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Editorial

Contents

Mini Review - Initially, it might be hard to imagine how microorganisms contribute to the fermentation process. How can such small and microscopic cells unseen to the naked eyes produce such high volumes of products? How such microorganisms can produced such a rich diversity of fermentation products which are useful to human beings?
The diversity of fermentation products produced by the microorganisms is attributed to the rich diversity of microorganisms which have diverse metabolisms that can yield various types of fermentation products.
The success of using microorganisms for fermentation lies in their very microscopic and metabolic characteristics. It is good being small!

Current Trends - The EPA requires manufacturers of disinfectants to prove efficacy prior to sale. Current FDA guidance suggests that validation studies should be as realistic as possible. For clean rooms and aseptic manufacturing environments, studies should include surface types found within the facility as well as recent isolates from facility monitoring programs.
The FDA requires drug manufacturers to validate disinfection procedures on a facility-by-facility basis, because facilities vary in terms of products used, surface types and predominant microbial contaminants.

Bug of the Month – ***Bacillus coagulans*** is a lactic acid-forming bacterial species within the genus *Bacillus*. *B. coagulans* is a Gram-positive rod (0.9 by 3.0 to 5.0 μm in size), catalase positive, spore-forming, motile, and a facultative anaerobe.
Bacillus coagulans has been added by the EFSA to their Qualified Presumption of Safety list and has been approved for veterinary purposes as GRAS by the U.S. Food and Drug Administration's Center for Veterinary Medicine, as well as by the European Union, and is listed by AAFCO for use as a direct-fed microbial in livestock production.

In Profile - Fanny Hesse (Born **Angelina Fanny Elishemius,** June 22, 1850 – December 1, 1934) is best known for her work in microbiology alongside her husband, Walther Hesse. Together they were instrumental in developing Agar as a medium for culturing microorganisms.

Did You Know? - An allergy is when your immune system reacts to a foreign substance, called an allergen. It could be something you eat, inhale into your lungs and inject into your body or touch. This reaction could cause coughing, sneezing, itchy eyes, a runny nose and a scratchy throat. In severe cases, it can cause rashes, hives, low blood pressure, breathing trouble, asthma attacks and even death.
There is no cure for allergies. You can manage allergies with prevention and treatment. More Americans than ever say they suffer from allergies. It is among the country's most common, but overlooked, diseases.

Best Practices - A risk factor is a proven disease indicator. Dental risk factors include a diet high in sugars and carbohydrates as well as dry mouth; while protective factors include fluoride in the local water supply, toothpaste, and vitamin supplements. Protective factors can also include good saliva flow and dental sealants.

"Laughter is the music of the soul" so ease your mind with the humour in our Relax Mood section.
So go on, enjoy reading & don't forget to give us your valuable inputs & feedback.

Microbes and Fermentation

Fermentation is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology.

The first solid evidence of the living nature of yeast appeared between 1837 and 1838 when three publications appeared by C. Cagniard de la Tour, T. Swann, and F. Kuetzing, each of whom independently concluded as a result of microscopic investigations that yeast is a living organism that reproduces by budding. It is perhaps because wine, beer, and bread were each basic foods in Europe that most of the early studies on fermentation were done on yeasts, with which they were made. Soon, bacteria were also discovered; the term was first used in English in the late 1840s, but it did not come into general use until the 1870s, and then largely in connection with the new germ theory of disease.

Louis Pasteur (1822–1895), during the 1850s and 1860s, showed that fermentation is initiated by living organisms in a series of investigations. In 1857, Pasteur showed that lactic acid fermentation is caused by living organisms. In 1860, he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization. In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, "*Etudes sur la Bière*", which was translated into English in 1879 as "Studies on fermentation". He defined fermentation (incorrectly) as "Life without air", but correctly showed that specific types of microorganisms cause specific types of fermentations and specific end-products.

Fermentation is the enzymatic decomposition and utililization of foodstuffs, particularly carbohydrates, by microbes. Fermentation takes place throughout the gastrointestinal tract of all animals, but the intensity of fermentation depends on microbe numbers, which are generally highest in the large bowel. Thus, the large intestine is quantitatively the most important site of fermention, except for species with forestomachs (ruminants). Further, there are major differences in the contribution of fermentation to energy production of different species. In carnivores like dogs and cats, and even in omnivores like humans, fermentation generates rather few calories, but in herbivores, fermentation is a way of life.

Large intestinal epithelial cells do not produce digestive enzymes, but contain huge numbers of bacteria which have the enzymes to digest and utilize many substrates. In all animals, two processes are attributed to the microbial flora of the large intestine:

- Digestion and metabolism of carbohydrates not digested in the small intestine (e.g. cellulose, residual starch)
- Synthesis of vitamin K and certain B vitamins

Cellulose is common constituent in the diet of many animals, including man, but no mammalian cell is known to produce a cellulase. Several species of bacteria in the large bowel synthesize cellulases and digest cellulose. Importantly, the major end products of microbial digestion of cellulose and other carbohydrates are volatile fatty acids, lactic acid, methane, hydrogen and carbon dioxide. Fermentation is thus the major source of intestinal gas.

Volatile or short-chain fatty acids (especially acetic, propionic and butyric acids) generated from fermentation are not only metabolized within intestinal epithelial cells, but can be absorbed by diffusion and thereby contribute fuel to systemic energy metabolism. The concentration of volatile fatty acids in the large gut is similar among mammals, but because of the enormous differences in the relative size of the large gut, the importance of microbial fermentation to energy production varies considerably among species. As examples, it has been estimated that the contribution to maintenance energy of volatile fatty acids produced in the hindgut is 6-9% in humans, 10-30% in pigs and only 2% in dogs, reflecting the relative size of their fermentation vats.

Synthesis of vitamin K by colonic bacteria provides a valuable supplement to dietary sources and makes clinical vitamin K deficiency rare. Similarly, formation of B vitamins by the microbial flora in the large intestine is useful to many animals. They are not absorbed in the large intestine, but are present in feces. The behavior of coprophagy or eating feces seen particularly in rodents, rabbits and other animals is thought to be a behavioral adaption to recovery of these valuable resources.

The success of using microorganisms for fermentation lies in their very microscopic and metabolic characteristics.

1. High surface area to volume ratio

Microorganisms are very very tiny creatures. Taking an example of a rod bacterium, we can see that it has six free surfaces that surrounds the bacterium. These six free surfaces interfaced with the surrounding environment from where they obtained their nutrients or to where they throw away their metabolic waste products.

With such a high number of free surface areas in a tiny volume of cell, it confers upon the bacterial cell a very high surface area to volume ratio. This very high surface area to volume ratio allows maximum or optimum surfaces for diffusions or molecular exchanges to occur between the microbial cell and the environment. No matter where the molecules are, they are easily accessible for diffusion into the microbial cell.

Once the nutrient molecules diffuse through the cell wall and membrane, it can be easily transported to where its needed in the cell. The size of the cell is so microscopic that distance covered in the transportation of the molecules in the cell is a convenience!

With such an ease of diffusion of nutrient molecules from the environment into the cell, and the diffusion of waste products from within the cell outwards to the surrounding environment we can see that metabolism will be at optimum state.

The efficient nutrient uptake coupled with the small size of the cell will allow for rapid synthesis and reproduction of new cells. Microorganisms under ideal state will double up within hours. Animals like elephants and human may take months to reproduce themselves.

2. Mode of nutrients transportation

The nutrients which diffuse into the microbial can either use simple diffusion process which is powered by the differences in the concentration gradients between the environment and within the cell. For very small nutrient molecules, most would diffuse by the mechanism of passive diffusion. Larger and complex molecules use active or group transport which requires expansion or utilization of energy

Microbes easily reproduce asexually. They will just as easily split their cells into two daughter cells which will later grow into larger cells and repeat the cycle.

