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Editorial

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Mini Review Section – Agrobacterium - mediated gene transfer has been of great help in modern plant molecular genetics and genetic engineering. Nowadays, plant transformation by A. tumefaciens has become one of the most used methods for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants.

Current Trend Section - In the retail food and foodservice industry, sanitization is a routine, common practice defined and recommended in the U.S. Food and Drug Administration (FDA) Food Code. Hence, sanitizers, rather than disinfectants, are the main antimicrobial products used in these settings. Sanitizers and disinfectants are not interchangeable products, but due to complex regulatory frameworks and lengthy labels, they may be inadvertently misused. Therefore, it is important to understand the differences in when, why, and how both can be properly used in retail food and foodservice establishments.

In Profile Scientist - Guthrie's method to diagnose individuals with PKU was a bacterial inhibition assay. Bacterial inhibition assays are tests that detect the presence of a specific substance in a sample. Guthrie's test required a few drops of blood from a finger prick. To conduct the bacterial inhibition assay, Guthrie coated agar culture gel, a substrate used to grow bacteria, with β-2-Thienylalanine, an amino acid that inhibits the growth of the bacteria Bacillus subtilis. Then, he collected a spot of blood on a filter paper disc and placed the disc on the surface of the agar culture gel. The presence of the amino acid phenylalanine in the blood, an indicator of PKU, reversed the inhibitory effects of β-2-Thienylalanine, causing B. subtilis to grow. Thus, a culture from an individual with PKU would show bacterial growth, while a culture from an individual without PKU would not.

Bug of The Month - On average, for each person infected with RSV, it is estimated that 5 to 25 uninfected people will become infected. RSV can spread when an infected individual coughs or sneezes, releasing contaminated droplets into the air. When these particles come into contact with another person's mouth, nose, or eyes, transmission typically takes place. Much like all other respiratory diseases that were originally thought to spread through respiratory droplets, the aerosols produced while normal breathing, speaking, and even singing are most likely the means of transmission. Additionally, RSV can survive for up to 25 minutes on infected skin, such as hands, and several hours on other surfaces, such as doorknobs and countertops. It has an incubation period of 2 to 8 days. Once infected, people are usually contagious for 3 to 8 days.

Did You Know – A team led by researchers at Tokyo Medical and Dental University (TMDU) elucidated the molecular details of how an enzyme called Tank-binding kinase 1 (TBK1) participates in a disease-relevant mitophagy mechanism.

Best Practices - GMP requires a quality approach to manufacturing, enabling companies to minimize or eliminate instances of contamination, mix-ups, and errors. This in turn, protects the consumer from purchasing unsafe and poor-quality products. Failure of firms to comply with GMP can result in very serious consequences including recall, seizure, fines, and imprisonment. It addresses issues including recordkeeping, personnel qualifications, sanitation, cleanliness, equipment verification, process validation, and complaint handling.

Importance of *Agrobacterium tumefaciens* in genetic manipulation

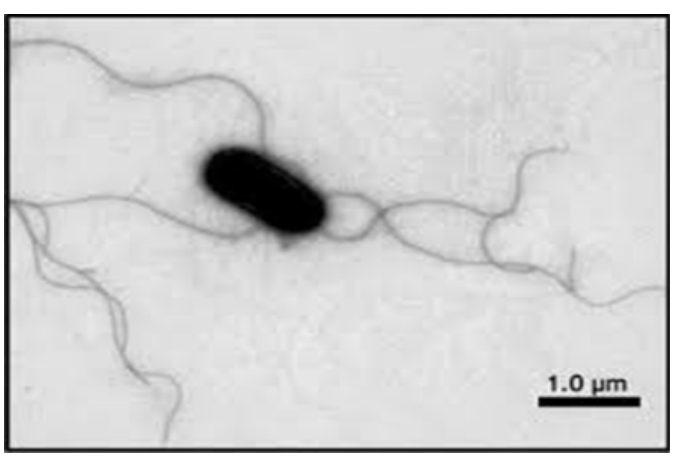
Today, many commercially important species are routinely transformed by different biotechnological methods. Methods available for plant transformation are arranged in three main groups: using biological vectors (virus- or bacteria-mediated transformation), direct DNA transfer techniques (chemical-, electrical-or laser-induced permeability of protoplasts or cells) and non-biological vector systems (microprojectiles, microinjection or liposome fusion). Today in many countries several transgenic important crops such as soybean, maize, cotton, canola, sugar beet, sugarcane and alfalfa are available, and the mostly preferred method is Agrobacterium -mediated transformation.

Agrobacterium- mediated gene transfer has been of great help in modern plant molecular genetics and genetic engineering. Nowadays, plant transformation by *A. tumefaciens* has become one of the most used methods for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants. In addition, the genus Agrobacterium can transfer a piece of its plasmid DNA into a remarkably broad group of organisms including numerous dicotyledonous and monocotyledonous species.

Furthermore, Agrobacterium can transform fungi, including yeasts, ascomycetes, and basidiomycetes.

Characteristics of Agrobacterium tumefaciens

Agrobacterium tumefaciens is a Gram negative, motile, rod-shaped soil bacterium, which is non-sporing, and is closely related to the N- fixing rhizobium bacteria, which form root nodules on leguminous plants. It is also a soil pathogen initiating tumors on plants. The bacterium is surrounded by a small number of flagella.



Crown Gall Disease

A. tumefaciens infects the wounded sites in dicotyledonous plants and some monocotyledonous as well, causing the formation of crown gall tumors. Crown gall disease is a common plant disease, affecting more than 600 types of plants. It affects nearly all dicotyledonous plants, woody and herbaceous plants, and many commercially important and valuable crops such as brambles, rose, willow, grapes, rice, and sugar beet.

The disease can be identified by the appearance of tumors or galls of varying size and shape on the lower stem where it meets the soil (crown), main roots and sometimes branches of the plant.

Infected plant cells contain bacterial genes (plasmid) that replace some of the normal plant cell genes. When they are young, the galls can be white or cream coloured and spongy or wart-like; as they age, they become dark and woody. The tumor usually either appears as a swelling of the plant tissue, or as a separate mass of tissue close to the plant surface, joined only by a narrow neck of tissue. Some of them can reach up to 30 cm in diameter, though 5–10 cm is more common.

When plants are infected with the bacterium, they become stunted, produce small chlorotic leaves, and become more susceptible to extreme environmental conditions such as winter cold and wind. Galls can interfere with the plant's ability to move water and nutrients through the stem, which may result in stunting or decline of the plant. Plant tumors normally do not kill the plant, but crown gall disease can be fatal if the tumors become too enlarged.

