

Editorial

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Mini review section: *Corynebacterium diphtheriae* is a globally important Gram-positive aerobic Actinobacterium capable of causing the toxin-mediated disease, diphtheria. Diphtheria was a major cause of childhood mortality prior to the introduction of the toxoid vaccine. New *Corynebacterium* species and new human infections, caused by this group of bacteria, has been described recently. However, identification of *Corynebacteria* is still a challenge despite application of sophisticated laboratory methods.

Current Trends Section: Chlorine has been used as a disinfectant for the treatment of drinking water for more than 100 years. It is by far the most commonly used means of disinfecting water, and its effectiveness as a microbicide has been widely assessed (AWWA, 2000). While most conventional systems in developed countries treat water with chlorine gas, other common alternatives include calcium hypochlorite, sodium hypochlorite, lithium hypochlorite and chloro isocyanurates. Until recently, the isocyanurates were used chiefly in the disinfection of water for swimming pools and industrial cooling towers. They are also a common microbial agent in cleaning and sanitizing applications, including baby bottles and contact lens.

In Profile: Eva Engvall, born 1940, is one of the scientists who invented ELISA in 1971. Engvall, Eva was born on March 11, 1940 in Stockholm, Sweden. Bachelor of Science, University Stockholm, 1964.

Bug of the Month: *Micrococcus luteus* is a common isolate found in pharmaceutical clean rooms during environmental monitoring. The likely source is humans. Its isolation in clean rooms can point to the need for better aseptic technique and gowning practices.

Did you Know: A new perspective in the treatment of bacterial infections is offered by the use of nanomaterials and nanoparticles as novel and nontraditional antibacterial agents. In particular, more recently, graphene has been proposed as a novel antimicrobial material. Graphene is a single-layer sheet of carbon atoms that are packed closely in a two-dimensional (2D) honeycomb lattice. It has unique physicochemical properties including a high surface area, extraordinary electrical and thermal conductivity, and strong mechanical strength.

Best Practices: Milk is virtually sterile when it is synthesized in a healthy cow's udder (mammary gland). Cows, like humans, are natural reservoirs of bacteria. Many of these bacteria are not harmful to humans, but some may be harmful to humans even though the cows are not affected and appear healthy. The area of dairy microbiology is large and diverse. The bacteria present in dairy products may cause disease or spoilage. Some bacteria may be specifically added to milk for fermentation to produce products like yogurt and cheese.

“Laughing is and will always be, the best form of Therapy” 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* (Issue II)

Corynebacterium diphtheriae is a globally important Gram-positive aerobic Actinobacterium capable of causing the toxin-mediated disease, diphtheria. Diphtheria was a major cause of childhood mortality prior to the introduction of the toxoid vaccine. The number of opportunistic infections caused by Corynebacterias increasing due to increase in number of immunocompromised patients. New *Corynebacterium* species and new human infections, caused by this group of bacteria, has been described recently. However, identification of Corynebacteria is still a challenge despite application of sophisticated laboratory methods.

Introduction:

The genus *Corynebacterium* consists of a diverse group of bacteria including animal and plant pathogens, as well as saprophytes. Some corynebacteria are part of the normal flora of humans, finding a suitable niche in virtually every anatomic site, especially the skin and nares. Corynebacteria are Gram-positive, aerobic, nonmotile, rod-shaped bacteria classified as Actinobacteria. Corynebacteria are related phylogenetically to mycobacteria and actinomycetes. "Diphtheroids" or "coryneform" bacteria are recognized as causing opportunistic disease under specific circumstances, such as in patients who are immunocompromised, have prosthetic devices, or have been in hospitals/nursing homes for long-term periods of time.

Staining

1. Gram Stain

Corynebacteria are Gram-positive, catalase positive, non-spore-forming, non-motile, rod-shaped bacteria that are straight or slightly curved, with clubbed ends.



Fig No.1 *Corynebacterium diphtheriae* -Gram stain

Corynebacteria are pleomorphic rods that occur in angular arrangements. They undergo snapping movements just after cell division, which brings them into characteristic forms resembling "Chinese-letters" or "palisades". This is due to the incomplete separation of the daughter cells after binary fission. Their size falls between 2-6 micrometers in length and 0.5 micrometers in diameter. Corynebacteria do not form spores or branch as do the actinomycetes.

2. ALBERT'S STAIN (METACHROMATIC GRANULES)

Metachromatic granules are irregularly sized granules found in the protoplasm of numerous bacteria. It stains a different color from that of the dye used. Granules are usually present in

Corynebacteria, representing stored phosphate regions. They are composed of complex polyphosphate, lipid, and nucleoprotein molecules (volutin) and serve as an intracellular phosphate reserve. Called also Babès-Ernst body or granule.

Albert's stain Procedure

1. Prepare a smear and fix it by gently heat over a flame.
2. Cover the heat fixed smear with methanol and keep for 15 secs.
3. Drain and air dry the smear.
4. Cover the smear with Albert's Stain A (Cat no.: 207010500100) solution for 5 minutes.
5. Drain the solution. Do not wash.
6. Cover the smear with Albert's Stain B (Cat No.: 207010510100) solution for 2 minutes.
7. Pour off the stain after 2 minutes. Do not wash.
8. Blot to dry the smear.
9. Examine the smear under microscope, 40X and 100X in oil immersion lens

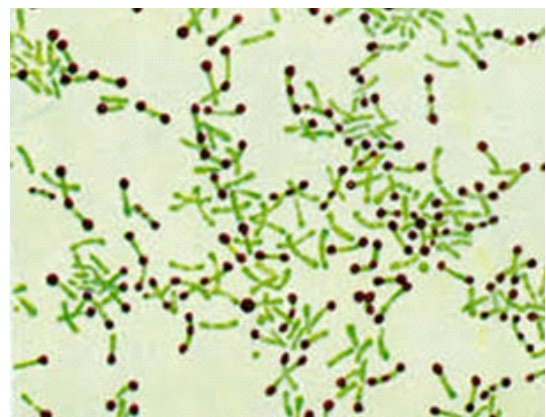


Fig No. 2: *Corynebacterium diphtheriae*-Albert's stain

Corynebacterium diphtheriae appears green coloured rod shaped bacteria with bluish black metachromatic granules at the poles.

Culture media

Most of the *Corynebacterium* species can be isolated from a 5% sheep blood agar-based selective medium containing 100 µg/ml of Fosfomycin as this group of bacteria is highly resistant to this compound. For lipophilic Corynebacteria, 0.1–1.0% Tween 80 should be added to the medium. Special recommendations for isolation of *C. diphtheriae* and other potentially toxigenic species (*C. ulcerans* and *C. pseudotuberculosis*) exist. Selective Tellurite blood agar media, such as Hoyle's agar medium and Clauberg's agar medium, are recommended. But recently published studies revealed that standard initial screening with Tellurite-selective medium for diphtheria bacilli identification may lead to false-negative results due to significant differences in tellurite resistance level of *C. diphtheriae* strains independent of origin, biotype or toxigenicity status. For confirmation of suspicious colonies, Tinsdale's medium, which enables detection of the enzyme cystinase, can be used as only *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* produce the characteristic black colonies surrounded by a brown halo after overnight incubation.

When potentially toxigenic species of *Corynebacterium* is detected, toxin testing should be performed. Toxin production can be investigated using *in vitro* Elek's test.

