JOURNAL OF HYGIENE SCIENCES

Committed to the advancement of Clinical & Industrial Disinfection & Microbiology

VOLUME - XIII ISSUE - VI FEB - MAR 2021

Editorial

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Mini review section – Extraction of DNA, RNA, and protein is the basic method used in molecular biology. In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labor-intensive, and limited in terms of overall throughput. Currently, there are many specialized methods that can be used to extract pure biomolecules, such as solution based and column-based protocols.

Current Trends section – To understand why it is important to Pre-clean and Disinfect the Surgical Instruments, it is necessary to know the past incidents that occurred when this criterion was neglected and the problems encountered. Also, learn about their sources and the different ways by which these microorganisms have survived the disinfection procedure and caused infection. Though the problem has been recognized for decades, the number of reports of pathogenic mycobacterial disease caused by the use of contaminated devices or from an invasive procedure has been increasing.

In Profile Scientist – Charusita Chakravarty was an Indian academic and scientist. She was a professor of chemistry at the Indian Institute of Technology, Delhi since 1999. In 2009 she was conferred Shanti Swarup Bhatnagar Prize for Science and Technology in the field of chemical science.

Bug of the month – Norovirus is a highly contagious virus. Norovirus infection causes gastroenteritis (inflammation of the stomach and intestines). This leads to diarrhea, vomiting, and stomach pain. Norovirus illness is often called by other names, such as food poisoning and stomach flu. Noroviruses can cause food poisoning, as can other germs and chemicals. Norovirus illness is not related to the flu (influenza). Though they share some of the same symptoms, the flu is a respiratory illness caused by influenza virus.

Did You Know? – **Luminol** - The compound that detectives spray at crime scenes to find trace amounts of blood may be used one day to kill the malaria parasite. Luminol glows blue when it encounters the hemoglobin in red blood cells. And now, researchers at Washington University School of Medicine in St. Louis have shown that they can trick malaria-infected red blood cells into building up a volatile chemical stockpile that can be set off by luminol's glow. To achieve this, the scientists gave infected red blood cells an unusual amino acid and used luminol's glow to trigger the chemical, killing the parasite.

Best Practices – It is possible to become infected with a pathogen simply by touching contaminated surfaces in the laboratory without personal protective equipment (PPE). According to the Clinical and Laboratory Standards Institute (CLSI), in the laboratory “telephones, doorknobs and handles, computer terminals, and other surfaces are considered contaminated.” One important way to minimize this exposure risk to lab employees is to utilize good decontamination practices. The purpose of the decontamination process is to reduce the number of these contaminants in order to minimize the possibility of transmission or infection.

Unwind your mood with some light humour in our **Relaxed Mood section**.

Feedback & suggestions are always welcomed.

Nucleic acid extraction: The past and the present

Extraction of DNA, RNA, and protein is the basic method used in molecular biology. In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labor-intensive, and limited in terms of overall throughput. Currently, there are many specialized methods that can be used to extract pure biomolecules, such as solution based and column-based protocols. Manual method has certainly come a long way over time with various commercial offerings which included complete kits containing most of the components needed to isolate nucleic acid. Automated systems designed for medium-to-large laboratories have grown in demand over recent years. It is an alternative to labor-intensive manual methods. The technology allows a high throughput of samples; the yield, purity, reproducibility, and scalability of the biomolecules as well as the speed, accuracy, and reliability of the assay is maximal, while minimizing the risk of cross-contamination.

1. Introduction

The extraction of biomolecules, DNA, RNA, and protein, is the most crucial method used in molecular biology. It is the starting point for downstream processes and product development including diagnostic kits. DNA, RNA, and protein can be isolated from any biological material such as living or conserved tissues, cells, virus particles, or other samples for analytical or preparative purposes. Two categories that involved in purifying DNA include the isolation of recombinant DNA constructs such as plasmids or bacteriophage and the isolation of chromosomal or genomic DNA from prokaryotic or eukaryotic organisms. Generally, successful nucleic acid purification required four important steps: effective disruption of cells or tissue; denaturation of nucleoprotein complexes; inactivation of nucleases, for example, RNase for RNA extraction and DNase for DNA extraction; away from contamination. The target nucleic acid should be free of contaminants including protein, carbohydrate, lipids, or other nucleic acid, for example, DNA free of RNA or RNA free of DNA. Quality and also integrity of the isolated nucleic acid will directly affect the results of all succeeding scientific research. On the other hand, RNA is an unstable molecule and has a very short half-life once extracted from the cell or tissues. There are several types of naturally occurring RNA including ribosomal RNA (rRNA) (80%–90%), messenger RNA (mRNA) (2.5%–5%) and transfer RNA (tRNA). Special care and precautions are required for RNA isolation as it is susceptible to degradation. RNA is especially unstable due to the ubiquitous presence of RNases which are enzymes present in blood, all tissues, as well as most bacteria and fungi in the environment. Strong denaturants has always been used in intact RNA isolation to inhibit endogenous RNases. RNA extraction relies on good laboratory technique and RNase-free technique. RNAse is heat-stable and refolds following heat denaturation. They are difficult to inactivate as they do not require cofactors. The most common isolation methods can be divided into two classes: utilization of 4M guanidinium thiocyanate and utilization of phenol and SDS.

2. History

2.1. Nucleic Acid Extraction.

The very first DNA isolation was done by a Swiss physician, Friedrich Miescher in 1869. He hoped to solve the fundamental principles of life, to determine the chemical composition of cells. He tried to isolate cells from lymph nodes for his experiment but the purity of lymphocytes was hard and impossible to be obtained in sufficient quantities. Therefore, he switched to leucocytes, where he obtained them from the pus on collected surgical bandages.