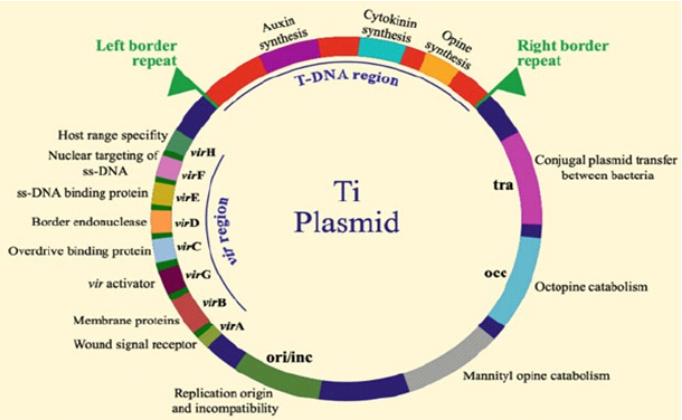
Crown gall can also be eradicated using creosote based chemical compounds, copper-based solutions, and strong oxidants such as sodium hypochlorite.

However, these are costly to apply in terms of both labour and buying the product. They are also very harmful to the surrounding environment and accumulation of large quantities of copper in the soil can have a disastrous impact on other plants in the area. Therefore, chemical controls are rarely used against *A. tumefaciens*.

Crown gall on blackberry cane

Crown gall on tomato

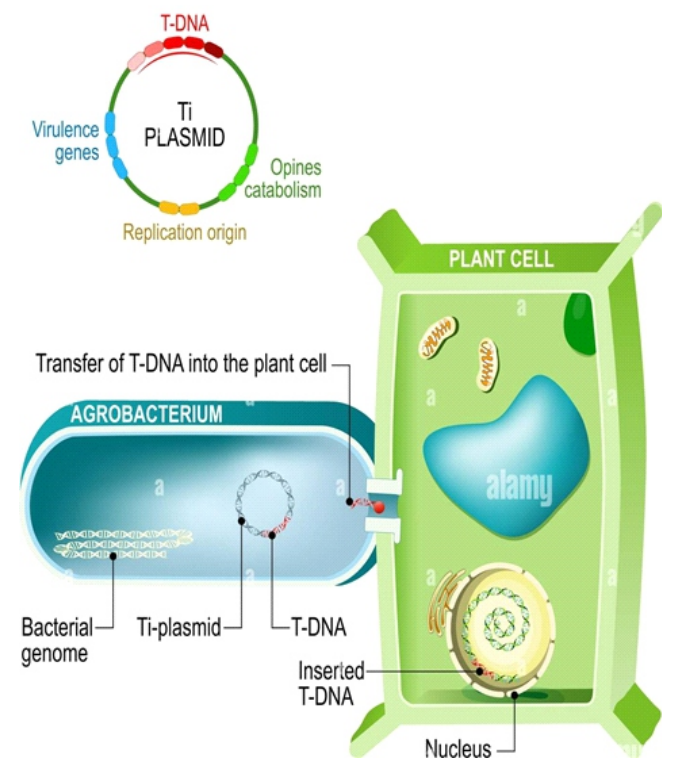
Genome Structure of Agrobacterium



A. tumefaciens, which has the exceptional ability to transfer a particular DNA segment (T-DNA) of the tumor-inducing (Ti) plasmid into the nucleus of infected plant cells. The molecular basis of genetic transformation of plant cells by *A. tumefaciens* is
(1) transferring some genes from the bacterium and
(2) their integration into plant nuclear genome located in a large tumor-inducing Ti plasmid of bacterium**.**

The virulent strain of *A. tumefaciens*, which induces crown gall disease, contains a large mega-plasmid (more than 200 kbp) called Ti plasmid. In genus Agrobacterium, Ti plasmids are classified according to opines which are produced and excreted by the tumors they induce.

During infection, the T-DNA that is a mobile segment of Ti plasmid, is transferred into the plant cell nucleus and integrated into the plant genome.



T-DNA region generally represents less than 10% of the Ti plasmid. Some Ti plasmids contain one T-DNA whereas others contain multiple T-DNA regions. The processing of T-DNA from Ti plasmid and its subsequent export from bacterium into plant cell result in large part from the activity of virulence (vir) genes carried by the Ti plasmid.
In *A. tumefaciens*, the T-DNA contains two types of genes;
(a) oncogenic genes which are encoding for enzymes involved in the synthesis of auxins and cytokinins that are responsible for tumor formation, and
(b) the genes which are encoding synthesis of opines that are responsible for the formation of the novel amino acid sugar conjugates.
The amino acids and sugars are synthesized and excreted by the crown gall cells and consumed by *A. tumefaciens* as carbon and nitrogen sources.

The specific DNA region acting as a cis element signal for the transfer apparatus from the Ti plasmid is generated by 25-bp direct repeats. Through the cooperative action of proteins encoded by the genes defined in Ti plasmid virulence region (vir genes) and in the bacterial chromosome, the process of T-DNA transfer is carried out.

Functions of Vir genes:

Vir Gene	Function
Vir A	A receptor activated by phenolic compounds, autophosphorylated and inturn phosphorylates VirG
VirG	VirG is Transcriptial factor necessary for expression of other virulence genes.
VirD2	Endonuclease; cuts T-DNA at right border to initiate T-strand synthesis and also it has an important role in integration of T DNA into host chromosomal DNA
Vir D1	Topiosomerase; Helps Vir D2 to recognise and cleave within the 25bp RB sequence
VirD4	VirD4 is the ATP-dependent linkage of protein complex necessary for T-DNA translocation
Vir C1	Promote high efficiency T-strand Synthesis; involved in spatial localization of the T-complex to the cell poles.
Vir E2	Binds to T-strand protecting it from nuclease attack, and intercalates with lipids to form channels in the plant membranes through which the T-complex passes.
Vir E1	Acts as a chaperone which stabilizes Vir E2 in the *Agrobacterium*.
Vir B & Vir D4	Assemble into a secretion system which spans the inner and outer bacterial membranes. Required for Export of the T-complex and Vir E2 into the plant cell

There are six operons organized in the vir region of Ti Plasmid (30 kb) essential (vir A, vir B, vir D, and vir G) or increasing of transfer efficiency (vir C and vir E) for the T-DNA transfer. In addition, there are also two more operons, which are not necessary for T-DNA transfer called vir F and vir H. The protein products of these genes, respond to the specific compounds secreted by the wounded plant to generate a copy of the T-DNA and mediate its transfer into the host cell. The vir A, vir G and vir F operons carry one gene, while vir E, vir C and vir H operons carry two genes. The protein products of these genes, respond to the specific compounds secreted by the wounded plant to generate a copy of the T-DNA and mediate its transfer into the host cell. The vir A, vir G and vir F operons carry one gene, while vir E, vir C and vir H operons carry two genes. Vir D includes four and vir B includes 11 genes. Vir gene expression is induced by chemical signal molecules (phenolic substances and sugar) released from wounded plant cells. Recognition of these signals by the sensor protein VirA leads to phosphorylation of VirG protein.
A. tumefaciens, is an important model system in understanding pathogen-host interactions including recognizing and delivering macromolecules into target cells resulting in disease, *A. tumefaciens* has been established by the intense mechanistic analysis of Agrobacterium -mediated transformation. From these studies, a basic model for the cellular transformation of plants by *A. tumefaciens* has been developed.