1. Blood agar

The causative agent of diphtheria is an aerobe or a facultative aerobe. The optimal temperature for growth is 37°C and the organism does not grow at temperatures below 15°C and above 40°C. The pH of medium is 7.2-7.6. *Corynebacterium diphtheriae* usually grows on media with blood with a weak beta-hemolysis (*C. diphtheriae* biotype *mitis* and *gravis*) or is nonhemolytic (biotype *intermedius*).



Fig No. 3: *Corynebacterium diphtheriae*-Blood agar culture

Three strains of *Corynebacterium diphtheriae* are recognized, *gravis*, *intermedius* and *mitis*. All strains produce the identical toxin and are capable of colonizing the throat. The differences in virulence between the three strains can be explained by their differing abilities to produce the toxin in rate and quantity, and by their differing growth rates. The *gravis* strain has a generation time (in vitro) of 60 minutes; the *intermedius* strain has a generation time of about 100 minutes; and the *mitis* strain has a generation time of about 180 minutes. The faster growing strains typically produce a larger colony on most growth media. In the throat (in vivo), a faster growth rate may allow the organism to deplete the local iron supply more rapidly in the invaded tissues, thereby allowing earlier or greater production of the diphtheria toxin. Also, if the kinetics of toxin production follow the kinetics of bacterial growth, the faster growing variety would achieve an effective level of toxin before the slow growing varieties.

2. TELLURITE AGAR AND TINSDALE AGAR

Tellurite Blood Agar—in which 0.4% tellurite inhibits other bacteria, diphtheria bacilli reduce tellurite to metallic tellurium which is incorporated in the colonies giving them a grey or black colour.

Tinsdale Agar (TIN) is used for the primary isolation and identification of *Corynebacterium diphtheriae*. The medium differentiates between *C. diphtheria* and diphtheroids found in the upper respiratory tract. It contains L-cysteine and sodium thiosulfate that are H₂S indicators.



Fig No. 4: *Corynebacterium diphtheriae*- Tellurite blood agar

Potassium tellurite is the selective agent (inhibits most of the upper respiratory tract normal flora) that turns the media brown-black as a result from the reduction of potassium tellurite to metallic tellurite. This differentiation is based on the ability of *C. diphtheriae* to produce black (or brown) colonies, surrounded by a brown/black halo. The dark halo is due to the production of H₂S from cystine, interacting with the tellurite salt (cystinase activity).

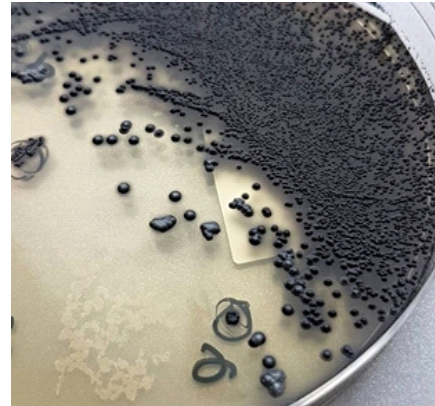


Fig No. 5: *Corynebacterium diphtheriae*-Tinsdale agar

ELEK TEST

The Elek culture plate precipitating test is routinely used for the detection of exotoxin from toxigenic strains of *Corynebacterium diphtheriae*. The test for toxigenicity, which detects the potent exotoxin, a phage-encoded protein, is the most important test and should be done without delay on any suspect isolate that is found by routine screening or while investigating a possible case of diphtheria. The toxigenic species *C. diphtheriae* acquire this characteristic when infected by the family of β-phages or other families of corynephages. The Elek test was first described in 1949 and replaced the in vivo virulence test in guinea pigs, a test that was used by many countries at that time.

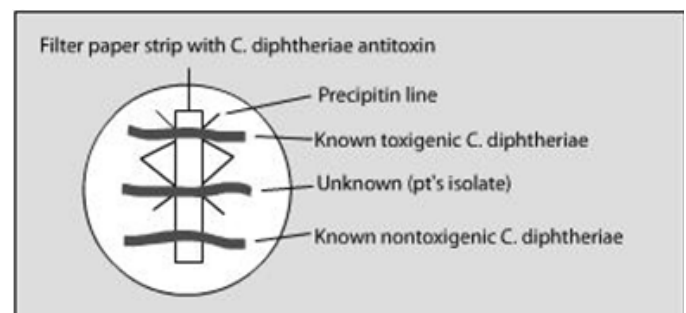


Fig No. 6: Elek's Test

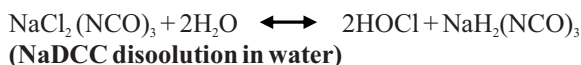
The Elek test principle

A filter paper strip impregnated with diphtheria antitoxin is buried just beneath the surface of a special agar plate before the agar hardens. Strains to be tested, known positive and negative toxigenic strains are streaked on the agar's surface in a line across the plate, and at a right angle to the antitoxin paper strip. After 24 hours of incubation at 37° C, plates are for the presence of fine precipitin lines at a 45-degree angle to the streaks. The presence of precipitin lines indicated that the strain produced toxin that react with the antitoxin.

SODIUM DICHLOROISOCYANURATE (NaDCC)

Chlorine has been used as a disinfectant for the treatment of drinking water for more than 100 years. It is by far the most commonly used means of disinfecting water, and its effectiveness as a microbicide has been widely assessed (AWWA, 2000). While most conventional systems in developed countries treat water with chlorine gas (delivered as a liquid in pressurized systems), other common alternatives include calcium hypochlorite, sodium hypochlorite, lithium hypochlorite and chloroisocyanurates (sodium dichloroisocyanurate or trichloroisocyanuric acid). Until recently, the isocyanurates were used chiefly in the disinfection of water for swimming pools and industrial cooling towers. They are also a common microbial agent in cleaning and sanitizing applications, including baby bottles and contact lens (Dychdala, 2001).

All of these compounds disinfect water by releasing free available chlorine (FAC) in the form of hypochlorous acid (HOCl). For example,



FAC (chlorine in the +1 oxidation state) is an effective biocide against a wide range of bacteria, fungi, algae, and viruses (White, 1998). Regardless of the original source of the available chlorine, the active microbicidal agent is hypochlorous acid. This also means that the most common method used in the field to assess the safety of drinking water—measuring FAC using the DPD reagent—is equally applicable with respect to water treated with NaDCC.

While both NaOCl and NaDCC rely on HOCl as the active agent, there are important differences in the performance of the two compounds. Unlike NaOCl which releases all of its chlorine as FAC, NaDCC releases only approximately 50% of the chlorine as FAC, the balance remaining as “reservoir chlorine” (bound) in the form of chlorinated isocyanurates (Bloomfield and Miles, 1979). When the FAC is used up, the equilibrium is disturbed, immediately releasing further FAC from the “reservoir” until the total available is used up. Thus, as shown in Figs. 1 and 2, the stabilized chlorine in NaDCC acts as a reservoir of HOCl which is rapidly released when the free available chlorine is depleted (Kuechler 1997, 1999).

