necessary for epidemiologic purposes, but this is rare and should be discussed thoroughly with the infection control program first. The cost of cultures and other tests performed for epidemiologic purposes is usually not charged to the patient. Designate at least one person from the microbiology laboratory to be the consultant to the infection control program and to serve as a member of the infection control committee. Any activity of the infection control program that involves the laboratory should be coordinated through a designated person. Conversely, this representative should keep the infection control program informed about changes in the laboratory that may affect surveillance and other aspects of the program. This person should be selected for his or her knowledge of and interest in infection control. Make laboratory test results available in an organized, easily accessible, and timely manner. The infection control program depends on the cooperation of the laboratory in making laboratory data accessible. The design of the laboratory's record-keeping system should accommodate the needs of the infection control program and should be developed in collaboration. Provide training on basic microbiology for the infection n control program staff. Most beginning ICPs do not have a working knowledge of microbiology and will require training before they are able to effectively use the laboratory services for the infection control program. The ICP will need to be taught how to interpret the results of cultures and other tests in order to conduct surveillance. Monitor laboratory results for unusual findings. The laboratory should watch for clusters of pathogens that may indicate an outbreak, the emergence of multidrug-resistant organisms, and the isolation of highly infectious, unusual, or virulent pathogens. The laboratory staff is usually the first to recognize these unusual events or trends, and reporting them early to the infection control program may avert a more serious problem. Use environmental cultures judiciously. Microbiology laboratories are often asked to perform environmental cultures to assess microbial contamination of inanimate objects or the level of contamination

in certain areas of the hospital. Such culturing must be coordinated with the infection control program to ensure that it is performed only when indicated and that the specimens are processed appropriately. In the past, environmental cultures were performed extensively in most hospitals. Routine environmental cultures are recommended only for monitoring autoclaves and water used to prepare dialysis fluid. Environmental cultures, including personnel cultures, should not be done unless epidemiologic evidence clearly indicates an environmental source of the pathogen. Under these circumstances, information about the etiologic agent can often lead to a clearer understanding about the source of the infection and mode of transmission. Occasionally, a culture of a device used on an infected patient can locate the source of the infection; for example, the semi quantitative method for culturing intravascular catheter tips to determine a vascular site infection has been found to be useful. When associated with local infection, colony counts of more than 15 CFU have a 15 to 40% association with concordant BSI. Store isolates that may require further identification for epidemiologic purposes. In collaboration with the infection control program, the laboratory should develop a system for storing epidemiologically important strains of pathogens from nosocomial infections by sub culturing them and maintaining them in a viable state. The collection should be reviewed frequently, and isolates should be discarded when they are no longer needed. Take proper action when contamination of a commercial product is suspected. Contamination of commercially produced products or devices during manufacture or transportation is rare. If intrinsic contamination is suspected, the hospital laboratory should not attempt to culture the product or device, since special techniques and equipment are required.

If substantial patient disease or mortality is occurring, notify your state/national health department.

Epidemiologic uses of laboratory findings

Laboratory findings are used to support epidemiologic evidence of the spread of a common organism between patients, employees, and the environment. Strain clonality permits the infection control program to confirm the association between patients (hosts) and reservoirs for the microorganisms of interest and to determine possible modes of transmission. The mode of transmission, reservoir, and nature of the susceptible hosts are easier to determine if a single strain (clone) is involved, because the mode of transmission or reservoir may not be the same for multiple strains. The determination of strain clonality may lie in routine tests performed by the microbiology laboratory or the variety of techniques that molecular biology offers to infection control. However, the use of these techniques should support an epidemiologic investigation rather than lead it.

For example, laboratory resources to assess colonization of hospital personnel (or patients) should never be used unless epidemiologically indicated. The degree to which organism identification is routinely carried out can be important. In general, identifying an isolate as *Pseudomonas cepacia* provides more useful epidemiologic information than identifying the organism only as "*Pseudomonas* species," since a variety of related bacilli could be included in the latter group but have different reservoirs or modes of transmission. Reporting of the biotype of microorganisms, i.e., pattern of response in biochemical reactions, is occasionally valuable in differentiating frequently encountered organisms.

Whenever a new procedure for the identification of microorganisms is introduced, the laboratory should consider the procedure's potential ability to assist or hinder the infection control program in tracking the incidence of infections. For example, nucleic acid probes are useful for direct detection of pathogens in clinical samples but do not provide information about antimicrobial agent susceptibility or strain type, which are often important to the infection control program. Therefore, if the pathogen is epidemiologically important, it may be necessary to culture a specimen. Serologic testing is a technique that most infection control programs are not using fully and appropriately. The laboratory should assist the infection control program by making clear the strengths and weaknesses of different assays when they use them for epidemiologic purposes.

Epidemiologic typing of microorganisms

To investigate whether microorganisms are clonal or not, the laboratory usually examines the results of species identification and biochemical tests and patterns of susceptibility to antimicrobial agents. However, more specialized techniques are occasionally required to type certain organisms. Two of these, biotyping and antimicrobial agent susceptibility testing, were discussed earlier. Another technique, phage typing, is based on an organism's susceptibility to bacteriophages and is used most often for *S. aureus*. Because only a limited number of nosocomial pathogens exhibit bacteriophage susceptibility, this procedure has a relatively narrow application. Furthermore, because considerable experience is required to reliably perform phage typing, the procedure should be done by a reference laboratory. Another technique, serotyping, is used for the typing of gram-negative bacilli, especially *P. aeruginosa*. Still other typing techniques that use molecular biology have added to the variety of typing techniques available. Among the most common are plasmid profiles and the digestion of plasmid or genomic material with restriction endonucleases. The appropriate use of these typing methods, some of which are redundant, is important. The key factor in deciding which method to use involves examination of how much discrimination the method can add. Surprisingly, some of the simplest, least expensive, and most available typing methods may be the best. For example, in a study of infections with CoNS, antimicrobial agent susceptibility profiles, biotyping, phage typing, and plasmid profiling were performed. The antimicrobial agent susceptibility profiles proved to be the most discriminatins. Test results may vary when tests are performed by inexperienced technicians or when specimens are processed in different batches. The microbiology laboratory should decide which of the typing tests it can do reliably on site and which should be sent to appropriate reference laboratories.

Advantages of Microbiology Laboratory Participation In Infectious Disease Surveillance

The participation of routine microbiology laboratories in surveillance has clear advantages for infectious disease surveillance. Technical methodologies and reporting of results can lead to better analysis, and all the information can be centralized, particularly when laboratory computing systems are connected to regional, national and supra-national systems. Automatic 'expert systems' can thus be developed to augment the analysis of surveillance data, and automatic alerts can be centralized for response strategies. Microbiology laboratories and microbiology resources may also facilitate temporal and spatial analysis of surveillance data, which would ensure the complete collection of notifiable diseases, as well as the detection of changing patterns.

Stains & their Application in Microbiology

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells.

In biochemistry it involves adding a class-specific (DNA, proteins, lipids, carbohydrates) dye to a substrate to qualify or quantify the presence of a specific compound. Staining and fluorescent tagging can serve similar purposes. Biological staining is also used to mark cells in flow cytometry, and to flag proteins or nucleic acids in gel electrophoresis.

Staining is not limited to biological materials, it can also be used to study the morphology of other materials for example the lamellar structures of semi-crystalline polymers or the domain structures of block copolymers.

In vivo staining (Intra Vital Staining) is the process of dyeing living tissues—in vivo means "in life" (compare with in vitro staining). By causing certain cells or structures to take on contrasting colour, their form (morphology) or position within a cell or tissue can be readily seen and studied. The usual purpose is to reveal cytological details that might otherwise not be apparent; however, staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells or tissues.