3. Genetic adaptability

Microorganisms generally show the ability to adapt to new environment. They can get easily adapted to living under different environmental conditions and also adapting to new sources of carbon or substrate. This ability is the result of various genetic adaptation which selects "successful" strains through mutation and genetic recombination. Some of the bacteria are even equipped with plasmids which can synthesize new enzymes that help the microorganisms exploit the new environment. The very short generation times and the high population generation will aid the selection and recombination process.

4. Metabolic diversity

The unique thing about microbes are their metabolic diversity shown by various members of the microorganisms. They have the ability to use different energy sources and to use different types terminal electron acceptors

Their ability to use different substrates is also correlated with the microbes ability to produce a diversity of fermentation products.

Fermentation is the process involving the biochemical activity of organisms, during their growth, development, reproduction, even senescence and death. Fermentation technology is the use of organisms to produce food, pharmaceuticals and alcoholic beverages on a large scale industrial basis. The basic principle involved in the industrial fermentation technology is that organisms are grown under suitable conditions, by providing raw materials meeting all the necessary requirements such as carbon, nitrogen, salts, trace elements and vitamins.The end products formed as a result of their metabolism during their life span are released into the media, which are extracted for use by human being and that have a high commercial value. The major products of fermentation technology produced economically on a large scale industrial basis are wine, beer, cider, vinegar, ethanol, cheese, hormones, antibiotics, complete proteins, enzymes and other useful products.

Fermentation Methodology:

Fermentation process is carried out in a container called the fermentor or bioreactor. The design and nature of the fermentor varies depending upon the type of fermentation carried out. Invariably all the fermentors have facilities to measure some of the fermentation parameters like temperature, pressure, pH, elapsed fermentation time, liquid level, mass etc.

The different types of fermentors are:

(1) External recycle airlift fermentor—for producing bacterial biomass, with methanol as substrate.
(2) Internal recycle airlift fermentor—for producing yeast with oil as substrate.
(3) Tubular tower fermentor—Used for making beer, wine, vinegar etc.
(4) Nathan fermentor—used in brewing industry.
(5) Stirred fermentor—used for making antibiotics.

Types of Fermentation Processes:

There are three different process of fermentation viz.:

(1) Batch fermentation
(2) Feb-batch fermentation and
(3) Continuous culture.

Batch fermentation:

This term is attributed to that type of fermentation wherein there is change in culture medium, number of microorganisms and the amount of the product produced (i.e. the metabolite or target protein). In batch fermentation six phases of the microbial growth are seen.

(a) Lag phase:

Immediately after inoculation, there is no increase in the numbers of the microbial cells for some time and this period is called lag phase. This is in order that the organisms adjust to the new environment they are inoculated into.

(b) Acceleration phase:

The period when the cells just start increasing in numbers is known as acceleration phase.

(c) Log phase:

This is the time period when the cell numbers steadily increase.

(d) Deceleration phase:

The duration when the steady growth declines.

(e) Stationary phase:

The period where there is no change in the microbial cell number is the stationary phase. This phase is attained due to depletion of carbon source or accumulation of the end products.

(f) Death phase:

The period in which the cell numbers decrease steadily is the death phase. This is due to death of the cells because of cessation of metabolic activity and depletion of energy resources. Depending upon the product required the different phases of the cell growth are maintained. For microbial mass the log phase is preferred. For production of secondary metabolites i.e. antibiotics, the stationary phase is preferred.

Feb-batch fermentation:

In this type of fermentation, freshly prepared culture media is added at regular intervals without removing the culture fluid. This increases the volume of the fermentation culture. This type of fermentation is used for production of proteins from recombinant microorganisms.

Continuous fermentation:

In this type of fermentation the products are removed continuously along with the cells and the same is replenished with the cell girth and addition of fresh culture media. This results in a steady or constant volume of the contents of the fermentor. This type of fermentation is used for the production of single cell protein (S.S.P), antibiotics and organic solvents.

Procedure of Fermentation:

(a) Depending upon the type of product required, a particular bioreactor is selected.
(b) A suitable substrate in liquid media is added at a specific temperature, pH and then diluted.
(c) The organism (microbe, animal/plant cell, sub-cellular organelle or enzyme) is added to it.
(d) Then it is incubated at a specific temperature for the specified time.
(e) The incubation may either be aerobic or anaerobic.
i. Aerobic conditions are created by bubbling oxygen through the medium.
ii. Anaerobic conditions are created by using closed vessels, wherein oxygen cannot diffuse into the media and the oxygen present just above is replaced by carbon dioxide released.
(f) After the specified time interval, the products are removed, as some of the products are toxic to the growing cell or at least inhibitory to their growth. The organisms are re-circulated. The process of removal of the products is called downstream processing.

Lactic acid production

The use of lactic acid fermentation as a good preservation method is another ancient art of unknown origin. Lactic acid fermentation was investigated by Pasteur as one of his first microbiological problems. Lactic acid is commonly produced from the usual cheap sources of fermentable carbohydrates such as acid or enzyme hydrolysed corn and potato starches, molasses, and *whey. Whey,* the watery part of milk separated from curd during cheese making, is widely used in the manufacture of lactic acid. Whey represents a satisfactory medium for the growth of certain bacteria. It contains a relatively large amount of lactose and proteiuaceous substances, minerals, and some essential vitamins. The homofermeutative *lactobaeilli* such as *Lactobacillus bufgariau, L. delbrueckii,* etc., grow rapidly and convert the lactose to the single end product, lactic acid.

The typical fermentation process involved in making commercial calcium lactate and the principal grades of lactic acid is described in brief. Pasteurized whey is inoculated with a starter containing *L. bulgaricus.* To prepare a sufficient amount of inoculum the culture is built up by successive transfers in sterile skim milk, pasteurized skim milk, and finally, when fermentation is carried out at a temperature of 43^0C to discourage the growth of undesirable organisms. Fermenters and accessory equipment are fabricated with type 316 stainless steel to resist the corrosiveness of lactic acid. Combinations of glass and fluorocarbon resins*(teflon)* are also employed in the design of piping system, valve, filters, etc. During the fermentation, lime ($Ca(OH)_2$) is added intermittently to neutralize the acid and to promote a good yield of calcium lactate, At the end of fermentation, the lactalbumin is coagulated by heat, when lactalbumin settles, the solution of calcium lactate is decanted off and filtered. It is then treated with decolourizing carbon and filter aids, filtered, evaporated, and crystallized. The crystals are further purified and sold as calcium lactate or converted to lactic acid. Various procedures are followed in producing the different grades of lactates and lactic acid.

Lactic acid has many uses. It is used as ail acidulant in confectionery, fruit juices, and essences. It may be used in the curing of meat and in canned vegetable and fish products. Lactic acid is used in various chemical industries. The lactates also have important uses. Calcium lactate is used in baking powders and bread, and in the treatment of calcium deficiency. Iron lactate is used in the treatment of anemia. Sodium lactate is used to help in the retention of moisture by such products as tobacco and as a *plasticizer.* Lactic acid fermentation from whey also helps in the removal of pollution of our environment.

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Disinfectant Validation

The EPA requires manufacturers of disinfectants to prove efficacy prior to sale. Current FDA guidance suggests that validation studies should be as realistic as possible. For clean rooms and aseptic manufacturing environments, studies should include surface types found within the facility as well as recent isolates from facility monitoring programs.

The FDA requires drug manufacturers to validate disinfection procedures on a facility-by-facility basis, because facilities vary in terms of products used, surface types and predominant microbial contaminants.

"Each manufacturer must have a formal program governing the qualification, use and disposal of disinfectants."

Validation Method

Qualification Tests

A?Suspension method ?Validation of sanitizer?

B? Surface spray / immersion or wipe method ?Validation of Sanitization method?

Suspension Method

Objective:

To establish the test concentration and the contact time a suspension test is generally applied. The suspension test estimate the in vitro bactericidal activity of the disinfectant under precise experimental conditions including

- Microbial strain
- Preparation of inoculum
- Volume of inoculum vs. Disinfectant
- Temperature
- Disinfectant concentration and contact period
- Interfering substances ?i.e. inorganic, organic matter?

