

Editorial

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Mini review section: *Corynebacterium diphtheriae* is a human pathogen, responsible for causing Diphtheria. It was once an important cause of death worldwide. It is also known as the Klebs-Löffler bacillus, because it was discovered in 1884 by German bacteriologists Edwin Klebs (1834–1912) and Friedrich Löffler (1852–1915).

Current Trends Section: Hand sanitizer, also called hand antiseptic or handrub, agent applied to the hands for the purpose of removing common pathogens (disease-causing organisms). Hand sanitizers typically come in foam, gel, or liquid form. Although the effectiveness of hand sanitizer is variable, it is employed as a simple means of infection control in a wide variety of settings. Depending on the active ingredient used, hand sanitizers can be classified as one of two types: alcohol-based or alcohol-free.

In Profile: Asima Chatterjee was born on 23 September 1917 in Bengal. She was an Indian organic chemist and an astounding scientist noted for her work in the fields of organic chemistry and phytomedicine. Her most notable work includes research on vinca alkaloids, the development of anti-epileptic drugs, and the development of anti-malarial drugs.

Bug of the Month: *Acinetobacter baumannii* is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. An opportunistic pathogen, *A. baumannii* has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged (> 90 d) hospital stay. Commonly associated with aquatic environments, it has been shown to colonize the skin as well as being isolated in high numbers from the respiratory and oropharynx secretions of infected individuals. In recent years, it has been designated as a “red alert” human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum.

Did you Know: A study conducted by a team of researchers at LSU Health New Orleans has shown for the first time that chronic exposure to inhaled nicotine alone increases blood pressure (hypertension), in both the body's general circulation and in the lungs that can lead to pulmonary hypertension. The study also found that nicotine-induced pulmonary hypertension is accompanied by changes in the size, shape and function (remodeling) of the blood vessels in the lung and the right lower chamber of the heart.

Best Practices: Aseptic media-fill testing is used to quantify the aseptic technique of compounding personnel or processes and to ensure that the processes used are able to produce a sterile product without microbiological contamination. During this test, a microbiological growth medium, such as soybean-casein digest medium (SCDM), also known as trypticase soy broth (TSB), is substituted for the actual drug product to simulate admixture compounding.

“There is nothing in the world so irresistibly contagious as laughter and good humor.” so ease your mind with some light humour in our Relax Mood section.

Looking forward for your feedback & suggestions.

Bacteriology - Elementary identification of *Corynebacterium diphtheriae*

Corynebacterium diphtheriae is the pathogenic bacterium that causes diphtheria. It is also known as the Klebs-Löffler bacillus, because it was discovered in 1884 by German bacteriologists Edwin Klebs (1834–1912) and Friedrich Löffler (1852–1915).



Corynebacterium diphtheriae is a human pathogen, responsible for causing Diphtheria. It was once an important cause of death worldwide. The mortality rates gradually decreased with time in the twentieth century in countries where living standards were improved, and then intensely fell once after

the introduction of immunization programs. Though, even today, despite these events it remains a substantial pathogen in many parts of the world. A variety of mechanisms are responsible for causing death. However, the name 'strangling angel' of children arose from the wing-shaped pseudo-membranes that are formed in the oropharynx. Displacement and impaction of these pseudo-membranes starts acute airway obstruction and sudden death.

Introduction

Corynebacterium diphtheriae is a non-motile, non-capsulated, club-shaped, Gram-positive bacillus. Toxigenic strains are lysogenic for one of a family of corynebacteriophages that carry the structural gene for diphtheria toxin, *tox*. Most strains require nicotinic and pantothenic acids for growth; some also require thiamine, biotin, or pimelic acid. For optimal production of diphtheria toxin, the medium should be supplemented with amino acids and must be de-ferrated (From which, iron atoms and ions have been removed).

Taxonomy

Four subspecies are recognized: *C. mitis*, *C. intermedius*, *C. gravis*, and *C. belfanti*. The four subspecies differ slightly in their colonial morphology and biochemical properties, such as the ability to metabolize certain nutrients, but all may be toxigenic (and therefore cause diphtheria) or not toxigenic.

C. diphtheriae produces diphtheria toxin which alters protein function in the host by inactivating the elongation factor EF-2. This causes pharyngitis and 'pseudomembrane' in the throat. The diphtheria toxin gene is encoded by a bacteriophage found in toxigenic strains, integrated into the bacterial chromosome.

Scientific Classification

Domain : Bacteria

Phylum : Actinobacteria

Order : Actinomycetales

Family : Corynebacteriaceae

Genus : *Corynebacterium*

Species : *C. diphtheriae*

Characteristics

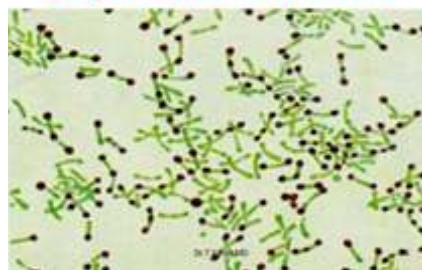
Corynebacterium diphtheriae is a Gram-positive non-motile, club-

shaped bacillus. Strains growing in tissue, or older cultures in vitro, contain thin spots in their cell walls that allow decolorization during the Gram stain and result in a Gram-variable reaction. Older cultures often contain metachromatic granules (polymetaphosphate) which stain bluish-purple with methylene blue. The cell wall sugars include arabinose, galactose, and mannose. In addition, a toxic 6,6'-diester of trehalose containing corynemycolic and corynemycolenic acids in equimolar concentrations may be isolated. Three distinct cultural types, *mitis*, *intermedius*, and *gravis* have been recognized. Most strains require nicotinic and pantothenic acids for growth; some also require thiamine, biotin, or pimelic acid. For the optimal production of diphtheria toxin the medium should be supplemented with amino acids and must be de-ferrated.

To accurately identify *C. diphtheriae*, a Gram stain is performed to show Gram-positive, highly pleomorphic organisms with no particular arrangement. Special stains like "Alberts stain and Ponder's stain are used to demonstrate the metachromatic granules formed in the polar regions. The granules are called polar granules, Babes Ernst granules, volutin, etc. An enrichment medium, such as Löffler's medium, is used to preferentially grow *C. diphtheriae*. After that, a differential plate known as tellurite agar, allows all *Corynebacteria* (including *C. diphtheriae*) to reduce tellurite to metallic tellurium. The tellurite reduction is calorimetrically indicated by brown colonies for most *Corynebacteria* species or by a black halo around the *C. diphtheriae* colonies.