Sanitizers and Disinfectants: A Retail Food and Foodservice Perspective

The coronavirus disease 2019 (COVID-19) pandemic has brought heightened attention to the importance of cleaning, sanitizing, and disinfecting in retail food and foodservice establishments. In the retail food and foodservice industry, sanitization is a routine, common practice defined and recommended in the U.S. Food and Drug Administration (FDA) Food Code. Hence, sanitizers, rather than disinfectants, are the main antimicrobial products used in these settings. Sanitizers and disinfectants are not interchangeable products, but due to complex regulatory frameworks and lengthy labels, they may be inadvertently misused. Therefore, it is important to understand the differences in when, why, and how both can be properly used in retail food and foodservice establishments. When used properly, sanitizers and disinfectants are powerful tools that can keep retail food and foodservice operations safe.

Antimicrobial products: sanitizers and disinfectants

Sanitizers and disinfectants are often complex formulations that contain at least one or more active ingredient(s). These active ingredients provide the intended antimicrobial effect (i.e., reduction or elimination of targeted microorganisms).

Attributes of common sanitizer and disinfectant active ingredients

Sanitizer	Spectrum of activity	Advantages	Disadvantages
Free available chlorine (chlorine, hypochlorous acid, sodium hypochlorite)	Vegetative bacteria and enveloped and nonenveloped viruses	Broad spectrum of activity. Good hard water tolerance.	May be incompatible with some soft metals. Rapidly inactivated by soil. Limited shelf life that varies with pH. Can generate chlorine gas if mixed with acid or ammonia. Can be inactivated by organic matter.
Quaternary ammonium compounds	Vegetative bacteria and enveloped and nonenveloped viruses	Broad spectrum of activity. Compatible with most surfaces. Very stable with long shelf lives. Less reactive with soil.	Can be inactivated by hard water. Can be inactivated by some surfactants used in cleaners. May bind to cleaning cloths, reducing active levels in a solution. Food Code requires use above 24°C (75°F).
Peroxides	Vegetative bacteria and enveloped and nonenveloped viruses	Minimal residue. Formulated for good hard water tolerance.	May require elevated levels to be effective against catalase-positive organisms. May be incompatible with some soft metals.
Peracids	Vegetative bacteria and enveloped and nonenveloped viruses	Broad spectrum of activity (note that antifungal activity may require a mixture of peracid). Compatible with most surfaces Minimal residue.	Pungent odor. Limited shelf life. Inactivated by some types of soil. May be incompatible with some metals.
Acid anionics	Vegetative bacteria and enveloped and nonenveloped viruses	Compatible with residual cleaners if rinsing is incomplete. Good cleaning performance. Good material compatibility. Good hard water tolerance.	May be incompatible with some soft metals and some plastic surfaces. Can generate chlorine gas if mixed with chlorine products.
Alcohol	Vegetative bacteria and enveloped viruses	Can be used in environments where aqueous sanitizers or disinfectants are undesirable. No residue Limited impact on organic matter.	High flammability. Some alcohols display poor compatibility with certain plastic materials. RTU format only.

Sanitizers and Disinfectants:

A **sanitizer** is defined as a substance, or mixture of substances, that reduces the bacteria population in the inanimate environment by significant numbers but does not destroy or eliminate all bacteria.

A **disinfectant** is defined as a substance, or mixture of substances, that destroys or irreversibly inactivates bacteria, fungi, and viruses, but not necessarily bacterial spores, in the inanimate environment.

Overview:

Disinfectant use is confined to places or surfaces where there may be a greater risk of human or animal pathogen transfer, such as high-touch surfaces (door handles, light switches, dispenser buttons, dining room chairs, and tables) and bathrooms.

In some instances, food-contact surfaces should be disinfected after certain contamination events. Traditional food-contact surface sanitizers are not designed to meet the decontamination challenges presented by viruses that may have contaminated surfaces during these events. If virus control or generally higher-level microbial control is required, it is necessary to disinfect (not sanitize) the contaminated food-contact surface.

For surfaces that are visibly dirty, the general protocol is to clean, rinse with potable water, disinfect according to label instructions for the disinfectant, rinse again with potable water, and then sanitize with a food contact sanitizer before reusing the surface. The rinse step before disinfection of a food-contact surface is essential to prevent reducing the efficacy of the disinfectant, and rinsing after disinfection is important to prevent chemical cross contamination with foods attributed to disinfectant residue and to prevent potential inactivation of sanitizer with residual disinfectant. If the surface is visibly clean and the product is labelled as a one-step disinfectant, one can eliminate the cleaning step, so the general protocol is disinfected, rinse with potable water, and sanitize with a food-contact sanitizer.

Sanitizers and disinfectants can be purchased in a range of formats-wipes, aerosols, sprays, concentrated liquids, and tablets. Wipes, aerosols, and sprays are typically ready-to-use (RTU) formats, and concentrates (liquids or tablets) require dilution with water. Concentrates are advantageous because they require less storage, use far less packaging, and are easier to ship than RTU products. However, safety of concentrated chemicals and the equipment and training needed for proper dilution of these products should be considered.

Cleaning frequencies of food contact surfaces and utensils

Temp	Cleaning frequency
<50°C (4145°F)	24h
>5.0°–7.2°C (>41–45°F)	20h
>7.2 –10°C (>45–50°F)	16h
>10–12.8°C (>50–55°F)	10h
>12.8°C (>55°F)	4h

Antimicrobial resistance:

Increased use of antimicrobial products, such as disinfectants and sanitizers, have centred around the potential risks associated with the misuse of these products. Concerns have been raised about the possibility of the development of reduced antimicrobial susceptibility.