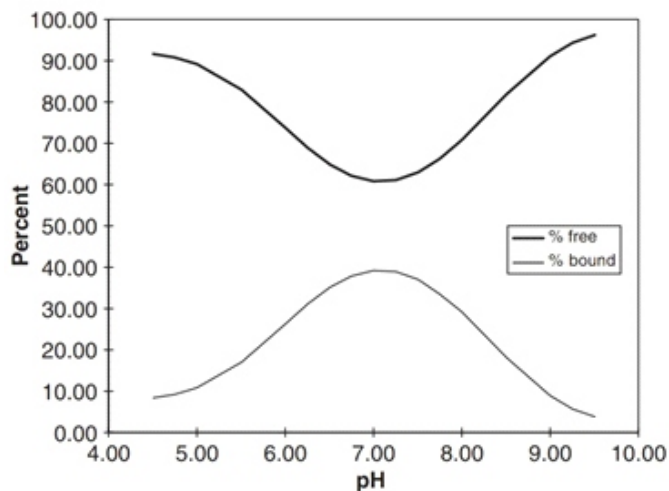


Fig. 1. Free and bound available chlorine in a solution of 1 mg/l NaDCC

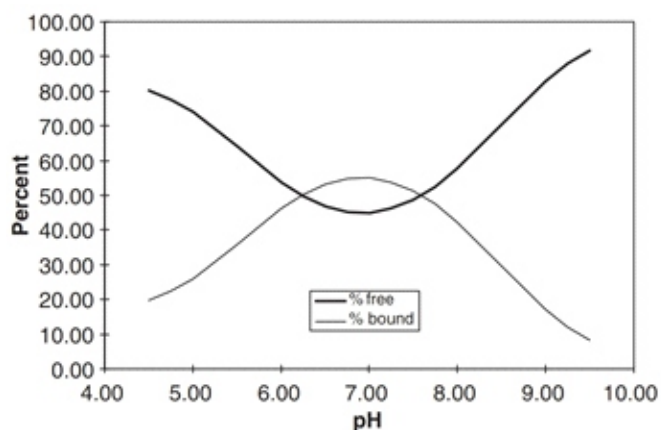


Fig. 2. Free and bound available chlorine in a solution of 3 mg/l NaDCC

This “reservoir” of FAC also enhances the biocidal protection over NaOCl when water is subject to high or variable organic loads (Bloomfield and Uso, 1985). Such conditions are common in some remote settings, forcing the use of more costly point-of-use water treatments (Crump et al., 2004).

NaDCC also presents certain advantages over NaOCl in those settings where the pH is high or variable. Hypochlorous acid is a weak acid, which tends to dissociate in water at increasing pH:



It is well known that chlorine loses its effectiveness to disinfect water at higher levels of pH, due to the dissociation of HOCl (Hurst, 2001). While 78% of chlorine exists in the active HOCl at neutral pH 7, at pH 8 the level drops to 26%. The capacity of NaDCC to continue to release significant amounts of HOCl

allows it to operate over a wider pH range (Dychdala, 2001). Moreover, in so far as NaDCC tablets are acidic in solution, (the effervescent base contributes to their acidity), they tend to reduce the pH of water favouring the formation of undissociated HOCl; hypochlorites, being alkaline, tend to disadvantageously increase the pH and, therefore, the dissociation of HOCl (Macedo and Barra, 2002). This is another parameter that is difficult to control or adjust for in household treatment in the field.

Even in a tightly closed opaque bottle, NaOCl has a recommended life of only 6 months after opening. Decomposition produces undesirable by-products (chlorite or chlorate ions). Internal testing under industry standards has shown that tabulated and strip packaged NaDCC, on the other hand, has a shelf life of 5 years in temperate and tropical climates. The stability and retention of chlorine activity has been cited as an advantage of NaDCC not only over NaOCl but also over other donors of free chlorine (Macedo and Barra, 2002).

Finally, the different presentation of the chlorine sources makes effervescent (self-dissolving) NaDCC tablets considerably more convenient to use than NaOCl. Bleach, though less hazardous than elemental chlorine, is a corrosive liquid subject to spillage. For water treatment, users typically measure out their commended dose using the bottle cap. NaDCC, on the other hand, is delivered as a solid tablet specifically sized to treat a given volume of water, typically 10 or 20 l in household applications. While liquid NaOCl (bleach) contains approximately 5% available chlorine, anhydrous NaDCC contains about 62%, roughly the equivalent of calcium hypochlorite. A single 67 mg NaDCC tablet, for example, can treat 20 l of clear water at a FAC dosage of 2 mg/l. (Two 67 mg NaDCC tablets are recommended for turbid waters, at a dosage of 4 mg/l FAC.) The potential for mis-dosing is minimized with the use of tablets, whereas the use of a bottle cap can lead to over or under dosing. Excess dosing would lead to an unpalatable level of residual chlorine and higher concentrations of potentially toxic chlorinated aromatic compounds (Crump et al., 2004). Investigators have found NaDCC to be advantageous to NaOCl in the production of trihalomethanes (Macedo, 1997).

Toxicity and Regulatory Issue

All chlorine products have some level of toxicity; this is what renders them such effective microbicides. When chlorinated water is ingested, however, the available chlorine is rapidly reduced by saliva and stomach fluid to harmless chloride ions salts (Kotiah et al., 1992). This is true for all sources of chlorine, including both NaOCl and NaDCC. The unique characteristic of the isocyanurates is cyanuric acid, the carrier that allows the chlorine to be contained in a solid, stable and dry form. It is the potential toxicity of such cyanuric acid therefore, that required review by regulatory agencies prior to the approval of NaDCC for the routine treatment of drinking water.

Cyanuric acid ($H_3C_3N_3O_3$), while confusingly similar in name, is not chemically related to cyanide. The toxicity of NaDCC and cyanuric acid have been extensively studied and documented in support of the registration of isocyanurates with the US EPA. These have been summarized (Hammond et al. 1986; US

Environmental Protection Agency (US EPA), 1992). Studies performed on acute toxicity and irritancy were intended to assess the safety of handling the dry product. These studies found chlorinated isocyanurates no more than slightly toxic and not corrosive. Chronic and sub-chronic toxicity studies also found no toxicity. Developmental toxicity studies have also established that the compound is not fetotoxic, teratogenic (causing birth defects), mutagenic or carcinogenic. Chlorinated isocyanurates are not metabolized in the body and do not bioaccumulate.

Under the US Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the manufacturer or distributor of disinfectants sold in the United States must be registered with the US EPA in a process required to demonstrate their safety and effectiveness. In July, 2001, OxyChem Corporation, the largest producer, secured such a registration for certain of its brands of isocyanurates for the routine treatment of drinking water. The US EPA approved label claims for NaDCC can be found at <http://oaspub.epa.gov/pestlabl/ppls.home> (registration number 935-41). NaDCC (up to 30 mg/l) is also certified by NSF International under NSF/ANSI Standard 60 (Drinking Water Treatment Chemicals-Health Effects), which extends to the health impact of water treatment additives (<http://www.nsf.org>).

In 2002, the WHO requested a review of the use of NaDCC as a disinfectant for drinking water as part of the rolling revisions of its Guidelines for Drinking Water Quality. The review was conducted by the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives (JECFA) and, like the EPA review, required the submission of detailed toxicological data. In June, 2003, JECFA recommended that the tolerable daily intake (TDI) for anhydrous NaDCC from treated drinking water be set at 0–2.0 mg per kg of body weight per day (WHO, 2004). Using standard methods (WHO, 1993) guideline values (GVs) for NaDCC can be derived from the TDI. This translates into a GV for adults (60 kg, with a daily drinking water consumption of 2 l) of 60 mg/l NaDCC; a GV for children (10 kg, with a daily consumption of 1l) of 20 mg/l NaDCC; and a GV for infants (5 kg, with a daily consumption of 0.75 l) of 13 mg/l. The dosage rate for Aquatabs, for example, is between 3.5 and 7 mg/l NaDCC (2–4 mg/l FAC), well within the JECFA value for daily intake (TDI).