Initially, Miescher focused on the various type of protein that make up the leukocytes and showed that proteins were the main components of the cell's cytoplasm. During his tests, he noticed that a substance precipitated from the solution when acid was added and dissolved again when alkali was added. This was, for the first time he had obtained a crude precipitate of DNA.

To separate DNA from the proteins in his cell extracts, Miescher developed new protocol to separate the cells nuclei from cytoplasm and then isolated DNA. However, his first protocol failed to yield enough material to continue with further analysis. He had to develop a second protocol to obtain larger quantities of purified nuclein, which had been named as 'nucleic acid' later by his student, Richard Altman.

3. Current Tendency

After the fated event where Miescher managed to obtain DNA from cell, many others have followed suit which lead to further advancement in the DNA isolation and purification protocol. The initial routine laboratory procedures for DNA extraction were developed from density gradient centrifugation strategies. Meselson and Stahl used this method in 1958 to demonstrate semiconservative replication of DNA. Later procedures made use of the differences in solubility of large chromosomal DNA, plasmids, and proteins in alkaline buffer. Currently, there are many specialized method of extracting out pure DNA, RNA, & protein. Generally, they are divided into solution-based or column-based protocols.

Most of these protocols have been developed into commercial kits that ease the biomolecules extraction processes.

3.1. Type of Nucleic Acid Extraction

3.1.1. Conventional Method

Guanidinium Thiocyanate-Phenol-Chloroform Extraction.

Salt is the common impurity in nucleic acid samples. It has always been required to be removed from nucleic acid samples before any downstream processes and analysis can be done. Therefore, single or multiple separation and/or purification steps are needed to desalt the sample comprising the nucleic acid. The general steps of nucleic acid purification include cell lysis, which disrupts the cellular structure to create a lysate, inactivation of

cellular nucleases such as DNase and RNase, and separation of desired nucleic acid from cell debris. Organic solvent—phenol chloroform extraction is one of the examples, which is widely used in isolating nucleic acid.

Although phenol, a flammable, corrosive, and toxic carbolic acid can denature proteins rapidly, it does not completely inhibit RNAse activity. This problem can be solved by using a mixture of phenol: chloroform: isoamyl alcohol (25:24:1). Proteins, lipids, carbohydrates, and cell debris are removed through extraction of the aqueous phase with the organic mixture of phenol and chloroform.

A biphasic emulsion forms when phenol and chloroform are added. The hydrophobic layer of the emulsion will then be settled on the bottom and the hydrophilic layer on top by centrifugation. The upper phase which contained DNA is collected and DNA can be precipitated from the supernatant by adding ethanol or isopropanol in 2:1 or 1:1 ratios and high concentration of salt. DNA precipitate is collected by centrifugation, and excess salt is rinsed with 70% ethanol and centrifuged to discard the ethanol supernatant. The DNA pellet is then dissolved with TE buffer or sterile distilled water.

The use of guanidinium isothiocyanate in RNA extraction was first mentioned by Ulrich et al. (1977). The method was laborious. Therefore, it has been displaced by a single step technique, which is known as Guanidinium thiocyanate-phenol-chloroform extraction, by Chomczynski and Sacchi(1987), whereby the homogenate is extracted with phenol/chloroformat reduced pH. Guanidinium thiocyanate is a chaotropic agent used in protein degradation. The principle of this single-step technique is that RNA is separated from DNA after extraction with acidic solution consisting guanidinium thiocyanate, sodium acetate, phenol, and chloroform. In the acidic conditions, total RNA will remain in the upper aqueous phase of the whole mixture, while DNA and proteins remain in the interphase or lower organic phase. Recovery of total RNA is then done by precipitation with isopropanol.

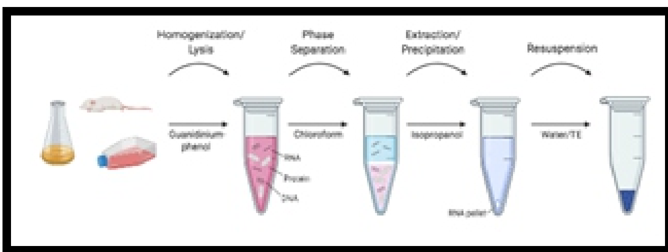

Fig 1 : RNA Extraction by Guanidinium Thiocyanate-Phenol-Chloroform Extraction Method.

Alkaline Extraction Method

Alkaline lysis has been used to isolate plasmid DNA and *E. coli*. It works well with all strains of *E. coli* and with bacterial cultures ranging in size from 1mL to more than 500mL in the presence of Sodium Dodecyl Sulfate (SDS). The principle of the method is based on selective alkaline denaturation of high molecular weight chromosomal DNA while covalently closed circular DNA remains double stranded. Bacterial proteins, broken cell walls, and denatured chromosomal DNA enmeshed into large complexes that are coated with dodecyl sulfate. Plasmid DNA can be recovered from the supernatant after the denatured material has been removed by centrifugation.

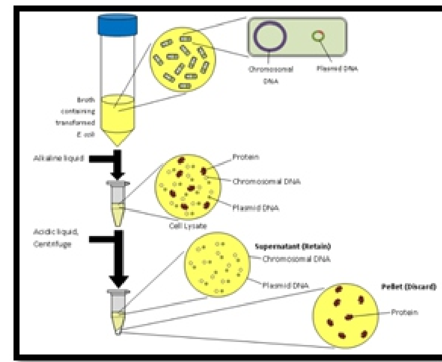

Fig 2: Alkaline Extraction Method

<u>CTAB Extraction Method</u>

Cetyltrimethylammonium bromide (CTAB) is a non-ionic detergent that can precipitate nucleic acids and acidic polysaccharides from low ionic strength solutions. Meanwhile, proteins and neutral polysaccharides remain in solution under these conditions. In solutions of high ionic strength, CTAB will not precipitate nucleic acids and forms complexes with proteins. CTAB is therefore useful for purification of nucleic acid from organisms which produce large quantities of polysaccharides such as plants and certain Gram-negative bacteria.