Surface spray / Wipe method

With the Spray / immersion or wipe method the following surfaces shall be taken for validation.

- Stainless steel ?SS?
- Epoxy
- Panel
- PU Paint wall ?Poly Urethane Paint?

Acceptance criteria

The decrease in the bacterial load to the exposed disinfectant indicates that the disinfectant is capable of reducing the contaminant when used in the area. That shall be minimum of 4-long reduction for non-spore forming microorganisms, Yeast and minimum of 3-log reduction shall achieve for Spore forming organisms, molds with the decided concentration.

Determine the contact period where the above said population log reduction of microorganisms achieved.

Validation Matrix

Following test matrix is prepared for the initial analytical method validation and revalidation criteria for Efficacy of disinfectants.

Sr. No	Test Description	Initial Validation	Revalidation
1	Suspension Method	All disinfectants used	Every new disinfectant
			Change in Disinfectant Concentration
			Change in Contact time
2	Surface Spray / Wipe Method	All disinfectants used	Every new disinfectant
			Change in Disinfectant Concentration
			Change in Contact time

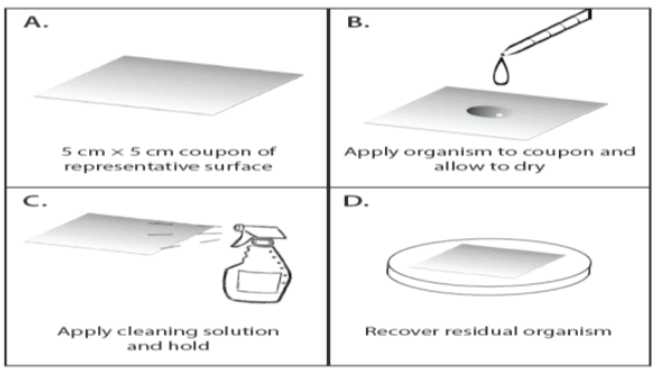
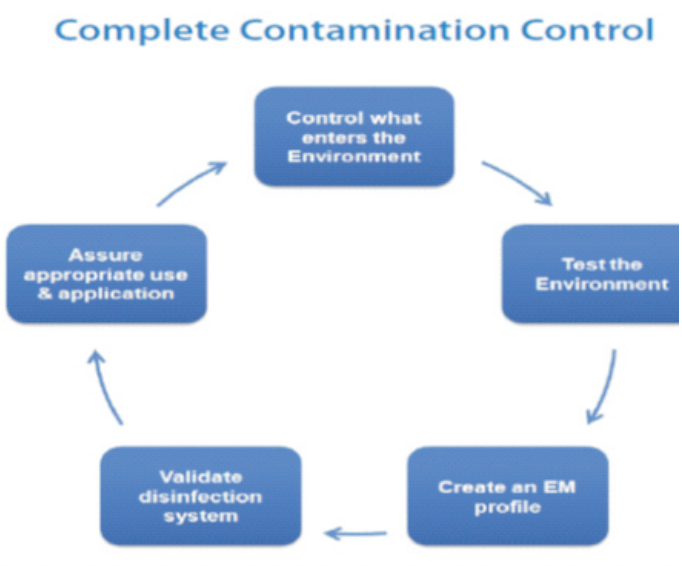


Figure 2. Overview of steps in a disinfectant efficacy study: A.) obtaining coupons representative of the surfaces that are disinfected; B.) drying challenge agent onto the coupon surface (with or without representative "soil"); C.) accurately mimicking the cleaning procedure using worst-case processing conditions; D.) recovery of any residual challenge agent.



Considerations for Disinfectant Efficacy Studies

Pre-Studies

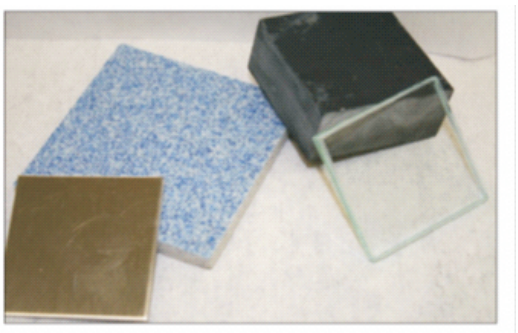
Prior to the initiation of the challenge experiments, it is essential to demonstrate whether the samples to be tested in the disinfectant efficacy study interfere with the detection of the challenge agents. These data verify that any decrease in the challenge agent is due to the disinfection procedure and is not the result of the disinfectant interfering with the endpoint assay used to detect the challenge agent.

Surfaces

5cm × 5cm (2" × 2") coupons of representative facility surfaces

are used in disinfection efficacy studies. It is important that the coupons are representative of the surfaces in the facility.The types of surfaces as well as the condition of the surfaces should be representative. For example, new stainless steel does not represent the same disinfection challenge as well as 'used' stainless steel, which can be pitted and can be more difficult to disinfect. Typical surfaces include:

- Stainless Steel
- Glass
- Vinyl flooring
- Epoxy coated wallboard
- Fiberglass
- Lexan (plexiglass)
- Vinyl curtain
- Tyvek
- Terrazzo tiles
- Plastic, polycarbonate



Coupons of surfaces representing the type and condition of those present in a manufacturing facility are used for the disinfectant efficacy study.

Disinfectants

The disinfectants evaluated in a disinfectant efficacy study must represent those that are in use in your facility. They may include formulated and ready-to-use agents. For a disinfectant efficacy study, formulated disinfectants will be prepared as they are routinely prepared in your facility, using a similar quality of water and/or following similar sterile filtration procedures. In order to represent a "worst-case", as is typically recommended for efficacy studies, suggests aging disinfectants to just beyond their expiration date before use in the study.

Challenge Spike

Microorganisms display varying levels of susceptibility to disinfection, and some require high level disinfectants for inactivation. There is a general hierarchy of disinfection; however, this is not an absolute representation. There are instances where a generally less resistant microorganism may display uncharacteristic resistance under certain disinfection conditions. Environmental isolates are typically more resistant to disinfection than the related laboratory strains. Some disinfectants are unlikely to be effective against resistant microorganisms. Typical challenge spikes include mycoplasma, bacteria, fungi, spores, viruses and transmissible spongiform encephalopathies (TSEs), but the selection of agents to use as a challenge for your cleaning procedure should be tailored to your product and your manufacturing facility. The selection should be based on the nature and origin of the raw materials used in your manufacturing process and their potential contaminants.

In Vitro Options for Testing

AOAC→Use-dilution Test→Sporicidal Activity of Disinfectants Germicidal →Spray Products as Disinfectants→ ASTM→Time Kill Method→Spray Slide→Sanitizer method (E1153)→Wipe method→Quantitative Carrier Method (E2111 & E2197)→Biofilm Method (E1427) Viral Testing (Suspension E1052)→Viral Testing (Carrier E1053)→Standard Guide for Evaluation of Cleanroom Disinfectants (E2614-08) →Variations of all of the above

More In Vitro Options for Testing

EN→1276 (bacterial suspension test)→1040 (bacterial suspension test)→1650 (fungal suspension test)→13704 (sporicidal suspension test)→13697 (Carrier test)→14476 (Viral Testing)→14348 (TB Testing)→

AFNOR (France)→NFT 72-150 Suspension→NFT 72-190 Carrier Test

DGHM Suspension Test (Carrier & Suspension Tests)→

TGA (Australia)

Testing at Contract Labs & Industry

European Testing is generally EN-13697 Hard Surface Testing Method – Log Reduction Values commonly used are from USP 35 (3 Log for Vegetative Bacteria and 2 Log for Bacterial and Fungal spores) – Some contract labs will conduct ASTM Methods E2111 or E2197 ● Some companies develop their own coupon testing methods ● Some companies use Modified AOAC testing methods ● Costs at contract labs includes contact time, surface, and test conditions. Viral testing more expensive. There are no currently harmonized disinfectant efficacy testing methods between the US and EU.