Two points need to be emphasized. First, under pandemic conditions, such as the COVID-19 pandemic, it is imperative that antimicrobial products be used according to the viricidal disinfection directions and not the sanitization directions if the product can be used as both a sanitizer and a disinfectant. Second, it is highly recommended that, during the COVID-19 pandemic, those within the retail food and foodservice industry should continue to use their sanitizers for routine procedures and use disinfectants where necessary, such as treating high-touch surfaces, cleaning bathrooms, and decontaminating the facility when there is known exposure.

Current and future trends in sanitizing and disinfecting

The SARS-CoV-2 pandemic has emphasized the importance of sanitizing and disinfecting unlike anything seen before in the retail food and foodservice industry. Even before the pandemic, efforts were underway to enhance cleaning, sanitizing, and disinfecting through innovative formulation and application. Retail food and foodservice establishments can be challenged by the complexities of sanitization programs, including multistep processes, the availability or need for multiple products with different use instructions, and low-moisture cleaning processes. The additional pressures of limited time and space for complicated procedures, high staff turnover, and the necessity for frequent training make time saving or simplification of sanitization (and disinfection) very desirable. Enhanced cleaning during operation have led to an increase in the availability and popularity of large area application techniques, such as fogging, misting, and electrostatic spray.

Robert Guthrie

Robert Guthrie developed a method to test infants for phenylketonuria or PKU in the United States during the twentieth century. PKU is an inherited condition that causes an amino acid called phenylalanine to build to toxic levels in the blood. Untreated, PKU causes mental disabilities. Before Guthrie's test, physicians rarely tested infants for PKU and struggled to diagnose it. Guthrie's test enabled newborns to be quickly and cheaply screened at birth and then treated for PKU if necessary, preventing irreversible neurological damage. After developing the test, Guthrie travelled the world to advocate for mass screening for PKU in newborns. Along with his PKU test, Guthrie developed newborn screens for maple syrup urine disease and for galactosemia. Guthrie's test for PKU and campaign for newborn screening led to the early diagnoses of PKU in thousands of infants, preventing those infants from developing mental disabilities.

Guthrie was born to Ina Florence Ledbetter Guthrie and Reginald Guthrie in Marionville, Missouri, on 28 June 1916. Due to his father's traveling job as a salesman, Guthrie's family moved from place to place throughout Guthrie's childhood before settling down in Minneapolis, Minnesota, in 1922. According to his biographer Jean Koch, Guthrie learned the skills and techniques of a salesman from his father. In Minneapolis, Guthrie graduated at the bottom of his high school class in 1935.

After graduation, Guthrie returned to high school to finish the requirements for admission to the University of Minnesota in Minneapolis, Minnesota. In the spring semester of 1936, Guthrie began studying at the University of Minnesota under the National Youth Administration program, which provided low-income students with federally subsidized jobs at state universities. Guthrie studied astronomy and microbiology when he started college, and he planned to major in astronomy and minor in microbiology. However, after taking a job with Charles Evans in the department of bacteriology and immunology, Guthrie majored in microbiology.

Evans encouraged Guthrie to attend medical school after graduation, and in 1939 Guthrie enrolled in the medical school program at the University of Minnesota. Guthrie disliked medical school and transferred to the University of Maine in Orono, Maine, less than a year later, to work towards a master's degree in bacteriology. In 1941 during his time at the University of Maine Guthrie met Margaret Flagstad, who sat next to him in organic chemistry, and the couple married in August of 1941. The same year, the couple returned to Minneapolis, where Guthrie finished his medical degree at the University of Minnesota. In 1945, Guthrie and his wife had their first son, Tom, in Minneapolis while Guthrie was in medical school. Guthrie spent an extra year at the University of Minneapolis to pursue a PhD in bacteriology before graduating in 1946 with a medical degree and a PhD. Between 1941 and 1946, Guthrie earned six degrees in six years: a bachelor's degree in bacteriology, a bachelor's of science, a bachelor of medicine degree, a master's degree in biochemistry, an MD, and a PhD in bacteriology.

One year after graduating, in 1947, Guthrie began his professional research career at the National Institutes of Health (NIH) in Bethesda, Maryland, studying the protozoan *Trichomonas fetus*, which causes spontaneous abortions in cattle. Guthrie's supervisor had no interest in his research project on protozoan, and Guthrie eventually dumped his cultures of protozoan down the sink and quit his job at the National Institutes of Health. In 1949, after his time at the National Institutes of Health, Guthrie took a position as chairman of the department of bacteriology at the University of Kansas in Lawrence, Kansas. Guthrie changed jobs several times between 1950 and 1954. During those four years, he moved from supervising the diagnostic bacteriology laboratory at the Staten Island Public Health Hospital in Staten Island, New York, to supervising the diagnostic bacteriology laboratory at the Sloan Kettering Institute in Manhattan, New York. He then moved to Roswell Park Cancer Institute in Buffalo, New York, to develop chemotherapeutic agents to treat cancer.

Between 1947 and 1954, Guthrie and his wife had five more children. Their second child, John Guthrie, was mentally disabled but hadn't been diagnosed with a birth defect or disease, though he visited numerous physicians and underwent several tests. His son's mental disability prompted Guthrie to work with the National Association for Retarded Children, headquartered in New York City, New York, a national organization that advocated for and served people with intellectual and developmental disabilities and their families.

In 1954 Guthrie's son, who was seven years old, attended a private school run by the Eric County chapter of the New York State Association for Retarded Children. Most public schools did not have programs for mentally disabled students. Guthrie participated in the local Williamsville Parent Teacher Association, where he collaborated with other parents to create a class for mentally disabled children in public schools. In 1957, the Academy School for children, a public school in Williamsville, New York, organized a special class for children with mental disabilities.

In 1957, Guthrie and his wife took their mentally disabled son to a children's rehabilitation center directed by Robert Warner, the director of the Children's Rehabilitation Center at the University of Buffalo Children's Hospital in Buffalo, New York. Although Warner did not diagnose Guthrie's son, he talked with Guthrie about phenylketonuria, a congenital metabolic disease hypothesized to cause neurological damage. In 1934 Ivar Folling, a physician in Norway, had described PKU. PKU People with PKU lack phenylalanine hydroxylase, the enzyme that breaks down the amino acid phenylalanine, which is present in protein-rich foods, such as meat or fish, as well as aspartame, a commonly used artificial sweetener. Because individuals with PKU cannot break down phenylalanine, toxic levels of the amino acid build up in their blood when they eat foods with phenylalanine, causing

neurological damage. Guthrie's niece had been diagnosed with PKU several years before.