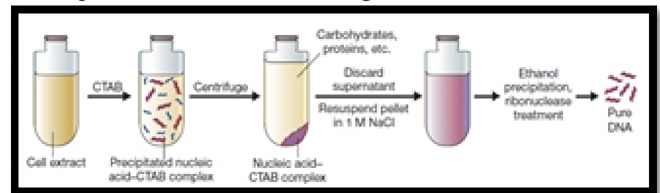

Fig 3: CTAB Extraction method for purification of DNA

This method also uses organic solvents and alcohol precipitation in later steps. Insoluble particles are removed through centrifugation to purify nucleic acid. Soluble proteins and other material are separated through mixing with chloroform and centrifugation. Nucleic acid must be precipitated after this from the supernatant and washed thoroughly to remove contaminating salts. The purified nucleic acid is then resuspended and stored in TE buffer or sterile distilled water.

<u>Ethidium Bromide (EtBr)-Cesium Chloride (CsCl) Gradient Centrifugation</u>

CsCl gradient centrifugation is a complicated, expensive, and time-consuming method compared to other purification protocols. It requires large scale bacterial culture. Therefore, it is not suitable for the mini preparation of plasmid DNA. Nucleic acids can be concentrated by centrifugation in an EtBr-CsCl gradient after alcohol precipitation and resuspension. Intercalation of EtBr alters the swimming density of the molecule in high molar CsCl. Covalently closed circular molecules will accumulate at lower densities in the CsCl gradient because they incorporate less EtBr per base pair compared to linear molecules.

The hydrophobic EtBr is then removed with appropriate hydrophobic solvents after extraction. The purified nucleic acid will be reprecipitated with alcohol.

The above mentioned are the Conventional methods in next issue we will discuss about the Solid-phase Nucleic Acid Extraction and Automated Extraction System.

Current Trends – Importance of Pre-Cleaning and Disinfection of Surgical Instruments

Introduction

To understand why it is important to Pre-clean and Disinfect the Surgical Instruments, it is necessary to know the past incidents that occurred when this criterion was neglected and the problems encountered. Also, learn about their sources and the different ways by which these microorganisms have survived the disinfection procedure and caused infection. Though the problem has been recognized for decades, the number of reports of pathogenic mycobacterial disease caused by the use of contaminated devices or from an invasive procedure has been increasing.(Carson *et al.* ; Schulze-Röbbecke *et al.*; Fujita *et al.*). There are several excellent reviews on nosocomial outbreaks/pseudo-outbreaks that have been published by (Fraser 1981; Wallace *et al.*; Phillips & von Reyn 2001).

Potential situations associated with **Pathogenic Environmental Mycobacteria (PEM)** isolation from a clinical specimen is explained in this table. (Adapted from Phillips & von Reyn 2001).

Situation	Definition
Colonization	Isolation of potentially pathogenic EM without signs or symptoms of disease attributed to the organism.
Infection/disease	Clinical evidence of infection attributed to the organism.
Pseudo-infection	No evidence of infection or colonization and isolation in the laboratory has resulted from contamination of the environment (can occur at any point: from a contaminated device, in obtaining the specimen, or up through the final cultivation in the laboratory).

Investigation

From 1956 to 1979, CDC carried out 252 hospital outbreak investigations; these have been summarized by (Stamm and coworkers). In the ensuing 16 years through 1995, CDC assisted in another 193 outbreak investigations. In the early years (1956-1962), the two most common problems investigated were epidemics of gastrointestinal disease, primarily due to Salmonella species or enteropathogenic *E. coli*, or staphylococcal infections. Increasing numbers of HAI outbreaks were associated with the bloodstream, respiratory tract, urinary tract, and surgical wounds or medical devices. These included outbreaks of hepatitis A virus or hepatitis B virus infections; necrotizing enterocolitis in nurseries; sternal wound infections after open heart surgery, particularly those caused by rapidly growing mycobacteria; and nosocomial Legionnaires' disease. Also during this period, CDC recorded increasing numbers of outbreaks associated with microorganisms resistant to multiple antimicrobials, particularly aminoglycosideresistant *Enterobacteriaceae* and non-fermentative gram-negative bacilli, and MRSA.

Outbreaks of Infection

Fifty-two (46%) of the 114 outbreaks were associated with either an invasive device or invasive procedure. Dialyzers (10; 43%) were the most common invasive devices associated with outbreaks followed by needleless intravascular device use among patients in inpatient, outpatient, or home care settings (7; 29%). The most common invasive procedures were surgery (21; 50%), dialysis (16; 37%), or cardiac catheterization (3; 7%). Twenty (17.5%) of the 114 outbreak investigations were associated with contaminated products, including intravenous anesthetics (9; 8.0%), parenteral solutions (5; 4.4%), or blood products (2; 1.8%). Twenty-one (28.6%) of the infectious disease outbreaks were associated with multidrug-resistant organisms, including multidrug-resistant *M. tuberculosis*; VRE; *S. aureus* with reduced susceptibility to vancomycin, vancomycin-resistant *Staphylococcus* epidermidis, or extended spectrum beta-lactamase producing E. coli and K. pneumoniae.