The study design and method used for in vitro testing of disinfectants by a pharmaceutical manufacturer must be carefully planned and be scientifically justifiable to the regulatory authorities. USP <1072> provides very little guidance on how these studies should be performed. While USP <1072> does refer to AOAC (Association of Official Analytical Chemists) methods, these are not necessarily appropriate when qualifying a disinfectant for use in a pharmaceutical facility and moreover, some AOAC tests, such as Use-Dilution Method require exceptional expertise as they are very technique dependent and often difficult to perform consistently. Unfortunately, there is not one perfect testing method. However, there are several published methods that do provide good general information for performing these studies and that can be modified and adapted for use in disinfectant qualification testing.

Such examples include, the ASTM E2197- 02(American Society for Testing and Materials) Quantitative Carrier Test (QCT) and the European Norm EN13697. These methods utilize stainless steel disks (other surfaces can be adapted) inoculated with the challenge microorganism that are treated with the disinfectant followed by neutralization and quantitation of survivors in order to establish the activity of the product.

In-situ testing

In situ testing demonstrates that the disinfectant or sporicidal agent in conjunction with preparation procedures and application procedures used by the facility and employees are effective at maintaining the environmental microbial levels deemed necessary for production of the target product. Efficacy of the disinfection program is demonstrated through evaluation of environmental monitoring data both over time and during "worst-case" remediation events. For example, many firms will compare environmental data pre and post decontamination after a preventative maintenance shut-down, when the room is more likely to show relatively high levels of environmental contamination. It is critically important that the procedures used to decontaminate the area during the in situ evaluation reflect the

written SOPs, as evidenced by regulatory feedback, "There is a lack of written procedures assigning responsibility, providing cleaning schedules, and describing in sufficient detail the method, equipment and materials to be used for sanitation. Specifically, your firm does not maintain written and approved procedures for the cleaning/disinfection of equipment and materials." (FDA 483, June 11, 2013). Clearly, the personnel who are assigned to perform these functions, must have sufficient training and oversight. Failure to have and/or to follow written procedures, problems with cleaning, sanitization, and maintenance, and failure to provide sufficient training are amongst the most frequently occurring FDA 483 observations.

USP 1072 provides some general guidance for in situ testing, "To demonstrate the efficacy of a disinfectant within a pharmaceutical manufacturing environment, it may be necessary to conduct the following tests...a statistical comparison of the frequency of isolation and the numbers of microorganisms isolated prior to and after the implementation of a new disinfectant." Further, the FDA Aseptic Processing Guide from 2004 states, "the effectiveness of these sanitization procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces (i.e., via obtaining samples before and after sanitization)." It is clear that evaluation of surfaces in order to compare contamination levels before and after sanitization or disinfectant treatment is an expectation in substantiating disinfectant performance.

Environmental monitoring and trending

Environmental monitoring practices, including frequency, location, and number of samples per sampling interval, should be based upon best available guidance documents and a valid scientific rationale suited to the type of product being manufactured. That being said, a single day of environmental monitoring data is but a snapshot in time, and cannot, alone, convey much useful intelligence about the state of control of a manufacturing area. Ongoing environmental monitoring, with data trending, is further validation that a holistic contamination control program is effective. It is recommended that any organisms detected be identified to the species level, and that they be stored for inclusion in future in vitro studies. Data should be reviewed periodically for negative trends; once a month is a common frequency. Additionally, criteria must be established for identifying a negative trend. "Procedures do not define how data must be presented in the trend reports generated by... The investigations include environmental data for the aseptic area that is reviewed for trends.

Summary

Disinfectant validation is a process that includes in vitro studies, where the disinfectant or sporicidal agent can be evaluated under highly controlled conditions; in situ evaluations which demonstrate how effective the disinfectant or sporicidal agent is under actual use conditions (typically conducted in a worst-case environment); and routine environmental monitoring with trending and assessment of negative trends. While there is no single regulatory or advisory document available that offers a blueprint for development of a disinfectant validation study, there are several documents and references, including FDA 483 observations and Warning Letters, which both highlight pitfalls and offer solid input on study design.

http://www.a3p.org/index.php/articles-techniques-et-scientifiques/1374-disinfectant-validation-a-roadmap-for-regulatory-compliance-la-vague-45.html

What do you know? What the Vendor tells you

- Chemical makeup
- Recommended prep method
- Efficacy using AOAC International
- Against ATCC organisms
- Usually 10 minute contact time
- In vitro testing

– Suspension testing (also called Time Kill Study)
– Carrier Testing (also called Coupon Testing)

- In situ testing
- Environmental monitoring

– Data trending (6-12 months, reviewed monthly)
– Identification of organisms (mold, yeast, and bacteria)

End-User Disinfectant

Validation Components

- Estimate the in vitro bactericidal activity of the disinfectant under precise experimental conditions

including

– Microbial strain
– Preparation of inoculum
– Volume of inoculum vs. disinfectant
– Temperature
– Disinfectant concentration and contact period
– Interfering Substances (i.e. inorganic-hard water, organic-soil load: serum or skimmed milk)
– Subculturing techniques
– Neutralization

- Chemical
- Dilution (alcohols) and washing

Suspension Testing

- Estimate the in vitro bactericidal efficacy when reproducing surface disinfection conditions including

– Substrate
– Application technique
– Spray, immersion or wipe
– Drying time
– Surface Area vs. Inoculum
– Interfering substances (i.e. inorganic-hard water, organic-soil load: serum or skimmed milk)

- Issues

– Recovery from surface
– Surface condition (i.e. rusting, pitting, flaking)

Most Common Causes for Failures in Efficacy Testing

Testing alcohol against bacterial endospores

- Using AOAC test methods for coupon studies
- Last second planning
- Contact time of <5 minutes for bacterial spores and fungal spores

Viability of Inoculum

- Making sure the bacteria are in the Log phaseof growth
- Making sure to isolate the fungal spores with a glass gauze fritted filter (testing spores and not mycelia or mycelial mat)
- Checking the viability of the culture and making sure no cross contamination is present

Surface Type and Condition

Visually smooth surfaces can be irregular
Older or damaged surfaces can be more challenging
Glass and stainless steel typically the least challenging

Recovery Method Issues

Typical surface recovery methods

– Contact plates (rarely used)
– Swabs
– Direct inoculation of coupons into neutralizing media
- Requires sterile coupons
- May include manual or automated dislodging

– Stomacher bags
- Recovery method must be validated
- Final plates must be countable to calculate log reduction

Recovery Issues
- Going for to high of a log reduction 10-6
- Drying to long leads to higher log reductions
- Final plates are TNTC

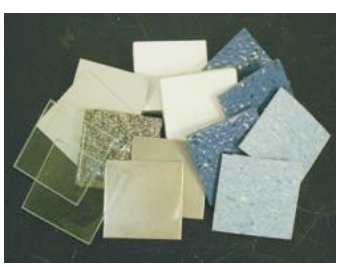
Disinfection Qualification

Two of the most common methods suggested for disinfection qualification in these environments are:
- Tube method: This method evaluates disinfectants by inoculating dilutions of the disinfectant and determining the microbial reduction. It would most commonly be used as a simple screening to determine the type of disinfectant most effective against a specific set of organisms before performing a comprehensive disinfectant qualification.
- Coupon method: This method is more comprehensive and uses coupons made from actual facility surfaces. The surfaces are inoculated and exposed to the disinfectant. The inoculum is then removed from the surfaces and the $\log_{10}$ reduction determined.

Determine the Surface Types to be Tested

Each of the construction materials used in the clean room and/or other controlled areas should be tested separately. Examples of common materials are stainless steel, glass, plastic, and Plexiglas®. Normally 2-inch by 2-inch square coupons are used for the qualification.