In vitro staining involves colouring cells or structures that have been removed from their biological context. Certain stains are often combined to reveal more details and features than a single stain alone. Combined with specific protocols for fixation and sample preparation, scientists and physicians can use these standard techniques as consistent, repeatable diagnostic tools. A counterstain is stain that makes cells or structures more visible, when not completely visible with the principal stain.

While ex vivo, many cells continue to live and metabolize until they are "fixed". Some staining methods are based on this property. Those stains excluded by the living cells but taken up by the already dead cells are called vital stains (e.g. trypan blue or propidium iodide for eukaryotic cells). Those that enter and stain living cells are called supravital stains (e.g. New Methylene Blue and Brilliant Cresyl Blue for reticulocyte staining). However, these stains are eventually toxic to the organism. To achieve desired effects, the stains are used in very dilute solutions ranging from 1:5000 to 1:500000.

Staining Techniques

Because microbial cytoplasm is usually transparent, it is necessary to stain microorganisms before they can be viewed with the light microscope. In some cases, staining is unnecessary, for example when microorganisms are very large or when motility is to be studied, and a drop of the microorganisms can be placed directly on the slide and observed. A preparation such as this is called a wet mount. A wet mount can also be prepared by placing a drop of culture on a cover-slip (a glass cover for a slide) and then inverting it over a hollowed-out slide. This procedure is called the hanging drop.

In preparation for staining, a small sample of microorganisms is placed on a slide and permitted to air dry. The smear is heat fixed by quickly passing it over a flame. Heat fixing kills the organisms, makes them adhere to the slide, and permits them to accept the stain.

Simple stain techniques: Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Such a procedure is the simple stain procedure. An alternative is to use a dye such as nigrosin or Congo red, acidic, negatively charged dyes. They are repelled by the negatively charged cytoplasm and gather around the cells, leaving the cells clear and unstained. This technique is called the negative stain technique.

Differential stain techniques: The differential stain technique distinguishes two kinds of organisms. An example is the Gram stain technique. This differential technique separates bacteria into two groups, Gram-positive bacteria and Gram-negative bacteria.

Another differential stain technique is the acid-fast technique. This technique differentiates species of Mycobacterium from other bacteria.

Other stain techniques seek to identify various bacterial structures of importance. For instance, a special stain technique highlights the flagella of bacteria by coating the flagella with dyes or metals to increase their width. Flagella so stained can then be observed.

A special stain technique is used to examine bacterial spores. Malachite green is used with heat to force the stain into the cells and give them color. A counterstain, safranin, is then used to give color to the nonsporeforming bacteria. At the end of the procedure, spores stain green and other cells stain red.

Types of Staining techniques used in Microbiology and their applications

Stains have been used for diagnosing infectious diseases since the late 1800s. The Gram stain remains the most commonly used stain because it detects and differentiates a wide range of pathogens. The next most commonly used diagnostic technique is acid-fast staining that is used primarily to detect *Mycobacterium tuberculosis* and other severe infections. Many infectious agents grow slowly on culture media or may not grow at all; stains may be the only method to detect these organisms in clinical specimens. In the hands of experienced clinical microscopists, stains provide rapid and cost-effective information for preliminary diagnosis of infectious diseases. A review of the most common staining methods used in the clinical microbiology laboratory is presented here.

Gram staining: Gram stain is a very important differential staining techniques used in the initial characterization and classification of bacteria in Microbiology. Gram staining helps to identify bacterial pathogens in specimens and cultures by their Gram reaction (Gram positive and Gram Negative) and morphology (Cocci/Rod).

The Gram stain is almost always the first step in the identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. This gives rise to Gram-variable and Gram-indeterminate groups as well.

Gram stains are performed on body fluid or biopsy when infection is suspected. Gram stains yield results much more quickly than culture, and is especially important when infection would make an important difference in the patient's treatment and prognosis; examples are cerebrospinal fluid for meningitis and synovial fluid for septic arthritis.

The Basic Method

1. First, a loopful of a pure culture is smeared on a slide and allowed to air dry. The culture can come from a thick suspension of a liquid culture or a pure colony from a plate

suspended in water on the microscope slide. Important considerations:
- Take a small inoculum—don't make a thick smear that cannot be completely decolorized. This could make gram-negative organisms appear to be gram-positive or gram-variable.
- Take a fresh culture—old cultures stain erratically.

2. Fix the cells to the slide by heat or by exposure to methanol. Heat fix the slide by passing it (cell side up) through a flame to warm the glass. Do not let the glass become hot to the touch.
3. Crystal violet (a basic dye) is then added by covering the heat-fixed cells with a prepared solution. Allow to stain for approximately 1 minute.
4. Briefly rinse the slide with water. The heat-fixed cells should look purple at this stage.
5. Add iodine (Gram's iodine) solution (1% iodine, 2% potassium iodide in water) for 1 minute. This acts as a mordant and fixes the dye, making it more difficult to decolorize and reducing some of the variability of the test.
6. Briefly rinse with water.
7. Decolorize the sample by applying 95% ethanol or a mixture of acetone and alcohol. This can be done in a steady stream, or a series of washes. The important aspect is to ensure that all the color has come out that will do so easily. This step washes away unbound crystal violet, leaving Gram-positive organisms stained purple with Gram-negative organisms colorless. The decolorization of the cells is the most "operator-dependent" step of the process and the one that is most likely to be performed incorrectly.
8. Rinse with water to stop decolorization.
9. Rinse the slide with a counter stain (safranin or carbol fuchsin) which stains all cells red. The counter stain stains both gram-negative and gram-positive cells. However, the purple gram-positive color is not altered by the presence of the counter-stain, its effect is only seen in the previously colorless gram-negative cells which now appear pink/red.
10. Blot gently and allow the slide to dry. Do not smear.

Bacteria have a cell wall made up of peptidoglycan. This cell wall provides rigidity to the cell, and protection from osmotic lysis in dilute solutions. Gram-positive bacteria have a thick mesh-like cell wall, gram-negative bacteria have a thin cell wall and an outer phospholipid bilayer membrane. The crystal violet stain is small enough to penetrate through the matrix of the cell wall of both types of cells, but the iodine-dye complex exits only with difficulty.

The decolorizing mixture dehydrates cell wall, and serves as a solvent to rinse out the dye-iodine complex. In Gram-negative bacteria it also dissolves the outer membrane of the gram-negative cell wall aiding in the release of the dye. It is the thickness of the cell wall that characterizes the response of the cells to the staining procedure. In addition to the clearly gram-positive and gram-negative, there are many species that are "gram-variable" with intermediate cell wall structure. As noted above, the decolorization step is critical to the success of the procedure.

Gram's method involves staining the sample cells dark blue, decolorizing those cells with a thin cell wall by rinsing the sample, then counter staining with a red dye. The cells with a thick cell wall appear blue (gram positive) as crystal violet is retained within the cells, and so the red dye cannot be seen. Those cells with a thin cell wall, and therefore decolorized, appear red (gram negative).

