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Editorial

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'Mini Review' section - The elementary identification of medically important genera of the family Enterobacteriaceae based on few basic biochemical tests was discussed in previous issue. In this article, elementary identification of Enterobacteriaceae based on genomic level/ molecular level techniques available is elucidated.
Few of the commercially available identification systems offered are serotyping and molecular typing techniques. Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

Current Trends section - Sterilization, as a specific discipline, has been with us for approximately 120 years, since the invention of the steam autoclave by Charles Chamberland in 1879. Sterilization processes cannot be considered in isolation; rather, they are inextricably related to the product to be sterilized. There is no single sterilization process that is suitable for all medical products. The diversity in sterilization processes and of operating systems within each process has arisen as a consequence of the efforts made to optimize medical sterilization and to meet the differing needs imposed by the vast range of products to be sterilized.

In Profile Scientist - Suniti Solomon was an Indian physician and microbiologist who pioneered AIDS research and prevention in India after having diagnosed the first Indian AIDS cases among the Chennai sex workers in 1986 along with her student Nirmala Sellappan. She founded the Y R Gaitonde Centre for AIDS Research and Education in Chennai. The Indian government conferred the National Women Bio-scientist Award on her. On 25 January 2017, Government of India announced "Padma Shri" award for her contribution towards Medicine.

Bug of the month - "*Mycobacterium ulcerans*" The organism belongs to the family of bacteria that causes tuberculosis and leprosy. However, *M. ulcerans* is an environmental bacterium and the mode of transmission to humans remains unknown. *Mycobacterium ulcerans* grows at temperatures between 29-33 °C and a low 2.5% oxygen concentration to grow. The organism produces a unique toxin – mycolactone – which causes tissue damage and inhibits the immune response.

Did You Know? Laxatives are a type of medication used to treat constipation by loosening stool or encouraging bowel movements. If you try to use laxatives for weight loss, you may well see the number on the scale go down. But this apparent drop is deceiving because it's actually water weight that you're losing. The weight loss is temporary and is not actually changing your body fat composition.

Best Practices - Health care workers who use or may be exposed to needles are at increased risk of needlestick injury. Such injuries can lead to serious or fatal infections with bloodborne pathogens. These injuries can be avoided by eliminating the unnecessary use of needles, using devices with safety features, and promoting education and safe work practices for handling needles and related systems. These measures should be part of a comprehensive program to prevent the transmission of bloodborne pathogens.

Ease your mind with the light humour in our **Relaxed Mood section**…….

So go on, enjoy reading & don't forget to give us your valuable inputs & feedback.

Bacteriology - Elementary Identification of Enterobacteriaceae (Issue 3)

The elementary identification of medically important genera of the family Enterobacteriaceae based on few basic biochemical tests was discussed in previous issue. In this article, elementary identification of Enterobacteriaceae based on genomic level / molecular level techniques available is elucidated.

Few of the commercially available identification systems offered are serotyping and molecular typing techniques. Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

Serotyping

Serotyping is subtyping method based on the immuno-reactivity of various antigens. Microorganisms, viruses, or cells are classified together based on their cell surface antigens, allowing the epidemiologic classification of organisms to the sub-species level. A group of serovars with common antigens is called a serogroup or sometimes sero-complex. Serotyping often plays an essential role in determining species and subspecies. The *Salmonella* genus of bacteria, for example, has been determined to have over 2600 serotypes, including *Salmonella enterica* serovar *Typhimurium, S. enterica* serovar *Typhi,* and *S. enterica* serovar *Dublin*. A *Salmonella* serotype is determined by the unique combination of reactions of cell surface antigens. The "O" antigen is determined by the outermost portion of the Lipopolysaccharide (LPS) and the "H" antigen is based on the flagellar (protein) antigens. *Shigella* species are by definition non-motile, as such, only the somatic (O) antigens are utilized for the determination of serotype. Flagellar (H) antigens are not expressed. The majority of serotypes of *Salmonella* possess two phases of H (flagellar) antigens and serotyping of these species can also be done.

Serotyping deciphers the antigenic makeup of the organism by identifying the somatic (O) and flagellar (H) antigens through reactions with specific antisera. Its usefulness in surveillance programs has long been recognized, in spite of the fact that it does not have the capacity to fingerprint strains in a sensitive manner. For the latter purpose, pulsed-field gel electrophoresis (PFGE), it is considered the gold standard for *Salmonella* molecular subtyping.

Typing of *salmonella* by phenotypic methods

(i) Serotyping by slide agglutination (Kauffmann-White-Le Minor scheme)

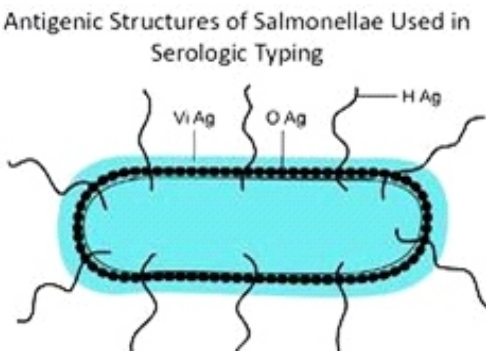

Fig. 1. Kauffmann-White-Le Minor scheme

This is the basis for naming the manifold serovars of *Salmonella*. To date, more than 2600 different serotypes have been identified. A *Salmonella* serotype is determined by the unique combination of reactions of cell surface antigens. The process to identify the serovar of the bacterium consists of finding the formula of surface antigens which represent the variations of the bacteria.

The traditional method for determining the antigen formula is agglutination reactions on slides. The agglutination between the antigen and the antibody is made with specific antisera, which reacts with the antigen to produce a mass. The antigen O is tested with a bacterial suspension from an agar plate, whereas the antigen H is tested with a bacterial suspension from a broth culture, Fig. 1. The scheme classifies the serovar depending on its antigen formula obtained via the agglutination reactions.

(ii) Serotyping by antibody microarrays

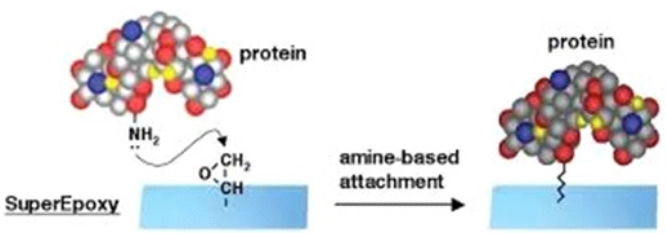

Fig. 2. SuperEpoxy microarray slides

Development of a serotyping assay based on SuperEpoxy microarray slides spotted with antibodies and fitting the KW scheme, Fig. 2. In this assay, the antibody-antigen reactions are conducted on a micro-volume scale on slides following fluorescent labeling of the investigated *Salmonella* strain. Detection is carried out with a common fluorescence scanner. The main advantages of antibody microarray-based serotyping over traditional serotyping are reduced analysis time, standardized agglutination detection, and simultaneous detection of the O and H antigens, for which the phase inversion step can be skipped thanks to the high sensitivity of detection. Further development of this promising assay is of course conditional upon its successful validation on a much larger number of serovars.

(iii) Other phenotyping methods

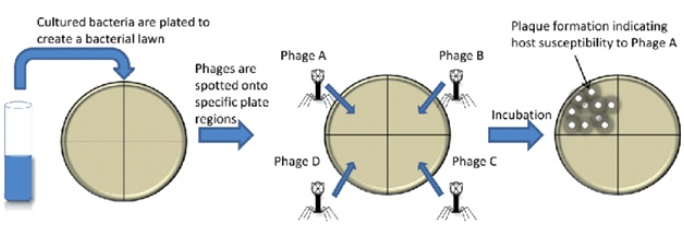

Fig. 3. Phage typing. The bacterial sample to be typed is plated together with a series of bacteriophages to create a bacterial lawn where clearings/plaque formation is indicative of bacteriophage replication and host susceptibility to the specific bacteriophage.

Phage typing is used to discriminate between Salmonella strains belonging to the same serovar. Phage types are assigned on the basis of the ability of a given phage to lyse the investigated strain, Fig. 3. The advantage of phage typing resides in the simplicity of its implementation, which requires only basic laboratory equipment. Ambiguous lysis reactions are common drawbacks, though, and careful coordination between reference laboratories is required in order to ensure reproducibility of the assay. The method is also limited by the number of available phages.

Molecular typing techniques - Rapid Molecular Methods

Most clinical microbiology laboratories still use traditional, culture-based diagnostic techniques for routine detection of bacterial enteric pathogens. These are both time-consuming and laborious and, in addition, have a prolonged delay in the reporting of results. Molecular detection of pathogens has, however, been shown to be faster and more sensitive than traditional culture. Faster diagnostic outputs allow earlier epidemiological investigations and infection control interventions. It is especially important where culture-based pathogen detection is problematic or lacks sensitivity.