In hospital-wide data, UTIs have accounted for approximately 40% of all HAIs, but UTIs make up a smaller proportion of HAIs occurring in the ICU setting. UTIs account for 15-21% of HAIs in pediatric ICU patients, 23% of HAIs in adult U.S. ICU patients, and 18% of ICU infections in the European EPIC study. The prevalence of UTI varies by ICU type; rates of CA-UTIs reported through the Centers for Disease Control and Prevention's (CDC) National Nosocomial Infections Surveillance (NNIS) system between January 2002 and June 2004 ranged from 3.0 infections/1,000 catheter-days in cardiothoracic ICUs to 6.7 infections/1,000 catheter-days in burn or neurosurgical ICUs. The rate of UTI in pediatric ICUs was 4.0 infections/1,000 catheter-days, lower than the rate seen in an equivalent adult medical ICU population of 5.1 infections/1,000 catheter-days. Nosocomial UTI is infrequently identified in neonatal ICUs. In data collected in a non-ICU setting in 42 German hospitals, the rate of infection was similar, 6.8 infections per 1,000 urinary-catheter days.

From October 1986 through June 1988, at a hospital in Wisconsin, USA, *Pseudomonas aeruginosa* of the biliary and respiratory tract, or bloodstream occurred in 16 (6.7%) of 240 patients undergoing Endoscopic Retrograde Cholangio-Pancreatography (ERCP) and in 99 (8.9%) of 1109 patients undergoing other upper gastrointestinal (UGI) endoscopic procedures. The endoscopes were routinely reprocessed in automated reprocessing machine that flushed with a detergent solution, disinfected with one of two liquid chemical germicides (2.45% glutaraldehyde; 2.45% glutaraldehyde/ 7.05% phenol/ 1.2% sodium phenate diluted 1:16 in tap water). An investigation performed by the hospital in June 1988 indicated that a thick biofilm of *P. aeruginosa* had formed in the detergent holding tank, inlet water hose, and air vents of the automated machine. Attempts to disinfect the machine by the manufacturer's instructions using commercial preparations of glutaraldehyde were unsuccessful.

Sources of Infection

Infection of intravascular catheters, pacemakers

Vascular devices such as indwelling venous access lines and vascular shunts can become infected with both **Rapidly Growing Mycobacteria (RGM)** and other species (Katz *et al.*; Schinsky *et al.*; Rodriguez-Gancedo *et al.*; Bouza *et al.*). Infections can result in skin and soft tissue involvement (including tunnel site infections) with or without bacteraemia. Pocket infections involving pacemakers have also been seen with PEM, with **MAC (both *M. avium M. intracellulare*)** and especially *M. abscessus* (Amin *et al.*; Katona *et al.*; Cutay *et al.*; Verghese *et al.*).

Dialysis releated infection

Dialysis related PEM infection has been reported in both intravascular and peritoneal mechanisms of renal replacement therapy. Rapidly growing mycobacteria species are the most commonly implicated. Contaminated aqueous solutions used to sterilize the re-usable dialysis filters have been involved in many cases. PEM representatives have been isolated from water supplies of haemodialysis centres (Carson *et al.*). In 1982, 27/140 patients receiving haemodialysis developed infection after being exposed to mycobacteria in water used to prepare dialysis fluids (Bolan *et al.*). Peritonitis can occur in patients undergoing chronic ambulatory peritoneal dialysis (Band *et al.*). In this setting it can involve the catheter insertion site, tunneling tract and/or the peritoneum itself. **MAC (both *M. avium M. intracellulare*)** and the rapid growers are the most commonly isolated species (Band *et al.*; Soriano *et al.*; Lowry *et al*. 1990; Vera & Lew 1999). Patients with end stage renal disease from any cause are likely to be more prone to infection due to impaired lymphocyte and neutrophil ctivity in the face of uraemia. Catheter removal improves the rate of cure and antibiotics are necessary to prevent clinical failure. Sequelae include adhesions and sometimes difficulty replacing the catheter (Hakim *et al.*).

Bronchoscopy and Endoscopy

An estimated 497,000 bronchoscopy procedures were performed in the United States in 1996. Several outbreaks have highlighted the problems of pulmonary infection and false-positive culture results because of inadequately cleaned fiberoptic bronchoscopes. So the need is realized for higher-level disinfection and sterilization of these scopes, especially after use on patients who could have tuberculosis. Although the hospital's procedures for disinfection, corresponded with most guidelines, the bronchoscope showed patient debris after disinfection, indicating that the manual cleaning was inadequate and was not approved for reprocessing in the hospital's automated endoscope reprocessor system. Failure to perform leak testing led to failure to discover a hole in the sheath of a bronchoscope, which led to inadequate disinfection and transmission of *M. tuberculosis* to patients via the bronchoscope resulting in infection and pseudoinfections. One outbreak was believed to be a result of a **manufacturing defect** of the biopsyport caps, and another was due to **incorrect connectors** joining the bronchoscope suction channel to the Steris System processor, obstructing peracetic acid flow through the bronchoscope lumen. Infection and pseudoinfection from bronchoscopic procedures eflect a number of problems including **ineffective cleaning** due to poor technique, damaged equipment, **difficult-to-clean accessories, ineffective reprocessing,** use of tap water to rinse the scopes, **inappropriate storage** (e.g., coiling the scopes), and **lack of familiarity** with national recommendations for reprocessing. (Srinivasan et al.) distributed a survey to practicing bronchoscopists regarding infection control issues related to bronchoscopy and specific reprocessing recommendations.