Corynebacterium diphtheria



As early as 1887, Loeffler described the isolation from healthy individuals of avirulent (non-toxigenic) *C. diphtheriae* that were indistinguishable from the virulent (toxigenic) strains isolated from patients. It is now recognized that avirulent strains of *C.*

diphtheriae may be converted to the virulent phenotype following infection and lysogenization by one of a number of distinct corynebacteriophages that carry the structural gene for diphtheria toxin, *tox*. Lysogenic conversion from the avirulent to virulent phenotype may occur in situ, as well as in vitro. The diphtheria toxin structural gene is not essential for either corynebacteriophage or *C. diphtheriae*. Despite this observation, genetic drift of diphtheria toxin has not been observed.

Pathogenesis

The pathogenesis of diphtheria is based upon two primary determinants:

- (1) The ability of a given strain of *C. diphtheriae* to colonize in the nasopharyngeal cavity and/or on the skin,
- (2) And its ability to produce diphtheria toxin. Since those determinants involved in colonization of the host are encoded by the bacteria, and the toxin is encoded by the corynebacteriophage, the molecular basis of virulence in *C. diphtheriae* results from the combined effects of determinants carried on two genomes. Non-toxigenic strains of *C. diphtheriae* are rarely associated with clinical disease; however, they may become highly virulent following lysogenic conversion to toxigenicity.

Colonization

Little is known of the colonization factors of *C. diphtheriae*. However, it is apparent that factors other than the production of diphtheria toxin contribute to virulence. Epidemiologic studies have demonstrated that a given lysotype may persist in the population for extended periods. It may later be supplanted by another lysotype. The emergence and subsequent predominance of a new lysotype in the population are presumably due to its ability to colonize and effectively compete in their segment of the nasopharyngeal ecologic niche. *Corynebacterium diphtheriae* may produce a neuraminidase that cleaves sialic acid from the cell surface into its pyruvate and N-acetylneuraminic acid components. Cord factor (6,6'-di-O-mycoloyl- α,α' -D-trehalose) is a surface component of *C. diphtheriae*, but its role in colonization of the human host is unclear.

Diphtheria Toxin Production

The structural gene for diphtheria toxin, *tox*, is carried by a family of closely related corynebacteriophages of which the β -phage is the most extensively studied. The regulation of diphtheria toxin expression is mediated by an iron-activated repressor, DtxR, which is encoded on the *C. diphtheriae* genome. The expression of *tox* depends on the physiologic state of *C. diphtheriae*. Under conditions in which iron becomes the growth-rate limiting substrate, iron dissociates from DtxR, the *tox* gene becomes depressed, and diphtheria toxin is synthesized and secreted into the culture medium at maximal rates.

Host Defenses

Immunity to diphtheria involves an antibody response to diphtheria toxin following clinical disease or immunization with diphtheria toxoid.

Medically importance

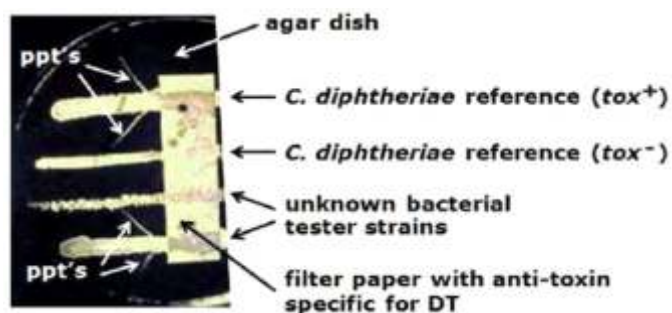
The disease occurs primarily in tropical regions and underdeveloped countries but has been known to appear throughout the world. Immuno-compromised individuals, poorly immunized adults, and unvaccinated children are at the greatest risk for contracting diphtheria. During the typical course of disease, the only affected body region is the upper respiratory system. A thick gray coating accumulates in the nasopharyngeal region, making it difficult for the individual to breathe and swallow. The disease remains contagious for at least two weeks following disappearance of symptoms but has been known to last for up to a month. The portals of entry for *Corynebacterium diphtheriae* are the nose, tonsils, and throat. Individuals suffering from the disease may experience, sore throat, weakness, fever, and swollen glands. Mode of transmission is person to person contact via respiratory droplets i.e. coughing or sneezing and less commonly, by touching open sores or contaminated surfaces. If left untreated, diphtheria toxin may enter the bloodstream causing damage to the kidneys, nerves, and heart. Extremely rare complications include suffocation and

partial paralysis. A vaccine, DTap, effectively prevents the disease and is mandatory in the United States for participation in education and many professions.

Diagnosis

The clinical diagnosis of diphtheria requires bacteriologic laboratory confirmation of toxigenic *C. diphtheriae* in throat or lesion cultures. For primary isolation, a variety of media may be used: Löffler agar, Mueller-Miller tellurite agar, or Tinsdale tellurite agar. Sterile cotton-tipped applicators are used to swab the pharyngeal tonsils or their beds. Calcium alginate swabs may be inserted through both nares (i.e. the nostrils) to collect nasopharyngeal samples for culture. Since diphtheritic lesions are often covered with a pseudomembrane, the surface of the lesion may have to be carefully exposed before swabbing with the applicator.

Following initial isolation, *C. diphtheriae* may be identified as mitis, intermedius, or gravis biotype on the basis of carbohydrate fermentation patterns and hemolysis on sheep blood agar plates. The toxigenicity of *C. diphtheriae* strains is determined by a variety of in vitro and in vivo tests. The most common in vitro assay for toxigenicity is the Elek immunodiffusion test. This test is based on the double diffusion of diphtheria toxin and antitoxin in an agar medium. A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and *C. diphtheriae* isolates are streak-inoculated at a 90° angle to the filter paper. The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar. Alternatively, many eukaryotic cell lines (e.g., African green monkey kidney, Chinese hamster ovary) are sensitive to diphtheria toxin, enabling in vitro tissue culture tests to be used for detection of



Elek Immunodiffusion Test

toxin. Several sensitive in vivo tests for diphtheria toxin have also been described (e.g., guinea pig challenge test, rabbit skin test).

Sensitivity

The bacterium is sensitive to the majority of antibiotics, such as the penicillins, ampicillin, cephalosporins, quinolones, chloramphenicol, tetracyclines, cefuroxime and trimethoprim.

Genetics

The genome of *C. diphtheriae* consists of a single circular chromosome of 2.5 Mbp, with no plasmids. The genome shows an extreme compositional bias, being noticeably higher in G+C near the origin than at the terminus.

Other Corynebacterium Species

In addition to *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*, *C. pseudodiphtheriticum* and *C. xerosis* may occasionally cause infection of the nasopharynx and skin. The last two strains are recognized by their ability to produce pyrazinamidase. In veterinary medicine, *C. renale* and *C. kutscheri* are important pathogens and cause pyelonephritis in cattle and latent infections in mice, respectively.