Molecular methods have had an enormous impact on the taxonomy of Enterobacteriaceae. Analysis of gene sequences has increased understanding of the phylogenetic relationships of Enterobacteriaceae and related organisms; and has resulted in the recognition of numerous new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), Matrix-Assisted Laser Desorption/Ionization - Time of Flight (MALDI-TOF) Mass Spectrometry, Multi-locus Sequence Typing (MLST), Multiple-Locus Variable-Number Tandem-Repeat Analysis (MVLA), SNP assays and Whole Genome Sequencing (WGS). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility. However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

(i) Pulsed Field Gel Electrophoresis (PFGE)

PFGE is a molecular typing method which detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel, Fig. 4. PFGE is known to

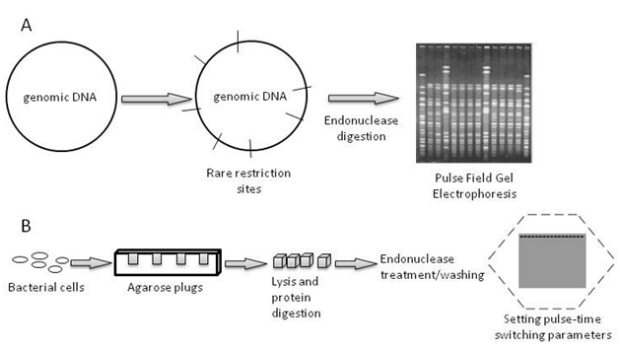

Fig. 4. Schematic representation of pulse field gel electrophoresis. (A.) PFGE of Sma I-digested genomic DNA of bacterial isolate. (B.) Sequence of steps involved in PFGE.

be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. The technique is useful for fingerprinting strains in outbreak situations and is relatively inexpensive to perform. This has been used successfully to identify and discriminate between species of the family Enterobacteriaceae – for example, it has been used in tracking the source of *Salmonella* infections for different serotypes and is considered the gold standard for *Salmonella* molecular typing and also for typing of *Klebsiella* species. This has been used successfully to discriminate between *Yersinia enterocolitica* strains and will still be useful for surveillance of the sources and transmission routes of sporadic *Yersinia enterocolitica* strains in future.

However, PFGE is time-consuming and labor-intensive and does not display equal sensitivity with different serovars. The stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. Hence, due to its time-consuming nature (30 hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories.

(ii) Matrix-Assisted Laser Desorption/Ionization - Time of Flight (MALDI-TOF) Mass Spectrometry

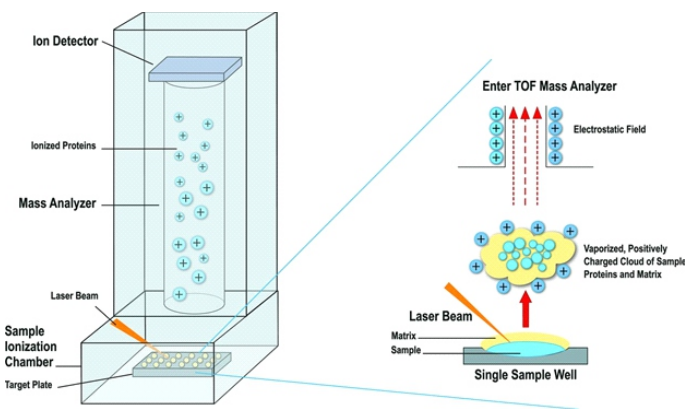

Fig. 5. MALDI-TOF mass spectrometer. The target plate is placed into the chamber of the mass spectrometer.

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS), which can be used to analyze the protein composition of a bacterial cell, has emerged as a new technology for species identification, Fig. 5. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use.

This has been utilized to aid in both the detection and species-level identification of *Salmonella* species. It has also been used successfully for the identification and characterization of the *Proteus mirabilis, Plesiomonas shigelloides* and *Enterobacter cloacae* isolates except that an improved MALDI-TOF MS database needs to be compiled in order to be able to determine species-level identifications, eg. *Enterobacter cloacae* complex. This technique has equally been used for the identification of *Pantoea* species but the database would benefit from additional

entries being added to further populate it with entries of both environmental and clinical interest. MALDI-TOF MS has the ability to accurately discriminate between the two clinically relevant and highly genetically similar organisms with identical 16S rRNA gene sequences, for e.g. *Y. pestis* and *Y. pseudotuberculosis*. The method of inactivation used for these pathogenic organisms does not have any influence on the MALDI-TOF MS spectra generated. This has also been used to identify and subtype *Yersinia enterocolitica* isolates. MALDI-TOF MS is able to derive genus- and species-level identifications for *Cronobacter* isolates or identify them as non-*Cronobacter* isolates in the case of non-target strains and to identify different biovars within the *C. sakazakii* species but according to a study by Cetinkaya et al., molecular techniques such as 16S rRNA and *fus*A gene sequencing and multilocus sequence typing (MLST) are more reliable mechanisms of *Cronobacter* identification. As a tool for subspecies and serovar typing, MALDI-TOF MS shows significant promise but will require additional studies and modifications to existing protocols before the method can be used as a stand-alone mechanism.

One of the limitations is the current inability of MALDI-TOF MS to reliably distinguish pathogenic from non-pathogenic *E. coli* isolates, in addition, numerous reports have also described the difficulty encountered when trying to discriminate *E. coli* from *Shigella* species. Differentiation of pathogenic *E. coli* strains from *Shigella* species is challenging because of the close genetic relatedness of the organisms and so biochemical and serology testing is essential in identification.

(iii) Multi-locus Sequence Typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple; the technique involves PCR amplification followed by DNA sequencing, Fig. 6. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the protein product. One of the advantages of MLST over other molecular typing methods is that

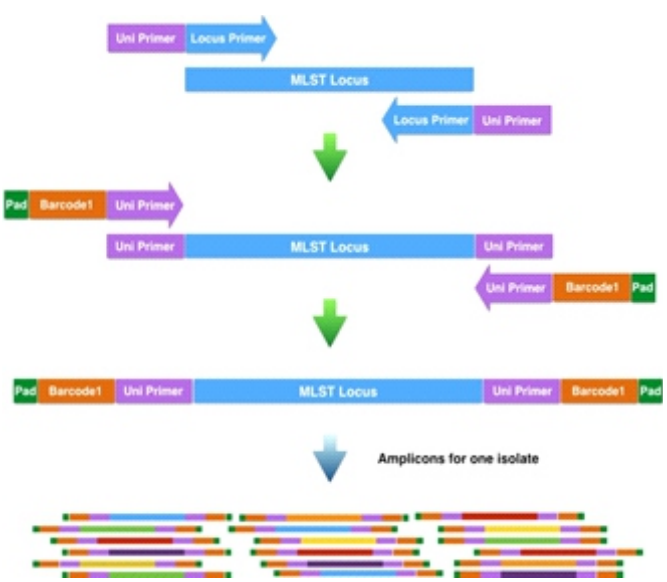

Fig. 6. Multi-locus Sequence

sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the Internet.

MLST has been extensively used as one of the main typing methods for analyzing the genetic relationships within the Enterobacteriaceae population especially the genus *Salmonella*. MLST was found to provide better discrimination of *Salmonella* serotype Enteritidis strains than PFGE and accurately differentiate outbreak strains and clones of the *Salmonella* serovars most commonly associated with human disease. It has also been useful for typing non-typhoidal *Salmonella* strains. This has also been used to identify Shiga-toxin producing *E. coli* and *Shigella* species as well as *Klebsiella* species.

The drawbacks of MLST are the substantial cost and laboratory work required to amplify, determine, and proof read the nucleotide sequence of the target DNA fragments, making the method hardly suitable for routine laboratory testing.

(iv) Multiple-Locus Variable Number Tandem Repeat Analysis (MVLA) also known as VNTR

Multiple-Locus Variable number tandem repeat Analysis (MLVA) is a method used to perform molecular typing of particular microorganisms. It utilizes the naturally occurring variation in the number of tandem repeated DNA sequences found in many different loci in the genome of a variety of organisms, Fig. 7. The molecular typing profiles are used to study transmission routes, to assess sources of infection and also to assess the impact of human intervention such as vaccination and use of antibiotics on the composition of bacterial populations. It is technically simple and inexpensive to perform.

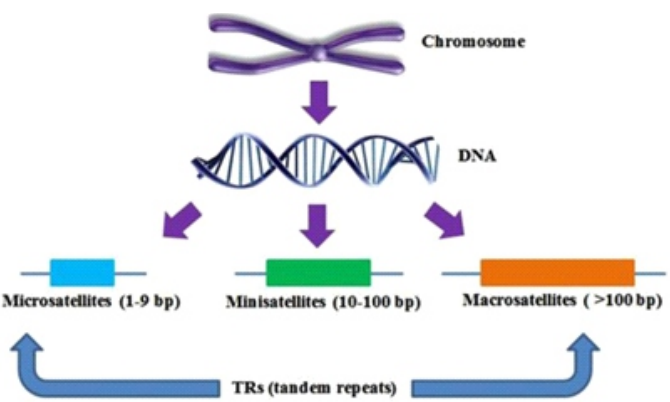

Fig. 7. Diagram illustrating the different types of tandem repeats (TRs)Typing (MLST).