Medical directors of bronchoscopy suites or attending bronchoscopists completed 46 surveys. “Of the respondents, 65% were not familiar with national reprocessing recommendations, and 39% did not know what reprocessing procedure was used at their own institution.” In addition, some parts of the bronchoscopes (e.g., reusable spring-operated suction valves) could require autoclaving if they become heavily contaminated with microbes that are relatively resistant to disinfection such as mycobacteria. Working and suction channels of 241 flexible gastrointestinal endoscopes at 80 healthcare facilities, it was found that 47% (38/80) of facilities had at least one patient-ready endoscope whose suction or biopsy channels were visibly encrusted with debris, and 11% (26/241) of endoscopes had severely scratched channels that provided pockets for debris. Only 5.4% (3/56) of facilities that attempted to dry their endoscopes between procedures were successful. Because high-level disinfectants require clean surfaces, flexible endoscopes must be carefully cleaned of all mucus, blood, and other biologic materials before subjecting them to a high-level disinfectant. To further complicate endoscope care, automated machines developed for endoscope reprocessing have been flawed. Users should adhere carefully to the manufacturer's protocols but also should be aware of the possibility that colonization of the washer holding tanks is not reversible despite use of the manufacturer's recommended disinfection protocol. Surveillance for endoscope-related infection and pseudoinfection is important, and infection control practitioners must educate their endoscope users (e.g., endoscopy suite personnel and physicians) about problems discussed in this section; the users also must be vigilant to monitor best practice for a complicated cleaning procedure because there are many opportunities for inadequate disinfection.

Other medical and surgical procedures

Post-surgical infections with PEM have been reported in many settings (Robicsek *et al.*, 1978, 1988; Hoffman *et al*. 1981; Safranek *et al.*; Wallace *et al.*; Jarvis 1991; Grange 1992; Syed *et al.*). They typically arise from solutions or instruments that have been inadequately sterilized (Phillips & von Reyn 2001). Mediastinitis and sternal wound infections due to *M. fortuitum M. chelonae* been reported after cardiothoracic surgery (Hoffman *et al.*; Kuritsky *et al.*). Infection due to *M. chelonae* felt to have resulted by transmission between patients from contaminated instruments (Lowry *et al.*). Spinal infections have been reported after epidural injections and also after spinal surgery. A large outbreak after discovertebral surgery in France involving 58 patients in a single hospital in Paris occurred over a period of more than 10 years. This was a result of chronic contamination of the hospital water supply with *M. xenopi* (Astagneau *et al.*). Other surgical procedures reported to result in PEM infection include augmentation mammoplasty, liposuction, laser vision correction surgery and other plastic surgical procedures (Murillo *et al.*). *M.*

abscessus, M. chelonae M. fortuitum major. In addition to solutions, instrumentation and implants that have been implicated include lacrimal duct probes, tympanostomy tubes, epidural catheters and graft materials.

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Charusita Chakravarty

Early Life and Education

Charusita Chakravarty was born on 5th May 1964 to her parents Sukhamoy and Lalita Chakravarty in Cambridge, Massachusetts, USA. The only child of two leading economists, Chakravarty grew up in a liberal environment that gave her the fire to break boundaries from an early age. She was exposed to a wide variety literature while growing up – so while she had a definitive partiality to science, and chemistry, in particular, she by no means restricted herself and her interests to purely scientific fields. In a day and age when the boundaries between arts and sciences were clearly drawn, she straddled an important intersection between the two, and demonstrated the value of both by simply taking pleasure in what each subject had to offer.

An early achiever, she topped the Delhi Higher Secondary Board before deciding to pursue B. Sc in Chemistry from *St. Stephens College*, leaving *Delhi University* as a gold medalist in 1985. She went on to pursue a Natural Science Tripos from *Cambridge* and graduated in 1987 before settling down to work on her PhD, also in *Cambridge*, on quantum scattering and spectroscopy under the guidance of David Clary. Charusita then became a Post Doctoral Scholar at the *University of California* at Santa Barbara, under Professor Horia Metiu. After a brief visit to India, she returned to Cambridge as a Gulbenkian junior research fellow in an independent post-Doctoral position.

Career

After marrying fellow scientist Ram Ramaswamy, Charusita relocated to India in 1994. The IITs were reluctant to give her a job, despite her PhD, because she had not done a Masters. She was then offered a position by IIT Kanpur, in their Chemistry Department. She finally accepted a job from IIT Delhi in their Chemistry Department where rose from an Assistant Professor to Professor in 2006, where she continued to teach there till her death.

Chakravarty was an academic through and through and submitted her research proposal to the Department of Science and Technology in New Delhi soon after joining IIT Delhi. With one referee calling it the best-written proposal he had ever seen, she managed to secure funding easily and carried on with her research even when funding became scarce. Her initial work had to with atomic and molecular clusters and over the course of her career, she became famous for her specialised application of path integral Monte Carlo simulation to unravel quantum mechanical effects in the properties of atomic and molecular clusters.

Her fields of interest also included theoretical chemistry and chemical physics, the structure and dynamics of Liquids, water and hydration, nucleation and self-assembly. International and national journals have published her articles and she was widely known for her single-author papers, published extensively over the course of her career. A few of her famous co-written works include, '*Multiple Time-scale Behaviour of the Hydrogen Bond Network in Water*' (2004), '*Estimating the entropy of liquids from atom-atom radial distribution functions: silica, beryllium fluoride and water*' (2008), and '*Excess entropy scaling of transport properties in network-forming ionic melts*' (2011).

Chakravarty was known for speaking out against the dearth of women in science and openly discussed the double burden females faced. While men in their thirties only had to worry about their career, women had the additional task of balancing family life and their work commitments. She managed to do a stellar job managing both, and became an accomplished academician while embracing motherhood in 2000 when her daughter, Krithi, was born.