All coupons must be sterilized or disinfected before use in the qualification. Depending on the material, sterilization may be accomplished through steam, ethylene oxide (EO), or chemical methods.

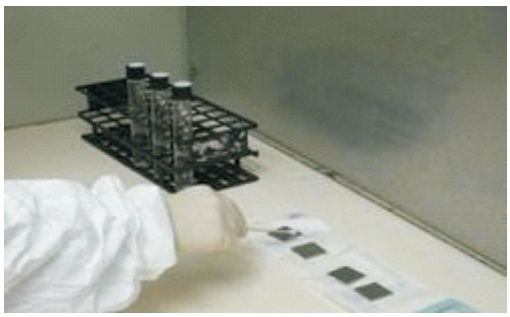


Method Validation

Method validation is a critical step to verify that the testing method allows adequate recovery of the challenge organisms in the presence of the disinfectants. Regardless of the method being used, the test system must be inoculated with a low level of challenge organism, with and without (control) exposure to the disinfectant for the designated contact time. Typically, the recovery of the challenge organisms should be within a factor of two of the positive controls for that organism. If the recovery is not satisfactory, the testing method should be repeated using a different neutralization system and/or additional dilutions. –

Efficacy Testing

Efficacy testing is the actual testing of the disinfectant. Per the USP General Chapter <1072> Disinfectants, the test system is inoculated with sufficient inoculum to demonstrate at least a two $\log_{10}$ reduction for bacterial spores and a three $\log_{10}$ reduction for vegetative bacteria and allowed to dry. The inoculated system is then exposed to the desired concentration of the disinfectant for the desired contact time.



Swabbing the inoculated coupons

The surviving population in the test system is determined and the $\log_{10}$ reduction calculated. The log reduction data should be used to establish a scientifically supported disinfection program for the client's facility.

What do you know?

What the Vendor tells you
- Chemical makeup

Label lists actives/concentration, MSDS lists only hazardous ingredients
- Recommended prep method (use-dilution)
- Efficacy using AOAC

Tested against ATCC organisms
- Usually 10 minute contact time

What you need to know

How the disinfectant performs:
- in YOUR facility
- prepped by YOUR procedures
- applied by YOUR methods
- with YOUR contact time
- on YOUR surfaces
- against YOUR resident microbes

Qualification and Validation
- Qualification could involve assessing vendor data against ATCC microbes, i.e. disinfectant is "qualified for use"
- May be verified with a suspension test against ATCC recommended microbes
- Validation typically involves coupon studies with in-house environmental isolates from the facility
- To FDA, "validation" typically refers to a process
- In-house isolates should include yeast, bacteria, spore forming bacteria and mold, and possibly Viruses

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http://www.cbinet.com/sites/default/files/files/Polarine_Jim_pres.pdf
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Fanny Hesse

The forgotten woman who made microbiology possible

In the earliest days of microbiology, scientists were stumped about how to isolate bacteria. That is, until the family cook—a woman named Angelina—changed everything by bringing her culinary insight into the lab. Before Angelina, the work of classifying different bacteria seemed hopelessly complex. Unable to differentiate them, Linnaeus classified all bacteria in the order Chaos in 1763. (Today, *Chaos* is a genus of giant amoebae.) In the 1800s, scientists studying the spots of fungus growing on moldy bread and meat began to realize that each spot was an individual species of microorganism, which could be transferred to a fresh piece of food and grown in isolation. Inspired by these early food-based studies, Robert Koch used thin slices of potatoes as naturally occurring "Petri dishes" when he began his studies of bacterial pathogens.

New techniques to isolate grow and study the behavior of individual species of microorganisms were developed in Koch's lab in the last decades of the 19th century. In a 1939 article, Arthur Hitchens and Morris Leikind described the history of these crucial microbiological techniques and the development of the solid medium still used in labs today. They begin by writing that Robert Koch's "genius lay in his ability to bring order out of chaos. Starting as it were with a box of miscellaneous beads, varying in size and shape, each bead a scientific fact, he found a thread on which the beads could be strung to form a perfect necklace." But they continue to highlight not only the genius "bead stringers" but also the numerous and talented "bead collectors" who help to build the tools and collect the data that the bead stringers use. For Koch's legendary discoveries of the bacteria that cause diseases like tuberculosis and cholera to be possible, he needed new techniques to effectively isolate bacteria beyond carefully sliced potatoes. He needed the tools that were developed by his less-celebrated laboratory assistants, like Julius Richard Petri's dishes and Walther Hesse's solid growth medium.

But behind the talented laboratory technicians that supported Robert Koch's genius was an even more unsung heroine of microbiology. It was Walther Hesse's wife (who was often an assistant and scientific illustrator for the lab) Angelina Fanny Hesse who made the isolation of bacteria possible. In the early 1880's, Walther was struggling to find the right sort of gel for Petri's dishes. He was experimenting with using gelatin to congeal the nutrient broth that the bacteria ate, but bacteria also liked to eat the proteins that congealed the gelatin, chewing through the gel and ruining the experiments. Gelatin also had another major drawback: it would soften and begin to melt at the incubation temperatures required for growing the bacteria.

Angelina, who cooked both the family's meals and the beef stock that the bacteria ate in her kitchen, suggested that Walther use agar-agar, which is more heat-stable than gelatin and used to make soups, desserts, and jellies, particularly in Asia. (She had learned about it from Dutch friends who had lived in Indonesia, which was a colony of the Netherlands at the time.) Agar is a sugar polymer derived from algae that most bacteria can't digest. Once it's boiled and cooled, it forms a tough matrix that stays solid at much higher temperatures than gelatin.

With agar, many of the technical problems hindering Hesse's—and therefore Koch's—experimental progress were solved. Koch briefly mentioned the development (though he fails to mention either Walther or Angelina) in his 1882 paper announcing the identification of the bacteria that causes tuberculosis: "The tubercule bacilli can also be cultivated on other media...they grow, for example, on a gelatinous mass which was prepared with agar-agar, which remains solid at blood temperature, and which has received a supplement of meat broth and peptone."

Angelina Hesse's creative insight was thus written out of history with the ever-present passive voice of the scientific literature. Even today, the Wikipedia article about Robert Koch masks Angelina's contribution to microbiological history, simply stating that Koch "began to utilize agar to grow and isolate pure cultures." In the late 19th century, the use of agar to isolate bacteria was initially referred to as "Koch's plate technique," but since the early 1900s only Petri's name remains in common use. In their article, Hitchens and Leikind suggested (seventy five years ago) that "plain agar" be referred to as "Frau Hesse's medium" to acknowledge her forgotten "service to science and to humanity." Perhaps it's finally time that we remember Frau Hesse and celebrate all the ignored "bead collectors" working in the laboratories and kitchens that make science possible.

JOKES

Sam walks into his boss's office and says:
"Sir, I'll be straight with you, I know the economy isn't great, but I have three companies after me, and I would like to respectfully ask for a raise."
After a few minutes of haggling the boss finally agrees to a 5 percent raise, and Sam happily gets up to leave.
"By the way," asks the boss as Sam is getting up, "which three companies are after you?"
"The electric company, water company, and phone company," Sam replied.

A young businessman had just started his own firm.
He rented a beautiful office and had it furnished with antiques. Sitting there, he saw a man come into the outer office. Hoping to look like a hot shot, the businessman picked up the phone and started to pretend he was working on a big, important business deal.
He threw huge figures around and made giant commitments. Finally he hung up and asked the visitor, "Can I help you?"
The man said, "Yeah, I've come to activate your phone lines."

One day a man goes to a pet shop to buy a parrot. The assistant takes the man to the parrot section and asks him to choose one.
The man asks, "How much is the yellow one?"
The assistant replies that it costs $2,000. The man is shocked and asks the assistant why it's so expensive. "This parrot is a very special one. He can type really fast."
"What about the green one?" the man asks. "He costs $5,000 because he can type, answer incoming phone calls and takes notes."
"What about the red one?" the man asks. The assistant says, "That one's $10,000."
Curious, the man asks, "What does he do?" The assistant says, "I don't know, but the other two call him boss."