Microbiocidal Effectiveness

As noted above, NaDCC is an alternative source of FAC (HOCl). Accordingly, the significant body of evidence on the antimicrobial action of chlorine is as relevant to NaDCC as it is to NaOCl and other sources of chlorine (White, 1998; Dychdala, 2001; CDC, 2005). While certain bacterial spores have shown greater resistance to NaDCC (Bloomfield and Arthur, 1992), thus at least suggesting the potential for differences in activity based on the chlorine donor, no differences have been reported in respect to waterborne pathogens. Susceptibility to hypochlorous acid has been established with respect to a wide variety of bacteria, including *Escherichia coli*, *Salmonella dysenteriae*, *Shigella sonnei*, *Campylobacter jejuni*, *Yersinia enterocolitica*; viruses including hepatitis A, poliovirus (type 1), rotavirus, adenovirus and calicivirus; helminthes; and protozoa, including cysts of *Entamoeba histolytica* and *Giardia lamblia* (Dychdala, 2001).

Microbicidal activity is a function of chlorine concentration and contact time (White, 1998; Bloomfield, 1996). At doses of a few mg/l and contact time of about 30 min, free chlorine inactivates more than 4 logs of most waterborne pathogens. *Cryptosporidium* has demonstrated considerable resistance to chlorination (Korich et al., 1990; Venczel et al., 1997) and *Mycobacterium* has also been reported as resistant (Taylor et al., 2000; Le Dantec et al., 2002). It should also be noted that in some cases, certain viruses have also exhibited greater resistance to chlorine and chlorine compounds than common bacterial indicators of faecal contamination (Hurst, 2001). This may have implications for determining the required concentration and contact time required to kill or deactivate potential pathogens in the untreated water collected for use in emergency and development settings.

A number of studies have compared the biocidal effectiveness of NaDCC with NaOCl and other disinfectants against a variety of microbes. D'Auria et al. (1989) assessed the antimicrobial activity of NaDCC among 29 Gram-positive and 29 Gram-negative bacteria, as well as 66 fungi. They reported good activity and significantly, no adverse influence by temperature and pH. Nascimento et al. (2003) found that at concentrations of 200 ppm, NaDCC yielded superior results compared to NaOCl and certain other agents used to sanitize fresh vegetables against aerobic mesophiles, molds and yeasts, total coliforms, *E. coli* and *Salmonella* sp. In another study at concentrations of 100 ppm, NaDCC was more effective than NaOCl against *Vibrio cholerae* (Eiroa and Porto, 1995). NaDCC has also been reported effective against encysted forms of *Acanthamoeba castellanii* (Khunkitti et al., 1996). Mazzola et al. (2003) compared the efficacy of NaDCC/sodium salt tablets with various chemical disinfectants, including a 10% solution of NaOCl on a variety of bacteria relevant to hospital settings. They recommended NaDCC over NaOCl for certain hospital applications due to its biocidal effectiveness, its slow decomposition and liberation of HOCl, its capacity to maintain an appropriate level of available chlorine without affecting the pH of the water, its low level of toxicity and its lower corrosivity against metal, plastic and rubber.

While NaDCC was shown to be comparable or superior to NaOCl in these studies of non-water treatment applications, we found few studies that compared the microbiological performance of NaDCC with other agents in respect of the treatment of drinking water. In one study, Aquatabs™ tablets containing 3.5 mg of NaDCC in an effervescent base were compared to Drinkwell™ (25 mg/ml NaOCl), and Hydroclonazon® (12.2 mg chloramine) and a generic solution of 2% iodine in ethanol. Except for the Hydroclonazon®, the agents performed comparably in removing all coliforms and *E. coli* from low turbidity water (NTU<1) and 1.8–2.8 logs of viable bacteria from raw river water (NTU>10) (Schlosser et al., 2001). The unimpressive results on more turbid water demonstrate a general weakness of chemical disinfectants. Notably, however, the required contact time for the NaDCC and iodine was 30 min compared to 60 min for the hypochlorite and chloramine based agents. In a further study, NaDCC tablets were recommended over chloramine tablets for use by the military owing to superior microbiological performance under a variety of polluted water conditions and lack of toxicity (Baylac et al., 1996).

Owing to its wide spread use by defense forces, water and sanitation departments and ministries of health in developing countries, the microbiological effectiveness of NaDCC tablets has been assessed by governmental investigators in Brazil, El Salvador, France, Honduras, Portugal, South Africa, Tanzania, Vietnam, and Zimbabwe. However, only one study has assessed the microbiological performance of the disinfectant in the field in the context of a household-based water treatment intervention (AfrozMolla, 2005). In that study, which involved a pilot program in Dhaka, 84% of samples from households using NaDCC tablets to treat their water were free of fecal coliform (FC) and the maximum level was 23 FC/100 ml, compared to 1000–2400 FC/100 ml in pre-intervention source water.

BioShields offers NaDCC in the brand name of Puresafe with 2 g and 20 g sachets. We are the first in healthcare industries, who launched NaDCC in powder form.

Applications of Puresafe are:

1. Water purification
2. Surface Disinfection
3. Dairy /Vegetables / Fruit Washing / Food Processing
4. Hospital Biowaste
5. Swimming pool usage
6. Poultry farm usage, etc.



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- BioShields Data

Eva Engvall

Engvall, Eva was born on March 11, 1940 in Stockholm, Sweden. Bachelor of Science, University Stockholm, 1964. Doctor of Philosophy in Immunology, University Stockholm, 1975.

Her postdoctoral work was done at the University of Helsinki and City of Hope National Medical Center in California, where she was subsequently appointed to staff. In 1979, Doctor Engvall was recruited to Sanford-Burnham Medical Institute in Louisiana Jolla, California (then called Louisiana Jolla Cancer Foundation). Foreign 1993-1996, Doctor Engvall held joint appointments at Sanford-Burnham Medical Institute and as Chairperson of the Department of Developmental Biology at Stockholm University.

Doctor Engvall received an honorary degree in Medicine from the University of Copenhagen in November 1994.

Enzyme-linked immunosorbent assay (ELISA) uses antibodies to detect proteins and other different immunogens. The ELISA technique was conceptualized and developed by two Swedish scientists: Peter Perlmann (principal investigator) and Eva Engvall at Stockholm University.

Engvall and Perlmann published their first paper on ELISA in 1971 and demonstrated its quantitative value using alkaline phosphatase as the reporter. Perlmann's further research included cytotoxicity of human lymphocytes and immunogen selection and epitope mapping for malaria vaccine development.

Peter Perlman died in 2005, but Eva Engvall continued her track as research scientist.

Engvall's group applied the ELISA measurement tool to parasitology, microbiology, and oncology. Engvall then focused her scientific interests on the biochemistry of tissues, e.g., fibronectin, laminin, integrins, and muscular dystrophies at Sanford-Burnham Medical Institute. Engvall's laboratory also tested the use of differentiation factors for muscle regeneration and myogenic cells from nonmuscle tissues for muscle cell replacement.

Since its invention ELISA now has a staggering number of analytical and clinical applications.



Jokes

An Investment Banker Was Getting Married.
During Wedding, The Wife Vomits.
Husband: "What Happened?"
Wife: "Capital Gains Arising Out Of Previous Investment."
Husband: "U cheated me.."
Wife: "U should know, mutual fund investments are subject to market risks!"