Achievements

Her work received widespread acclaim and she was the recipient of several prizes and honors. Chakravarty's earliest award came in 1996 when she received the *Medal for Young Scientists* from the *Indian National Science Academy* (INSA), a leading society for science and technology in India. From 1996 to 2003, she served as a member of the *Abdus Salam International Center* for Theoretical Physics, Trieste, Italy, an institute dedicated to scientific research and excellence. In 1999 she bagged two awards, the *B.M. Birla Science Award* and *Anil Kumar Bose Memorial Award* from the *Indian National Science Academy*. In 2003, she received the *Swarnajayanti Fellowship* from the Department of Science and Technology and in 2006 a Fellowship from *Indian Academy of Sciences*. She also received the prestigious *Shanti Swarup Bhatnagar Award* in 2009 and was an Associate Member of the *Centre for Computational Material Science*, *Jawaharlal Nehru Centre for Advanced Scientific Research*, Bangalore.

Death and Legacy

Unfortunately, we lost Dr Chakravarty too early, after a long and valiant fight against cancer. Despite the fact that she was diagnosed in 2013, she kept authoring and publishing articles up till her death, a testament to her indomitable will. She even received a Fellowship in 2015 from INSA, an insight to the brilliant mind she had right till the end. Charusita continued to take classes on days when she well enough and made it a point to make time for her friends and family, despite the side-effects of the treatments and the horrific pain. She passed away in 2016, at the age of fifty-one.

While we lost her too soon, she displayed a kind of genius that is hard to forget. Her work made her a brilliant academic and exemplary role model that prompted numerous women to cross the threshold and enter laboratories.

Jokes

Teacher :Tomorrow There Will Be A Lecture On Sun.Everyone Must Attend It.
Raju:No Mam! I Will Not Be Able To Attend It.
Teacher :Why?
Raju:My Mother Will Not Allow Me To Go So Far!!!

Banta Built 2 Swimming Pools.
And He Left One Of Them Unfilled Y?
When Asked Him, He Said,
“Oye, That's For Those Who Don't Know Swimming.”

Teacher:Can Anyone Give Me An Example Of Coincidence?
Sunny:Sir, My Mother And Father Got Married On The Same Day Same Time.
Teacher: How Old Is Ur Father.
Sunny:As Old As I Am.
Teacher:How Is It Possible?
Sunny:He Became Father Only After I Was Born.

A Police Officer Jumps Into His Squad Car And Calls The Station.
“I Have An Interesting Case Here,” He Says.
“A Woman Shot Her Husband For Stepping On The Floor She Just Mopped.”
“Have You Arrested Her?” Asks The Sergeant.
“No, Not Yet. The Floor's Still Wet.”

A guest is ordering at a restaurant, “Do you think you c
Wait For Me Honey, I'm Just Finishing My Make-Up.
You Don't Need Make-Up, Jane.
Oh, Richard…. Really? That Is So Sweet Of You!
You Need Plastic Surgery.

Wife: Can U Help Me In The Gardening?
Husband: What Do U Think I Am…A Gardener?
Wife: Can U Fix The Door Handle?
Husband: What Do You Think I Am… A Carpenter?
In The Evening, When Husband Came From Work, He Saw Everything Has Been Fixed.
Husband: Who Did All This?
Wife: Our Neighbour. But He Gave Me 2 Options…..Either I Should Give Him A Burger Or A Kiss.
Husband: I Am Sure U Must Have Given Him A Burger.
Wife: What Do U Think I Am…….McDonalds?

Norovirus—The Stomach Bug

Norovirus is a highly contagious virus. Norovirus infection causes gastroenteritis (inflammation of the stomach and intestines). This leads to diarrhea, vomiting, and stomach pain. Norovirus illness is often called by other names, such as food poisoning and stomach flu. Noroviruses can cause food poisoning, as can other germs and chemicals. Norovirus illness is not related to the flu (influenza). Though they share some of the same symptoms, the flu is a respiratory illness caused by influenza virus.

Anyone can get norovirus illness

Norovirus is the most common cause of acute gastroenteritis in the U.S. Each year, norovirus causes 19 to 21 million cases of acute gastroenteritis in the U.S. There are many types of norovirus and you can get it more than once.

Norovirus illness can be serious

Norovirus illness can make you feel extremely sick with diarrhea and vomiting many times a day. Some people may get severely dehydrated, especially young children, the elderly, and people with other illnesses. Each year, norovirus causes 56,000 to 71,000 hospitalizations and 570 to 800 deaths, mostly in young children and the elderly.

Norovirus spreads very easily and quickly

It only takes a very small amount of norovirus particles (fewer than 100) to make you sick. People with norovirus illness shed billions of virus particles in their stool and vomit and can easily infect others. You are contagious from the moment you begin feeling sick and for the first few days after you recover. Norovirus can spread quickly in enclosed places like daycare centers, nursing homes, schools, and cruise ships. Norovirus can stay on objects and surfaces and still infect people for days or weeks. Norovirus can survive some disinfectants, making it hard to get rid of.

Norovirus can spread in many ways

Norovirus can spread to others by having direct contact with an infected person, for example, touching an infected person while caring for them, eating food or drinking liquids that are contaminated with norovirus, touching objects that have norovirus on them and then putting your fingers in your mouth, for example, touching a countertop that has vomit droplets on it and then putting your fingers in your mouth and sharing utensils or cups with people who are infected with norovirus.

There's no vaccine to prevent norovirus infection and no drug to treat it

Antibiotics will not help with norovirus illness because antibiotics do not work on viruses. When you have norovirus illness, drink plenty of liquids to replace fluid loss and prevent dehydration. If you or someone you are caring for is dehydrated, call a doctor.