This has been used successfully in the subtyping of *Salmonella enterica* subsp *enterica* serovar *Typhimurium, Enteritidis, Typhi, Infantis, Newport, Paratyphi A, Saintpaul,* and *Gallinarum* isolates. The method has proven very useful for detecting and investigating outbreaks, since it has the capacity to differentiate closely related strains. It is technically simple and inexpensive to perform. However, it has no usefulness for serovar assignment or for global phylogenetic studies because the scope of each MLVA is commonly restricted to a unique serovar. This has also been used successfully to identify *Shigella* and *Escherichia* strains, suggesting that it could significantly contribute to epidemiological trace-back analysis of *Shigella* infections and pathogenic *Escherichia* outbreaks. MLVA has been used successfully to identify and discriminate between *Yersinia enterocolitica* strains and it has been found to be a more effective

method than PFGE. This method is also less labor intensive and the results from it are easier to analyze. It has also been used to genotype *Yersinia pestis*. However, the scope of each MLVA is commonly restricted to a unique serovar, making MLVA a second-line typing method with no usefulness for serovar assignment or for global phylogenetic studies. A drawback of MLVA commonly observed during assessing of small tandem repeat motifs is genetic homoplasy, leading to apparent relatedness of the investigated strains.

(vi) SNP discovery and typing - rpoB Single Nucleotide Polymorphism (rpoB SNP) assay

rpoB gene is a single-copy chromosomal gene encoding the RNA polymerase β-subunit. This gene has been previously used in phylogenetic analysis for bacteria species and genus delineation, since it is highly conserved across organisms. However, the 16SrRNA gene has been used widely and its usefulness has been greatly enhanced through the establishment of public domain databases but its sensitivity has been questioned particularly among Enterobacteriaceae and so when the rpoB gene was used as an alternative for detection based on a Single Nucleotide Polymorphism, it was found to be more compatible with the currently accepted classification of Enterobacteriaceae and a powerful identification tool which may be useful for universal bacterial identification. This has been used to demonstrate that the genus *Klebsiella* is polyphyletic and to detect *Salmonella enterica* serotype *Typhimurium*. High-density array platforms assessing 1,500 SNPs were used in two studies to type *Salmonella* serovar *Typhi* isolates at an unprecedented level of sensitivity. A simpler and cost-effective SNP typing method was developed by Octavia and Lan. This method assesses each SNP individually using hairpin-shaped primers formatted to preferentially amplify the actual nucleotide found at a given SNP position. Bishop et al. are designing a method to detect SNPs in *Salmonella* Paratyphi A, based on single-base extension of DNA probes combined in four multiplex PCR assays. The originality resides in the analysis of the 47 generated amplicons, which are passed through a mass spectrometer. Finally, a low-density microarray assessing 62 markers directly amplified from purified genomic DNA by ligation detection reaction was recently developed with the aim of identifying virulence-associated gene repertoires within common serovars.

The distinct advantage of SNP and other nucleotide sequence-based methods over profile-generating methods is that genetic relationships can be established on the basis of discrete data that are directly suitable for bio-computing and statistical analysis.

The discovery of SNPs has been facilitated by the genome sequencing of several strains belonging to either different or identical serovars. Much effort has been focused on *Salmonella* serovar *Typhi* for obvious public health reasons. Given the highly monomorphic character of this human-adapted serovar, clonal fingerprinting of *Salmonella* serovar *Typhi* strains requires that single-nucleotide variants be discovered and sensitive techniques used for their discrimination. Such sequence polymorphisms were identified by nucleotide sequence comparison of *Salmonella* serovar *Typhi* strains or by denaturing high-performance liquid chromatography (dHPLC). Methods that can identify critical nucleotides in a subset of polymorphic markers, sometimes in a highly multiplexed manner, have arisen. Ben-Darif et al. developed a multiplex primer extension (MPE) assay to genotype multiple known SNPs selected from the MLST scheme of Kidgell et al. Fig. 8.

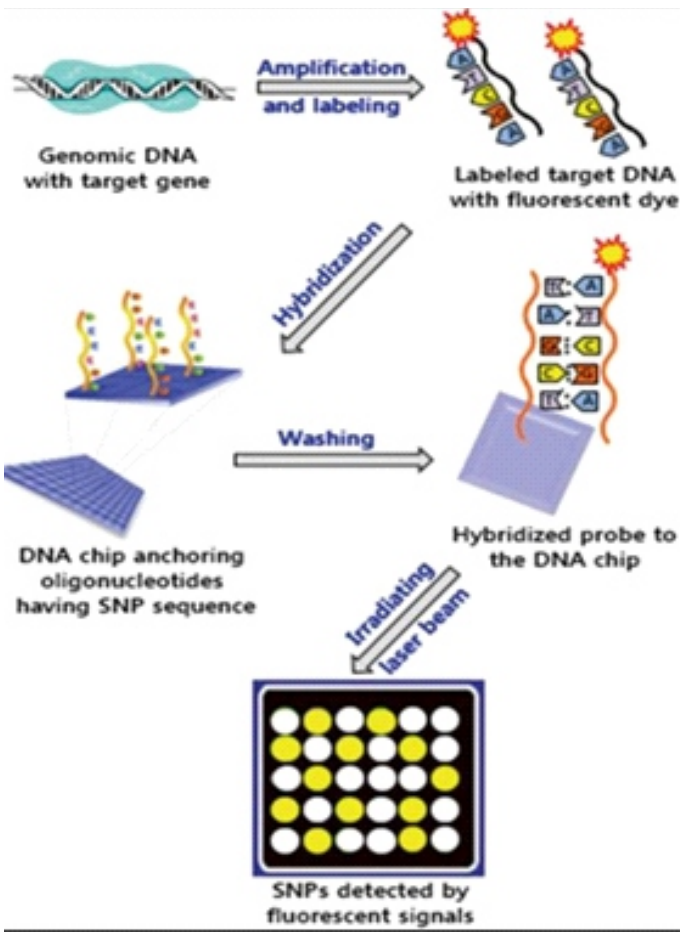

Fig. 8. DNA chips to analyze single nucleotide polymorphisms (SNPs). A DNA chip is repaired by attaching DNA fragments of ~25 bp in length from a species to a glass or metal plate. These fragments contain the SNP variants to be analyzed. Fluorescence-labelled probes are hybridized with the DNA fragments on the plate, and the DNA chip is washed. Finally, the probes are exposed to light of a specific wavelength to induce fluorescence.

(vii) Whole Genome Sequencing (WGS)

Whole Genome Sequencing (WGS) is also known as "full genome sequencing, complete genome sequencing, or entire genome sequencing". It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time, Fig. 9. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nano-pore technology, IIIumina sequencing, Ion Torrent sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs. This technique has equally been used to characterize

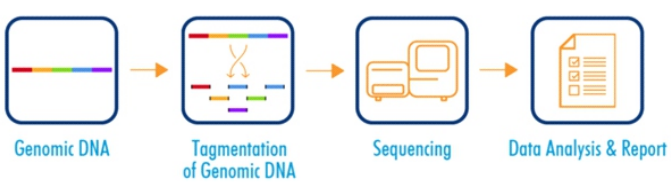

Fig. 9. Whole Genome Sequencing (WGS).

Salmonella enterica serovar *Typhi* and to discover its recently acquired genes, such as those encoding the Vi antigen, by horizontal transfer events and it has provided new insights into how this pathogen has evolved to cause invasive disease in humans.

This has been used successfully to explore the genome of *Shigella* species and *E. coli* O157:H7 to identify candidate genes responsible for pathogenesis, and to develop better methods of strain detection and to advance the understanding of the evolution of *E. coli*. With this technique, lateral gene transfer of *E. coli* was discovered and found to be very extensive. WGS has also been used to provide important insights into the pathology of *Yersinia enterocolitica* and, more broadly, into the evolution of the genus and other human entero-pathogens.

Conclusion and perspectives

Many methods have been and are still being developed for the subtyping of *Salmonella*. Each of them has its own advantages and drawbacks in terms of cost, speed, robustness, and sensitivity. Molecular methods developed as alternatives to classical serotyping have generally proven very successful, even if they do not deliver results that exactly and completely mirror those obtained by the current reference method, which probes surface antigens with antisera. The observed discrepancies result from the intrinsic nature of molecular versus phenotypic methods, which cannot be perfectly correlated due to the multiple differences that characterize genes on the one hand and antigens exposed at the bacterial surface on the other. These minor differences certainly do not affect the usefulness of the alternative molecular methods. However, it may preclude their use in contexts where strict observations of the reference methodology and classification rules are imposed. Whenever it has been assessed by classical or modern methodologies, the historical classification into serovars has been shown to be useful, and it is generally accepted that this classification should be maintained.