Importance of Raw Material used in Hand Sanitizers



Gel Hand Sanitizer – A person using gel hand sanitizer

Hand sanitizer, also called hand antiseptic or handrub, agent applied to the hands for the purpose of removing common pathogens (disease-causing organisms). Hand sanitizers typically come in foam, gel, or liquid form. Their use is recommended when soap and water are not available for hand washing or when repeated hand washing compromises the natural skin barrier (e.g., causing scaling or fissures to develop in the skin). Although the effectiveness of hand sanitizer is variable, it is employed as a simple means of infection control in a wide variety of settings, from day-care centers and schools to hospitals and health care clinics and from supermarkets to cruise ships.

Types of Hand Sanitizers

Depending on the active ingredient used, hand sanitizers can be classified as one of two types: alcohol-based or alcohol-free. Alcohol-based products typically contain between 60 and 95 percent alcohol, usually in the form of ethanol, isopropanol, or n-propanol. At those concentrations, alcohol immediately denatures proteins, effectively neutralizing certain types of microorganisms. Alcohol-free products are generally based on disinfectants, such as benzalkonium chloride (BAC), or antimicrobial agents, such as triclosan. The activity of disinfectants and antimicrobial agents is both immediate and persistent. Many hand sanitizers also contain emollients (e.g., glycerin) that soothe the skin, thickening agents, and fragrance.

Difference between Isopropyl alcohol and Ethyl Alcohol

Isopropyl alcohol or isopropanol is a colorless liquid with a bitter taste. It is used in the manufacture of acetone and glycerin. It is often used as the solvent in rubbing alcohol, and some antifreeze and windshield wiper fluid. Isopropyl alcohol, also called 2-propanol, one of the most common members of the alcohol family of organic compounds. Isopropyl alcohol was the first commercial synthetic alcohol; chemists at the Standard Oil Company of New Jersey (later Exxon Mobil) first produced it in 1920 while studying petroleum by-products.

Ethanol, also called ethyl alcohol, grain alcohol, a member of a class of organic compounds that are given the general name alcohols; its molecular formula is C_2H_5OH . Ethanol is an important industrial chemical; it is used as a solvent, in the synthesis of other chemicals, and as an additive to automotive gasoline (forming a mixture known as gasohol). Ethanol is also the intoxicating ingredient of many alcoholic beverages such as beer, wine, and distilled spirits.

Hence, ethyl alcohol is made from sugarcane or corn, while isopropyl alcohol is made from Propene - compound comes from fossil fuels – petroleum, natural gas, and even coal.

Isopropyl Alcohol vs Ethyl Alcohol (Ethanol)

Isopropyl Alcohol	Ethyl Alcohol
Isopropyl alcohol is secondary alcohol since -OH is attached to the 2nd carbon.	Ethyl alcohol is primary alcohol since -OH is attached to the 1st carbon.
Boiling point – 82.4°C Melting point – 89.5°C	Boiling point – 78°C Melting point – 115°C
Not drinkable	Used as a beverage
Used as a solvent, disinfectant, and a cleaning agent	Used as a solvent, disinfectant, biological specimen preservative, etc.

Grades of Purity

AR (ANALYTICAL REAGENT): Reagents essential for analytical purpose and research work having high purity. The certificate of guarantee gives the minimum assay and maximum limits of trace impurities. These reagents comply with maximum tests of ACS grades, GR, ANALAR, P.A. (Proanalysis) which are marketed by international companies.

FOR SYNTHESIS: General purpose reagents having many potential applications in laboratories.

LR (LABORATORY REAGENTS): This grade consists of Pure, Extra Pure, Purified organic, and inorganic chemicals having reliable accuracy in Routine Laboratory Analysis.

EP, EXTRA PURE, PURIFIED, PURE: Products suitable for qualitative and semi- quantitative work. Most of “extra pure” products comply with “Pharma Copoeia” grades of all countries and preferentially used in the Pharmaceutical industries and making of hand sanitizers.

CP (CHEMICALLY PURE): Chemicals being used for regular practical having its original purity.

MICROSCOPY (M.S.): Highly purified Reagent for use in biochemical research and analysis. They are free from inhibitors such as traces of heavy metals and tested with a view for biochemical work.

HPLC GRADE: This grade includes solvents, ion pair reagents, and buffers of appropriate purity to be used as mobile phases in HPLC. Solvents undergo highly efficient distillation, solid-phase absorption, and other chemical treatments. This allows eliminating impurities that could interfere in HPLC analysis. In this grade, intensive quality control using UV spectroscopy.

SPECTROSCOPY GRADE: Nuclear magnetic resonance spectroscopy is the most commonly used technique in the structural analysis of compounds obtained by organic synthesis. It normally requires the sample to be dissolved in a solvent whose hydrogen atoms have been replaced with deuterium atoms. It offers deuterated solvents of differing isotopic purities. The most usual is 99.8%, but it can offer purities of up to 99.98%.

COMMERCIAL GRADE: Ample amount of impurities is present in this grade. Most of the manufacturers are using commercial grade alcohol to produce hand sanitizers, and quality standards of these hand sanitizers cannot be guaranteed. It does not provide any valid documentation such as the certificate of analysis.



Foam Hand Sanitizer – A person using foam hand sanitizer

Effectiveness

The effectiveness of hand sanitizer depends on multiple factors, including how the product is applied (e.g., the quantity used, duration of exposure, frequency of use) and whether the specific infectious agents present on the person's hands are susceptible to the active ingredient in the product. In general, alcohol-based hand sanitizers, if rubbed thoroughly over fingers and hand surfaces for 30 seconds, followed by complete air-drying, can effectively reduce populations of bacteria, fungi, and some enveloped viruses (e.g., coronavirus, influenza A viruses). Similar effects have been reported for certain alcohol-free formulations, such as SAB (surfactant, allantoin, and BAC) hand sanitizer. Most hand sanitizers, however, are relatively ineffective against bacterial spores, nonenveloped viruses (e.g., norovirus), and encysted parasites (e.g., Giardia). They also do

not fully cleanse or sanitize the skin when hands are noticeably soiled prior to application.

Despite the variability in effectiveness, hand sanitizers can help control the transmission of infectious diseases, especially in settings where compliance with handwashing is poor. For example, among children in elementary schools, the incorporation of either an alcohol-based or an alcohol-free hand sanitizer into classroom hand-hygiene programs has been associated with reductions in absenteeism related to infectious illness. Likewise, in the workplace, the use of alcohol-based hand sanitizer has been associated with reductions in illness episodes and sick days. In hospitals and health care clinics, increased access to alcohol-based hand sanitizer has been linked to overall improvements in hand hygiene.

Safety Concerns

Agencies such as the World Health Organization and the U.S. Centers for Disease Control and Prevention promote the use of alcohol-based hand sanitizers over alcohol-free products. Indeed, the use of alcohol-free products has remained limited, in part because of WHO's and CDC's focus on alcohol-based products but also because of concerns about the safety of chemicals used in alcohol-free products. Disinfectants and antimicrobials also can potentially contribute to the development of antimicrobial resistance.