Warner asked Guthrie for help to develop a simple and inexpensive method to diagnose individuals with PKU. To test his patients for PKU, Warner had to ship venous blood samples to California to determine whether or not the blood had higher than average levels of phenylalanine. At the time, a standard test for PKU was the ferric chloride urine test, which detected the presence of a phenylalanine in urine samples. Phenylalanine was present in urine of those with PKU because their bodies couldn't decompose phenylalanine. Guthrie agreed to help Warner and reported a few days later that he had developed a new method to replace the ferric chloride urine test.

Guthrie's method to diagnose individuals with PKU was a bacterial inhibition assay. Bacterial inhibition assays are tests that detect the presence of a specific substance in a sample. Guthrie's test required a few drops of blood from a finger prick. To conduct the bacterial inhibition assay, Guthrie coated agar culture gel, a substrate used to grow bacteria, with β-2-Thienylalanine, an amino acid that inhibits the growth of the bacteria *Bacillus subtilis*. Then, he collected a spot of blood on a filter paper disc and placed the disc on the surface of the agar culture gel. The presence of the amino acid phenylalanine in the blood, an indicator of PKU, reversed the inhibitory effects of β-2-Thienylalanine, causing *B. subtilis* to grow. Thus, a culture from an individual with PKU would show bacterial growth, while a culture from an individual without PKU would not.

After developing his test for PKU, and with a mentally disabled son and PKU-affected niece, Guthrie pushed for mandatory nationwide newborn screening of PKU. However, before every infant could undergo the Guthrie test for PKU diagnosis, the test had to go through clinical trials to prove its safety and efficacy. Near Rochester, New York, Guthrie tested his method on about 3,000 residents of a state school for the mentally disabled. His test detected twenty-three cases of PKU when the traditional test detected only nineteen cases. Thus, Guthrie showed his method was not only simpler and more convenient, but also more accurate than the traditional urine test.

After validating his test for PKU, Guthrie travelled the world to campaign for universal screening of PKU in infants. In 1960, Guthrie attended the International Association for Scientific Study of Mental Deficiency in London, England, where he introduced his PKU test. Guthrie also became involved in the International League of Societies for Persons with Mental Deficiency, which hosted a symposium for scientist studying mental disabilities. In 1961, the US Children's Bureau in Washington, D.C., which worked to improve the overall health and well-being of the nation's children and families, funded a trial to test Guthrie's technique on 400,000 infants nationwide. Guthrie assembled warehouses to produce test kits and distributed them to twenty-nine states that agreed to use the test. In two years, thirty-seven cases of PKU were diagnosed in the group of 400,000 infants tested, an incidence of about one per 10,000.

In 1962, Guthrie presented his PKU test at the International Association for Pediatrics in Lisbon, Portugal. Guthrie travelled to Spain, Japan, and New Zealand, among other places, to introduce his PKU test. In the US, Massachusetts was the first state to mandate the newborn screening for PKU in 1963. However, many scientists opposed Guthrie and his test, claiming that the test was inaccurate. In 1963, an article in *The Atlantic Monthly* argued that Guthrie and Warner should be prosecuted for wrongly diagnosing infants with PKU. Guthrie's test received acceptance among many scientists after Guthrie and his colleague Ada Susi published an article in *Pediatrics* about the Guthrie test in 1963.

In 1964, Guthrie further simplified his test so that infants could be screened at birth. He developed what he called Guthrie cards, pieces of cardstock on which physicians collected blood directly from a small heel puncture in the infant. Guthrie cards enabled doctors to save the dried blood samples of newborns for testing, hole punch them out, and place them onto the gel cultures for screening. Once the punched out discs holding dried blood were placed on the gel cultures, lab technicians performed the Guthrie test, watching for bacterial growth as an indicator of PKU. The convenience of Guthrie cards enabled doctors to diagnose in newborns so that the newborns could avoid ingestion of phenylalanine and prevent any damage to their body. By 1966, most states in the US mandated Guthrie's newborn screen for PKU, while Guthrie attempted to develop additional tests to prevent mental retardation.

After developing and marketing his PKU test, Guthrie developed newborn tests for galactosemia and for maple syrup urine disease also using bacterial inhibition assays. Galactosemia is a disorder in which the body cannot metabolize galactose, a sugar primarily part of lactose, leading to life-threatening symptoms such as lethargy, jaundice, liver damage, and abnormal bleeding. Maple syrup urine disease is an inherited disease in which the body cannot process specific amino acids, and it causes a sweet odor in the infant's urine and developmental delays. If untreated, maple syrup urine disease can lead to seizures, coma, and death. Eventually, Guthrie's laboratory developed tests for more than thirty different treatable conditions that cause mental disabilities or death. Guthrie's grant for the PKU test from the US Children's Bureau was extended until 1968 to test his other tests and add them to the list of routine newborn screens.

In 1975, Guthrie became a consultant for the California Department of Health in Sacramento, California. There, he urged for the investigation of lead poisoning in children. The Sonoma State Hospital in Eldridge, California, an institution for the mentally disabled, found a serious problem of lead poisoning. After that finding, Guthrie persuaded the Centers for Disease Control in DeKalb County, Georgia, to lower the maximum level of lead in the blood that was considered safe from 30 milligrams per 100 milliliters to 10 milligrams per 100milliliters.

In the early 1970s, Guthrie also supervised the research of two scientists working in his laboratory, William Murphey and Adam Orfanos, who tested dried-blood spot specimens from residents of the West Seneca Developmental Disability Center in Buffalo, New York. Murphey and Orfanos found high levels of lead in the residents' blood samples. Guthrie contacted the Newark Institution for the Mentally Retarded in Newark, New York, because the residents had earlier lived at that institution. After an investigation, the institution found lead-based paint in the Newark Institution. Consequently, New York State facilities were all tested for lead-based paint and, if necessary, repainted to prevent possible damages in residents from exposure to lead.

Toward the end of his life Guthrie also participated in Physicians for Social Responsibility, an organization that actively opposed nuclear weapon testing. Guthrie served as vice president and later president of the National Association for Retarded Children, Eric County chapter in New York. Throughout his life, Guthrie improved public schools by adding special classes for the mentally disabled and worked to educate the public about the dangers of lead poisoning. Guthrie died on 24 June 1995 in Seattle, Washington.

Jokes

(A man talking to God)
The man: God, how long is a million years?
God: To me, it's about a minute.
The man: God, how much is a million dollars?
God: To me it's a penny.
The man: God, may I have a penny?
God: Wait a minute.

A man walks into a shop and sees a cute little dog.
He asks the shopkeeper- "Does your dog bite?"
The shopkeeper says,- " No, my dog does not bite."
The man tries to pet the dog and the dog bites him.
"Ouch!" He says, " I thought you said your dog does not bite!"
The shopkeeper replies,- " That is not my dog!"