Molecular subtyping methods have proven highly valuable in characterizing and differentiating Salmonella strains at a sensitive level for epidemiological studies. Housekeeping genes, virulence determinants, antibiotic resistance markers, mobile genetic elements, prophage genomes, and sequence repeats have all been used as targets, either alone or combined. To evaluate the level of sensitivity reached by these new methods, PFGE is often used as the reference method. For many of them, this level relies on the number of markers included in the assay and on the capacity of the chosen platform to analyze them in a single run. In this respect, microarrays achieved a significant breakthrough by allowing dozens of such markers to be analyzed at once. Methodological challenges remain for multiple SNP amplification and typing. In addition, high costs often preclude the use of many such methods for routine use. However, as costs fall and automation improves, global effectiveness and speed may soon favor a more systematic use of these new DNA-based assays.

STERILIZATION

Sterilization, as a specific discipline, has been with us for approximately 120 years, since the invention of the steam autoclave by Charles Chamberland in 1879. Since that time, we have seen progressive refinement in steam sterilizers: from the early, manually operated equipment to modern microprocessor-controlled, automatic machines. Although the efficiency, reliability, and performance monitoring of modern equipment is continually improving, the fundamental process remains essentially the same.

Sterilization processes cannot be considered in isolation; rather, they are inextricably related to the product to be sterilized. They are also related to the packaging of the sterilized product. Except for the rare instances when the sterilizer can be located where the sterile goods are to be used, there is a need for the sterilized products to be packaged in a manner that will preserve their sterility during storage, handling, and transport. The majority of sterile goods produced in the medical device industry and in healthcare facilities are terminally sterilized that is, they are sterilized already packaged. They may be packaged only in their primary packaging or in multiple layers of packaging such as a unit pack, shelf pack, and shipping carton.

For a product sterilized in its packaging, the packaging material must be compatible with the sterilization process. This requires both that the packaging tolerates the process without adverse effects on its performance characteristics, and that it permits the attainment of the specified sterilization conditions in the product to be sterilized.

There is no single sterilization process that is suitable for all medical products. The diversity in sterilization processes and of operating systems within each process has arisen as a consequence of the efforts made to optimize medical sterilization and to meet the differing needs imposed by the vast range of products to be sterilized.

Traditional Processes

The sterilization processes that have traditionally been used for medical products include steam, ethylene oxide (EtO), ionizing radiation (gamma or E-beam), low-temperature steam and formaldehyde, and dry heat (hot air). These methods can be divided into three categories, based on the nature of the sterilant and its reaction with microorganisms: physical processes (ionizing radiation, dry heat); physicochemical processes (steam, steam/formaldehyde); and chemical processes (EtO, glutaraldehyde).

Chemical and physicochemical processes depend on direct physical contact between molecules of the sterilant and the microorganism to be killed. In consequence, access must be available to the surfaces of the product to be sterilized and the packaging material (for terminally sterilized products) must be porous or permeable to these molecules. For example, in the case of steam sterilization of dry products, the air must be removed from the package and replaced with steam in direct contact with the product. This is necessary both to provide the required thermal energy by condensation of the steam and the required water for the protein hydrolysis reaction to occur. At the same temperature in the absence of water, the degradation of proteins would occur at the much slower rate characteristic of dry-heat processes.

Purely physical processes such as ionizing radiation may be used for product designs and packaging materials that are impermeable to gases as long as they are "transparent" to energy of the wavelengths employed in the sterilization process.

New Processes

What do we mean by a new sterilization process? New is often a marketing description for the latest outcome in a gradual development and refinement of an existing process. Continued development or refinement in one area allows, and sometimes requires, development in another. This advance may be driven by the product, the sterilization process, the packaging, economics, or other external forces.

Over the past two or three years, the development of the art of sterilization seems to have accelerated, with the introduction of several new processes. At least one reason for this is the potential decline in the use of EtO in hospitals. This is a result both of increased concern over the toxicity of residuals and of the need to eliminate the use of chlorofluorocarbons (CFCs), which had previously been employed to minimize the flammability and explosion risks of the EtO. Another prominent trend is the proliferation of various minimally invasive therapies, and the need for appropriate sterilization protocols for the equipment used in these procedures.

It is important to note that one must consider two very different fields of application for sterilization processes industrial and hospital. Although the same level of sterility assurance should be provided in each case, the operational circumstances are sufficiently different that the two fields need to be considered separately. In industry, a sterilizer will typically be used to process virgin product with a known bioburden. The range of products will be limited and may be a single product type or single product family of closely related types. There will normally be good engineering and analytical laboratory support, and the process will be subjected to in-depth validation and routine monitoring. All of the personnel involved will be specifically trained in the process.

Contrast this with the situation found, all too often, in hospitals. A diverse range of reusable products will be processed after being subjected to a largely invalidated cleaning process. The extent and nature of residual soiling and bio burden will be unknown. The process will be subject to minimal validation certainly not covering the diversity of products processed and only rarely will there be adequate support from an analytical laboratory. The expectation will be that the process should be simple to operate and safe for use by personnel with minimal training.

Chemical Processes (Gas/Liquid)

Whereas the most widely used traditional chemical processes were based on alkylating agents such as EtO and the various aldehydes, most of the new methods are oxidative processes based on "peroxy" compounds. These include sterilants based on compounds such as hydrogen peroxide, peracetic acid, peroxysulphates, chlorine dioxide, and ozone. For the most part, the microbicidal action of these chemicals has been recognized for many years.

Peracetic Acid - Peracetic acid is currently used in a number of sterilization processes. Examples include liquid systems such as the Steris machine (Steris Corp., Mentor, OH) for endoscopes, or

use as a liquid sterilant in suitable disinfectors (e.g., Nu-Cidex from Johnson & Johnson Medical Ltd., Skipton, UK) for sterilization of thermolabile endoscopes. Vapor-phase generators employing peracetic acid are being sold for the decontamination, disinfection, or sterilization of products such as isolators.

The process itself is not new, as the bactericidal activity of peracetic acid was noted by Greenspan and MacKellar in 1951.[2] It was used in solution as a sterilization process as early as 1955, and, in the vapor phase, by Portner and Hoffman in 1968.[3] Aerosolized peracetic acid for the sterilization of surgical instruments was considered by Werner and others in the early 1970s.

Peracetic acid is a colorless liquid with a pungent odor, miscible with water. Commercially available as a 35% or 40% solution, it is generally unstable, decomposing to give oxygen, acetic acid, and other degradation products, which include hydrogen peroxide and water.

Acetic acid and hydrogen peroxide are invariably present in low concentration. Peracetic acid is corrosive to certain materials and it is lachrymatory, an irritant, and a vesicant (causing blistering) on prolonged contact. Its use as a sterilant therefore relies on obtaining formulations that inhibit corrosion of sensitive materials and stabilize the solution to give it a usable shelf life.

The Steris system currently available for the sterilization of endoscopes is like the glutaraldehyde system it is intended to replace a wet system. This limits its applicability, since it becomes difficult to provide a system for packaging and storage of the sterile product. In common with any other liquid chemical system, water is used to flush out any residual chemical at the end of the process. Ensuring that this water is of suitable chemical purity and microbial quality to prevent recontamination of the processed goods requires thorough control. The vapor-phase process has found applications within industry for decontamination of environmental spaces, but there has been no move toward the development of a general-purpose peraceptic-acid sterilization process, and none seems likely.

Hydrogen Peroxide - Hydrogen peroxide as a 3% aqueous solution has long been used as an antiseptic. For example, hydrogen peroxide potentiated by ultraviolet light has been used in the production-line sterilization of commodity items such as cartons for food products. The use of hydrogen peroxide as a vapor-phase sterilant was developed by Amsco in the United States as the VHP system. This process was originally developed in several formats, including a cassette system for endoscopes, a freestanding system for environmental decontamination, a system for use in sterilizing lyophilizers and isolators, and a general-purpose unit for the sterilization of medical devices. (Following the recent acquisition of Amsco by Steris, it appears that the company will emphasize the development of peracetic acid for endoscope sterilization and hydrogen peroxide for environmental and general-purpose applications.)

The deep-vacuum hydrogen peroxide process operates in a manner analogous to gas sterilization processes. Initial air removal allows for rapid diffusion, and humidity is controlled to optimize the microbicidal effect. The process is compatible with a wide range of materials, but traditional packaging is likely to interfere with the process because of its reaction with, or high absorption of, the hydrogen peroxide. Although the process is finding extensive use in the sterilization of lyophilizers, a general-purpose unit is not yet available.

Ozone - The bactericidal and sporicidal effect of ozone has long been recognized. Its use as a sterilant, however, has been limited because of its instability, which precludes storing it ready for use, and because of the difficulty of generating pure ozone. Ozone is produced naturally by the effect of sunlight or ultraviolet light on oxygen, and also by electrical discharge. Recent technological advances have made the generation of ozone a more practicable proposition, and commercially available sterilizers have been developed.[7] The Cyclops Co. has introduced a machine for sterilizing endoscopes that pumps humidified ozone through the unit. Advantages of the system are said to include freedom from long-term toxic residuals and ease of use, with only medical-grade oxygen and electrical connections required. Potential disadvantages include the reactivity of ozone with certain materials.