By comparison, concerns over the use of alcohol-based hand sanitizer have centered primarily on product flammability and ingestion, both unintentional (e.g., by young children) and intentional (by individuals seeking to abuse alcohol). With proper storage and strategies that limit access to alcohol-containing sanitizer (e.g., issuing hand sanitizer to individuals), the risk of fire or poisoning from accidental or intentional ingestion of alcohol-based hand sanitizers is considered to be low.

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Asima Chatterjee

Asima Chatterjee was born on 23 September 1917 in Bengal. She was an Indian organic chemist and an astounding scientist noted for her work in the fields of organic chemistry and phytomedicine. Her most notable work includes research on vinca alkaloids, the development of anti-epileptic drugs, and the development of anti-malarial drugs.

Asima Chatterjee was the eldest of the two children of a medical doctor Indra Narayan Mukherjee and his wife, Kamala Devi. She grew up in Calcutta in a middle-class family. Her father was very interested in botany and Chatterjee shared in his interest. She graduated with honours in chemistry from the Scottish Church College of the University of Calcutta in 1936. Asima Chatterjee received a master's degree (1938) and a doctoral degree (1944) in organic chemistry from the University of Calcutta. She was the first Indian woman to earn a doctorate in science. Her doctoral research focused on the chemistry of plant products and synthetic organic chemistry.

She joined the Lady Brabourne College of the University of Calcutta and founded the department of chemistry there. In the year 1954, Asima Chatterjee joined the University College of Science of the University of Calcutta, as a reader in pure chemistry.

Asima Chatterjee has made many great achievements and contributions to science. She initiated a chemical investigation of alkaloids in *Rauwolfia canescens*, which is commonly known as the be still tree or devil-pepper. Growing as a bush or small tree. Investigated the chemistry of almost all principal types of indole alkaloids. She carried out synthetic studies on several complex indoles, quinoline and isoquinoline alkaloids. Developed procedures for the preparation of beta-phenylethanolamines in connection with alkaloid synthesis. Introduced the use of periodic acid as a reagent for the detection and location of both terminal and exocyclic double bonds in organic compounds. And has made many such contributions.

Asima Chatterjee received many awards and recognitions in her life. She was a Premchand Roychand Scholar of the University of Calcutta. She was Khaira Professor of Chemistry from the year 1962 to 1982, one of the most prestigious and coveted chairs of the University of Calcutta. In the year 1972, she was appointed as the Honorary Coordinator of the Special Assistance Program to intensify teaching and research in natural product chemistry, sanctioned by the Indian University Grants Commission (UGC).

She was elected a Fellow of the Indian National Science Academy in New Delhi. In the year 1961, she received the Shanti Swarup Bhatnagar Award in chemical science, becoming the first female who received this award. In the year 1975, she was granted the prestigious Padma Bhushan and became the first female scientist to be elected as the General President of the Indian Science Congress Association. She was granted the D. Sc. (honoris causa) degree by several universities.

She was nominated by the President of India as a Member of the Rajya Sabha.

Asima Chatterjee proved to be a great inspiration. She contributed her knowledge for the betterment of society. When women in society were rarely encouraged for their career, she made her name in the field of science and became the first women scientist. She made India proud in the field of organic chemistry.

Awards and recognition:

- ◆ She was a Premchand Roychand Scholar of the University of Calcutta.
- ◆ From 1962 to 1982, she was the Khaira Professor of Chemistry, one of the most prestigious and coveted chairs of the University of Calcutta.
- ◆ In 1972, she was appointed as the Honorary Coordinator of the Special Assistance Programme to intensify teaching and research in natural product chemistry, sanctioned by the Indian University Grants Commission. In 1960, she was elected a Fellow of the Indian National Science Academy, New Delhi.
- ◆ In 1961, she received the Shanti Swarup Bhatnagar Award in chemical science, becoming the first female recipient of this award.
- ◆ In 1975, she was conferred the prestigious Padma Bhushan and became the first female scientist to be elected as the General President of the Indian Science Congress Association.
- ◆ She was conferred the D. Sc. (honoris causa) degree by several universities.
- ◆ She was nominated by the President of India as a Member of the Rajya Sabha from February 1982 to May 1990.
- ◆ On 23 September 2017, the search engine Google deployed a 24-hour Google Doodle in honour of the 100th anniversary of Chatterjee's birth.



Jokes

Son - "Dad, Can You Write In The Dark?"

Dad- "I Think So. What Is It You Want Me To Write?"

Son - "Your Name On This Report Card."

An airplane was about to crash.

There were 4 passengers on board, but only 3 parachutes.

The 1st passenger said "I am Stephen Curry, the best NBA basketball player. The Warriors and my millions of fans need me, and I can't afford to die." So he took the 1st pack and left the plane.

The 2nd passenger, Donald Trump, said, "I am the newly-elected US President, and I am the smartest President in American history, so my people don't want me to die." He took the 2nd pack and jumped out of the plane.

The 3rd passenger, the Pope, said to the 4th passenger, a 10-year-old schoolboy, "My son, I am old and don't have many years left, you have more years ahead so I will sacrifice my life and let you have the last parachute."

The little boy said, "That's okay, Your Holiness, there's a parachute left for you.

America's smartest President took my schoolbag."

Once all the engineering professors were sitting in one plane.

Before the takeoff, one announcement came "This plane is made by your students"

Then all professors stood up, ran and went outside. But the principal was sitting.

One guy came and asked, "are you not afraid"?

Then the principal replied

"I trust my students very well and I am sure the plane won't even start".

My Chinese friend got really sick one day and had to go to a hospital.

I went to see him the next day.

He just kept whispering "yang qi guan" over and over and then died.

I was very sad and Googled his last message after the burial.

Apparently, it means "You're standing on my oxygen tube".

Today was my first day entering a court.

The judge shouted "Order, Order!!"

I was so excited,

So I shouted back "fried rice with chicken, five bottles of beer and a chilled glass of special ice mineral water."

I am now locked up in a dark room.

I am sure they will bring my order soon.

I visited my EX girlfriend and she gave me food. After a few second their dog came in and started to jump over and I said "this dog loves visitors" A child replied, "No! No! Uncle, the problem is that you are using its plate".

If a paper comes very tough in exam,

Just close your eyes for a moment,

Take a deep breath and say loudly,

"This is a very interesting subject; I want to study it again".

Acinetobacter baumannii

Acinetobacter baumannii is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. An opportunistic pathogen, *A. baumannii* has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged (> 90 d) hospital stay. Commonly associated with aquatic environments, it has been shown to colonize the skin as well as being isolated in high numbers from the respiratory and oropharynx secretions of infected individuals. In recent years, it has been designated as a “red alert” human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum.

This phenomenon of multidrug-resistant (MDR) pathogens has increasingly become a cause for serious concern with regard to both nosocomial and community-acquired infections. Indeed, the World Health Organization (WHO) has recently identified antimicrobial resistance as one of the three most important problems facing human health.⁶ The most common and serious MDR pathogens have been encompassed within the acronym “ESKAPE,” standing for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.