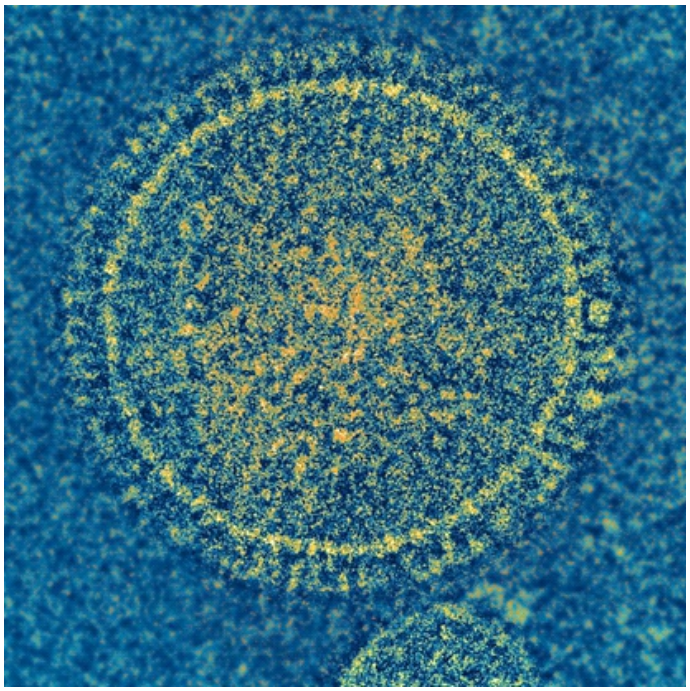
Teacher: Why are you sleeping in the class?
Student: Your voice is so soothing, I felt sleepy.
Teacher: Then why others are not sleeping?
Student: They aren't listening to you ma'am.

Teacher: Whoever answers my next question can go home.
One boy at the back of the class throws his bag out of the window.
Teacher: Who just threw that?
The boy replied: "Me… and I'm going home."

A: Just look at that young person with the short hair and blue jeans. Is it a boy or a girl?
B: It's a girl. She's my daughter.
A: Oh, I'm sorry, sir. I didn't realise that you were her father.
B: I'm not, I'm her mother.

A teenage girl had been talking on the phone for about half an hour, and then she hung up.
"Wow!" said her father, "That was short. You usually talk for two hours,
What happened?"
"Wrong number," Replied the daughter.

Respiratory syncytial virus



Respiratory syncytial virus (RSV), also called human respiratory syncytial virus (hRSV) and human orthopneumovirus, is a contagious virus that causes infections of the respiratory tract. It is a negative-sense, single-stranded RNA virus that usually causes mild, cold-like symptoms.

From moderate upper respiratory tract infections (URTI) to severe and potentially fatal lower respiratory tract infections (LRTI) that necessitate hospitalization and mechanical support, RSV infection can present with a wide spectrum of signs and symptoms. RSV is a common childhood infection that can cause respiratory tract infections in people of all ages, but how it manifests itself differs depending on the immune system and age group. Although reinfection is common throughout life, symptomatic infection is still a risk for infants and the elderly. Childhood RSV infections are fairly self-limited with typical upper respiratory tract signs and symptoms, such as nasal congestion, runny nose, cough, and low-grade fever. Inflammation of the nasal mucosa (rhinitis) and throat (pharyngitis), as well as redness of the eyes (conjunctival infection), may be seen on exam. Approximately 15–50% of children will go on to develop more serious lower respiratory tracts infections, such as bronchiolitis, viral pneumonia, or croup. Bronchiolitis is a common lower respiratory tract infection characterized by inflammation and obstruction of the small airways in the lungs. While several viruses can cause bronchiolitis, RSV is responsible for about 70% of cases. RSV reinfection is common throughout life. Adult reinfection frequently results in mild to moderate symptoms that are similar to those of a sinus infection or common cold. An infection might be asymptomatic. If present, symptoms are generally isolated to the upper respiratory tract: runny nose, sore throat, fever, and malaise. While RSV very rarely causes severe disease in healthy adults, it can cause morbidity and mortality in the elderly and in those with underlying immune compromise or cardiopulmonary disease. In particular, the elderly are more likely to experience pneumonia, respiratory distress, and death. In both adults and children, those who are immunocompromised are at an increased risk of severe infection with RSV. Infected individuals in this group are more likely to progress from upper to lower respiratory tract involvement and have prolonged viral shedding.

RSV is extremely contagious and can spread across hospitals as well as the community, leading to outbreaks. On average, for each person infected with RSV, it is estimated that 5 to 25 uninfected people will become infected. RSV can spread when an infected individual coughs or sneezes, releasing contaminated droplets into the air. When these particles come into contact with another person's mouth, nose, or eyes, transmission typically takes place. Much like all other respiratory diseases that were originally thought to spread through respiratory droplets, the aerosols produced while normal breathing, speaking, and even singing are most likely the means of transmission. Additionally, RSV can survive for up to 25 minutes on infected skin, such as hands, and several hours on other surfaces, such as doorknobs and countertops. It has an incubation period of 2 to 8 days. Once infected, people are usually contagious for 3 to 8 days. In infants and in people with weakened immune systems, however, the virus may continue to spread for up to 4 weeks (even after they are no longer showing symptoms).

After entering the body through the eyes or nose, RSV infects the ciliated columnar epithelial cells of the upper and lower airway. For almost 8 days, RSV keeps replicating inside these bronchial cells. RSV-infected cells will become more rounded and ultimately slough into the smaller bronchioles of the lower airway. The virus is also assumed to spread from the upper to the lower respiratory tract through this sloughing mechanism. Infection causes generalized inflammation within the lungs, including the migration and infiltration of inflammatory cells (such as monocytes and T-cells), necrosis of the epithelial cell wall, edema, and increased mucous production. Instead of being diffuse, inflammation and cell damage are typically patchy. Together, the sloughed epithelial cells, mucous plugs, and accumulated immune cells cause obstruction of the lower airway.

There are various tests available for diagnosis of RSV infection. Common identification techniques include antigen testing, molecular testing, and viral culture.

- **Antigen Testing**
  Antigen testing involves the detection of RSV antigen fragments, usually with a nasopharyngeal swab or aspiration. This can be achieved by looking at fluorescently labelled antigens under a microscope (direct fluorescence assay or DFA) or by using a commercially available rapid antigen detection test.
- **Molecular Testing**
  Molecular assays, such as nucleic acid amplification tests (NAATs), enable sensitive detection of very small amounts of virus in nasopharyngeal swabs and aspirates. NAAT assays such as polymerase chain reaction (PCR) detect virus-specific genetic material, rather than viral antigens. It can be used to detect virus in those with lower viral shedding, such as

older children and adults. It may also be used to detect the disease earlier in at-risk individuals (such as hospitalized or immunocompromised patients), when the viral burden may still be too low to be identified by traditional techniques. Because of its sensitivity, PCR can also often detect asymptomatic carriers and may remain positive even days after an infection has clinically resolved.