Chlorine Dioxide - Chlorine dioxide (ClO_2), which is a gas at temperatures above 11°C, was discovered by Sir Humphry Davey in 1811 and is another chemical that has long been known to have microbicidal properties and that has, like ozone, been used in water purification systems. The germicidal and sporicidal properties of chlorine dioxide have been recognized since 1936 (Leseurre) and 1949 (Ridenour et al.), respectively.[8,9]

Many disinfection technologies employ chlorine dioxide in aqueous solution and, when necessary, use nitrogen or air purging to remove the traces of residual gas. The major problems with this technology have always been that chlorine dioxide gas cannot be safely liquefied or stored under pressure for transport and subsequent use (since under these conditions it is explosive), and that as an aqueous solution it is unstable and corrosive. Recent developments have seen the use of both gaseous and liquid chlorine dioxide systems.

Gaseous Systems - In the system developed by Johnson & Johnson, the chlorine dioxide is generated in situ by the action of chlorine on sodium chlorite. The chlorine is presented as 2% Cl_2 in N_2, in a cylinder filled initially to 2700 psig and then emptied to 300 psig; the chlorine accounts for a pressure of 60 psig. The generator employs a two-column system, with discharge of the chlorine into the first column pressure controlled and monitored, and output from the generator monitored by a fiber-optic UV absorption system. The working life of the column is limited to 70% of its theoretical capacity, as established by validation studies, in order to ensure that the conversion process will always take place effectively. The second column is used as a backup.

The sterilizer is operated at slightly above room temperature (32°C), which allows for good control over the process. The process uses a cycle analogous to that of EtO sterilizers, with a vacuum air-removal stage followed by a dynamic conditioning stage to humidify the chamber and load to an RH of about 70%. At the end of the conditioning phase, ClO_2 gas is admitted to give a concentration of 30 mg/L. This is then topped off by the addition of N_2 at pressures of 80 kPa. A total gas exposure time of about 60 minutes is standard. At the end of the cycle, the ClO_2 is removed using a four-pulse dynamic air exchange.

Advantages of this process compared with EtO are that because ClO_2 does not have the chemical solubility of ethylene dioxide there are no significant levels of residual sterilant within the product material, and that ClO_2 is not flammable in air at the concentrations employed.

Gaseous ClO_2 may be removed from the effluent airstream by scrubbing with $Na_2S_2O_3$. Residual levels for discharge to the atmosphere can be well below 1 ppm and are usually undetectable.

The gaseous chlorine dioxide system is currently being used in several medical applications, including the sterilization of contact lenses and the secondary sterilization of overwrapped foil suture packages.

Liquid Systems - Solutions of chlorine dioxide are also commercially available as liquid sterilants under trade names such as Tristel and Medicide and as such compete with glutaraldehyde and peracetic acid solutions. While the microbicidal efficacy of chlorine dioxide has long been recognized, there have been two problems associated with the use of liquid systems. First, the solutions are unstable, with the concentration of ClO_2 rapidly diminishing; second, because chlorine dioxide is highly oxidative, it is potentially corrosive to many materials. The development of usable solutions has therefore required formulations that incorporate stabilizing agents, usually based on boron components and anticorrosion compounds. These comprise a base solution and an activator which, when mixed, yield a solution of approximately 0.1% chlorine dioxide, with a 14-day shelf life. Solutions of this type are increasingly being used for the sterilization of fiber-optic endoscopes.

Physicochemical Processes

Plasmas - Plasma is the fourth state of matter, and as such is distinguished from solids, liquids, and gases. Plasmas are produced at very high temperatures, or at low temperatures in strong electromagnetic fields (the so-called "glow-discharge" plasmas). The plasma usually consists of a reactive cloud of ions, electrons, free radicals, and other neutral species.

The plasma process seeks to produce a sterilizing effect using lower concentrations of sterilant with a higher reactivity than would be possible in a normal gas process.[10-12] Because the active species are only present when power is applied to the system and disappear quickly when the power is turned off, the very active species that act as the sterilant will not be present as a source of toxicity at the end of the process.

The precursor gas selected for plasma generation will determine which active species are present, and these may be expected to influence the comparative microbicidal activity of the system.

When a plasma contacts the surface of an item to be sterilized, the collisions between the active species and other molecules cause a significant proportion of the active species to return to the ground state. Packaging material can thus cause a serious depletion in the concentration of active species reaching the item to be sterilized, soiling on the surface may have a significant inhibitory effect, and the extent of diffusion into narrow lumens may be limited.

The Sterrad Process - The Sterrad process (Advanced Sterilization Products, Johnson & Johnson Medical Inc., Arlington, TX) is a plasma system that uses hydrogen peroxide as the source of the active species. The process seeks to overcome the inhibitory effect of packaging materials by using a gas-diffusion phase to allow gas to penetrate to all parts of the load before the plasma is created. The adequacy of this approach depends on the certainty with which one can ensure that the hydrogen peroxide gas diffuses to all parts of the load and that the nature and construction of load items will not inhibit subsequent plasma formation. Although this system is becoming widely adopted, there are still reservations about its use in hospitals-where product cleaning prior to sterilization may not have been well controlled, where inappropriate products may be processed, and where parametric release may be used without supporting evidence comparable to that required for other processes.

Steam - The inclusion of steam sterilization in the context of recent developments in sterilization technology may at first seem strange. However, there is a continuing evolution of the equipment, packaging, and monitoring systems used for the process. The publication of EN 554 has stimulated renewed interest in ensuring appropriate steam purity for product contact. One continues to see progressive refinement of the microprocessor-based control systems and, in particular, of secondary or supporting functions, such as providing users with a prompt when maintenance is required. Control systems are becoming much more user-friendly, with touch screen systems becoming commonplace. Improvements continue to be made in related steam sterilization supplies such as packaging materials and biological and chemical indicators.

Synergetic Processes

Psoralens and UVA (PUVA) - An interesting example of the development of sterilization techniques for specific applications is the recently reported use of ultraviolet light in combination with psoralens to purge blood plasma and platelets of pathogenic organisms. Psoralens are naturally occurring substances found in a wide range of plants, in which their role is to fight infection from pathogenic fungi.

Irradiation of blood with UV light has been recognized as a method of treating otherwise intractable infections ever since its development in the 1930s for use with polio patients. It is reported that the fundamental effect of exposure to UVA is to stimulate the body's biochemical and physiological defenses.[13,14] Researchers have speculated that this is related to the low concentration of ozone produced from the oxygen circulating in the blood.

Ultraviolet blood-irradiation therapy is currently under investigation for the treatment of diseases such as HIV infection and hepatitis, and is the method of choice for the treatment of cutaneous T-cell lymphoma. The use of UV is also noted for its ability to inactivate viruses while preserving their antigenic properties for the preparation of vaccines.

The recent proliferation of novel blood-borne viruses has led to demands for better safety guarantees for blood products, and hence many methods of sterilization have been extensively examined.[15,16] It has become clear that most viruses are quite sensitive to UVB or to UVA when used with psoralens as photosensitizing agents. The psoralens form a labile bond with DNA and RNA which, upon exposure to UV light, becomes a firm bond. Recent work by Cerus Corp. (Concord, CA) appears to show that synthetic psoralens and UV irradiation can be used to destroy infectious agents such as HIV, hepatitis viruses, and toxemia-inducing bacteria. However, it has been thought that producing viral inactivation of sufficient magnitude was not feasible without causing intolerable damage to vital blood components-especially erythrocytes, in which hemoglobin blocks the penetration of UV light.

The absence of genetic material in platelets, however, means that these would remain unaffected by the PUVA mechanism, and it should therefore be possible to use the technique to sterilize plasma and platelets. This possibility is currently under investigation in clinical trials. Although the psoralens and dead microorganisms would remain in the product, it is considered unlikely that they would pose a risk, given the extensive clinical history of psoralens.

Microwave and Bactericide - Sterilization methods are being marketed that propose the use of microwaves in conjunction with a bactericidal solution-a modern version of the century-old

process of heating with a bactericide. The technique is being promoted for use with dental instruments and relies on heating a solution of a quaternary ammonium compound (benzylkonium chloride) to approximately 100°C. At present, these processes are applicable only to unpackaged instruments.

Low-Temperature Steam and Formaldehyde - Low-temperature steam in combination with formaldehyde is another traditional process that has continued to evolve. It is an example of synergism in that it brings together steam at subatmospheric pressure and formaldehyde gas-neither of which is markedly sporicidal-to produce a highly efficient sporicidal effect.

The process has been in and out of fashion several times over its 100-year history. Concerns over the toxicity and carcinogenicity of formaldehyde have limited its acceptance in the United States, despite potential advantages over ethylene oxide. More recently, improved process control has allowed the production of sterilizers with negligible environmental emissions and very low product residual levels. Other developments have included the use of operating cycles at temperatures comparable to those employed for ethylene oxide instead of in the 70°-80°C range that was traditionally employed.