1 property dealer gives an ads for Lake View Flats in Kolkata.
When pappu bought that Flat He found something else insted of Lake View.
Property dealer called to Pappu to change Flat.
Pappu said - I do want to change Flats.
Infact there is College Girls Hotel View in place of Lake View.

A man went to Renown lawyer and told him,
"My neighbor owes me 50,000 Rupees and he won't pay up. What should I do?"
"Do you have any proof he owes you the money?" asked the lawyer. "No," replied the man.
"OK, then write him a letter asking him for the 500000 Rupees he owed you," said the lawyer.
"But it's only 50,000," replied the man. "Precisely. That's what he will reply and then you'll have your proof!"

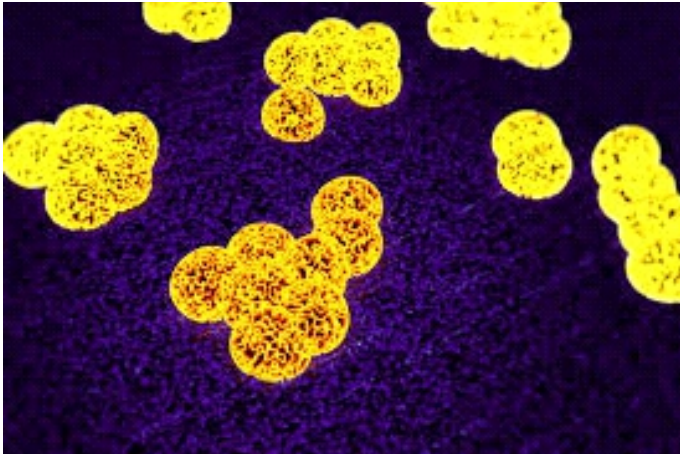
Boss hangs a poster in office(jokes)
'I am the boss, dont forget'
He returns from lunch,
finds a slip on his desk,
'ur wife called, she wants her poster back home..!!'

Quiz

1. The method in which the cells are frozen dehydrated is called
 - a) Pasteurization
 - b) Lyophilization
 - c) Disinfection
 - d) Dessication
2. Temperature required for pasteurization is
 - a) 50 degree celsius
 - b) Below 160 degree celsius
 - c) Above 200 Degree Celsius
 - d) Below 100 Degree Celsius
3. Which of the following method of sterilization has no effect on spores?
 - a) Autoclave
 - b) Hot Air oven
 - c) Drying
 - d) All the above
4. Temperature in pasteurization is
 - a) 62.8 Degree Celsius
 - b) 77 Degree Celsius
 - c) 68.2 Degree Celsius
 - d) 56 Degree Celsius
5. The condition required for autoclave
 - a) 121 degree Celsius temp. and 15 lbs. pressure for 20 min.
 - b) 120 degree Celsius temp. and 20 lbs. pressure for 30 min
 - c) 150 degree Celsius temp. for 1 h
 - d) 111 degree Celsius temp. for 20 mins
6. Temperature used for hot air oven is
 - a) 100 Degree Celsius for 1 hour
 - b) 120 Degree Celsius for 1 hour
 - c) 160 Degree Celsius for 1 hour
 - d) 250 Degree Celsius for 1 hour
7. Spores are killed by
 - a) 70% alcohol
 - b) Glutaraldehyde
 - c) Autoclaving
 - d) Both B & C

Answers: 1.b, 2.d, 3.c, 4.a, 5.a, 6.c, 7.d

Micrococcus luteus



Kingdom: Bacteria
 Phylum: Actinobacteria
 Order: Micrococcales
 Family: Micrococcaceae
 Genus: Micrococcus
 Species: *M. luteus*

As a pharmaceutical microbiologist, you are probably no stranger to *Micrococcus luteus* since it is among the most commonly found organisms in pharmaceutical environmental monitoring. The strain is typically associated with human skin, particularly from the head, arms and legs; consequently, inadequate aseptic techniques and gowning practices, and poor cleanroom management are frequent sources of *M. luteus* contamination.

Appearance:

Gram-stain positive, non-motile, non-spore-forming spherical cells. Cells occur in tetrads or irregular clusters.

Conditions for Growth:

Aerobic. Its optimal temperature range for growth is 25° to 37°C. It can grow at 45°C and in 10% Sodium Chloride. It will grow on a variety of media including Tryptic Soy Agar, Standard Methods Agar, Nutrient Agar, and Sheep Blood Agar.

Colony Morphology:

Colonies are circular, yellow, convex and smooth.

Habitat:

Found on the skin of humans and other mammals. It has also been isolated from foods such as milk, goat cheese and cassava fish.

Special Features:

Catalase positive. Lysostaphin resistant (*Staphylococcus aureus* is lysostaphin sensitive). *Micrococcus luteus* degrades the compounds in sweat into ones producing unpleasant odors. It also has the ability to degrade pollutants such as petrol.

Pathogenicity:

Although generally a harmless saprophyte, *Micrococcus luteus* can act as an opportunistic pathogen. It has been associated with a variety of illnesses including meningitis, septic arthritis, endocarditis, chronic cutaneous infections in HIV positive patients, and catheter infections.

Contamination Potential:

Micrococcus luteus is a common isolate found in pharmaceutical clean rooms during environmental monitoring. The likely source is humans. Its isolation in clean rooms can point to the need for better aseptic technique and gowning practices.

Family:

Micrococcaceae. Genus: Micrococcus. The family, *Micrococcaceae*, has been shrinking. Many members of the genus have been reclassified into other genera. For example, *Micrococcus varians* is now known as *Kocuria varians*. An interesting remaining family member, *Micrococcus antarcticus*, was isolated from Antarctica, and is capable of growing at 4°C.

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Graphene-based Nanomaterials as Novel Antimicrobial Drugs

A new perspective in the treatment of bacterial infections is offered by the use of nanomaterials and nanoparticles as novel and nontraditional antibacterial agents. In particular, more recently, graphene has been proposed as a novel antimicrobial material. Graphene is a single-layer sheet of carbon atoms that are packed closely in a two-dimensional (2D) honeycomb lattice. It has unique physicochemical properties including a high surface area, extraordinary electrical and thermal conductivity, and strong mechanical strength. Graphene and its derivatives (like graphene nanoplatelets, multilayer graphene flakes, graphene oxide, and reduced graphene oxide) are considered graphene-based nanomaterials (GFNs) and have been studied extensively in material science, chemistry, biotechnology, and nanomedicine for a wide range of applications including biosensing/bioimaging, disease diagnostics, drug delivery, and photothermal therapy. GFNs vary in shape, size, surface area, layer number, lateral dimensions, surface chemistry, stiffness, defect density or quality of the individual graphene sheets, and purity; and all these properties significantly influence the interaction of GFNs with biological systems. Generally, GFNs with small size, sharp edges, and rough surfaces easily internalize into the cell as compared to larger, smooth GFNs. GFNs, particularly monolayer graphene, have the theoretical maximum surface area because every atom lies on the surface, providing an extremely high capacity for drug delivery. In particular, more recently, graphene has been proposed as a novel antimicrobial material, with a strong cytotoxic effect on both Gram-positive and Gram-negative bacteria and fungi but a very low cytotoxic effect on human cells and animal models. In general, with respect to carbon nanotubes, graphene-based nanomaterials are preferable due to the lower production cost and ease of manipulation. With reference to antimicrobial application, graphene-based materials are preferred with respect to carbon nanotube due to their better efficiency against bacteria and ease of use. Reports indicate that GFNs exert cytotoxicity in both in vitro and in vivo studies in various types of bacteria, mammalian cells, and animal models. Among them, graphene oxide (GO) has good antibacterial effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus* compared to benzalkonium chloride one, a common surface disinfectant. Reduced GO (rGO) can be also used as an antibacterial surface when it is activated by solar near-infrared irradiation that gives it the ability to kill the majority of airborne bacteria on contact, proving to be a very efficient coating nanomaterial. It has been shown that these nanostructures also have a remarkable antimicrobial activity against some multidrug-resistant bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, and *P. aeruginosa*. Most published studies have evaluated graphene oxide (GO) and reduced GO (rGO) due to their better solubility/dispersibility/stability in water and under physiological conditions compared to other GFNs. However, it is demonstrated that GO and rGO induce formation of reactive oxygen species (ROS), which are representative of an induced oxidative stress on the cell. On the contrary, it is demonstrated