While in the 1970s *A. baumannii* is thought to have been sensitive to most antibiotics, today the pathogen appears to exhibit extensive resistance to most first-line antibiotics. More recently, *A. baumannii* has become a major cause for concern in conflict zones, and has gained particular notoriety in the recent desert conflicts in Iraq, earning it the moniker “Iraqibacter.” In particular, high incidences of MDR bacteremia (bloodstream infections) have been noted among US Army service members following Operation Iraqi Freedom (OIF).⁹ Interest from the scientific community over the past 15 years has led to significant advances of our understanding of this organism.

Antibiotic Resistance

The rapid emergence of multi- and pandrug-resistant strains of *Acinetobacter* highlights the organism's ability to quickly acclimatize to selective changes in environmental pressures. The upregulation of the organism's innate resistance mechanisms coupled with the acquisition of foreign determinants have played a crucial role in the express route the organism has taken to becoming a multidrug-resistant pathogen.

A 2006 study undertaken by Fournier et al. compared the genome of AYE and SDF *Acinetobacter* using whole shotgun genome sequences. In France, the epidemic AYE strain had a 26% mortality rate in infected patients, while the SDF strain came from the same geographical region, but was associated with human body lice, and was fully susceptible to antimicrobial agents. The genomic comparisons revealed that the genome of the virulent AYE strain contained an 86 kb region called a resistance “island” that contained a cluster of 45 resistance genes. The homologous location in the susceptible

strain exhibited a 20 kb genomic island that is devoid of these resistance markers. This ability to “switch” its genomic structure goes some way to explaining the speed with which *Acinetobacter* captures resistance markers when under antibacterial pressure, such as may occur in a high risk environment, such as in hospital intensive care units⁵ (where broad spectrum antimicrobials are commonly used). Sequence similarity and phylogenetic analyses confirmed that most of the resistance genes found in the *Acinetobacter* strain AYE had been recently acquired from bacteria of the genera *Pseudomonas*, *Salmonella* or *Escherichia*.

All genomic variants of *A. baumannii* contain a non-inducible chromosomal AmpC cephalosporinase, also known as *Acinetobacter*-derived cephalosporinases (ADCs). The presence of an upstream IS element known as ISAbal determines the regulation of the AmpC gene. Overexpression of AmpC cephalosporinase and resistance to extended spectrum cephalosporin is intrinsically linked to the presence of ISAbal. Cefepime and carbapenems, however, appear to be stable in response to these enzymes.

A. baumannii also possess an intrinsic class D oxacillinase belonging to the OXA-51-like group of enzymes that constitutes over 40 sequence variants. The ubiquitous nature of OXA-51-like genes in *A. baumannii* has led to this gene becoming an important genetic marker in identification of the organism to the species level. OXA-51-like enzymes are able to hydrolyze penicillins (benzylpenicillin, ampicillin, ticarcillin and piperacillin) and carbapenems (imipenem and meropenem) but do so only very weakly. A significant contribution to lactam resistance by OXA-51-like enzymes therefore requires the presence of an insertion element ISAbal upstream of the gene, which acts as a strong transcriptional promoter. The most common enzymatic mode of carbapenem resistance is the production of oxacillinases encoded by genes of the blaOXA-23, blaOXA-40 and blaOXA-58-like lineage.

In Europe, the spread of multidrug-resistant *Acinetobacter* is not confined to hospitals within a city but also occurs on a national scale, mostly through inter-hospital patient transfers, for example the spread of the so-called Southeast clone and the Oxa-23 clones 1 and 2 in southeast England. International transfer of colonized patients has led to the introduction and subsequent epidemic spread of multidrug-resistant *A. baumannii* strains from southern into northern European countries, such as Belgium and Germany.

In an industry-supported surveillance report (MYSTIC) from 48 European hospitals for the period 2002–2004, just 73.1% of *A. baumannii* isolates were susceptible to meropenem and 69.8% were susceptible to imipenem. Susceptibility to other antibiotics was also very low, with 32.4%, 34.0% and 47.6% being susceptible to ceftazidime, ciprofloxacin and gentamicin, respectively.

There is a long history of multidrug-resistant *A. baumannii* infections occurring in the United States. In 1991 and 1992,

outbreaks of carbapenem-resistant *A. baumannii* were observed in a hospital in New York City. This followed an outbreak of infections due to ESBL-producing *Klebsiella pneumoniae* during which use of imipenem increased substantially. In a more recent industry-supported surveillance study including isolates of *Acinetobacter* collected between 2004 and 2005 from 76 centers throughout the United States, only 60.2% were susceptible to imipenem.

Numerous outbreaks of pandrug-resistant *A. baumannii* have been documented in Asian and Middle Eastern hospitals. Rates of non-susceptibility in SENTRY (Anti-microbial Surveillance Program) isolates (2001–2004) exceeded 25% for imipenem and meropenem, 40% for cefepime and ceftazidime, 40% for ampicillin-sulbactam, 35% for amikacin, and 45% for ciprofloxacin.⁴⁷ It is worth noting that resistance to tigecycline and polymyxin B (drugs relied on heavily to treat infection with *A. baumannii*) both already exist in this region.

Clinical Symptoms

A. baumannii infections are implicated across a wide range of anatomical regions and with varying severity and patient outcomes.⁴⁹ There is considerable debate relating to the actual clinical impact of infection and its relationship to patient mortality. While a number of studies have concluded that infection with *Acinetobacter* has a detrimental effect on patient outcome, other similar studies implied little or no effect on patient outcome as a result of infection.

The lack of consensus is most likely due to the difference in the approaches of the various studies; some being prospective while others have been of retrospective samples. The results generated by some studies have also only identified the organism to genus level but not to species level, with many referring to infection with *Acinetobacter calcoaceticus-baumannii* complex which could conceivably indicate colonization with the environmental species *Acinetobacter calcoaceticus* coupled with a polymicrobial infection, rather than a monomicrobial infection with a virulent *Acinetobacter* species such as MDR *Acinetobacter*.

Hospital-acquired pneumonia

Ventilator associated pneumonia (VAP) is commonly linked to infection. Longer periods of hospitalization, longer time on mechanical ventilation and prior use of antibiotics are the recognized factors increasing the risk of VAP due to *Acinetobacter*. Nosocomial outbreaks have also been described due to health care professionals with colonized hands and poor personal hygiene;⁵ such individuals may act as opportunist carriers of an epidemic strain. Contaminated ventilators or respiratory care equipment as well as intra-hospital transmission may also contribute to the beginning of an outbreak.

Community-acquired pneumonia

Pneumonia acquired outside of the hospital setting and caused by *Acinetobacter* has been noted in Australia and Asia. The source of infection may be throat carriage, which occurs in up to 10% of community residents with excessive alcohol consumption. It is characterized by a severe and sudden onset coupled with secondary bloodstream infection and has a mortality rate of between 40% and 60%.