Physical Processes

Microwaves - The inherent advantage of microwave heating compared with other forms of heating lies in its lower power requirements. The interaction between microwaves and biological materials does not of itself appear to be lethal: rather, the lethality obtained is directly derived from the heating effect, which in turn depends on the composition of the microorganism being targeted, including its water content. Limitations related to the specifics of microwave reflectance, transmittance, and absorbance may limit applicability for device sterilization.

Pulsed-Light Systems - A novel sterilization method introduced in the past several years uses high-power electrical energy to produce intense pulses of light that are claimed to provide unique bactericidal effects.[17] Called the PureBright system (PurePulse Technologies, San Diego), the technology rectifies and converts normal building ac to high-voltage dc and uses it to charge a capacitor, which is then discharged through a specially designed xenon lamp unit. The high-voltage, high-current pulse applied to the lamp causes it to emit an intense pulse of light, which typically lasts for a few hundred microseconds. The light produced by the lamp includes a broad spectrum of wavelengths, from ultraviolet to infrared, with an intensity some 20,000 times greater than sunlight.

The process is reported to be highly successful in killing microorganisms, viruses, and spores, as well as in deactivating enzymes. Its effectiveness depends in part on the ease with which the organisms to be killed can be directly illuminated. For example, organisms on porous surfaces or those suspended in turbid solutions will require higher treatment levels compared with those on smooth, continuous surfaces or transparent materials. Parametric release should be practicable, since the factors controlling the microbicidal activity can be directly, and continuously, monitored. These include both the energy output in the UV range and the lamp current, on which the intensity and spectrum of each flash depend. In use, the normal operating ranges for the system are from 0.1 to 3.0 J/cm^2 per flash, with total accumulated fluences of 0.1 to 12.0 J/cm^2. The number of lamps, their configuration, and the flash rate depend on the particular application. The economics of the process are encouraging, with costs as low as one cent per square meter of surface sterilized.

Potential applications include the surface sterilization of packaging materials for aseptic packaging or for bioburden reduction, and the terminal sterilization of parenterals packed in transparent plastic bags or bottles (e.g., from a blow, fill, seal machine). The photoproducts from treated substrates are reported to be generally similar to those induced by exposure to sunlight and similar to, but fewer than, those produced by thermal sterilization processes. When the process is used to sterilize the surface of opaque materials, any degradative effects would, of course, be restricted to the surface.

Validation of Sterilizer Processes

In the development of new sterilization methods, a key consideration is the data that are needed to demonstrate the efficiency of a process. Although in the United States there is a well-defined process for review of new types of hospital sterilizers, this is not the case worldwide. Furthermore, the FDA approval system does not apply to sterilizers for use in industry.

The lack of any universally accepted approach to validation or process approval for new sterilizers has led to considerable difficulty for both manufacturers and users as new processes have been introduced. There is also a need for a suitable European standard providing a means for presumption of conformity to the Medical Devices Directive for sterilizers, since these have become devices within the scope of the directive. These factors have stimulated work on a standard for *Validation and Routine Control of Sterilization Processes General Requirements*, which is being developed within ISO TC 198 as a common international and European standard under the Vienna agreement. A draft for public comment is expected to be published some time this year. It is to be hoped that the standard will provide a format establishing a common standard for the acceptance or rejection of new sterilizing processes.

Suniti Solomon

Suniti Solomon (1938 or 1939 – 28 July 2015) was an Indian physician and microbiologist who pioneered AIDS research and prevention in India after having diagnosed the first Indian AIDS cases among the Chennai sex workers in 1986 along with her student Nirmala Sellappan. She founded the Y R Gaitonde Centre for AIDS Research and Education in Chennai. The Indian government conferred the National Women Bio-scientist Award on her. On 25 January 2017, Government of India announced "Padma Shri" award for her contribution towards Medicine.

Early life and education

Suniti Solomon, or Suniti Gaitonde as her maiden name, was born in a Maharashtrian Hindu family of the leather traders in Chennai. She was the seventh child in a family of eight and was the only daughter. In a 2009 interview she said she became interested in medicine from the yearly health officer visits to their home for vaccinations.

She studied medicine at Madras Medical College and then was trained in pathology in the UK, the U.S. and Australia until 1973 when she and her husband, Victor Solomon, returned to Chennai, because "she felt her services were more needed in India." She did her doctorate in microbiology and joined the faculty of the Institute of Microbiology in Madras Medical College afterwards.

Personal Life

Solomon met her husband, Victor Solomon, a cardiac surgeon, when studying medicine at Madras College. She followed his travels to the UK, US and Australia. He died in 2006. Their son Sunil Solomon is an epidemiologist at Johns Hopkins University in Baltimore. She was diagnosed with pancreatic cancer 2 months before her death on July 28, 2015, in her home in Chennai, at the age of 76.

Career

In her earlier career life abroad, Solomon had worked as a junior physician at King's College Hospital, London. After returning to India, Solomon worked as a microbiologist at Madras Medical College and rose to the rank of professor. She followed the literature about the clinical descriptions of AIDS in 1981, discovery of HIV in 1983 and by 1986 decided to test 100 female sex workers, as India had no openly gay community. Six of the one hundred blood samples turned out to be HIV positive. Solomon later sent the samples to Johns Hopkins University in Baltimore for a retest which confirmed the result. This discovery became the first HIV documentation in India. Since then, Solomon decided to dedicate her life working on HIV/AIDS research, treatment, and awareness. She has described how people shunned HIV infected persons; even her husband did not want her "to work with HIV-positive patients," most of whom at that time were homosexuals, those who self-injected drugs and sex workers. Solomon replied by "you have to listen to their stories and you wouldn't say the same thing." Solomon was one of the first people who spoke openly about HIV and the stigma along it, she once stated "what is killing people with AIDS more is the stigma and discrimination."

From 1988 to 1993, Solomon set up the first AIDS Resource Group in India founded at the MMC and ran a variety of AIDS research and social services. The group was also the first comprehensive HIV/AIDS facility in India before any private and public sectors. In 1993, Solomon established the 'Y R Gaitonde Centre for AIDS Research and Education' (YRG CARE) after the name of her father. It was one of India's first places for voluntary HIV counselling and testing. As of 2015, 100 outpatients were seen there daily and 15000 patients were on regular follow-up. The centre and her work there have been described as "significant factors in slowing the [HIV] epidemic". She also provide education to other doctors and students about HIV and its treatment She obtained the name of "the AIDS doctor of Chennai" and served as the President of the AIDS Society of India.

Solomon also collaborated in international research studies, including a multi-country HIV/STD Prevention Trial at the US National Institute of Mental Health, the HIV Prevention Trials Network run by the US National Institute of Allergy and Infectious Diseases, an NIH study of the HIV stigma in health care settings in Southern India, and a Phase III study of 6% CS GEL, a candidate microbicide of CONRAD (organization).

REWARDS AND HONORS

- Solomon received the following awards:
- In 2001, award for pioneering work on HIV/AIDS by the state run medical varsity.
- In 2005 a Lifetime Achievement Award for her work on HIV by Tamil Nadu State AIDS Control Society
- In 2006, DMS (Honoris Cusa) by Brown University, USA
- In 2009, 'National Women Bio-scientist Award' by the Indian ministry of science and technology.
- In 2010, Fellowship of the National Academy of Medical Sciences.
- In 2012, 'Lifetime Achievement Award for Service on HIV/AIDS' by the state-run Dr MGR Medical University in Chennai.
- and several other awards, like the 'Mother Teresa Memorial Award' for education and humanitarian services.
- In 2017 Government of India announced "Padma Shri" award (posthumous) for her distinguished service in the field of medicine.

What is *GENERATION GAP*

Father used to walk 20 mins to save 20rs.

Son spends 20rs. to save 20 mins.

(Surprisingly both r correct!)

Cultural Gap

If *electricity* goes in *America* they call the power house.

In *Japan*, they test the fuse,

But In *India*, they check neighbour's house, "power gone there too, then ok!"

Sense of Responsibility

A man goes to library n asks for a book on Suicide. Librarian looks at him & says: "hello who will return the book?"

Grandfather to Grandson:

Go hide! Your teacher is coming as u bunked school today!

Grandson: U go hide I told her you passed away!

Sister to brother: What r u going to gift grandma on her b'day?

Brother: A football

Sister: But grandma does not play!

Brother: On my B'day she gave me Bhagavat Gita.

Judge: Why did you steal the car?

Man: I had to get to work

Judge: Why didn't you take the bus?

Man: I don't have a driver's license for the bus.

The Perfect Son.

A: I have the perfect son.

B: Does he smoke?

A: No, he doesn't.

B: Does he drink whiskey?

A: No, he doesn't.

B: Does he ever come home late?

A: No, he doesn't.

B: I guess you really do have the perfect son. How old is he?