Excessive Decolorization

It is clear that the decolorization step is the one most likely to cause problems in the gram stain. The particular concerns in this step are listed below-

1. Excessive heat during fixation: Heat fixing the cells, when done to excess, alters the cell morphology and makes the cells more easily decolorized.
2. Low concentration of crystal violet: Concentrations of crystal violet up to 2% can be used successfully, however low concentrations result in stained cells that are easily decolorized. The standard 0.3% solution is good, if decolorization does not generally exceed 10 seconds.
3. Excessive washing between steps: The crystal violet stain is susceptible to wash-out with water (but not the crystal violet-iodine complex). Do not use more than a 5 second water rinse at any stage of the procedure.
4. Insufficient iodine exposure: The amount of the mordant available is important to the formation of the crystal violet – iodine complex. The lower the concentration, the easier to decolorize (0.33% – 1% commonly used). Also, QC of the reagent is important as exposure to air and elevated temperatures hasten the loss of Gram's iodine from solution. A closed bottle (0.33% starting concentration) at room temperature will lose >50% of available iodine in 30 days, an open bottle >90%. Loss of 60% iodine results in erratic results.
5. Prolonged decolorization: 95% ethanol decolorizes more slowly, and may be recommended for inexperienced technicians while experienced workers can use the acetone-alcohol mix. Skill is needed to gauge when decolorization is complete.
6. Excessive counter staining: As the counter stain is also a basic dye, it is possible to replace the crystal violet—iodine complex in gram- positive cells with an over-exposure to the counter stain. The counter stain should not be left on the slide for more than 30 seconds.

Acid fast stain (Ziehl-Neelsen technique): The Ziehl–Neelsen (ZN) stain is a common standard stain which is readily performed in a majority of histopathology laboratories around the world. It was first described by Dr. Franz Ziehl and Dr Friedrich Neelsen, a German bacteriologist and a German pathologist respectively. Acid-fast stain (Ziehl-Neelsen method) is specifically stains for all members of the genera Mycobacteria. The procedure utilizes heat and phenol (carbolic acid) to help the penetration of the dye, basic fuchsin, to the inside of mycobacterial cells, which are impermeable to basic rosaniline dyes in routine stains such as the Gram stain. The high lipid and wax content of the mycobacterial cell walls is thought to be the reason for such impermeability.

The stain is the gold standard procedure for diagnosis of tuberculosis and leprosy. Being unassociated with the human flora (except *Mycobacterium smegmatis* found in human smegma), finding of acid-fast bacilli in human specimens such as sputum and nasal scrapings is strongly indicative of an active infectious process, namely of tuberculosis and leprosy. Acid-fast pathogens other than mycobacteria include very few genera such as the bacterium Nocardia and the fungus Cryptosporidium. Ziehl-Neelsen stain can also be used for the primary identification of these other acid-fast pathogens.

Procedure:

1. Drop suspension onto slide
2. Air dry slide 10 minutes at 60 °C, heat-fix slide 10 minutes at 90 °C
3. Flood slide with Carbol Fuchsin
4. Hold a flame beneath the slide until steam appears but do not allow it to boil
5. Allow hot slide to sit for 3 to 5 minutes, rinse with tap water
6. Flood slide with 3% hydrochloric acid in isopropyl alcohol
7. Allow to sit 1 minute, rinse with tap water

8. Flood slide with Methylene Blue
9. Allow to sit 1 minute, rinse with tap water
10. Blot dry
11. View under oil immersion lens

Giemsa stain: Giemsa stain is a Romanowsky stain. It is widely used in cytogenetics and for the histopathological diagnosis of Malaria and other blood parasites. Giemsa's solution is a mixture of methylene blue, eosin, and Azure.

- It is specific for the phosphate groups of DNA and attaches itself to regions of DNA where there are high amounts of adenine-thymine bonding. Giemsa stain is used in Giemsa banding, commonly called G-banding, to stain chromosomes and often used to create an idiogram. It can identify chromosomal aberrations such as translocations and rearrangements.
- Giemsa stain is also a differential stain. It can be used to study the adherence of pathogenic bacteria to human cells. It differentially stains human and bacterial cells purple and pink respectively. It can be used for histopathological diagnosis of malaria and some other spirochete and protozoan blood parasites. It is also used in Wolbach's tissue stain.
- Giemsa stain is a classic blood film stain for peripheral blood smears and bone marrow specimens. Erythrocytes stain pink, platelets show a light pale pink, lymphocyte cytoplasm stains sky blue, monocyte cytoplasm stains pale blue, and leukocyte nuclear chromatin stains magenta.
- Giemsa stain is also used to visualize chromosomes.
- Giemsa stains the fungus Histoplasma, Chlamydia bacteria, and can be used to identify Mast cells.

A thin film of the specimen on a microscope slide is fixed in pure methanol for 30 seconds, by immersing it or by putting a few drops of methanol on the slide. The slide is immersed in a freshly prepared 5% Giemsa stain solution for 20–30 minutes (in emergencies 5–10 minutes in 10% solution can be used), then flushed with tap water and left to dry.

Leishman stain: It is basically use to stain leukocytes, Malaria parasite and trypanosomas. Leishman stain contain 1st methylene blue dye, a basic dye, which gives color to an acidic component.2nd eosin dye,an acidic dye ,which gives color to a basic component. These dye differentiat the different component of blood.

Field stain: It is a histological method for staining of blood smears. It is used for staining thin blood films in order to discover malarial parasites. Field's stain is a version of a Romanowsky stain, used for rapid processing of the specimens.

Field's stain uses methylene blue and Azure 1 dissolved in phosphate buffer solution and Eosin Y in buffer solution.

Acridine Orange Stain: Acridine orange is a nucleic acid selective fluorescent cationic dye useful for cell cycle determination. It is cell-permeable, and interacts with DNA and RNA by intercalation or electrostatic attractions respectively.

Acridine orange can be used to differentiate between deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) very easily. When acridine orange bonds with DNA and forms a complex, the emitted radiation is green. When it bonds with RNA and forms a complex, the emitted light is orange.

Auramine-Rhodamine technique: The auramine-rhodamine stain (AR), also known as the Truant auramine-rhodamine stain, is a histological technique used to visualize acid-fast bacilli using fluorescence microscopy, notably species in the Mycobacterium genus. Acid-fast organisms display a reddish-yellow fluorescence. Although the auramine-rhodamine stain is not as specific for acid-fast organisms (i.e. *Mycobacterium tuberculosis* or Nocardia) as the Ziehl-Neelsen stain, it is more affordable and more sensitive, therefore it is often utilized as a screening tool.

AR stain is a mixture of Auramine O and Rhodamine B.

Calcofluor White Staining: It is commonly used to directly detect fungal element and to observe the subtle characteristics of fungi grown in culture. The cell walls of fungi will bind the stain calcofluor white, which greatly enhances visibility of fungal element in tissue or other specimens.

Capsule stain: The capsule stain employs an acidic stain and a basic stain to detect capsule production. *Streptococcus pneumoniae, Neisseria meningitides, Haemophilus influenzae, Klebsiella pneumoniae* are common capsulated bacteria.

Most capsules are composed of polysaccharides, but some are composed of polypeptides. The capsule differs from the slime layer that most bacterial cells produce in that it is a thick, detectable, discrete layer outside the cell wall. Some capsules have well-defined boundaries, and some have fuzzy, trailing edges. Capsules protect bacteria from the phagocytic action of leukocytes and allow pathogens to invade the body. If a pathogen loses its ability to form capsules, it can become avirulent.

Bacterial capsules are non-ionic, so neither acidic nor basic stains will adhere to their surfaces. Therefore, the best way to visualize them is to stain the background using an acidic stain and to stain the cell itself using a basic stain. We use India ink and Gram crystal violet. This leaves the capsule as a clear halo surrounding a purple cell in a field of black.

The medium in which the culture is grown as well as the temperature at which it is grown and the age of the culture will affect capsule formation. Older cultures are more likely to exhibit capsule production. When performing a capsule stain on your unknown, be sure the culture you take your sample from is at least five days old.

Cytoplasmic inclusion stains: Identifies intracellular deposits of starch, glycogen, polyphosphates, hydroxybutyrate, and other substances. E.g. Albert staining is used to stain the volutin or metachromatic granules of *C. diphtheria*.