that multilayer graphene flakes and graphene nanoplatelets have cytotoxic effect on bacteria cell but without induction of ROS. Like in other types of nanocompound, the antimicrobial effect of graphene derivatives is defined by mechanical interactions damaging cell walls and by its chemical oxidation that lead to the generation of reactive oxygen species (ROS). Several works report the development and production of graphene-based nanomaterials, zinc-oxide nanostructures, and zinc-oxide-decorated graphene nanoplatelets for use as severe antibacterial and antibiofilm agents but without exerting relevant cytotoxic effect on human cells in vitro. Physical interaction between nanomaterial and bacterial cell leads to a direct damage of the cell wall, whereas chemical interaction leads primarily to formation of reactive oxygen species (ROS), which are representative of an induced oxidative stress on the cell. In particular, this developed nanomaterial does not induce ROS production on the cell, and for this reason it can produce a cytotoxic effect on bacteria and fungi but not on human cells and animal models. In general, the antibacterial action of nanomaterials and nanoparticles involves both physical and chemical effects. The interaction mechanisms that can be considered as the main cause of the antimicrobial effects of nanoparticles and graphene-based materials cannot be understood or expected without taking into consideration the fact that phenomena intrinsic to the nanoscale are governed by quantum effects and by the domain of the phenomena of surface and interface. It is known that nanostructures and nanoparticles are characterized by an increasing ratio between surface and volume atoms, as their size decreases. Therefore, nanoparticles are characterized by a much stronger surface interaction capability with other objects than micro-sized particles. In graphene, volume approaches zero and surface area infinity; it is thus understood that nanostructures have a much higher probability to get in touch and interact strongly with bacterial cell than microparticles. There are several interaction mechanisms between nanomaterials and cell walls. Among them is bacterial wrapping: this mechanism characterizes the interaction, for example, of graphene nanoplatelets with bacteria. The D-nanostructure adheres to the bacterium surface and induces mechanical stress. Several results report on the important role of 2D basal planes rather than edges in antimicrobial properties, in which completely flat Langmuir-Blodgett films act against bacterial cells having few contacts with sheet edges. This antimicrobial mechanism is a valuable alternative to biocide-releasing surfaces that uses antibiotics or silver, which are depleted from the surface over time. Antimicrobial GFN surfaces also avoid the release of toxic biocides, relevant in the design of antimicrobial surfaces for environmental applications.

Another antimicrobial mechanism is based on membrane puncturation: nanostructures adhere to the cell wall and penetrate through the membrane with their sharp edges. This mechanism is a characteristic of both 2D and 1D nanostructures (like GNPs and ZnO-NRs) and it is particularly effective in case of GNPs

decorated with ZnO-NRs (ZNGs), because the 2D shape of the supporting GNP enables the 1D ZnO-NRs decorating its surface to penetrate the cell wall [72]. Strategy to decorate GO nanosheets with structure-featured metal oxides was also addressed. In fact, these nanomaterials took advantage of the large specific surface area and morphological features from graphene, but also introduced the bactericidal activities of metal oxides simultaneously. Recently, the synthesis of Zn–CuO@GO nanosheets to apply as disinfectants has been carried out and demonstrated their activities to combat against multi-drug-resistant bacteria strains, such as a *E. coli* multidrug-resistant strain and a methicillin-resistant *S. aureus* strain. The nanosheets, inhibiting bacterial growth via physical damage, function as effective antibacterial agents. In this way, possible genetic mutation and development of other drug-resistant mechanisms might not be applicable.

Graphene is also able to induce antiadhesion of the bacterial cells over the substrate for biofilm formation and it is particularly effective in order to prevent biofilm formation.

The mechanism based on ROS generation does not seem to be activated by the nanostructures because graphene is fully reduced and ZnO is a biocompatible material. This limits the cytotoxicity induced on human cells *in vitro* or on animal models like *Caenorhabditis elegans* as it was observed in the case of GO. Moreover, the development of bacterial resistance to such nanomaterials is improbable because of their multiple and simultaneous bactericidal mechanisms. Various medical devices such as synthetic fibers, venous catheters, and surgical instruments have already been treated with nanoantimicrobial coatings, using silver nanoparticles in order to fight nosocomial infections and subsequent bacterial resistance.

Dairy Microbiology

Dairy is the term given to a facility for the extraction and processing of animal milk, mostly from goats or cows and sometimes also from buffaloes, sheep, horses or camels primarily for human consumption.

Milk is the secretion of the mammary gland of female mammals and is often the sole source of food for the very young mammal. The role of milk is to nourish and provide immunological protection. It is a complex biological fluid containing different components including water, lactose, fat, proteins, and minerals. Milk in its natural state is a highly perishable material because it is susceptible to rapid spoilage by the action of naturally occurring enzymes and contaminating microorganisms. However, it is desirably converted into a wide variety of milk products using a range of advanced processing technologies. The products include a variety of cheeses, yogurts, butter and spreads, ice cream, and dairy desserts.

In order to assess the quality of the final product; milk or processed milk, it is essential to know:

Initial Microflora of Raw Milk

The numbers and types of microorganisms in milk immediately after production (i.e., the initial microflora) directly reflect microbial contamination during production, collection, and handling. The microflora in the milk when it leaves the farm is influenced significantly by the storage temperature and the elapsed time after collection. Where milk is stored at -4°C , this low temperature normally will delay bacterial multiplication for at least 24 hours. The microflora, therefore, is similar to that present initially. However, if unsanitary conditions exist with the milking equipment or storage tank, the low temperature could mask these conditions.

A useful indicator for monitoring the sanitary conditions present during the production, collection and handling of raw milk is the "total" bacterial count or standard plate count (SPC). The SPC is determined by plating (or using equivalent procedures) on a standardized plate count agar followed by aerobic incubation for 2 or 3 days at 32°C or 30°C , respectively. Microorganisms failing to form colonies, of course, will not be counted. The SPC does not indicate the source/s of bacterial contamination or the identity of production deficiencies leading to high counts. Its sole value is to indicate changes in the production, collection, handling and storage environment.

Follow-up microbial assessments for psychrotrophs or thermotrophic bacteria, spore-forming bacteria, streptococci, and coliforms can assist in determining sanitary deficiencies.