A: He will be six months old next Wednesday.

During a job interview :

Boss : What's the highest level of education you obtained?

Candidate : PHD

Boss : Great! So that means you have a Doctor degree ...

Candidate : Wellll, No... That means

Passed Highschool with Difficulties (P.H.D.)

An Investment Banker Was Getting Married.

During Wedding, the Wife Vomits.

Husband: "What Happened?"

Wife: "Capital Gains Arising Out Of Previous Investment."

Husband: "U cheated me."

Wife: "U should know, mutual fund investments are subject to market risks!"

Mycobacterium ulcerans

Buruli ulcer, caused by *Mycobacterium ulcerans* is a chronic debilitating disease that affects mainly affects the skin and sometime bone. The organism belongs to the family of bacteria that causes tuberculosis and leprosy. However, *M. ulcerans* is an environmental bacterium and the mode of transmission to humans remains unknown. Currently, early diagnosis and treatment are crucial to minimizing morbidity, costs and prevent long-term disability.

Buruli ulcer has been reported in 33 countries in Africa, the Americas, Asia and the Western Pacific. Most cases occur in tropical and subtropical regions except in Australia, China and Japan. Out of the 33 countries 13 regularly report data to WHO.

The majority of cases are reported from West and Central Africa, including Benin, Cameroon, Côte d'Ivoire, Democratic Republic of the Congo and Ghana. There are no predictable trends in the number of cases reported yearly. In 2015, 13 countries reported 2046; in 2016 there were 1920 cases and partial data for 2017 is 2209 cases. Australia and Nigeria have been reporting increasing numbers of cases. Côte d'Ivoire which used to report the highest number of cases has seen a progressive decline since 2011.

Clinical and epidemiological characteristics of cases

The clinical and epidemiological aspects of cases vary considerably within and across different countries and settings. In Africa, for example, about 48% of those affected are observed to be children under 15 years, whereas in Australia, 10% are children under 15 years and in Japan, 19% are children under 15 years. No significant difference exists between the rates of affected males and affected females.

Lesions frequently occur in the limbs: 35% on the upper limbs, 55% on the lower limbs, and 10% on the other parts of the body.

In terms of severity, the disease has been classified into three categories: Category I single small lesion (32%), Category II non-ulcerative and ulcerative plaque and oedematous forms (35%) and Category III disseminated and mixed forms such as osteitis, osteomyelitis, joint involvement (33%). In Australia and Japan, most lesions (>90%) are diagnosed in Category I. Since 2013, severe cases are being reported in Australia and it is unclear the reasons for this observation. In all countries, at least 70% of all cases are diagnosed in the ulceration stage.

Causative organism

Mycobacterium ulcerans grows at temperatures between 29-33 °C (*Mycobacterium tuberculosis* grows at 37°C) and a low 2.5% oxygen concentration to grow. The organism produces a unique toxin – mycolactone – which causes tissue damage and inhibits the immune response.

Transmission

The exact mode of transmission of *M. ulcerans* is still unknown.

Signs and symptoms

Buruli ulcer often starts as a painless swelling (nodule). It can also initially present as a large painless area of induration (plaque) or a diffuse painless swelling of the legs, arms or face (oedema). Local immunosuppressive properties of the mycolactone toxin enable the disease to progress with no pain and fever. Without treatment or sometimes during antibiotics treatment, the nodule, plaque or oedema will ulcerate within 4 weeks with the classical, undermined borders. Occasionally, bone is affected causing gross deformities.

Diagnosis

Clinical

In most cases, experienced health professionals in endemic areas can make a reliable clinical diagnosis.

Depending on the patient's age, the patient's geographical area, the location of lesions, and the extent of pain experienced, other conditions should be excluded from the diagnosis. These other conditions include tropical phagedenic ulcers, chronic lower leg ulcers due to arterial and venous insufficiency (often in the older and elderly populations), diabetic ulcers, cutaneous leishmaniasis, extensive ulcerative yaws and ulcers caused by *Haemophilus ducreyi* (1).

Early nodular lesions are occasionally confused with boils, lipomas, ganglions, lymph node tuberculosis, onchocerciasis nodules or other subcutaneous infections such as fungal infection.

In Australia, papular lesions may initially be confused with an insect bite.

Cellulitis may look like oedema caused by *M. ulcerans* infection but in the case of cellulitis, the lesions are painful and the patient is ill and febrile.

HIV infection is not a risk factor, but in co-endemic countries HIV infection complicates the management of the patient. The weakened immune system makes the clinical progression of Buruli ulcer more aggressive, and as a result the treatment outcomes are poor.

Due to international travel, cases can appear in non-endemic areas. It is therefore important that health workers are knowledgeable about Buruli ulcer and its clinical presentations.

Laboratory

Four standard laboratory methods can be used to confirm Buruli ulcer; IS2404 polymerase chain reaction (PCR), direct microscopy, histopathology and culture. PCR is the most commonly used method. WHO has recently published a manual on these 4 methods to guide laboratory scientists and health workers.

Treatment

Treatment consists of a combination of antibiotics and complementary treatments (under morbidity management and disability prevention/rehabilitation). Treatment guidance for health workers can be found in the WHO publication "*Treatment of Mycobacterium ulcerans disease (Buruli ulcer).*"

Prevention

There are currently no primary preventive measures that can be applied. The mode of transmission is not known. Bacillus Calmette–Guérin (BCG) vaccination appears to provide a limited protection.

Control

The objective of Buruli ulcer control is to minimize the suffering, disabilities and socioeconomic burden. Early detection and antibiotic treatment is the cornerstone of the WHO Buruli ulcer control strategy.

Research priorities

There are three research priorties for Buruli ulcer:

1. **Development of oral antibiotic treatment**
   A randomized clinical trial coordinated by WHO started in Benin and Ghana in 2013 with the objective of developing an oral-based treatment for Buruli ulcer. The recruitment was completed at the end of 2016 and the one-year follow-up ended in December 2017, with manuscript preparation in progress.
2. **Development of rapid diagnostic tests**
   In March 2018, WHO and Foundation for Innovative New Diagnostics (FIND) organized a meeting to assess progress in the development of rapid diagnostic test for Buruli ulcer. The meeting agreed on further work to detect mycolactone as a diagnostic test, including improvements to the fluorescent thin layer chromatography technique, currently being piloted in selected countries.
3. **Mode of transmission**
   Despite extensive studies to determine the mode of transmission, there is no clear understanding how people acquire the disease from the environment. In order to design an effective public health intervention, understanding the mode of transmission remains a critical research priority.

WHO and global response

WHO provides technical guidance, develops policies, and coordinates control and research efforts. WHO brings together all major actors involved in Buruli ulcer on a regular basis to share information, coordinate disease control and research efforts, and monitor progress.

These efforts have also helped to raise the visibility of Buruli ulcer, and mobilized resources to fight it. Under WHO's leadership and with support of nongovernmental organizations, research institutions and governments of affected countries, steady and impressive progress has been made.

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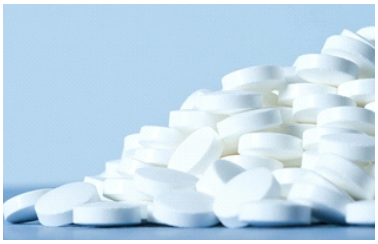
5 Things You Need To Know About Using Laxatives for Weight Loss



A lot of women have used laxatives to try to lose weight. Here is why it's a bad idea. Of all the health myths in the world, the idea that there is a silver bullet for weight loss may be among the most persistent and pernicious. From detox teas to trendy diets, we've seen countless products and practices that people claim are quick, easy, and harmless ways to lose weight. Using laxatives for weight loss is another one of those practices, but it's hardly harmless. Unfortunately, this might be one of the longest running and most popular misguided methods for weight loss, especially among young women.

One study looking at 13,000 people published in the journal Pediatrics in July 2016, found that 10.5 percent of women aged 23 to 25 have used laxatives to try to lose weight. Misusing laxatives is an all-around bad idea. Here's what you need to know about laxatives, including why you don't ever want to use them for weight loss.

1. First things first: What are laxatives?

Laxatives are a type of medication used to treat constipation by loosening stool or encouraging bowel movements, according to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Nearly everyone experiences constipation at one point or another. There are approximately a zillion causes, including dietary issues (too little fiber, too much dairy), certain medications (antidepressants), lifestyle changes (not pooping when you have to go, traveling), medical conditions (hypothyroidism, IBS), and even stress. Not only does constipation feel miserable—it can cause complications like hemorrhoids or anal fissures if you strain too hard to poop.

Lifestyle modifications like eating more fiber-rich foods, exercising regularly, and drinking enough water should be your first move, as SELF previously reported. But sometimes, you might need a little extra push. This is where laxatives come in. For the occasional treatment of constipation, they can do the trick and are generally pretty harmless.