Endospore stain: It demonstrates spore structure in bacteria as well as free spores. Relatively few species of bacteria produce endospores, so a positive result from endopore staining methods is an important clue in bacterial identification. *Bacillus* spp and *Clostridium* spp are main endospore producing bacterial genera.

Bacterial endosporesare metabolically inactive, highly resistant structures produced by some bacteria as a defensive strategy against unfavorable environmental conditions. The bacteria can remain in this suspended state until conditions become favorable and they can germinate and return to their vegetative state. The primary stain applied is malachite green, which stains both vegetative cells and endospores. Heat is applied to help the primary stain penetrate the endospore. The cells are then decolorized with water, which removes the malachite green from the vegetative cell but not the endospore. Safranin is then applied to counter stain any cells which have been decolorized. At the end of the staining process, vegetative cells will be pink, and endospores will be dark green.

Flagella stain: Bacterial flagella are appendages used for motility. Their presence is a useful tool for identification and differentiation of prokaryotes. Since flagella are too thin to be seen by compound light microscopy, staining methods employ the use of a mordant (often tannic acid) to make them thick enough to see using an oil immersion objective. Two protocols are described. Basic Protocol 1 is a modified Leifson method and is the one that many microbiologists have adapted. Basic Protocol 2 is a wet-mount stain using a Ryu stain and is included because the stain is stable at room temperature. Both of these methods are fairly time-consuming, taking from 15 to as long as 60 min to perform.

Joseph Lister

Born: 5-Apr-1827
Birthplace: Upton, Essex, England
Died: 10-Feb-1912
Location of death: Walmer, Kent, England
Gender: Male
Religion: Anglican/Episcopalian
Race or Ethnicity: White
Occupation: Doctor
Nationality: England

Executive summary: Pioneer of antiseptic surgery

Joseph Lister is the surgeon who introduced new principles of cleanliness which transformed surgical practice in the late 1800s.

Lister came from a prosperous home in Upton, Essex, son of Joseph Jackson Lister, a pioneer of achromatic object lenses for the compound microscope.

At Quaker schools, he became a fluent reader of French and German, which were also the leading languages of medical research. As a teenager, Lister attended Grove House School Tottenham, studying mathematics, natural science, and languages.

He attended University College London, one of only a few institutions which accepted Quakers at that time. He initially studied botany and obtained a bachelor of Arts degree in 1847. He registered as a medical student and graduated with honours as Bachelor of Medicine, subsequently entering the Royal College of Surgeons at the age of 26.

To realize Lister's work it is necessary to remember the condition of surgical practice at that date.

Until Lister's studies of surgery most people believed that chemical damage from exposures to bad air (see "miasma") was responsible for infections in wounds. Hospital wards were occasionally aired out at midday as a precaution against the spread of infection via miasma, but facilities for washing hands or a patient's wounds were not available. A surgeon was not required to wash his hands before seeing a patient because such practices were not considered necessary to avoid infection. Despite the work of Ignaz Semmelweis and Dr. Oliver Wendell Holmes, Sr., hospitals practised surgery under unsanitary conditions.

About the middle of the 19th century the introduction of anaesthetics had relieved the patient of much of the horror of the knife, and the surgeon of the duty of speed in his work. The pain of the operation itself no longer counted, and the surgeon was enabled not only to be as cautious and sedulous as dexterous, but also to venture upon long, profound and intricate operations which before had been out of the question. Yet unhappily this new enfranchisement seemed to be but an ironical liberty of Nature, who with the other hand took away what she had given. Direct healing of surgical wounds ("by first intention"), far from being the rule, was a piece of luck too rare to enter into the calculations of the operator; while of the graver surgical undertakings, however successful mechanically, the mortality by sepsis was ghastly. Suppuration, phagedaena and septic poisonings of the system carried away even the most promising patients and followed even trifling operations. Often, too, these diseases rose to the height of epidemic pestilences, so that patients, however extreme their need, dreaded the very name of hospital, and the most skillful surgeons distrusted their own craft.

While he was a professor of surgery at the University of Glasgow, Lister became aware of a paper published by the French chemist Louis Pasteur, showing that rotting and fermentation could occur under anaerobic conditions if micro-organisms were present. Pasteur suggested three methods to eliminate the micro-organisms responsible for gangrene: filtration, exposure to heat, or exposure to chemical solutions. Lister confirmed Pasteur's conclusions with his own experiments and decided to use his findings to develop antiseptic techniques for wounds.

As the first two methods suggested by Pasteur were inappropriate for the treatment of human tissue, Lister experimented with the third method, that of exposing the wound to chemicals.

Friedlieb Runge (1797–1867) discovered creosote, which later was processed into carbolic acid. Although Runge had no understanding of how decomposition occurred, the chemical had been used to treat the wood used for railway ties and ships since it protected the wood from rotting. Later, it was used for treating sewage in England, Belgium and Holland. The same chemical was also used to fight parasites and reduce the odors during cholera and cattle plague. In 1867, Lister championed the use of carbolic acid as an antiseptic, such that it became the first widely used antiseptic in surgery. He first suspected it would prove an adequate disinfectant because it was used to ease the stench from fields irrigated with sewage waste. He presumed it was safe because fields treated with carbolic acid produced no apparent ill-effects on the livestock that later grazed upon them.

Therefore, Lister tested the results of spraying instruments, the surgical incisions, and dressings with a solution of it. Lister found that carbolic acid solution swabbed on wounds remarkably reduced the incidence of gangrene. In August 1865, Lister applied a piece of lint dipped in carbolic acid solution onto the wound of an eleven-year-old boy at Glasgow Infirmary, who had sustained a compound fracture after a cart wheel had passed over his leg. After four days, he renewed the pad and discovered that no infection had developed He chose dressings soaked with carbolic acid (phenol) to cover the wound and the rate of infection was vastly reduced. He subsequently published his results in The Lancet in a series of 6 articles, running from March through July 1867, entitled: "On a new method of treating compound fracture, abscess, etc.: with observation on the conditions suppuration". Later, on 9 August 1867, he read a paper before the British Medical Association in Dublin, on the Antiseptic Principle of the Practice of Surgery, which was reprinted in The British Medical Journal.

He instructed surgeons under his responsibility to wear clean gloves and wash their hands before and after operations with 5% carbolic acid solutions. Instruments were also washed in the same solution and assistants sprayed the solution in the operating theatre. His lowered infection rate was very good and Listerian principles were adopted throughout many countries by a number of surgeons.

Lister left Glasgow in 1869, returning to Edinburgh as Professor of Surgery at the University of Edinburgh, and continued to develop improved methods of antisepsis and asepsis. His fame had spread by then, and audiences often came to hear him lecture. As the germ theory of disease became more widely accepted, it was realised that infection could be better avoided by preventing bacteria from getting into wounds in the first place. This led to the rise of sterile surgery. Some consider Lister "the father of modern antiseptics".

Enjoy the humour

One day a girl went to visit an art gallery,
She saw a photo & said to the owner.
Girl: You call this horror photo a modern artfi
Owner: Oh dear! Don't take stress,
Go home, this is a mirror!!!

A man waiting at the doctor's clinic
Another patient comes in and asks "W w why are y y y you hh herefi"
The man replies "Well, if you must know. I have a prostate problem"
"A pp prostate ppp problem w what's ttthatfi"
" Well, I pee like you talk."

Foreigner: In India where does the,
Ice fall morefi
Smart answer by Santa…..
Santa: Before 8 p.m. in Kashmir,
& after 8 p.m. in glass of whiskey…

Boy: Where are you goingfi
Girl: For committing suicide.
Boy: Then why have you put on
So much of make up…fi
Girl: Oh! Stupid, tomorrow my,
Photo will come in the newspaper…

A mosquito & a fly get married…
Next day the fly was crying,
Her friend asked: What happened sisterfi
She said: Yesterday night I put on the
GOODNIGHT & your jijaji died….