An employee goes to see his supervisor in the front office.
"Boss," he says, "we're doing some heavy house-cleaning at home tomorrow, and my wife needs me to help with the attic and the garage, moving and hauling stuff."
"We're short-handed," the boss replies. "I can't give you the day off."
"Thanks, boss," says the employee "I knew I could count on you!"

The owner of a company tells his employees:
"You worked very hard this year, therefore the company's profits increased dramatically. As a reward, I 'm giving everyone a check for $5,000."
Thrilled, the employees gather round and high five one another.
"And if you work with the same zeal next year, I'll sign those checks!"

The sales chief, the HR chief, and the boss of a company are on their way to lunch when they stumble upon a beat up, but valuable looking brass container.
The sales chief picks it up and starts cleaning it with his handkerchief. Suddenly, a genie emerges out of a curtain of purple smoke. The genie is grateful to be set free, and offers them each a wish.
The HR chief is wide-eyed and ecstatic. She says, "I want to be living on a beautiful beach in Jamaica with a sailboat and enough money to make me happy for the rest of my life."
Poof! She disappears.
The sales chief says, "I want to be happily married to a wealthy supermodel with penthouses in New York, Paris, and Hong Kong."
Presto! He vanishes.
"And how about you?" asks the Genie, looking at the boss. The boss scowls and says, "I want both those idiots back in the office by 2 PM."
Moral of the Story: Always let your boss speak first.

Bacillus coagulans (Lactobacillus sporogenes) a probiotic?

In Europe, Asia and the US several products are marketed with the possible probiotic bacterium *Bacillus coagulans*, often marketed wrongly as '*Lactobacillus sporogenes'*. Other designations of this strain in products are 'Lactospore', 'spore forming *Lactobacillus*' or 'spore forming lactic acid bacteria'.

In several countries there has been a debate whether this is a valid bacterial name and whether the product can indeed be considered a probiotic.

The bacterial species, now known as *B. coagulans*, was isolated in 1932 by Horowitz-Walssowa and Nowotelnow as *Lactobacillus sporogenes* and subsequently reclassified in 1939 as *Bacillus coagulans*. The name " *Lactobacillus sporogenes"* thus has no further scientific status and should not be used in either scientific publications or consumer information.

Legally the European Food Law states that consumers may not be mislead by the producer using labeling and advertising, that ingredients should be indicated by their specific name, which is to be the legal name in the country and " which is clear enough to let the purchaser know its true nature and distinguish it from other products with which it might be confused".

The law thus states clearly that any ingredient should be labeled with its official legal name. And that the product may not be confused with other (similar) products.

Unfortunately there is no legal list of bacterial names, however, there is a list of accepted scientific names, which is widely acknowledged by the international scientific community and scientific journals as the official list of bacterial names.

The use of the name '*L. sporogenes'* also may be confusing to consumers, as it would indicate that there are Lactobacillus species present, which is not the case. The use of '*L. sporogenes',* thus is in violation with the EU law.

Furthermore, as probiotic claims should be substantiated with scientific claims, a name that is not scientifically accepted should not be used. If someone would like to investigate the claims, no information will be found if a non scientifically accepted name is being used. This can be elaborated with L-ascorbic acid (Vitamin C), which may also be listed as E300 on the label (in Europe). There are plenty scientific studies with L-ascorbic acid or vitamin C, but there are no studies with E300. In this case E300 is a legally accepted synonym for L-ascorbic acid when used as a food additive (antioxidant). This means that producers can legally use E300 on the label for use as an antioxidant, but not for use as a vitamin supplement.

It is stated by some producers that the name is retained 'in honour of the discoverers'. This may be a good idea, but to use the old name is not the proper way to do so. A well known probiotic bacterium, LGG, is also named after the discoverers and has a much more complex taxonomic history :

- Originally discovered by Goldin and Gorbach, as *Lactobacillus acidophilus*

  Marketed as *Lactobacillus species* GG (taxonomic position unclear)
- Renamed as *Lactobacillus casei* subspecies *rhamnosus* GG (sometimes referred to as Lactobacillus casei GG)
- Present name *Lactobacillus rhamnosus* GG

The strain indication GG thus clearly honours the discoverers. An alternative for '*L. sporogenes'* could thus be *B. coagulans* HW-N.

Another argument by some producers is that they retain the old name 'for historical reasons'. As most bacteria have changed taxonomic position during the years, it would be most confusing as this would be made common practice. Probiotic bacteria such as *Lactobacillus acidophilus* or *Bifidobacterium* species, were originally designated *Bacillus acidophilus* and *Bacillus bifidus* respectively. All *Bacillus bifidus* (later also known as *Lactobacillus bifidus)* are now within a genus of more than 20 species…. The well known *E.coli* would still be *Bacillus coli* etc.

In any case, the name *L. sporogenes* was in use only for 7 years, with practically no publications (and thus no historical 'value'), whereas *B. coagulans* has been in use for 70 years with thousands of publications. For historical reasons, the name *B. coagulans*would thus be much more suitable!

Finally in several publications and in many product descriptions provided by manufacturers of products with this bacterium, it is stated that *B. coagulans* is not a proper *Bacillus* and has some characteristics in common with the genus *Lactobacillus.* Considering the characteristics of the species it can be stated that it indeed bears characteristics of both genera. There are as many arguments to place the species in *Lactobacillus* as in *Bacillus.* There is, however, no conclusive argument to place the strain under *Lactobacillus,* whereas the spore-forming capacity definitely places the strain within the genus *Bacillus.* By definition, the genus*Lactobacillus* does not form any spores (5), which makes it impossible to name the strain *Lactobacillus.* As there are more bacteria that make lactic acid, the fact that it produces lactic acid is not conclusive to place the strain in the genus *Lactobacillus.*

Therefore, unless there is a taxonomic change in the genus *Bacillus,* the official name remains *B. coagulans.*

Of the other designations 'Lactospore' and 'spore forming lactic acid bacteria' can be used freely in advertising, but not as an ingredient. Lactospore is a brand/market name and thus can not be a listed ingredient (6). It can be used properly when the strain is listed as *B. coagulans* (Lactospore). 'Spore forming lactic acid bacteria' is a general description and the *B. coagulans* may be part of that group. On a label it is as valid as stating 'Colour' or 'Emulsifier'. The general description is correct, but not as an ingredient.

The designation 'spore forming *Lactobacillus'* is definitely incorrect, as by definition *Lactobacillus* species do not form spores and, as stated above, this may be confusing and thus illegal. It is like saying 'a barking cat'; some cats may look like dogs, but they do not bark.

Concluding, the name *Lactobacillus sporogenes* has no scientific or legal status and thus does not 'exist' scientifically and therefore should not be used on product labels. The official name is *Bacillus coagulans.*

Safety:

Lactobacillus species are normally considered safe for human consumption, even in large doses (Donohue). Among the genus *Bacillus,* however, there are many species that produce toxins, including the well known *Bacillus anthracis* (anthrax) and

Bacillus cereus (food poisonings). *B. coagulans* has been implicated in human infections, but not from oral administration. *B. coagulans* is thus not known to be a pathogen or to produce endotoxins. *B. coagulans,* however, does not have a GRAS status of the FDA, nor has there been a thorough independent safety evaluation of this species (7). *Bacillus* species are, unlike *Lactobacillus* species, not considered a normal part of the intestinal microflora. They can be isolated from faeces, but only as transient species. There are no indications that *Bacillus*species can colonise the human gut.