2. There are several types of laxatives.

There are actually five main types, and they all get things moving in different ways, Marc Leavey, M.D., an internist at Baltimore's Mercy Medical Center, tells SELF. And some of them are available in both an oral form and a rectal suppository. Here is how they work, as explained by the Mayo Clinic:

- **Stimulants (Dulcolax, Senokot):** This class of laxatives triggers contractions of intestinal wall muscles in order to move stool along the GI tract, resulting in elimination. These are available in oral forms and as a rectal suppository.
- **Osmotics (Milk of Magnesia, Miralax):** They work by drawing water from nearby body tissue into the colon in order to soften the stool and spur bowel action.
- **Bulking agents (Metamucil, Benefiber, Citrucel):** These fiber supplements absorb liquid in the intestines and swell up to form a large, soft, bulky stool, the presence of which prompts a normal bowel movement.
- **Lubricants (Fleet):** These use oil, like mineral oil, to coat both the bowel and the stool, keeping the stool moist and soft and helping it pass through the GI tract more easily. These also come in rectal suppository form.
- **Stool softeners (Colace, Surfak):** These help reduce straining by helping moisture mix into dry, hard stools.

3. Laxatives will not help you actually lose fat.

If you try to use laxatives for weight loss, you may well see the number on the scale go down. But this apparent drop is deceiving because it's actually water weight that you're losing, women's health expert Jennifer Wider, M.D., tells SELF. The weight loss is temporary and is not actually changing your body fat composition. “Very little to no fat can be lost [with laxatives],” Dr. Wider explains.

While weight and weight loss are highly individual and complex issues, what's clear is that they depend on a number of factors in and out of your control. This includes your diet and exercise routine, yes, but also things like your metabolism, hormones, genetics, other health issues you have going on, or medications you're taking. In any case, as Dr.Leavey puts it,your body weight has to do with so much more than "excess poop.”

4. Long-term use of laxatives can actually perpetuate your constipation issues.

Stimulant laxatives, the kind most commonly used for weight loss, are “relatively harsh” and shouldn't be used for a long period of time, says Dr. Leavey. Why? “The bowel can get used to them, leading to more constipation,” he explains. Your system develops a dependence on them, according to the Mayo Clinic, meaning your ability to have natural bowel movements declines and need more and more laxatives. It's a nasty cycle best avoided. (That said, if you do think you have developed a dependence on laxatives, talk to your doctor.) According to the NIDDK, you should only use stimulant laxatives if your constipation is severe or other laxatives have not helped.

While of course, if you are experiencing persistent constipation, you should talk to your doctor first to see if there's an underlying health issue, generally bulk-forming laxatives are the gentlest on your body and safest to use long term, according to the Mayo Clinic.

5. When used over the long-term, laxatives can actually be extremely harmful.

While it's usually fine to take a laxative here and there if you're stopped up, ongoing and unnecessary laxative use—such as using them in an attempt to lose weight—can negatively impact your health in a few ways.

Prolonged laxative use can irritate the lining of your bowel and cause all sorts of gastrointestinal issues, Dr. Leavey says. It can also cause dehydration and electrolyte and mineral imbalances, Dr. Wider says. Since electrolytes such as calcium and sodium are crucial to several body functions, an imbalance can cause dizziness, fainting, blurry vision, and even death, Dr. Wider explains. These imbalances can also cause symptoms like abnormal heart rhythms, weakness, confusion and seizures, per the Mayo Clinic.

What's more, osmotic laxatives can cause your blood pressure to drop and even cause permanent kidney damage, Dr. Leavey adds.

Bottom line: This is not a weight-loss method you want to try. “There is no rational basis to try to lose weight with laxatives, and there is a clear potential for harmful side effects,” says Dr. Leavey. “Don't do it.”

Best practices for prevention of needle stick injuries

Recommended practices

Use safety-engineered medical devices with protection mechanisms

Use medical devices that incorporate safety-engineered protection mechanisms that eliminate or reduce the risk of occupational exposure to the lowest feasible extent

- Passively activated protection mechanisms should be used where practical and feasible.
- A passive safety device activates itself during the course of normal clinical use and provides protection
- Implementation of safety-engineered devices must be combined with relevant training and education on the use of the technology.

Use needle-free intravenous access systems

- Needle-free systems should be used to access intravascular devices
- It is acknowledged that needle stick injuries that occur from needles used to access intravenous lines are low risk however, uniformity of access leads to less confusion and increased compliance with other safety devices.

Product selection

When considering safety-engineered medical devices the following selection criteria should be considered:

- The device must not compromise patient care
- The device must perform reliably
- The safety mechanism must be an integral part of the safety device, not a separate accessory
- The device must be easy to use and require little change of technique on the part of the health professional
- The activation of the safety mechanism must be convenient and allow the care-giver to maintain appropriate control over the procedure
- The device must not create other safety hazards or sources of blood exposure
- A passive activation is preferable
- The activation of the safety mechanism must manifest itself by means of an audible, tactile or visual sign to the health professional
- The safety mechanisms should not be easily reversible once activated.

Retractable syringes

When introducing retractable syringes keep in mind that they may not be suitable for some purposes, and therefore some non-safety devices may be required for specific purposes, for example:

- Aspiration
- Administration of local anaesthetic (LA) >3 mL
  - Most procedures require no more than 1-2 mL of LA for effective anaesthesia
  - Using low volume syringes also reduces the chance of an excess volume being used; excessive volume may cause more pain
- Pre-packed medications +/- administration needle
- Procedures where non-retractable needles form part of the procedure kit/pack.

Conventional needles that may still be required:

- 18 gauge (blunt) needles for drawing up medication
- 21 gauge needles for injection of medication into infusion bags and blood collection (only if necessary)
- For the administration of LA >3 mL.

Sharps disposal

- All hollow-bore needles including those with safety features must be disposed of into an approved sharps disposal container that complies with AS4031 or AS/NZS4261
- Ensure sharps containers being used are large enough to accommodate the types of devices being used in the area
- Point of use disposal of sharps is encouraged by ensuring adequate placement of sharps containers in areas where sharps are generated
- Sharps containers should be either wall mounted or fixed to a trolley/similar
- Sharps containers should be fixed at a height between 900mm and 1100mm, as per the Australasian Health Facility Guidelines Standard Components Room Layout Sheets for; Dirty Utility 10m2, Patient Bay - Recovery, Stage 1, and Patient Bay - Resuscitation.

Disposable Multiple-Dose Injecting Devices (i.e. injector pens)

- Hospital in patients who are able to self-administer should ideally do so using safety engineered pen needles.
  - Note: safety-engineered pen needles are not routinely used by people with diabetes in their homes so will need to be provided and their use demonstrated by the facility.
- Healthcare workers should only administer medication using a disposable multiple-dose injecting device (i.e. injector pen) if a safety-engineered needle is used.
- If safety-engineered pen needles are not available:
  - The patient may self-administer as normal provided they can remove and dispose of their pen needle.
  - If the patient is unable to self-administer
  - and if the medicine is dose specific (e.g. High concentration insulin (e.g.Toujeo), exenatide (Byetta), liraglutide (Victoza)), the medical officer must be informed and an alternate medication should be sourced.
  - If the medication is not dose specific, healthcare workers should use a single-use safety-engineered needle/syringe.
- Product information for disposable insulin pen devices, pen cartridges and vials indicates that they are for SINGLE PATIENT USE. Pen devices, cartridges or vials of insulin must NOT be used for more than one person.

Safety device failure / product complaint management

Product complaints should be documented to ensure potential statewide problems are identified.

If a device fails:

- Report through normal facility product complaint process
- The product complaint should be investigated at the local level to determine if it was due to:
  - product failure/quality assurance problem
  - User technique requiring in-service/remedial training.

Product complaints should be reported via your local internal complaints/feedback channels.

Trends in product complaints should be reported to the company for quality assurance and analysis of failed product for manufacturing/design faults.

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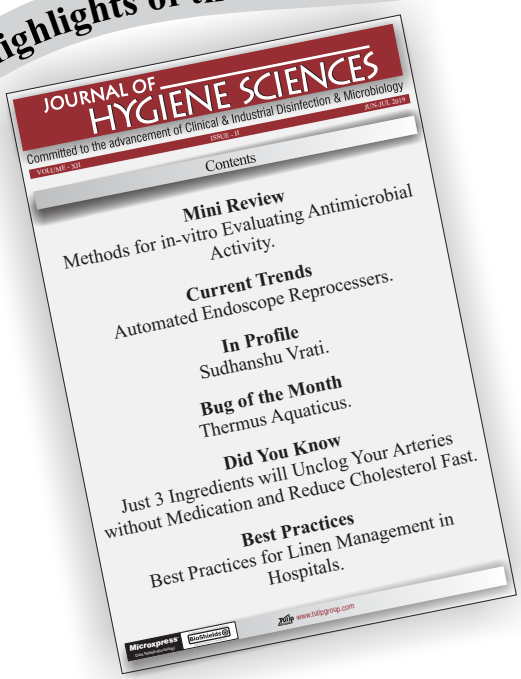
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