- **Viral Culture**
  In traditional viral culture, a sample of the virus is introduced to different cell lines and allowed to replicate so it can be studied. Benefits of this technique include the ability to perform genetic characterization, strain typing, and antiviral susceptibility testing.
- **Serologic Testing**
  Serology (the measurement of virus-specific antibodies in the serum) is not often used to diagnose RSV. The time it takes for the body to produce a significant serological response (and to show a significant increase in detectable antibodies in the serum) is generally not useful in managing the patient's treatment. Up to 30% of patients with documented RSV infection have negative serological results. As such, this method is usually reserved for research.
- **Image Findings**
  Chest X-rays findings in children with RSV bronchiolitis are generally nonspecific and include perihilar markings, patchy hyperinflation, and atelectasis. In children, chest X-ray is sometimes considered when the diagnosis of bronchiolitis is unclear or when there is an unexpected worsening. In adults with RSV infection, chest films are often normal or demonstrate nonspecific changes consistent with viral pneumonia, such as patchy bilateral infiltrates.
- **Differential Diagnosis**
  The differential diagnosis for individuals presenting with signs and symptoms of upper and lower respiratory tract infection includes other viral infections (such as rhinovirus, metapneumovirus, and influenza) and primary bacterial pneumonia. In children, inhaled foreign bodies and congenital conditions such as cystic fibrosis or asthma are typically considered.

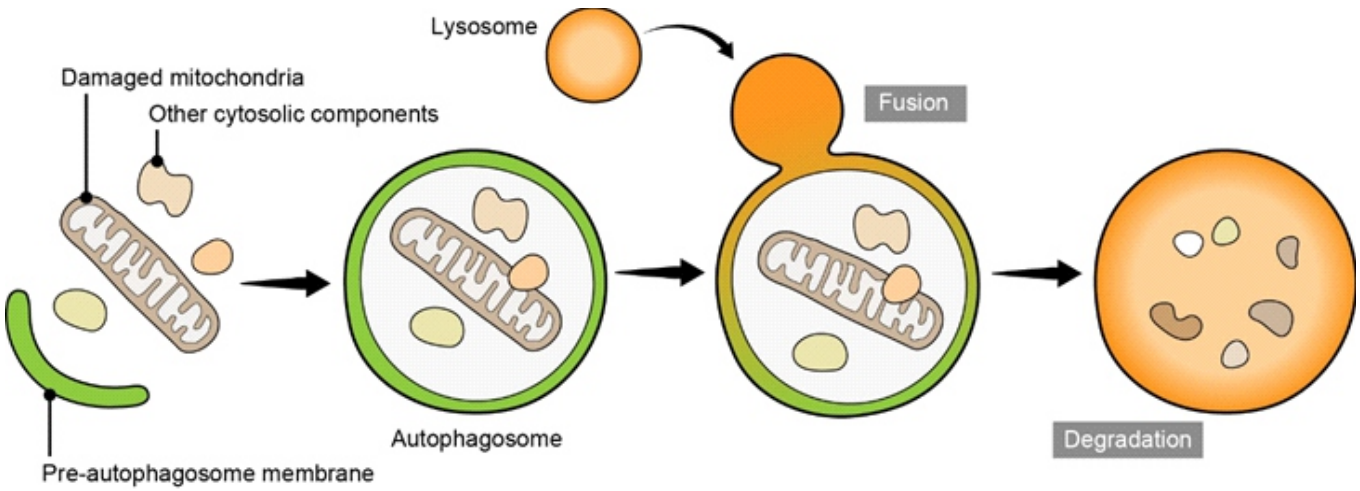
The general measure of prevention is to avoid close contact with infected individuals. Airborne precautions such as respirators, ventilation, and HEPA/high MERV filters, are likely protective against RSV-laden aerosols. Practitioners, parents, and those in close contact with a child with RSV should practice good hand washing technique and repetitive hand washing. Patient isolation of those with RSV in a hospital environment, infection control measures, and cleaning of toys and other objects contaminated with RSV will also aid in decreasing viral transmission.

CDC recommends immunizations to protect those most at risk of getting very sick with RSV: infants, toddlers, and adults 60 years and older. There are also prevention actions that all people can take to help reduce the spread of RSV. A single dose of an RSV vaccine helps protect adults 60 years and older from severe RSV illness. Older adults are at greater risk than young adults for serious complications from RSV because immune systems weaken with age. Older adults who have chronic medical conditions, are elderly or frail, or are living in nursing homes are at increased risk of getting very sick from RSV and benefit the most from RSV vaccination. To protect newborn, RSV vaccine is either given during pregnancy or an RSV immunization that provides antibodies to the baby after birth.

There are currently no specific antiviral medications or treatments for RSV disease. The primary treatment for RSV is supportive care and may include oxygen and respiratory care treatments.

RSV seasonality varies around the world. In temperate climates, infection rates tend to be highest during the cold winter months. This is often attributed to increased indoor crowding and increased viral stability in the lower temperatures. In tropical and arctic climates, however, the annual variation is less well defined and seems to be more prevalent during the rainy season. Annual epidemics are generally caused by the presence of several different viral strains.

Research uncovers specific protein interactions needed for cells to break down and remove damaged mitochondria



Autophagy is a process used by cells as a recycling system to transport and break down organelles and other cytosolic components, which become enveloped in a membrane called the autophagosome. When this involves the removal of damaged mitochondria, it is known as mitophagy.

In a recent article published in The EMBO Journal, a team led by researchers at Tokyo Medical and Dental University (TMDU) elucidated the molecular details of how an enzyme called Tank-binding kinase 1 (TBK1) participates in a disease-relevant mitophagy mechanism.

Although autophagy has been characterized as a more general process meant to degrade and clear various cellular components, recent data have suggested that certain pathways are specifically involved in the autophagy of particular organelle types that are damaged or no longer needed. The researchers became interested in mitophagy mediated by molecules called PINK1 and Parkin, as they are proteins that have been pathologically linked to Parkinson's disease.

"Mitophagy-related defects have been directly implicated in the neurodegeneration observed in Parkinson's disease patients," says Koji Yamano, lead author of the study. "Normally, PINK1 and Parkin work together to mark damaged mitochondria for removal by adding a chain of molecules called ubiquitin. This mark allows proteins called autophagy adaptors to associate with the mitochondria and bring in the autophagy machinery for autophagosome development."