Use as probiotic:

To be considered a probiotic the joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of probiotics in food (2001) recognized the need for guidelines to set out a systematic approach for the evaluation of probiotics leading to the substantiation of health claim. As a consequence, a consensus panel on selection criteria for probiotics was developed in which base requirements for probiotics were stated. They include a correct identification at strain level of the microorganism, as well as in vitro tests to determine physiologic and functional health characteristic of the strain and in vivo trials to substantiate efficacy in humans or in animals.

A large review has been published in 2003 on the use of spore forming bacteria as human probiotics, which included *B. coagulans.* It was concluded that there are only very few studies in which *B. coagulans* was successfully used as human probiotic. This conclusion was confirmed by De Vecchi and Drago.

A recent search in the scientific databases Medline, Current Contents, PubMed and Food Science and Technology Abstracts (nov 2006) did not result in many new publications since 2003 on the use of *B. coagulans* (or *L. sporogenes)* in humans.

These studies included a study on the t reatment of irritable bowel syndrome in humans (10) In this study a complex product, including three bacteria (*B. coagulans,L. acidophilus* and *S. salivarius* ssp *thermophilus)* and several plant extracts was used. The effects were minimal and could not be attributed to either the bacteria or the other (chemical) components in the mixture (10).

Another study from 2003 showed a reduction in diarrhea in children. In this study both *B. coagulans* and fructo-oligosaccharides were used (11)

Another recent publication (12) shows a positive effect of *B. coagulans* on vaginitis, but this obviously is not a probiotic application.

Finally there have been some recent publications on the use of *B. coagulans* as a probiotic in shrimp larvae (13, 14) and pigs (15).

In conclusion, there is very little evidence of the use of *B. coagulans* as a probiotic in humans. There are several studies that show positive effects, but, comparing these studies with those of more well known probiotics, these are very limited. It can thus be stated that there are indications that *B. coagulans* may act as a probiotic in humans, but many more studies are needed to state that *B. coagulans* is a probiotic (9).

There are no indications that *B. coagulans* has any side effects either.

Contamination:

The effectivity of a probiotic preparation obviously is correlated to the purity of the product. It is noteworthy that in several occasions there have been other bacteria isolated from products with spore forming bacteria in high numbers. This has not (yet) been reported for products with lactobacilli or bifidobacteria.

Sanders et al (7) mention 7 products in which other species were present as mentioned on the label, only one of these was labeled *L. sporogenes,* but contained *B. subtilis.* In an experiment with only one product in 2000 it was found that the product contained only *B. licheniformis* (16). In a study by the Dutch Consumer Organisation (17) several other *Bacillus* species were isolated from products with *B. coagulans.*

Whether or not this is a coincidence, it may be a warning sign for the producer(s) to be more aware of quality control. So far, there are no indications that these contaminations form a health risk.

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Allergy - Facts and Figures

An allergy is when your immune system reacts to a foreign substance, called an allergen. It could be something you eat, inhale into your lungs and inject into your body or touch. This reaction could cause coughing, sneezing, itchy eyes, a runny nose and a scratchy throat. In severe cases, it can cause rashes, hives, low blood pressure, breathing trouble, asthma attacks and even death.

There is no cure for allergies. You can manage allergies with prevention and treatment. More Americans than ever say they suffer from allergies. It is among the country's most common, but overlooked, diseases.

How Many People Do Allergies Affect?

- Researchers think nasal allergies affect about 50 million people in the United States.
- Allergies are increasing. They affect as many as 30 percent of adults and 40 percent of children.
- Allergic disease, including asthma, is the fifth leading chronic disease in the U.S. in people of all ages. It is the third most common chronic disease in children under 18 years old.

How Many People Get Sick from Allergies?

- Allergic conditions are the most common health issues affecting children in the U.S.
- In 2012, 11.1 million people were diagnosed with allergic rhinitis.
- People visit the emergency room about 200,000 times each year because of food allergies. Almost 10,000 people stay in the hospital each year because of food allergies.

How Many People Die from Allergies?

- The most common triggers for anaphylaxis, a life-threatening reaction, are medicines, food and insect stings. Medicines cause the most allergy related deaths.
- African-Americans and the elderly have the most deadly reactions to medicines, food or unknown allergens.
- Deadly reactions from venom are higher in older white men. Over the years, deadly drug reactions have increased a lot.

What Are the Costs of Allergies?

- In 2010, Americans with nasal swelling spent about $17.5 billion on health costs. They have also lost more than 6 million work and school days and made 16 million visits to their doctor.[6]

  Food allergies cost about $25 billion each year.

What Are Indoor and Outdoor Allergies?

Types of indoor and outdoor allergies include sinus swelling, seasonal and returning allergies, hay fever and nasal allergies. Many people with allergies often have more than one type of allergy. The most common indoor/outdoor allergy triggers are: tree, grass and weed pollen, mold spores, dust mites, cockroaches, and cat, dog and rodent dander.

- In 2012, 17.6 million adults and 6.6 million children had hay fever.
- Worldwide, allergic rhinitis affects between 10 percent and 30 percent of the population. In 2010, allergic rhinitis was diagnosed during 11.1 million doctor visits.
- In 2010, white children were more likely to have hay fever than African-American children.
- The same triggers for indoor/outdoor allergies also often cause eye allergies.

How Common Are Skin Allergies ?

Skin allergies include skin inflammation, eczema, hives, chronic hives and contact allergies. Plants like poison ivy, poison oak and poison sumac are the most common skin allergy triggers. But skin contact with cockroaches and dust mites, certain foods or latex may also cause skin allergy symptoms.

- In 2012, 8.8 million children had skin allergies.
- Children age 0-4 are most likely to have skin allergies.
- In 2010, African-American children in the U.S. were more likely to have skin allergies than white children.

How Common Are Food Allergies ?

Children have food allergies more often than adults. Eight foods cause most food allergy reactions. They are milk, soy, eggs, wheat, peanuts, tree nuts, fish and shellfish.

- Peanut is the most common allergen. Milk is second. Shellfish is third.
- Researchers think about 6 million children in the U.S. have food allergies. Most of them are young children.
- Also, 38.7 percent of food-allergic children have a history of severe reactions.
- In children with food allergies, 30.4 percent are allergic to multiple foods.

How Common Are Drug Allergies?

- Penicillin is the most common allergy trigger for those with drug allergies. Up to 10 percent of people report being allergic to this common antibiotic.
- Bad drug reactions may affect 10 percent of the world's population. These reactions affect up to 20 percent of all hospital patients.

How Common Is Latex Allergy?

- About 1 percent of people in the U.S. have a latex allergy.
- Health care workers are becoming more concerned about latex allergies. About 8-12 percent of health care workers will get a latex allergy.

How Common Is Insect Allergy?

People who have insect allergies are often allergic to bee and wasp stings and poisonous ant bites. Cockroaches and dust mites may also cause nasal or skin allergy symptoms.

- Insect sting allergies affect 5 percent of the population.
- At least 40 deaths occur each year in the United States due to insect sting reactions.

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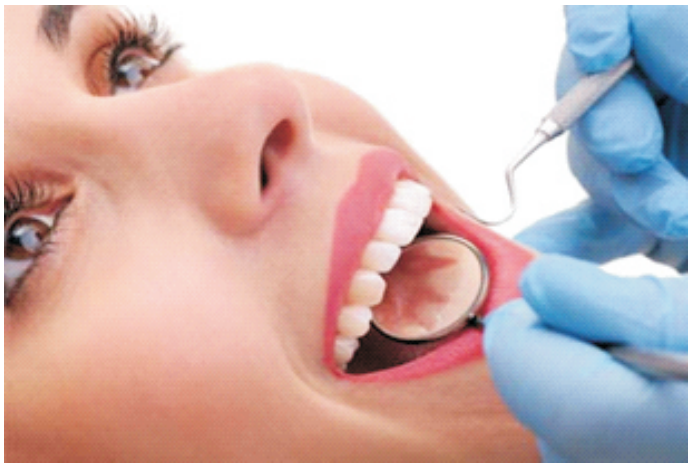
Best Practices in Dental Hygiene



Protective Factors

Caries protective factors are biologic or therapeutic measures that can be used to prevent or arrest the pathologic challenges posed by the caries risk factors. The higher the severity of the risk factors, the greater the intensity of protective factors must be in order to reverse the caries process. These protective factors include a variety of products and interventions that will enhance remineralization and keep the balance between pathology and protection of the patient's oral health. Protective factors also include living in a community with fluoridated water; regularly using fluoridated toothpastes, low-fluoride oral rinses and xylitol; and receiving topical applications of fluoride, chlorhexidine and calcium phosphate agents.