Bloodstream infections

In a seven year review (1995–2002) of nosocomial bloodstream infections in the United States, *Acinetobacter* accounted for 1.3% of all monomicrobial bloodstream infections. *Acinetobacter* was a more common cause of ICU-acquired bloodstream infection than of non-ICU-ward infection (1.6% vs. 0.9% of bloodstream infections, respectively, in those locations). Crude mortality figures overall from *Acinetobacter* bloodstream infection was 34.0% to 43.4% in the ICU and 16.3% outside the ICU. *Acinetobacter* bloodstream infection had the third highest crude mortality rate in the ICU, exceeded only by *P. aeruginosa* and *Candida* spp infections. It is notable that 102 patients had bloodstream infections at sites treating US military personnel injured in Iraq or Afghanistan from January 1, 2002 and August 31, 2004.

Battlefield trauma and other wounds

Acinetobacter is a well-documented pathogen of burns units and is difficult to treat in patients with severe burns. However, infection of the skin and soft tissue outside of a military environment is uncommon. A retrospective review of 57 patients with SSTI revealed that eight cases were infected with *Acinetobacter*. In this instance all patients were male, ranging in age from 13 to 55 and of both American and Iraqi nationality. The median time from trauma to diagnosis with *Acinetobacter* infection was 15 d. All eight patients had a similar clinical presentation of SSTI; characteristic cellulitis with “peau d'orange” appearance, severe infection resulted in formation of bullae on the skin surface. The mortality rate in this instance was 12.5% (i.e., one of the eight died; however given that the patient was admitted with a gunshot wound to the groin, mortality cannot be solely assigned to infection).

Meningitis

Nosocomial, post-neurosurgical *Acinetobacter* meningitis is becoming increasingly more common with many other Gram-negative bacteria also becoming problematic in post-operative care. Installation of an external ventricular drain becomes a site for opportunistic infection. The mortality rate may be as high as 70%; however it is not possible to discern the definitive cause of mortality.

Future therapies

Given the rapid and extensive development of antibiotic resistance, several attempts have been made to develop alternative control strategies for dealing with *A. baumannii* including, but not limited to the following:

Bacteriophage

Recently renewed interest in the area of antibacterial phage therapy has gained some traction. Due to the high specificity of phage and their ability to work quickly, bacteriophage therapy is being re-examined as an alternative treatment to help counteract the phenomenon of antibiotic resistance. Indeed, a recent study by Yang et al. has resulted in the isolation and characterization of the virulent AB1 bacteriophage which has been shown to be effective against *A. baumannii* and as such represents a novel therapeutic of some potential.

Bactericidal gene transfer therapy

The design and delivery of vectors containing bactericidal genes that can be introduced into recipient pathogenic

organisms by conjugation using attenuated donor cells is referred to as bactericidal gene transfer therapy. While the therapeutic potential of this approach is limited by the requirement for donor cells to be in contact with the pathogen (to facilitate vector transfer), positive effects have nonetheless been observed using murine burn infection models. Using this approach, Shankar et al. have shown that mice treated with a single dose of 10¹⁰ CFU of donor cells containing bactericidal genes had lower levels of *A. baumannii* in burn wounds compared with untreated mice.

Radioimmunotherapy

Although not yet not exploited as a therapeutic antimicrobial strategy in the clinic, radioimmunotherapy can target microorganisms as quickly and efficiently as cancer cells. This approach takes advantage of the specificity of antigen-antibody interactions to deliver radionuclides that emanate lethal doses of cytotoxic radiation directly to the target cell. Producing only transient hematological toxicity in experimental animals, radioimmunotherapy has been successfully adapted for the treatment of bacterial, fungal and viral infections. Given that previous studies have already described the development of antibodies against *A. baumannii*, the application of radioimmunotherapy as a novel therapeutic strategy for *A. baumannii* is a definite possibility.

Photodynamic therapy

Involves the combination of nontoxic photosensitizers (PSs) with oxygen and visible to produce reactive oxygen species that oxidize biomolecules thereby killing cells. The use of photodynamic therapy (PDT) to treat localized bacterial infections generally involves the topical application of a PS into the infected tissue, followed by illumination with red (or near-infrared) which is capable of penetrating the infected tissue. Using a murine burn wound model, this technique has previously been shown to be effective against *A. baumannii* while having no obvious effects on wound healing. Recently, Tsai et al. investigated the effect of chitosan, a polycationic biopolymer, on increasing the efficacy of PDT against a number of pathogens including *A. baumannii*. Under conditions in which hematoporphyrin-PDT exhibited a bacteriocidal effect on a 2- to 4-log scale, subsequent treatment with chitosan (0.025%) for a further 30 min completely eradicated the bacteria (at a starting inoculum of 10⁸ CFU/ml). Chitosan alone did not exert significant antimicrobial activity, without prior PDT, suggesting that the potentiated effect of chitosan worked only after the bacterial damage induced by PDT. Furthermore, the potentiated PDT effect of chitosan appears to be related to the level of PDT damage and the deacetylation level of the chitosan.

Nanoparticle Technology

Nitric oxide (NO) has been shown to exhibit potent antimicrobial activity as well as playing an important role in modulating immunity and regulating wound healing. Using nanotechnology based on a silane hydrogel, Friedman et al. have designed a stable nitric oxide (NO)-releasing nanoparticle (NO-np) platform. With the potential to serve as a novel, inexpensive and easily applied topical class of antimicrobials, this technology has been shown to be effective for the treatment of complex cutaneous infections such as those caused by *A. baumannii*. Indeed, Mihu et al. recently

demonstrated the effect of NO-np against *A. baumannii* using murine wound and soft tissue models. Compared with control animals, NO-np-treated mice exhibited significant reductions in bacterial burden, enhanced wound healing rates and less collagen degradation by bacterial collagenases.

Conclusions

In conclusion, *A. baumannii* is an important opportunistic and emerging pathogen that can lead to serious nosocomial infections. Its pathogenic potential includes the ability to adhere to surfaces, form biofilms, display antimicrobial resistance and acquire genetic material from unrelated genera, making it a versatile and difficult adversary to control and eliminate. The optimal treatment for *A. baumannii*, especially nosocomial infections resulting from multiple resistant strains, remains to be established. It is thus a clinical imperative that well-designed procedures are put in place to help guide clinicians on decisions regarding the current best therapeutic practice. Furthermore, new experimental approaches are warranted to develop and evaluate novel therapeutic strategies for dealing with *A. baumannii* infections.

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Nicotine exposure alone leads to pulmonary hypertension

A study conducted by a team of researchers at LSU Health New Orleans has shown for the first time that chronic exposure to inhaled nicotine alone increases blood pressure (hypertension), in both the body's general circulation and in the lungs that can lead to pulmonary hypertension. The study also found that nicotine-induced pulmonary hypertension is accompanied by changes in the size, shape and function (remodeling) of the blood vessels in the lung and the right lower chamber of the heart.