Certain groups can be enumerated selectively. For instance, psychrotrophs can be counted either by incubating SPC plates for 10 days at $5 - 7^{\circ}\text{C}$ or by using a preliminary incubation of the raw milk at 13°C for 16 hours followed by performing the SPC procedure. Thermotrophic bacteria can be determined by laboratory pasteurization of milk before plating. Selective or

diagnostic media can be used for coliforms, lactic acid bacteria, mastitis pathogens, Gram-negative rods, lipolytic, proteolytic and caseinolytic microbial types, and so on. An increased number of automated methods are now being employed for plating and enumerating bacteria. Also, rapid quantifying techniques are being used, such as the direct epifluorescent filtration technique, adenosine triphosphate method and impedance measurements.

Microbiology of Milk and Dairy

Types of microorganisms present in raw milk

Pathogens for Humans in Raw Milk

Raw milk may contain microorganisms that are pathogenic to humans, and their source may lie either within or outside the udder. Historically, the most serious human diseases disseminated by the consumption of contaminated raw milk are tuberculosis and brucellosis. In both the diseases, the causative organisms that may be excreted in milk from infected animals are *Mycobacterium bovis* or *M. tuberculosis* and *Brucella abortus*, *B. melitensis*, or *B. suis*. Often with Brucella infections, there is little change in the milk or udder (i.e., mastitis is not present), but in the case of tuberculosis mastitis, a pronounced and characteristic change in the milk and udder is observed.

Pathogenic bacteria also may be present in raw milk as a direct consequence of udder disease. Among the organisms commonly producing mastitis, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli* are pathogens known to humans. *Streptococcus agalactiae* can initiate a variety of clinical conditions, the most serious of which are bacteremia and meningitis in newborns, which are potentially fatal to infected infants.

However, for humans the pathogenicity of bovine strains of *Streptococcus agalactiae* is uncertain and is carried by a large proportion of the human population. While it seems likely that the consumption of contaminated raw milk may play a part in infections of the population at large, some researchers have reported higher rates of *S. agalactiae* among consumers of raw milk who do not experience symptoms of a milk-borne illness.

Staphylococcal mastitis of the cow poses a more direct threat to public health because some bovine strains produce enterotoxin. Consumption of food containing enterotoxin leads to asymptomatic illness, usually of approximately 24-hour duration, characterized by nausea, diarrhea, and abdominal pain. The production of enterotoxin usually associated with the multiplication of staphylococci under favorable growth conditions during the storage of the milk. Because enterotoxin is relatively heat stable, subsequent pasteurization of the contaminated milk will not make it safe for consumption.

Further biohazards stem from the adventitious contamination of raw milk by pathogenic bacteria from sources external to the udder. Salmonellae and thermotrophic Campylobacter strains fall into this category and have produced many outbreaks of enteritis. Human carriers also may be sources of infection in milk-borne

outbreaks. This has been reported for Salmonella infections and for cases of scarlet fever or septic sore throat attributed to *Streptococcus pyogenes*.

All of these pathogens are destroyed by pasteurization, except *Clostridium perfringens* and *Bacillus cereus*, which can survive the pasteurization process because of their ability to sporulate. It is improbable, however, that *C. perfringens* will germinate and multiply under the modern-day conditions of milk storage.

Contamination of milk

Contamination of milk can vary widely depending on milk handling practices ranging from milking a few cows by hand in the out of doors to milking 3000 cows by a complex, automated system in a well-equipped parlor. There are however three basic sources of microbial contamination of milk: (1) from within the udder, (2) from the exterior of the teats and udder, and (3) from the milk handling and storage equipment.

Milk is produced at ambient temperatures ranging from sub zero centigrade, where it is necessary to protect milk from freezing, to above 25°C, where refrigeration is needed. Furthermore, the duration of milk storage time on the farm can vary widely. Therefore depending on the duration of milk storage on the farm before leaving can differ, often unpredictably, even under similar conditions.

In most dairying areas, milk production methods, equipment, and on-farm storage have improved over time. However, udder disease remains widespread because of the presence of mastitis-associated microorganisms. Refrigeration on the farm all too often masks the effects of unsanitary practices, including the use of inadequately cleaned and sanitized milking equipment. As a result, the microbiological quality of raw milk supplies produced under apparently good sanitary conditions and stored under adequate refrigeration may produce off-flavors, yield poor product, and present a risk of food-borne infections to the consumer.

Environmental sources

While the lactating animal, the production environment, and the milk handling equipment remain the principal sources for microbial contamination of raw milk, other environmental sources include the following:

Air

Air is not considered a significant source for microbial contamination in raw milk. Through its movement, air transfers soil and dust particles from a microbial-laden source into exposed milk surface such as soil and microbes. The main sources of airborne microbes may include the activity of factory personnel, ventilation and air-conditioning systems, the inflow of outdoor air and packaging materials. The potential contamination from bacterial biofilms is also of major concern because microbial cells may attach, grow and colonize on open exposed wet surfaces.

Outdoor Environment

The control of airborne microorganisms in the immediate surroundings of dairy premises is more difficult than in closed, indoor environments where more controlled measures can be taken. Natural agents such as UV light, humidity, temperature, wind direction, and speed have a significant influence on the total number of airborne microorganisms in the outdoor atmosphere.

The Milk Handler

When cows are hand-milked, the milk handler can contribute to an increased microbial load in the raw milk by dislodging dirt particles from the udder, increasing aerial contamination through accelerated air movement, and contacting the milk with infected hands. Risks of contamination from the milk handler are much less with machine milking.

The Water Supply

Water used in the milk production process should be of potable quality. This means that the water supply must be from an approved source free from pathogens and fecal contamination.

One must recognize that a potable water supply can become contaminated within the dairy production environment, such as in a farm storage tank that is not properly protected from rodents, birds, insects, and dust. Bacteria also may be introduced into the water supply through dirty wash troughs, buckets, and hoses. If untreated water gains access into milk or is used for rinsing equipment and containers, the microbes present in the water eventually will contaminate the milk.

The reasons why and how milk gets contaminated are numerous, therefore it is also necessary to know the means that are adopted for the treatment of milk so that dairy products are free from pathogens and other contaminating microbes.

Many processes have been developed over the years to enhance its utilization and safety. These processes can be grouped and analyzed in a variety of ways.

- Fractionation
- Concentration
- Preservation

Fractionation: The term is used to describe the fractionation or disassembly of the components of milk, utilizing their various properties of the individual components.

- Centrifugal separation, utilizing the density difference of the components. The most common equipment used is the disc bowl separator, which allows the separation of light and heavy phases and also allows removal of any sediment.
- Membrane separation, utilizing size or charge difference. This is normally a pressure – motivated, flow – dependent process, involving the use of a selective membrane, with a wide range of fractionation possible, from simple water removal to separation of different proteins.
- Ion exchange, utilizing charge difference. In this process, tiny resin beads exchange charged ions on their surface with charged ions or larger charged molecules in solution, removing them for subsequent recovery.

- Precipitation and Crystallization, utilizing differences insolubility and suspension stability.
- Filtration, utilizing size difference, the principle is similar to that already mentioned with membrane separation but involves the separation of larger components.
- Homogenization is a process of size reduction of the fat globules to prevent fractionation of the cream and skim milk by density difference. A combination of a high-pressure pump and special valves provides high shear.

Concentration Process: This grouping involves the removal of one or more components resulting in a concentration of the remaining components. Many of these processes also involve fractionation. The processes include the following:

- Evaporation, utilizing phase change of the aqueous component. An evaporator is a specialized heat exchanger operating under vacuum, facilitating efficient water vapour generation and removal from a liquid with minimal thermal damage to the remaining liquid.
- Freeze concentration, also utilizing phase change. This involves freezing and crystallization of the aqueous component of a liquid by refrigeration followed by crystal removal. It is not widely used in dairy processing.
- Here the permeate, or material passing through the membrane, includes water, enabling concentration of the retentate or material retained.
- Drying, utilizing phase change. This is a very important process, particularly in the production of milk powder, casein, and whey products. It involves water removal from a liquid concentrate or solid by heating with hot air.