5 Tips to Prevent Norovirus from Spreading

1. Practice proper hand hygiene Always wash your hands carefully with soap and water after using the toilet and changing diapers, and before eating, preparing, or handling food. Alcohol-based hand sanitizers can be used in addition to hand washing. But, they should not be used as a substitute for washing with soap and water.
2. Wash fruits and vegetables and cook seafood thoroughly carefully wash fruits and vegetables before preparing and eating them. Cook oysters and other shellfish thoroughly before eating them. Be aware that noroviruses are relatively resistant. They can survive temperatures as high as 140°F and quick steaming processes that are often used for cooking shellfish. Food that might be contaminated with norovirus should be thrown out. Keep sick infants and children out of areas where food is being handled and prepared.
3. When you are sick, do not prepare food or care for others you should not prepare food for others or provide healthcare while you are sick and for at least 2 to 3 days after you recover. This also applies to sick workers in schools, daycares, and other places where they may expose people to norovirus.
4. Clean and disinfect contaminated surfaces After throwing up or having diarrhea, immediately clean and disinfect contaminated surfaces. Use a chlorine bleach solution with a concentration of 1000–5000 ppm (5–25 tablespoons of household bleach [5.25%] per gallon of water) or other disinfectant registered as effective against norovirus by the Environmental Protection Agency (EPA).
5. Wash laundry thoroughly immediately remove and wash clothes or linens that may be contaminated with vomit or stool (feces). You should handle soiled items carefully without agitating them, wear rubber or disposable gloves while handling soiled items and wash your hands after, and wash the items with detergent at the maximum available cycle length then machine dry them.

Luminol- A compound used in crime scene may combat malaria

The compound that detectives spray at crime scenes to find trace amounts of blood may be used one day to kill the malaria parasite.

Luminol glows blue when it encounters the hemoglobin in red blood cells. And now, researchers at Washington University School of Medicine in St. Louis have shown that they can trick malaria-infected red blood cells into building up a volatile chemical stockpile that can be set off by luminol's glow. To achieve this, the scientists gave infected red blood cells an unusual amino acid and used luminol's glow to trigger the chemical, killing the parasite.

"The light that luminol emits is enhanced by the antimalarial drug artemisinin," said senior author Daniel Goldberg, MD, PhD, professor of medicine and molecular microbiology. "We think these agents could be combined to form an innovative treatment for malaria."

The World Health Organization (WHO) estimated that in 2013, malaria infected 198 million people and killed 584,000, the majority of whom were African children.

The new therapy would have an advantage over current malaria treatments, which have become less effective as the parasite mutates. WHO recommends that artemisinin -- the most commonly used antimalarial drug -- only be used in combination with other treatments because the parasite is becoming resistant to it.

The new approach targets proteins made by human red blood cells, which the parasite can't mutate.

In the study, researchers led by first author Paul Sigala, PhD, a scientist in Goldberg's laboratory, worked with human red blood cells infected with the malaria parasite. They wanted to better understand how the parasite gets hold of heme, the deep red, nonprotein part of hemoglobin that carries oxygen. Heme is essential to the parasite's survival.

The malaria parasite opens an unnatural channel on the surface of red blood cells. When scientists put an ingredient of heme -- an amino acid -- into the solution containing the cells, the amino acid entered the cells through the channel and started the heme-making process.

The process led to a buildup of a molecule called protoporphyrin IX. When exposed to light, this molecule emits dangerous, chemically reactive compounds known as free radicals, killing the parasites.

The research team plans to test the approach in animal studies.

“All of these agents -- the amino acid, the luminol and artemisinin -- have been cleared for use in humans individually, so we are optimistic that they won't present any safety problems together," said Goldberg, who is co-director of the Division of Infectious Diseases. "This could be a promising new treatment for a devastating disease."

Journal Reference:

1. Paul A Sigala, Jan R Crowley, Jeffrey P Henderson, Daniel E Goldberg. **Deconvoluting heme biosynthesis to target blood-stage malaria parasites.** *eLife*, 2015; 4 DOI: 10.7554/eLife.09143

LABORATORY DECONTAMINATION TOOLS AND PRACTICES

Tracking the number of laboratory-acquired infections (LAIs) in the United States is no easy task. Not all incidents are reported, and sometimes the victim may not be aware of the source of the infection. Despite that, the United States Bureau of Labor Statistics (BLS) does collect available information about those reported exposures in labs. The numbers have been declining over the past decade, but exposures do still occur.

It is possible to become infected with a pathogen simply by touching contaminated surfaces in the laboratory without personal protective equipment (PPE). According to the Clinical and Laboratory Standards Institute (CLSI), in the laboratory "telephones, doorknobs and handles, computer terminals, and other surfaces are considered contaminated." One important way to minimize this exposure risk to lab employees is to utilize good decontamination practices.

Decontamination is the act of minimizing the overall pathogenic microbial presence. For labs that process routine patient specimens, that microbial presence may exist in the form of bloodborne pathogens, bacteria, fungi, and even prions. The purpose of the decontamination process is to reduce the number of these contaminants in order to minimize the possibility of transmission or infection.

Routine Decontamination

Routine decontamination of lab counter tops or work areas should occur frequently. Lab benches should be cleaned at a minimum after each working shift and after spills occur. This routine decontamination process should be documented as well. It is also a good idea to regularly include telephones, computer terminals, and other frequently handled surfaces in the lab in the disinfection process.

Typically, chemical germicides are recommended for the surface decontamination in the lab setting. These germicides are classified as high-level, intermediate, or low-level disinfectants based on their activity and strength. The most commonly recommended disinfectant for lab surfaces is a 10-percent solution of sodium hypochlorite (or bleach), which is considered an intermediate-strength chemical germicide. Intermediate-level disinfection will eliminate most bacteria (including Mycobacterium tuberculosis) and all fungi, and it inactivates viruses.