Although cigarette smoking is the single most important risk factor for developing cardiovascular and lung diseases, the role of nicotine in the development of disease has not been well understood. The researchers used a novel nicotine inhalation model in mice that closely mimics human smokers/e-cigarette users to examine the effects of chronic nicotine inhalation on the development of cardiovascular and pulmonary disease with a focus on blood pressure and cardiac remodeling.

The researchers documented that nicotine inhalation increased systemic systolic and diastolic blood pressure as early as the first week of exposure.

"The increase was transient, but was sufficiently long to pose potential health risks in individuals with preexisting cardiopulmonary conditions," notes Eric Lazartigues, PhD, Professor of Pharmacology at LSU Health New Orleans School of Medicine.

Pulmonary hypertension is also often associated with remodeling of the blood vessels of the lung. The study findings suggest that chronic nicotine inhalation leads to muscularization of previously non-muscular pulmonary arterioles (small branches of arteries leading to capillaries) consistent with increased right ventricular systolic pressure and pulmonary vascular resistance.

Right ventricle failure is a major cause of death in pulmonary hypertension. The researchers found an eight-week exposure to nicotine resulted in significantly higher right ventricular systolic pressure, as well as thickening of the walls and enlargement of the right ventricle.

"Interestingly, the adverse effects of inhaled nicotine

are largely isolated to the right heart, as we found no significant changes in left heart remodeling or protein expression," adds Xinping Yue, MD, PhD, Assistant Professor of Physiology at LSU Health New Orleans School of Medicine.

According to the Centers for Disease Control and Prevention, tobacco use is the leading cause of preventable disease, disability, and death in the United States. Based on 2018 data, about 34 million US adults smoke cigarettes. Every day, about 2,000 young people under age 18 years smoke their first cigarette, and more than 300 begin smoking cigarettes daily. Over 16 million people live with at least one disease caused by smoking, and 58 million nonsmoking Americans are exposed to secondhand smoke. In 2017, 25.2% of Louisiana high school youth reported currently using any tobacco product, including e-cigarettes. Among Louisiana high school youth, 12.3% reported currently smoking cigarettes.

"There is a frightening trend of increasing usage of e-cig and vape products in youths and young adults," says Jason Gardner, PhD, Associate Professor of Physiology at LSU Health New Orleans School of Medicine. "Recent high-profile cases of hospitalization and death following e-cig usage necessitate a greater understanding regarding the health impact of inhaled nicotine delivery systems. The current study clearly demonstrates the adverse effects of nicotine on both systemic and pulmonary blood pressure and cardiac remodeling. This study should help raise the awareness of the adverse effects of nicotine inhalation on the cardiopulmonary system and help formulate public health policies on e-cigarettes."

The LSU Health New Orleans research team also included Joshua Oakes, PhD, Postdoctoral Fellow; Jiayi Xu, PhD, Postdoctoral Fellow; Tamara Morris, BS, Research Associate; Nicholas Fried, BS, MD/PhD student; Charlotte Pearson, undergraduate student; Thomas Lobell, MS, Research Associate; and Nicholas Gilpin, PhD, Professor of Physiology.

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MEDIA-FILL TEST

'Sterile' is a powerful word, with harsh legal implications surrounding non-compliance. Global regulatory authorities would define sterile as 'free of viable organisms', and sterility assurance has become one of the most scrutinised areas of pharmaceutical and medical device manufacture. The favoured method of production of sterile pharmaceutical products includes a terminal sterilisation process, such as autoclaving or irradiation. Since it is not practical to examine every unit for confirmation of sterility, terminal sterilisation processes use biological indicators (BIs) to provide levels of sterility assurance. BIs are substrates carrying high loads of resistant micro-organisms, at levels far greater than the bioburden of the load being sterilised. If everything on the BI is killed, it is reasonable to assume that the load is also free of viable organisms and can be deemed sterile. However, many therapeutic agents would not withstand terminal sterilisation, so aseptic manufacture and aseptic filling processes are required.

Aseptic processing used to produce sterile parenteral drug products and Active Pharmaceutical Ingredients (APIs) involves the handling of pre-sterilised products in a highly controlled environment. Using the BI correlation approach is not applicable here, as aseptic processing involves ensuring a great deal of process control, with sensitive handling of products until they are sealed within their final containers.

All efforts are made to minimise the risk of contamination:

- ✦ Filling and support areas are engineered to minimise contamination
- ✦ Air in critical areas is supplied at point-of-use as high-efficiency particulate air (HEPA) filtered, laminar flow air at a velocity sufficient to sweep particles away from the filling and closing areas
- ✦ Positive air pressure is used to prevent ingress of airborne contamination: anything that can be sterilised must be rendered sterile before it can be taken into the clean area where the process is performed
- ✦ Human intervention is kept to a minimum
- ✦ Cleaning is thorough and validated
- ✦ Disinfection practices are tight and validated
- ✦ Monitoring is done to prove the process and environment are under control

Despite such measures, contamination is an ever-present threat, since there will always be a risk that materials and surfaces may carry organisms, and inefficiencies in air filtration may pose a risk. The largest source of potentially viable contamination comes from people – the operators running the filling process. Aseptic processing is a process being operated in a controlled – but not sterile – environment; the probability of non-sterility cannot be calculated. The industry works to recognised, accepted contamination levels, so the probability of viable contamination is recognised and calculated. Routine sampling for sterility testing is not sensitive enough to detect such low-level contamination. Sample numbers are too small, and only gross contamination is likely to be detected. Pharmaceutical manufacturers, therefore, need other means of guaranteeing the quality of their products. This is why process simulations (media fills) – supported by environmental monitoring and other related processes – are required. These are used to demonstrate control of the process to the industry standard for allowable contamination levels.

UNITED STATES PHARMACOPEIA (USP) GENERAL CHAPTER <797>: PHARMACEUTICAL

Compounding – Sterile Preparations recommends minimal requirements for personnel training and evaluation in aseptic manipulation skills. These guidelines apply to all organizations that prepare compounded sterile preparations (CSPs), and are enforceable by the FDA, individual state boards of pharmacy, and accreditation organizations. The aim of USP Chapter <797> is to set consistent compounding standards and increase patient safety.

What is a media-fill test?