One day a owner was having bath in the bathroom,
& the servant entered the bathroom,
The owner said:-
" Why did you enter the bathroom,
didn't you know I was having a bathfi "
The servant replied:-
" I am sorry Sir, I thought that
Mistress is inside, having a bath. "

A man went to a doctor for a
check up of his brain…
Doctor: You have a brain tumour.
Man: Oh! That's great!
Doctor: Why are you so happyfi
Man: By this it is proved that I have a brain…!

One day a man was passing through a jungle,
There a witch stopped him & said:-
" Hoo Hoo Haa Ha Ha, I am a witch. "
The man stared at her & replied:-
" I know, your twin sister is in my house!! "

One day father & his son were sitting on a beach
& talking to each other,
The son asks his father:-
" Father, why do we call it a 'beach'fi "
The father said:-
" Don't you knowfi "
The son said:-
" No. "
The father answered:-
" This is between the sky & land,
that's why we call it a beach. "

Husband: Can you be the moon of my lifefi
Wife: Wow! Yes sweet heart…!
Husband: Great! then….
Stay 9,955,887.6kms away from me…!!

What's the similarity between,
SUN & WIFE…fi
Very simple….
You can't stare at both of them…!

Wife: Tell me such a thing,
that I can become happy as well as sad…
Husband: You are my life,
& shame on such kind of life.

A police asked to a thief,
" Why did you steal the same rack 3 times from the storefi"
The thief replied,
" Sir, I stole one dress for my wife,
& I came to change it twice. "

Caulobacter crescentus

Classification
Higher order taxa
Cellular organisms; Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacteralus; Caulobacteraceae; Caulobacter
Species
Caulobacter crescentus

Description and significance
Caulobacter crescentus is a rod-shaped single-celled organism found in fresh water, soil and sea water. Often flourishing in low nutrient environments, this gram-negative bacterium undergoes a dimorphic life cycle that is beneficial in its competitive surroundings. It produces one swimming cell and one sessile cell at each cell cycle. (3,4) Prior to cell division, *C. crescentus* possesses a stalk (or prosthecae) at one cell pole and a flagellum at the other. Following cytokinesis, the flagellated motile daughter cell (swarmer) swims for about one-third of the cell cycle. The swarmer then sheds its polar flagellum and from the same pole produces an adhesive holdfast. The holdfast, to date the strongest measured biological glue, cements *C. crescentus* to surfaces in aquatic environments. (2, 5)
It is important to know the genomic sequence of the *C. crescentus* because the asymmetric processes in this organism provide useful models for cellular differentiation and development. Stalk synthesis is also a highly tractable model for testing current models for cell shape determination providing general information concerning the production of cell shape in any bacterium. (2)
Caulobacters are generally isolated from environments of low organic content; however, readily isolated strains from a variety of sewage treatment system designs and locations were found and 33 distinct strains were characterized. Most were morphologically similar, having the crescent-shaped cell body, short stalk, and hexagonally packed, paracrystalline surface (S) layer characteristic of several *Caulobacter crescentus* laboratory strains. (7)

Genome structure
The genome sequence of *C. crescentus* CB15 (wild-type strain) was determined by the whole genome random sequencing method. The genome consists of a single circular chromosome of 4,016,942 base pairs encoding 3,767 genes with an average G + C content of 67.2%. The completion of the full genome sequence of this organism provides access to the complete signal transduction network that controls differentiation and cell cycle progression within the context of a unicellular organism growing in a dilute nutrient environment. (6)
Genome analysis identified a large number of genes that would enable utilization of dilute carbon sources and provides a comprehensive picture of the strategies used by *C. crescentus* for survival in nutrient-limiting conditions. Unlike *E. coli* and *Vibrio cholerae*, *C. crescentus* has no OmpF-type outer membrane porins that allow the passive diffusion of hydrophilic substrates across outer membrane. However, it does possess 65 members of the family of TonB-dependent outer membrane channels that catalyze energy-dependent transport across the outer membrane. This is more than any other organism thus far characterized and with no other sequenced proteobacteria possessing more than 10. (6)
The genome contains multiple clusters of genes encoding essential proteins. Included are those involved in chemotaxis, outer membrane channel function, degradation of aromatic ring compounds, and the breakdown of plant-derived carbon sources, in addition to many extra cytoplasmic function sigma factors, providing the organism with the ability to respond to a wide range of environmental fluctuations. *C. crescentus* is the first free-living alpha-class proteobacterium to be sequenced and will serve as a foundation for exploring the biology of this group of bacteria. (6)

Cell structure and metabolism
C. crescentus cells are curved rods. They divide into two distinct types of cells, division-competent stalked cells and shorter, flagellated swarmer cells that cannot divide. Swarmer cells shed their flagellum and develop into stalked cells before dividing. (1)
Ideal growth conditions require the presence of oxygen and organic nutrients in aquatic environments at an optimal temperature of 35ºC. *C. crescentus* contains a number of energy-dependent transport systems, presumably enabling growth in the substrate-sparse aquatic environments that it lives in. A few other distinguishable characteristics of the *C. crescentus* include a short stalk, which are known to enhance nutrient uptake, and a surface (S) layer for protection. Stalk morphogenesis occurs specifically at one cell pole, the elongation is directional (stalk do not grow into the cell body), and it culminates in the formation of a tube that is approximately 100 nm in diameter, or about one-fifth the diameter of the cell body cylinder. (2) Stalks also produce an adhesive holdfast that is found at the tip of the *C. crescentus* stalk. *Caulobacter crescentus* is covered with a crystalline protein surface layer (S-layer) composed of the 98-kDa protein RsaA. (8) There is evidence that S-layers provide a protective barrier for cells that enables them to resist attack by lytic enzymes or possess key surface features that enable them to interact with host organisms to effect pathogenesis. It seems reasonable that to be a protective barrier, an S-layer must completely cover a cell. This seems to be the case in *Caulobacter crescentus*; although significant morphogenesis occurs, the entire bacterium, including the stalk, is covered at all times. (9)
The presence in *C. crescentus* of a 20-gene cluster for the metabolism of aromatic compounds, a pathway extensively characterized only in soil bacteria including *Pseudomonas* and *Streptomyces* species, highlights a shared biology between this aquatic species and various species of soil bacteria. It also suggests that *C. crescentus* may be exposed to diverse substrates of terrestrial origin in its natural habitat. As revealed by comparative genome analysis, this shared biology between *C. crescentus* and soil organisms extends to other cellular processes. The conservation of gene order and the sequence similarity of genes involved in intermediary metabolism again suggests that gene transfer between these species has taken place. Consistent with this concept, it has been experimentally demonstrated that *C. crescentus* is able to integrate, retain, and efficiently express plasmid encoded degradative pathway genes from Pseudomonas putida. The presence of genes for the breakdown of numerous plant polysaccharides, including cellulose, xylan, lignin, glucan, and pectin, as well as transporter systems for the import of the

resulting sugars, suggests that, unexpectedly, plant polymers are a significant source of metabolites for the central intermediary metabolism of this organism. (6)

Ecology

No extensive research was found regarding any interactions of *C. crescentus* with other organisms in its environment however, *C. crescentus* plays an important part in biogeochemical cycling of organic nutrients, thus exhibiting a significant role in the carbon cycle.