Laboratories should use an EPA-registered or approved disinfectant. Manufacturers list their products with the U.S. Environmental Protection Agency (EPA), and the agency verifies that the disinfectant is effective against common pathogens and complies with Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen standard requirements. These requirements include the ability of the product to effectively decontaminate contaminated surfaces and spills.

Many manufacturers offer pre-made bleach cleaning products, but many laboratories still make their own dilutions from concentrated bleach. That is an acceptable practice, but fresh bleach solutions should be made daily, as the efficacy of the solution wanes quickly. It is also important when making mixtures to consider the concentration of the purchased bleach. Traditionally, labs made a 1:10 solution, as most bleach products were sold in the past at a 5.25 percent concentration. Since many available commercial bleach concentrations now are at 8.25 percent, labs should make a 1:16 solution for disinfection purposes if they are using that bleach. Laboratorians should be sure to check the label of the bleach product purchased before making dilutions. Sodium hypochlorite is a corrosive chemical, and even in its diluted form it can cause damage to some surfaces over time. In order to prevent this, the disinfected surface should be rinsed off with sterile water or 70 percent ethanol (another intermediate chemical germicide).

Formaldehyde Use

In the Anatomical Pathology section of the laboratory, formaldehyde is sometimes used as a disinfectant. Depending on its concentration, formaldehyde can be considered a high-level disinfectant. The chemical, a known carcinogen, is placed inside a cryostat, and fumes generated buy a heat source are used to decontaminate the inside of the apparatus. However, the effectiveness of the disinfection is unclear, and the dangers of using a carcinogen mark this method as less than satisfactory. Check with the cryostat manufacturer for cleaning guidelines, but many instruments can be disinfected using 70 percent alcohol.

Chemical Concentration

While concentration of chemicals plays a role in effectiveness against pathogens, one final consideration with all lab disinfectant products is the contact time with the pathogen. Most disinfectants do not instantly kill the organisms on contact; the product must be left on the contaminated surface for a specified amount of time in order to complete its action. EPA-registered disinfectants all have designated contact times printed on the label. For example, a surface disinfectant may have a contact or kill time for hepatitis B virus of one to three minutes; however, its tuberculocidal contact time may be 10 minutes. Be sure to check labels and understand the contact time requirements. Using these products properly is the only way to ensure proper disinfection.

A Dangerous Place…

The laboratory setting is an intrinsically dangerous place where bloodborne and airborne pathogens are omnipresent, and specific actions must be taken in order to protect the people working in the department. In order to prevent LAIs, engineering controls, work practice controls, and PPE should always be employed. Decontamination of surfaces is another significant control that must be utilized properly. Understanding the proper types, concentrations, and contact times of the appropriate lab disinfectants is a practice that will reduce those threatening pathogens and make the laboratory a safer place to work.A list of BioShields' Disinfectants can be used in Laboratory Area:

S. No.	COMPOSITION	APPLICATION
1.	PHMB & DDAC (Novacide)	Cold Sterilant for Instruments
2.	Silver Nitrate & Hydrogen Peroxide (Silvicide)	Aerial Fumigant
3.	Sodium Dichloroisocynurate (NaDCC) in Powder Form (Puresafe)	Blood Spillage
4.	Benzalkonium Chloride (Microlyse)	Floor Mopping (Non-Critical Area)
5.	Glutaraldehyde & Benzalkonium Chloride (Acitar)	Floor Mopping (Critical Area)
6.	CHG, Triclosan, and Isopropyl Alcohol (Sterimax)	Hand Sanitizer
7.	BKC & Isopropyl Alcohol (Aerosept)	Surface Sprayable Disinfectant
8.	DDAC (Linosafe)	Linen Disinfectant
9.	Benzalkonium Chloride (Maxishine)	Glass window Sprayable Disinfectant
10.	Hydrochloric Acid (Exit)	Ready-To-Use Toilet Bowl Cleaner

References:

U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). 5th ed. Washington, DC: US Department of Health and Human Services; 2011.

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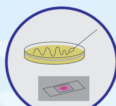

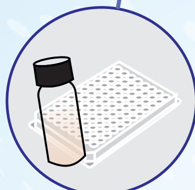

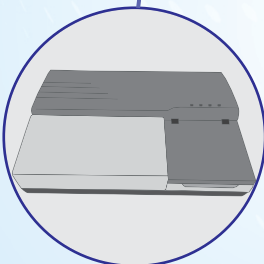

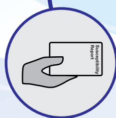

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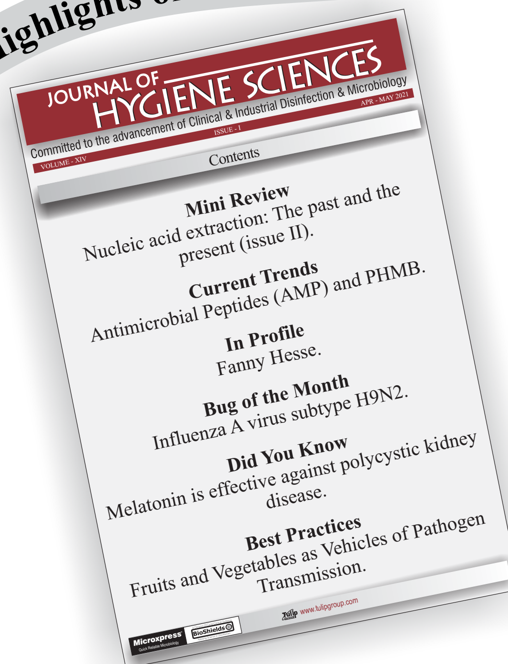

JOURNAL OF HYGIENE SCIENCES

Committed to the advancement of Clinical & Industrial Disinfection & Microbiology

VOLUME - XIV ISSUE - I APR - MAY 2021

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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.**

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