Preservation Process: This category is primarily concerned with reducing microbiological and chemical change. They include the following:

- Pasteurization, thermalization, and sterilization utilizing heat to kill microorganisms. All these processes involve the transfer of heat into the product in order to raise the temperature to achieve a closely controlled time–temperature process (e.g., 72°C, 15 sec.) for pasteurization.
- Chilling and freezing, to slow microbial growth and chemical change and is widely used both during or prior to processing or for final product storage. Heat exchangers of the type can be used for liquid products, with cool stores and freezing chambers for finished goods.
- Reduction of pH, to inhibit microbial growth. This may be achieved by addition of acids or by bacterial fermentation of lactose.
- Dehydration (drying), to inhibit microbial growth and chemical change. The water content of dairy products influences the microbial changes and hence drying also serves the purpose of storage and to prolong the shelf life of the product.
- Salting, to reduce water activity and inhibit microbial growth. Salt may be added as dry granular salt or by means of a brine solution, with the product being immersed for a period in a tank of concentrated brine.
- Packaging, to contain the product, protect it, and reduce microbiological and chemical changes.

Heat Treatment of Milk

Pasteurization

This process is done to eliminate all non-spore forming pathogens commonly associated with milk. This process also effectively destroys spoilage organisms and thus contributes to product keeping quality under required refrigeration storage. The process of heating every particle of milk to at least 143°F(61.7°C) and holding at such temperature for at least 30 minutes, or to at least 160°F (71.1°C) and holding at such temperature for at least 15 seconds.

Ultra-Pasteurization (Extended Shelf-Life Milk)

In this process, milk is 'thermally processed' at or above 138°C for at least 2 seconds, so as to produce a product that has an extended shelf life under refrigerated conditions.

Ultra-High-Temperature Sterilization

Ultra-High-Temperature (UHT) sterilization is a process that combines rapid heating of milk to very high temperatures followed by aseptic handling and packaging to produce a shelf-stable, commercially sterile product. Though heat treatments for UHT vary indifferent countries, temperatures of 130–150°C with holding time of 1 second or more are prescribed, with holding times of 2–8 seconds commonly applied.

In-container Sterilization

Conditions specified for in-container sterilization of milk include temperatures from 105°C to 120°C for 20–40 minutes. With this strategy, milk is prefilled into cans or bottles that are hermetically sealed, and then the milk is heated in an autoclave or a batch or continuous retort. In-container sterilized milk products have expected shelf lives of a year or more with no refrigeration required.

Hazard Analysis Critical Control Points (HACCP)

The 'Hazard Analysis Critical Control Points' system offers a structured approach to the control of hazard in food processing and, when properly applied, identifies areas of concern and appropriate control measures before product failure is experienced. It represents a shift from retrospective quality control through end-product testing to a preventative quality assurance approach.

The HACCP procedure is generally targeted at food safety management (pathogenic microorganisms and their toxins), but, as an approach in the context of broader quality management, it can be effectively applied to microbiological spoilage, foreign-body contaminations or pesticide contamination. It is preferable to conduct a HACCP program with a narrow scope (a single pathogen or possibly pathogens) rather than attempt to cover an extended list of hazard areas then documentation tends to become complex.

Processed Dairy Products

Here there is a brief description of the various processed products that are made from milk.

Fluid Milk Products

This group of products falls into the 'consumer products' family, competing in the beverage sector of the grocery business. Their manufacture is relatively simple, involving *fractionation* processes such as centrifugal separation to produce cream, skim milk, or reduced-fat milk, *concentration* processes such as membrane separation (ultra filtration) to produce high calcium milk, and *preservation* processes such as pasteurization, ultra high temperature (UHT), and refrigeration to extend the safety and shelf life of the product range. Homogenization is used to prevent separation of the fat in the liquid product.

Fermented Milk Products

There are two groups in this family: (a) cheese products in which part of the original liquid is removed during manufacture as whey and (b) products in which there is no whey drainage, such as yogurts.

Both groups have a very long history of preparation and were probably developed by accident as a means of preserving milk. All or some of the standard food preservation tools of *moisture* removal, *acid* development, *salt* addition, and *temperature* adjustment may be used. The first letters of the italic words spell the conveniently remembered acronym MAST.

Cheese is an example of a fermented processed milk and its manufacture is a highly complex process. The composition of milk is adjusted or standardized (fractionation) by centrifugal separation and possibly also ultra filtration. For most cheese types, the milk will then be pasteurized (72°C, 15 sec) to reduce the risk from pathogenic organisms, adjusted to the desired fermentation temperature, and then pumped into a cheese vat. Starter culture consisting of a carefully selected species of lactic acid bacteria and a coagulate (e.g., calfrennet) are then added and the milk is allowed to coagulate. This is by destabilization of the case in micelle. This permits the beginning of the fractionation and selective concentration processes that form the basis of cheese making. Once the coagulum is of sufficient strength, it is cut into small particles and, by a process of controlled heating and fermentation, syneresis or expulsion of moisture and minerals (whey) occurs. Separation of the curd from the whey over a screen (filtration) follows. Depending on the cheese type, the curd may be allowed to fuse together (e.g., cheddar) or may be kept in granules (e.g., Colby). Salt may then be incorporated into the curd for preservation, as dry granules or by immersion in brine. The curd is pressed into blocks by either gravity or mechanical compression, and the cheese then goes into controlled storage conditions for final fermentation and maturation.

For fermented milk products, the process of manufacture is some what simpler. For example, in yogurt manufacture, the milk to be used is fortified with additional protein (skim milk powder or concentrated milk), severely heated (e.g., 95°C, 5 minutes) to reduce the microbial load and to encourage whey protein/casein interaction, cooled to fermentation temperature (e.g., 36°C), and transferred to a fermentation vessel. Selected cultures are then added, and fermentation is continued until the desired pH of around 4.5 is reached. This causes the coagulation of the vessel contents, and the plain yogurt is then cooled prior to possible incorporation of fruit and flavoring agents, followed by packaging.

When all the raw materials and the necessary equipment that comes in contact with milk is properly treated and adequate measures are taken for safety, there is a drastic drop in the number of organisms in milk. Some processes, however, ensure that milk supplied has no microbes at all, which serves as an excellent starting material for any process and also making the milk safe for consumption without prior heating.

Quality control in the Dairy Industry

The quality of food, such as milk and dairy products, may be defined as that sum of characteristics, which enables the food to satisfy definite requirements and which determine its fitness for consumption. In this sense, quality can be judged by means of sensory evaluation, its nutritive value, and according to its chemical, physical, and microbiological characteristics.

Cases of food-borne disease and food poisoning are becoming more and more common throughout the world. Both of these public health problems and the microbiological spoilage of foods can be minimized by the careful choice of raw materials and correct manufacturing and storage procedures. Achievement of such objectives requires, in many cases, monitoring at various stages to assess microbiological load or to look for particular microbial types.

Monitoring procedures commonly used include the air and water supplies in a factory environment, the hygiene of packaging material, and the sampling and testing of raw materials and end products.

The analytical procedures will include standard and rapid methods for assessing microbiological load and for enumerating and detecting specific microbial genera or groups.

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