Although TBK1 is known to participate in PINK1/Parkin-mediated mitophagy, a detailed mechanism of how it activates remained unclear. Using various molecular biology techniques, the team found that deleting the gene encoding TBK1 prevented the association of an autophagy adaptor called optineurin (OPTN) during Parkin-mediated mitophagy. Additionally, deleting the OPTN gene prevented autophosphorylation of TBK1, which is necessary for it to function.

Further work suggested that the interactions between OPTN and ubiquitin, as well as between OPTN and the developing autophagosome, were all needed for OPTN and TBK1 to come together at the contact site between damaged mitochondria and the pre-autophagosome membrane. Without this contact site, TBK1 autophosphorylation could not occur.

The researchers also generated molecules called monobodies in their lab that could specifically bind OPTN and inhibit its physical interactions. The monobodies prevented OPTN accumulation at the mitophagy contact sites. This subsequently blocked TBK1 activation and, thereby, mitochondrial degradation. These experiments further emphasized the importance of the OPTN-TBK1 relationship to support proper mitophagy.

"Because PINK1 and Parkin are critical contributors to the molecular basis of Parkinson's disease, understanding the mechanistic details related to the mitophagy process mediated by these molecules is very important," explains Yamano.

This study demonstrates a positive and reciprocal relationship between OPTN and TBK1 that is necessary for autophagosomes to begin forming on damaged mitochondria. The impactful finding may lead to the development of novel drugs to treat Parkinson's disease.

Good Manufacturing Practices (GMP)

Good Manufacturing Practices (GMP) refers to the Practices which manufacturers, processors, and packagers should take as proactive steps to ensure that their products are safe, pure, and effective.

GMP requires a quality approach to manufacturing, enabling companies to minimize or eliminate instances of contamination, mix-ups, and errors. This in turn, protects the consumer from purchasing unsafe and poor-quality products. Failure of firms to comply with GMP can result in very serious consequences including recall, seizure, fines, and imprisonment. It addresses issues including recordkeeping, personnel qualifications, sanitation, cleanliness, equipment verification, process validation, and complaint handling.

Most GMP requirements are very general and open-ended, allowing each manufacturer to decide individually how to best implement the necessary controls. This provides much flexibility, but also requires that the manufacturer interpret the requirements in a manner which makes sense for each individual business.

There are several sets of GMP standards which have been endorsed by different governments. Fortunately, although they are nearly identical. Some versions of GMPs include:

- CGMP* (food) addresses the production of food for the US.
- CGMP* (drug) addresses the production of pharmaceutical drugs for the US.
- EU-GMP addresses the production of pharmaceutical drugs for the European Union.
- Guide 104 GMP addresses the production of pharmaceutical drugs for Canada.

GMP Certification Process

After a manufacturer aligns their operations with GMPs, they may consider going through the certification process through a private auditing firm. This is how the certification process typically works:

1. A manufacturer adopts the GMP standards and makes the required adjustments to align with the standards. Depending on the preexisting practices and conditions, this could take 3-12 months.
2. The manufacturer chooses a private auditing firm (there are many) to conduct the GMP audit.
3. The auditing firm conducts the audit, which may include an inspection of the facility and a review of records.
4. The manufacturer will correct any areas of non-compliance and, if they achieve a passing score, they will receive a certificate from the auditing firm.
5. The manufacturer can provide this certificate to prospective buyers as an indication of their alignment with industry standards.

Here are the 10 principles of GMP to be practiced:

1. **Written Procedures and Instructions:** Detailed, written procedures are essential for each process that could affect the quality of the finished product. There must be systems to provide documented proof that correct procedures are consistently followed at each step in the manufacturing process.

2. **Facilities and Equipment:** Facilities and equipment should be properly designed, maintained, and cleaned to ensure the quality of products. Equipment validation and calibration are also crucial for maintaining consistent operations.

3. **Materials:** All materials used in a manufacturing process must be of tested quality, clearly identified, and readily traceable. This includes both the product's ingredients and the specific containers and closures that will be used.

4. **Production:** Manufacturing processes are clearly defined, controlled, and validated to ensure consistency and compliance. Critical processes are validated to demonstrate that they are capable of consistently delivering quality products.

5. **Quality Control**: Products must be tested at different stages

of production to verify quality. These tests should ensure the identity, purity, potency, and quality of the product.

6. **Documentation:** Records, raw data, and documents related to production and distribution should be retained and available for review. They should be clear, comprehensive, and accurate.

7. **Personnel:** Qualified and adequately trained personnel are essential. Every person involved in manufacturing should have the education, training, and experience to perform their role effectively.

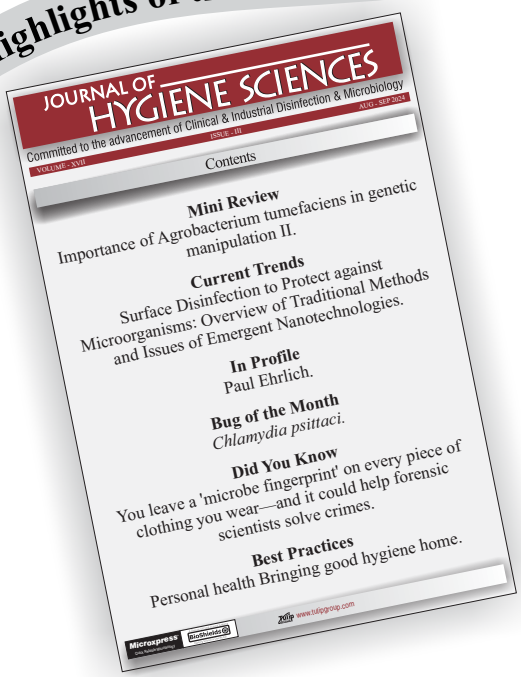
8. **Validation and Change Control:** Changes to the manufacturing process must be properly reviewed, validated, and documented to ensure the quality of the product isn't compromised.

9. **Complaints and Recalls:** There should be systems in place for handling complaints and product recalls. This involves reviewing and investigating complaints and taking appropriate corrective actions when necessary.

10. **Auditing (Self-inspection and Quality Audits):** Regular audits should be conducted to ensure that GMP guidelines are being followed. These audits can identify areas for improvement and ensure that corrective actions are implemented.

These 10 principles of GMP form the foundation of GMP and are designed to provide a structure for a strong quality management system (QMS) or Pharmaceutical Quality Management System (PQS). They help ensure that pharmaceutical products are consistently high in quality, safe for use, and fit for their intended use.

Highlights of the coming issue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Best Practices
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