In addition, due to its distinctive ability to survive in nutrient-limiting conditions, *C. crescentus* has been selected for extensive studies in broad environmental habitats where contamination may be present. For example, potentially hazardous levels of heavy metals have dispersed into subsurface sediment and groundwater in a number of metal contaminated DOE sites and represent a challenge for environmental restoration. Effective bioremediation of these sites requires knowledge of genetic pathways for resistance and biotransformation by component organisms within a microbial community, including *C. crescentus*. The recently completed sequence of the strain *C. crescentus* CB15 has provided the information to study genome wide response to heavy metal stress. (10)

Pathology

There are no known diseases that are caused by *C. crescentus*. It has not been found to be pathogenic.

Application to Biotechnology

Caulobacters are the most prevalent organisms adapted solely for survival in nutrient-poor aquatic and marine environments. The completion of the genomic sequence now lays the foundation for understanding, on a molecular level, how this bacterium's obligate differentiation and asymmetric division enable it to thrive in such dilute habitats. Furthermore, the tools developed for genetic manipulation of *C. crescentus* make it an attractive organism for development as a bioremediation agent. (6)

Current Research

Bacterial Morphogenesis

A remarkable example of bacterial morphogenesis is the elaboration of long, tubular extensions of the cell envelope of certain aquatic bacteria, such as *Caulobacter crescentus*. These appendages, also called prosthecae or stalks, possess features that make them particularly amenable models for experiments designed to uncover general principles of cell morphogenesis and of cell shape function. Recent evidence supports the hypothesis that stalk synthesis in *Caulobacter crescentus* is a specialized form of cell elongation that confers to the cell substantial advantages in nutrient uptake. Further insights into the mechanisms and function of stalk synthesis will require a multidisciplinary systems biology approach using principles and methodologies from ecology and evolutionary biology to biophysics and mathematical modeling. (2)

Stalk Synthesis

The identification of bona-fide 'stalk-less' mutants of *C. crescentus* would be a boon not only because they would provide clues about how a bacterial shape is determined, but also because a non-pleiotropic stalkless mutant could be utilized in experiments testing the relative fitness of cells with and without stalks under various selective pressures. Thus, we could test hypotheses concerning the possible function of a specific bacterial shape under different environmental conditions. (2)

Microfluidics

Even though there is a growing abundance of data suggesting that the stalk enhances nutrient uptake to the cell, there is a need for more sophisticated experiments to assay nutrient uptake by the stalk in a direct manner. This might be possible using microfluidics to deliver nutrients to precise locations in the cell. For example, microfluidics devices could be used to determine if *C. crescentus* cells are capable of growth when nutrients are directed only at stalks, if the stalk absorbs particular nutrients, or whether the stalk is competent to deliver nutrients to the cell body at all. The information we gain from studying the basic process of bacterial morphogenesis will increase our basic understanding of how bacterial cells are organized, but will also be useful for environmental, industrial, agricultural and medical applications.(2)

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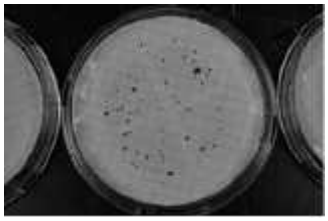
Indicator Bacteria

What are bacteria ?



Bacteria are microscopic, single-celled organisms that are the most numerous organisms on earth. They are so small that over five million could be placed on the head of a pin. Bacteria can live in numerous environments and perform many complex actions, some of which are beneficial and some harmful. Most bacteria, however, are not harmful and do not cause human health problems. Those that are disease producing is referred to as pathogenic. Viruses and some protozoans can also be pathogenic. Coliform bacteria are part of the Enterobacteriaceae family and individual cells cannot be seen with the naked eye due to their small size (but colonies can be seen.) While some coliform bacteria can be naturally found in soil, the type of coliform bacteria that lives in the intestinal tract of warm-blooded animals and originates from animal and human waste is called fecal coliform bacteria.

Escherichia coli (*E. coli*) is one subgroup of fecal coliform bacteria. Even within this species, there are numerous different strains, some of which can be harmful. However, the release of these naturally-occurring organisms into the environment is generally not a cause for alarm. But, other disease causing bacteria, which can include some pathogenic strains of *E. coli*, or viruses may also be present in these wastes and pose a health threat.

What are indicator bacteria ?

The use of an organism that can serve as a surrogate for another is called an indicator organism. Direct testing for pathogens is very expensive and impractical, because pathogens are rarely found in water bodies. Further, testing for pathogens requires large volumes of water, and the pathogens can often be difficult to grow in the laboratory and isolate. Instead, monitoring for pathogens uses "indicator" species—so called because their presence indicates that fecal contamination may have occurred. Each gram of human feces contains approximately ~100 billion (1×10^{11}) bacteria. These bacteria may include species of pathogenic bacteria, such as *Salmonella* or *Campylobacter*, associated with gastroenteritis.

Types of indicator organisms

Commonly used indicator bacteria include total coliforms, or a subset of this group, fecal coliforms, which are found in the intestinal tracts of warm blooded animals. Total coliforms were used as fecal indicators by public agencies in the US as early as the 1920s. These organisms can be identified based on the fact that they all metabolize the sugar lactose, producing both acid and gas as byproducts. Fecal coliforms are more useful as indicators in recreational waters than total coliforms which include species that are naturally found in plants and soil; however, there are even some species of fecal coliforms that do not have a fecal origin, such as *Klebsiella pneumoniae*. Perhaps the biggest drawback to using coliforms as indicators is that they can grow in water under certain conditions. *Escherichia coli* (*E. coli*) and enterococci are also used as indicators.

Why Monitor Bacteria ?

Pathogenic microorganisms (including bacteria, viruses, and protozoans) are associated with fecal waste and can cause a variety of diseases including typhoid fever, cholera, giardiasis (a parasitic infection of the small intestine), and hepatitis, either through the consumption of contaminated shellfish or ingestion of tainted water. Since these pathogens tend to be found in very low concentrations in the water, and there are many different pathogens, it is difficult to monitor them directly. Also, pathogens are shed into the waste stream inconsistently. For these reasons, direct testing for pathogens is expensive and nearly impossible.

E. coli bacteria are good indicator organisms of fecal contamination because they generally live longer than pathogens, are found in greater numbers, and are less risky to collect or culture in a laboratory than pathogens. However, their presence does not necessarily mean that pathogens are present, but rather indicates a potential health hazard.

The EPA (Environmental Protection Agency) has determined that *E. coli* are one of the best indicators for the presence of potentially pathogenic bacteria (EPA, 2002b). Because *E. coli* monitoring does not measure the actual pathogens, the assessment is not foolproof; however, it is a good approach for assessing the likelihood of risks to human health. Monitoring for these indicator organisms is an easy and economical method for citizens or professionals to assess health risks due to bacterial contamination of surface waters.

Common sources of *E. coli*

Bacteria in water can originate from the intestinal tracts of both humans and other warm-blooded animals, such as pets, livestock and wildlife. Human sources include failing septic tanks, leaking sewer lines, wastewater treatment plants, combined sewer overflow (CSOs), boat discharges, swimming "accidents" and urban storm water runoff. In urban watersheds, fecal indicator bacteria are significantly correlated with human density (Frenzel and Couvillion, 2002).

Animal sources of fecal coliform bacteria include manure spread on land, livestock in runoff or in streams, improperly disposed farm animal wastes, pet wastes (dogs, cats), wildlife (deer, elk, raccoons, etc.), and birds (geese, pigeons, ducks, gulls, etc.).

Common routes of bacteria to streams

Polluted water runoff from the land is the leading cause of water quality problems nationwide (USEPA, 2002a). Fecal material as well as other pollutants can be transported to waterways through runoff. How quickly they are transported partially depends on the type of land use. Non-developed lands including grasses and other vegetation tend to soak up rainfall, thereby increasing infiltration into the ground and reducing runoff to waterways. Developed lands such as streets, rooftops, sidewalks, parking lots, driveways, and other hard surfaces tend to create more impervious surfaces, and runoff increases. Lands that support domesticated animals, such as cattle, hogs, or horses, can also be a source of bacteria, particularly if animals enter the water for drinking or if heavy rains wash manure from the land into receiving waters.