Aseptic media-fill testing is used to quantify the aseptic technique of compounding personnel or processes and to ensure that the processes used are able to produce a sterile product without microbiological contamination. During this test, a microbiological growth medium, such as soybean-casein digest medium (SCDM), also known as trypticase soy broth (TSB), is substituted for the actual drug product to simulate admixture compounding. This process simulation, normally includes exposing the microbiological growth medium to product contact surfaces of equipment, container closure systems, critical environments, and process manipulations to closely simulate the same exposure that the product itself will undergo. After using TSB instead of an actual drug product to prepare a simulated sterile preparations (CSP), the final container is then incubated and checked for turbidity, which indicates the presence of microbial contaminants. Results are then interpreted to assess the potential for a unit of drug product to become contaminated during actual operations (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing). Environmental monitoring data from the process simulation can also provide useful information for the processing line evaluation. Process simulation studies should be designed to emulate the routine production process as closely as possible, including formulation, filtration, and filling stages. Processes will vary in relation to the type of product to be filled, e.g. liquid or solid dosage forms, and each process simulation is a unique event whereby extrapolation of outcomes cannot be directly linked to actual process contamination rates.

The study will be performed using microbiological growth media in place of active pharmaceutical ingredients (API). Microbiological growth medium, such as soybean casein digest medium, should be used. Use of anaerobic growth media (e.g., fluid thioglycolate medium) should be considered in special circumstances. The media selected should be demonstrated to promote growth of gram-positive and gram-negative bacteria, and yeast and mold (e.g., USP indicator organisms). The QC laboratory should determine if USP indicator organisms sufficiently represent production-related isolates. Environmental monitoring and sterility test isolates can be substituted (as appropriate) or added to the growth promotion challenge. Growth promotion units should be inoculated with a <100 CFU challenge. If the growth promotion testing fails, the origin of any contamination found during the simulation should nonetheless be investigated and the media fill promptly repeated (the cause of the growth promotion failure should also be investigated.) The production process should be accurately simulated using media and conditions that optimize detection of any microbiological contamination. Each unit should be filled with an appropriate quantity and type of microbial growth medium to contact the inner container closure surfaces (when the unit is inverted or thoroughly swirled) and permit visual detection

of microbial growth. Some drug manufacturers have expressed concern over the possible contamination of the facility and equipment with nutrient media during media fill runs. However, if the medium is handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary, sterilization of equipment, subsequently processed products are not likely to be compromised.

Modern culture media, designed for media fill trials, possess certain attributes that facilitate process simulations; they will be irradiated making them suitable for introduction into compounding areas, will dissolve in cold water and have known filtration performance as standard broth can be slow to filter or block the filter.

How is a media-fill test prepared?

USP Chapter <797> provides examples of media-fill test procedures that are considered an adequate representation of each

CSP Risk Level	Example of Media-Fill Test Procedure
Low	Within an ISO Class 5 environment, transfer, with same sterile 10-mL syringe and needle or dispensing pin, three sets of four 5-mL aliquots of sterile SCDM into three separate 30-mL sterile vials.
Medium	Within an ISO Class 5 environment, transfer six 100- mL aliquots of sterile SCDM by gravity through separate tubing sets into separate evacuated sterile containers. Arrange the six containers as three pairs, and use a sterile 10-mL syringe and 18-gauge needle to exchange two 5-mL aliquots of medium from one container to the other in the pair. Then, inject a 5-mL aliquot from each container into a sterile 10-mL clear vial (three total), using a sterile 10-mL syringe and vented needle or pin.
High	Dissolve 3 g of non-sterile commercially available SCDM powder in 100 mL of nonbacteriostatic water to make a 3% nonsterile solution. Withdraw 25 mL of the medium into each of three 30-mL syringes and transfer 5 mL from each syringe into separate sterile 10-mL vials. (These vials are positive controls and will generate exponential microbial growth, for comparison). Next, in an ISO Class 5 environment, affix a 0.2-micron filter and 20-gauge needle to the previously prepared syringes and inject 10 mL from each syringe into three separate 10-mL sterile vials. Repeat for three more vials, affix sterile adhesive seal, and label.

How is the prepared final container of media incubated?

The USP Chapter <797> Proposed Revisions indicate that vials should be incubated within a range of 20 to 35°C for 14 days. “Positive” test, is indicated by visible turbidity in the medium on or before 14 days. The American Society for Microbiology (ASM) asserts that in their “experience and opinion a range of 32°C ± 2°C covers a broader spectrum of potential contaminants and pathogens”. Therefore, a temperature range of 30 to 35°C would likely be acceptable to both the USP and ASM as a range of incubator temperature. It is suggested that a “media-fill results log” be kept, with results documented at days seven and 14 of the incubation process.

What does a turbid or “positive” media test mean?

A positive test could indicate that the compounding employee needs additional training and instruction regarding aseptic technique. Often, simple touch contamination can be the culprit, but a turbid test could also indicate that the controlled cleanroom environment was negatively compromised, possibly due to a malfunctioning blower/motor in a laminar air flow workstation (LAFW) or biological safety cabinet (BSC) or a leak in a hood or cleanroom HEPA-filter. In addition, for highrisk compounding, a positive test could indicate that the integrity of the sterilizing 0.2-micron filter was compromised.

What should be analyzed if the media-fill test is positive?

As part of the aseptic media-fill validation process, written policies and procedures should describe how your organization will meet the USP Chapter <797> requirements and provide employees with a step-by-step process for the media-fill activity. It is suggested that each employee performing media-fill activities have a

of the three risk levels – low, medium, and high – assigned to CSPs. Media is generally available in sterile vials and bags, and as sterile and nonsterile powder. Each organization should review the types of sterile compounding performed and mimic their own procedure as closely as possible. For example, the USP low- and medium-risk media-fill examples do not specifically mention the use of a sterile, lyophilized powder for reconstitution as part of the process. If any organization reconstitutes antibiotics from lyophilized powder in vials, this should be included in their media-fill validation process. The media-fill tests should mimic the most challenging or stressful conditions that might be encountered during the preparation (and sterilization, when applicable) of CSPs. Additionally, be sure to clear the compounding area of any real patient compounding records, labels, and drug vials, to assure that the TSB media will not be dispensed in error to a patient. Table 1 for the suggested examples listed in the current proposed revisions to USP Chapter <797>.

“mentor” for the activity – someone to verbalize the instructions, step-by-step throughout the activity, and to provide additional observation of aseptic technique. Additionally, the written policy should define the steps to take if a media-fill is positive. Recommended steps include re-training of aseptic technique (with a mentor) and a repeat media fill test. If tests continue to indicate microbial contamination, further testing of the hoods and cleanroom environment may be necessary. If highrisk compounding is performed during the media fill, filter integrity may need to be examined as a potential culprit. If environmental air sampling is being performed on a weekly (high risk) or monthly (low- or medium-risk) basis, review these results to see if any compelling trends are noticed in relation to areas where the positive media-fill activity was performed. All corrective actions and re-testing should be documented as part of the overall quality assurance process.

Along with other quality assurance measures, a robust media-fill program is a necessary step to validate the processes of organizations that prepare CSPs. Media-fill testing is just one part of a necessary overall quality assurance program. Alone, it may not provide enough data to fully validate compounding, but it is an important step in the overall pharmacy quality assurance process.

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