Another source of bacteria pollution to stream waters comes from

Combined Sewer Overflows (CSOs). Some sewer and storm water pipes are not separated. When a large storm event occurs, the wastewater treatment plants cannot handle the excess volume of water being pumped to them. As a result, untreated sewage along with storm water is dumped directly into rivers and streams. The presence and levels of *E. coli* in a stream do not give an indication of the source of the contamination. However, it can be a good first step in investigating the watershed for potential sources.

Risks to human health

Most people are concerned about the risk that bacteria may pose to human health. When numbers are above health standards, people exposed to water that contain bacteria may exhibit fever, diarrhea and abdominal cramps, chest pain, or hepatitis. While *E. coli* by itself is not generally a cause for alarm, other pathogens of fecal origin that are health threats include *Salmonella, Shigella,* and *Psuedomonas aeruginosa.* Non-bacterial pathogens that may be present with fecal material include protozoans, such as *Cryptosporidium* and *Giardia*, and viruses. There are some strains of *E. coli* that are pathogenic themselves. One that has received much attention is the *E. coli* strain named 0157:H7 that lives in the intestinal tract of cattle. This strain is primarily spread to people by eating contaminated, undercooked beef or drinking unpasteurized milk and is not generally found in surface waters.

Fecal indicator bacteria are used to assess the microbiological quality of water. Although these bacteria are not typically disease causing, they are associated with fecal contamination and the possible presence of waterborne pathogens. The density of indicator bacteria is a measure of water safety for body-contact recreation or for consumption. Fecal material from warm-blooded animals may contain a variety of intestinal microorganisms (viruses, bacteria, and protozoa) that are pathogenic to humans. For example, bacterial pathogens of the genera *Salmonella*, *Shigella*, and *Vibrio* can result in several types of illness and diseases in humans, including gastroenteritis and bacillary dysentery, typhoid fever, and cholera. Bacteriological tests for specific indicator bacteria are used to assess the sanitary quality of water and sediments and the potential public health risk from gastrointestinal pathogens carried by water. The suitability of indicator organisms for these purposes is ranked according to a specific set of criteria, described below.

Criteria for selecting an indicator of fecal contamination in water

The preferred fecal indicator:

- Can be tested for easily
- Is of human or other animal origin
- Survives as long as, or longer than, pathogens
- Is present at densities correlated with fecal contamination
- Can be used as a surrogate for many different pathogens
- Is appropriate for fresh and saline aqueous environments

Two methods can be used to test for indicator bacteria in the field: (1) the membrane-filtration method and (2) the liquid broth method, using the presence-absence format or the most-probable-number (MPN) format.

Water quality standards for bacteria

Drinking water standards

World Health Organization Guidelines for Drinking Water Quality state that as an indicator organism *Escherichia coli* provides conclusive evidence of recent fecal pollution and should *not* be present in water meant for human consumption. In the U.S., the EPA total coliform rule states that a water system is out of compliance if more than 5% of its monthly water samples contain coliforms.

Recreational standards

Early studies showed that individuals who swam in waters with geometric mean coliform densities above 2300/100 mL for three days had higher illness rates. In the 1960s, these numbers were converted to fecal coliform concentrations assuming 18% of total coliforms were fecal. Consequently, the National Technical Advisory Committee (NTAC) in the US recommended the following standard for recreational waters in 1968: 10% of total samples during any 30-day period should not exceed 400 fecal coliforms/100 mL or a log mean of 200/100 mL (based on a minimum of 5 samples taken over not more than a 30-day period). Despite criticism, the US Environmental Protection Agency (EPA) recommended this criterion again in 1976, however, numerous studies were initiated by the EPA in the 1970s and 80's to overcome the weaknesses of the earlier studies. In 1986, the EPA revised its bacteriological ambient water quality criteria recommendations to include *E. coli* and enterococci.

Single Sample Maximum Allowable Density per 100 mL							
Water Type	**Indicator**	**Acceptable Swimming-Associated Gastroenteritis Rate per 1000 Swimmers**	**Steady State Geometric Mean Indicator Density per 100 mL**	**Designated Beach Area (upper 75% C.L.)**	**Moderate Full Body Contact Recreation (upper 82% C.L.)**	**Lightly Used Full Body Contact Recreation (upper 90% C.L.)**	**Infrequently Used Full Body Contact Recreation (upper 95% C.L.)**
Freshwater	*E. coli*	8	126	235	298	409	575
	Enterococci	8	33	61	78	107	151
Marine Water	*E. coli*	19	35	104	158	276	501

Canada's National Agri-Environmental Standards Initiative's approach to characterizing risks associated with fecal water pollution bacterial water quality at agricultural sites is to compare these sites with those at reference sites away from human or livestock sources. This approach generally results in lower levels if *E. coli* being used as a standard or "benchmark" based on a study that indicated pathogens were detected in 80% of water samples with less than 100 cfu *E. coli* per 100 mL.

Risk assessment for exposure to pathogens in recreational waters

Most cases of bacterial gastroenteritis are caused by food-borne enteric microorganisms, such as *Salmonella* and *Campylobacter*; however, it is also important to understand the risk of exposure to pathogens via recreational waters. This is especially the case in watersheds where human or animal wastes are discharged to streams and downstream waters are used for swimming or other recreational activities. Other important pathogens other than bacteria include viruses such as rotavirus, hepatitis A and hepatitis E and protozoa like giardia, cryptosporidium and *Naegleria fowleri*. Due to the difficulties associated with monitoring pathogens in the environment, risk assessments often rely on the use of indicator bacteria.

Epidemiological studies

In the 1950s, a series of epidemiological studies were done in the US to determine the relationship between water quality of natural waters and the health of bathers. The results indicated that swimmers were more likely to have gastrointestinal symptoms, eye infections, skin complaints, ear, nose, and throat infections and respiratory illness than non-swimmers and in some cases, higher coliform levels correlated to higher incidence of gastrointestinal illness, although the sample sizes in these studies were small. Since then, studies have been done to confirm causative relations between swimming and certain health outcomes. A review of 22 studies in 1998 confirmed that the health risks for swimmers increased as the number of indicator bacteria increased in recreational waters and that *E. coli* and enterococci concentrations correlated best with health outcomes among all the indicators studied. The relative risk (RR) of illness for swimmers in polluted freshwater versus swimmers in unpolluted water was between 1-2 for the majority of the data sets reviewed. The same study concluded that bacterial indicators were not well correlated to virus concentrations.

Fate and transport of pathogens

Survival of pathogens in waste materials, soil, or water, depends on many environmental factors including temperature, pH, organic matter content, moisture, exposure to light, and the presence of other organisms. Fecal material can be directly deposited, washed into waters by overland runoff, transported through the ground, or discharged to surface waters via sewer lines, pipes, or drainage tiles. Risk of exposure to humans requires pathogens to (1) survive and be present (2) recreate in surface waters and (3) Individuals to come in contact with water for sufficient time, or ingest sufficient volumes of water to receive an infectious dose. Die-off rates of bacteria in the environment are often exponential, therefore, direct deposition of fecal material into waters generally contribute higher concentrations of pathogens than material that must be transported overland or through the subsurface.

Human exposure

In general, children, the elderly, and immunocompromised individuals require a lower dose of a pathogenic organism in order to contract an infection. Unfortunately, there are very few studies which are able to quantify the amount of time people are likely to spend in recreational waters and how much water they are likely to ingest. In general, children swim more often, stay in the water longer, submerge their heads more often, and swallow more water.

Quantitative microbiological risk assessment (QMRA)

Quantitative microbial risk assessments (QMRA) combine pathogen concentrations in water with dose-response relationships and data reflecting potential exposure to estimate the risk of infection. Data on water exposure are generally collected using questionnaires, but may also be determined from actual measurements of water ingested, or estimated from previously published data. Respondents are asked to report the frequency and timing and location of exposures, detailed information about the amount of water swallowed and head submersion, and basic demographic characteristics such as age, gender, socioeconomic status and family composition. Once sufficient data are collected and determined to be representative of the general population, they are usually fit with distributions, and these distribution parameters are then used in the risk assessment equations. Monitoring data representing occurrence of pathogens, direct measurement of pathogen concentrations, or estimations deriving pathogen concentrations from indicator bacteria concentrations, are also fit with distributions. Dose is calculated by multiplying the concentration of pathogens per volume by volume. Dose-responses can also be fit with a distribution.

Risk management and policy implications

The more assumptions that are made, the more uncertain estimates of risk related to pathogens will be. However, even with considerable uncertainty, QMRAs are a good way to compare different risk scenarios. In a study comparing estimated health risks from exposures to recreational waters impacted by human and non-human sources of fecal contamination, QMRA determined that the risk of gastrointestinal illness from exposure to waters impacted by cattle were similar to those impacted by human waste, and these were higher than for waters impacted by gull, chicken, or pig faeces. Such studies could be useful to risk managers for determining how best to focus their limited resources, however, risk managers must be aware of the limitations of data used in these calculations. For example, this study used data describing concentrations of *Salmonella* in chicken feces published in 1969. Methods for quantifying bacteria, changes in animal housing practices and sanitation, and many other factors may have changed the prevalence of *Salmonella* since that time. Also, such an approach often ignores the complicated fate and transport processes that determine bacteria concentrations from the source to the point of exposure.

Troubleshooting Guide for Disc Diffusion Test in Antimicrobial Susceptibility Testing

ERRONEOUS RESULT	PROBABLE CAUSE	CORRECTIVE ACTION
Tetracycline zone too large and clindamycin zone too small with *E. coli* or *S. aureus* control strains.	pH of medium too low.	Adjust pH 7.2 to 7.4 before pouring media. Commercial media should not have pH problems. Report to manufacturer.
Tetracycline zone too small and clindamycin zone too large with *S. aureus* or *E. coli* control strain.	pH of medium too high.	Get a new lot. (Incubation in CO_2 may alter agar surface pH.)
Aminoglycoside zone too small with *P. aeruginosa,* Acinetobacter control strain.	Calcium ion and/or Magnesium ion too high in medium.	Acquire a new lot of agar medium that will meet QC criteria.
Aminoglycoside zone too large with *P. aeruginosa* control strain.	Calcium ion and/or Magnesium ion too low in medium.	Acquire a new lot of agar medium that will meet QC criteria.
Zones universally too large on control plates.	Inoculum too light.	Adjust inoculum to a McFarland 0.5 turbidity standard.
	Nutritionally poor medium.	Use only Mueller Hinton Agar medium.
	Slow-growing organism. (not seen with controls)	Use minimum inhibtory concentration (MIC) procedure only.
	Improper medium depth. (too thin)	Use 4-5mm depth.
Zones universally too small on control plates.	Inoculum too heavy.	Adjust inoculum to a McFarland 0.5 turbidity standard.
	Agar depth too thick. (minor)	Use 4-5mm depth.
Methicillin zone decreasing over days or weeks with control organisms.	Methicillin degrading during refrigerator storage	Change methicillin discs or use oxacillin or nafcillin as the routine disc.
Methicillin zone indeterminate in disc test.	Methicillin being degraded by strong beta-lactamase producing staphylococci.	Change methicillin discs or use oxacillin or nafcillin as the routine disc.
Carbenicillin zone disappears with *Pseudomonas* control.	Resistant mutant has been selected for testing.	Change *Pseudomonas* control strain every two weeks and whenever resistant mutants appear within the carbenicillin zone.
S.aureus from a patient was resistant to methicillin one day and sensitive the next.	May be two different organisms. Temperature shift from 35-37ºC can dramatically alter the zone size in this case.	Check testing temperature. Test must be performed at 35ºC or 37ºC. for methicillin (oxacillin or nafcillin) and *S.aureus.*
A single disc result above or below the control limit.	Error in reading. Fuzzy zone edge. Transcription error. Bad disc.	Note error. Recheck error and ask for a second opinion.
	Disc may not be pressed firmly onto the agar surface. (Bad discs usually demonstrate a trend toward being out of control.)	Statistically, one may expect an occasional out-of-range result. Values usually fall within range on retesting.

Troubleshooting Guide for Disc Diffusion Test in Antimicrobial Susceptibility Testing

ERRONEOUS RESULT	PROBABLE CAUSE	CORRECTIVE ACTION
Colonies within zone of inhibition.	Mixed culture.	Isolate, identify and retest pure cultures only.
	Resistant mutants within zone.	Gram stain or do another test to rule out contamination.
Very large zones with anaerobes.	–	Do not use disc agar diffusion procedure to test anaerobes.
With colistin, growth seen immediately adjacent to disc, then larger zone at endpoint (Occurs with colistin when testing *Serratia* spp. and some *Enterobacter* spp.).	"Prozone-like" phenomenon.	Confirm with MIC.
The methicillin disc test shows "resistant" but an MIC shows "sensitive" for *S.aureus.*	Mueller Hinton Broth is inadequate in this case. A modified broth used in some commercial MIC systems frequently eliminates this problem.	No action necessary with disc test. To be expected if Mueller Hinton Broth is used in MIC test. Use broth with 2% NaCl if MIC testing is necessary.
	Low methicillin content in disc.	Use new discs
Zones overlap.	Discs too close together.	Use no more than 12 discs on a 150mm plate and 4 to 5 discs on a 100mm plate. Place discs no closer than 15mm from the edge of the plate.
"Zone within a zone"	Swarming / movement of Proteus spp.	Read the wide distinct zone and disregard the growth that swarmed over.
	Feather edges of zones around penicillin or ampicillin discs usually occur with beta-lactamase negative strains of S. aureus.	Take half the distance from the inner zone to outermost zone as measure mark.
	Sulfonamides	Disregard growth from disc margin to the major inner zone.
	Beta-lactamase-positive Haemophilus influenzae with penicillin or ampicillin.	Use inside zone.
Zones indistinct (hazy) with single colonies noted on the plate.	Poorly streaked plate. Inadequate inoculum.	Use properly adjusted inoculum and repeat test.
Indistinct zones with sulfamethoxazole with or without trimethoprim or with trimethoprimalone.	Thymidine in medium inhibits the action of these antimicrobics.	Use commercial thymidine-free plates. Disregard small amount of growth within the zone as with sulfonamides.

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   L.J. solid media based, ten secondary drug panel for MTB sensitivity tests with 2 control.
   CAT No.: M-20305202 Pack size: One Set
10. **Sensicult Secondary 2.0** (10 drugs)**
    (Ethionamide, Ciprofloxacin, Kanamycin, para-Aminosalicylic acid, Lomefloxacin, Rifabutin, Clarithromycin, Ofloxacin, D-cycloserine and Amikacin)
    L.J. solid media based, ten secondary drug panel for MTB sensitivity tests with 2 control.
    CAT No.: M-20305203 Pack size: One Set
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Highlights of the coming issue

Printed and published by D.G. Tripathi, Edited by Akshaya Tendulkar for and on behalf of Tulip Diagnostics (P) Ltd., Tulip House, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex, Post Office Goa - 403 202, India. Fax: 0832 2458544, **Website: www.tulipgroup.com.**