The Caries Imbalance model uses the acronym "SAFE" to describe the following four protective factors:

- Saliva and sealants
- Antimicrobials or antibacterials (including xylitol)
- Fluoride and other products that enhance remineralization
- Effective lifestyle habits

Best practices dictate that once the clinician has identified the patient's caries risk (low, moderate, high or extreme), a therapeutic and/or preventive plan should be implemented. Clinical intervention protocols have been developed based on research, and individualized treatment options should be presented to the patient.

Several of these protective agents are used off-label, meaning their use in caries management is not cleared for marketing by the Food and Drug Administration (FDA). While dental professionals are not regulated by the FDA, manufacturers are, and dissemination of off-label information about an FDA-regulated product is limited. If an individual dental professional decides to use a product off-label, he or she must first ascertain that the product is effective and safe for the intended use.

Saliva and Sealants

The protection that saliva provides to the oral cavity is often overshadowed by the emphasis on oral disease. An evaluation of the quantity and quality of saliva should be conducted on all patients at the initial exam and then periodically assessed for changes. At a minimum, during the clinical examination, the viscosity and flow should be evaluated. Saliva is 99% water and should look like water, not thick and stringy or frothy and bubbly. A quick and simple test to confirm function and duct patency is to "milk" one of the major glands, such as the parotid or submandibular gland. Massage or squeeze the duct until saliva is expressed. If it takes longer than one minute to express saliva from the duct or the clinician is unable to express any saliva, this could indicate salivary hypofunction. At this time there is an opportunity to test the pH of the expressed saliva by using a simple piece of litmus paper. Healthy saliva pH should measure no lower than 6.6. According to the CAMBRA clinical guidelines, saliva testing, including bacterial testing, is suggested at baseline for all new patients and if high levels of bacteria are suspected for patients who are at moderate risk for dental caries disease. High- and extreme-risk patients should have saliva testing conducted at every recare examination, provided they still have some functioning of the salivary glands. Compared to the total levels of calcium and phosphate in enamel, healthy saliva is supersaturated with these minerals. As the pH drops from bacterial acid challenges, the level of supersaturation of the calcium and phosphate also drops and the risk of demineralization increases. At the same time, the remineralization process redeposits calcium and phosphate ions back into the damaged tooth mineral to form new dental mineral that is stronger and more resistant to future acid challenges than the original tooth surface. Sealants are universally recognized as an evidence-based method to boost the tooth's resistance to carious lesions in pits and fissures of teeth. As long as the pits and fissures remain filled with sealant material, carious lesions will not occur, so it is critical that clinicians include sealant retention evaluation at the patient's periodic examination. Both unfilled and filled resin materials are available, and there are many sealant choices available in the marketplace. Fluoride-releasing sealants are gaining in popularity, with the premise that the low level of fluoride released from the sealant will assist with remineralization in the oral cavity and help prevent carious lesion formation at sealant margins.Glass ionomer cements may also be used as a sealant, and it has been suggested that due to their fluoride-releasing and hydrophilic nature, they are especially suitable for partially erupted teeth when a dry working field cannot be obtained. Because of their poor retention rate compared with that of resin-based sealants, glass ionomer sealants need to be closely monitored and their use be limited to a transitional sealant on tooth surfaces that cannot be adequately isolated to place a resin-based sealant. CAMBRA clinical guidelines recommend that the placement of sealants be based on the risk of the patient, and resin-based sealants and glass ionomers are optional for patients at lower risk for caries.

Antimicrobials

Antimicrobial agents destroy or suppress the growth or multiplication of microorganisms, including bacteria. CAMBRA clinical guidelines recommend the use of antimicrobials for patients over six years of age who are classified as being at high or extreme risk for caries, and for caregivers of noncompliant moderate through extreme risk children under the age of six. Antimicrobials require repeated applications at various intervals, depending on the agent. Chlorhexidine gluconate rinse has been widely studied, and in addition to being FDA-approved to treat

gingivitis, when used off-label as a 30-second rinse every day of the first week of every month, it is effective in reducing the levels of MS bacteria but is not as effective against LB. In the United States, chlorhexidine gluconate rinse is available as a 0.12% rinse with or without alcohol. The use of 0.12% chlorhexidine gluconate rinse in caries management is not without controversy, and the long-term effects of bacteria suppression have been questioned. Long-term use of chlorhexidine rinse can lead to discoloration of teeth, the mucous membrane, the tongue and composite restorations; it can also lead to taste disturbances. These undesirable side effects can be avoided by using a chlorhexidine-containing varnish. Chlorhexidine varnish, approved for desensitization in the United States, has also been shown to be effective against cariogenic bacteria, especially the highly susceptible S. mutans. It has been concluded that the most persistent reductions of MS have been achieved by chlorhexidine varnishes. Chlorhexidine gels are the next most efficacious, followed by oral rinses for patients at moderate to extreme risk. It has been shown that a 1% chlorhexidine diacetate and 1% thymol varnish, when applied and dried, contains approximately 10% chlorhexidine and 10% thymol and has been found in a systematic review to have a higher efficacy than other chlorhexidine varnishes.The side effects seen with chlorhexidine rinses are not seen with chlorhexidine varnishes, and the application of the varnish is easy and moisture tolerant. It has also been shown to reduce the incidence of root carious lesions in a geriatric population. The application of chlorhexidine varnish every three to four months may be a more viable option than the use of chlorhexidine rinses, especially for caregivers of children.

Xylitol

CAMBRA clinical guidelines recommend the use of xylitol to control the cariogenic bacteria S. mutans for patients over six years of age who are classified as being at moderate to extreme risk for caries. For children under six, xylitol wipes and xylitol products to replace sugary treats are recommended for children and all others who are classified as being at moderate to extreme risk, including caregivers. Xylitol has been well-studied, and it is generally accepted that this naturally occurring sugar alcohol reduces the amount of MS and the quantity of plaque biofilm when habitually consumed. Studies have also demonstrated that habitual consumption of xylitol by caregivers of young children has halted or slowed the transmission and colonization of MS. Xylitol is dose-dependent, and the minimum amount needed to provide a beneficial effect on the plaque biofilm has been shown to be 5-6 grams/day, divided into three to four doses, for no shorter than 5-10 minutes per exposure. Currently, it is suggested that no more than 6 to 10 grams/day be ingested as the effects of xylitol plateau between 6.44 g and 10.32 g xylitol/ day. Clinicians need to know the amount of xylitol present in the products being recommended, as it varies considerably. Simply telling a patient or caregiver to use xylitol gum or mints three to four times a day may not deliver the minimum amount shown to be effective.

Fluoride

The use of fluoride has been the cornerstone of prevention, and fluoridated toothpaste remains the most common and cost-effective form of dental caries control. A Cochrane Review on fluoride confirmed the benefits of daily toothbrushing with fluoridated toothpaste as a means to decrease dental caries, and for preventing caries in children and adolescents, toothpastes of at least 1,000 ppm fluoride should be used. For very young children, when brushing with concentrations greater than 1,000 ppm fluoride, a risk-benefit decision needs to be discussed with caregivers regarding the development of mild fluorosis. While research emphasizes the positive use of fluoridated toothpaste, other topical fluoride modalities such as mouth rinses, gels and varnishes have also been studied and their effectiveness has been confirmed. The American Dental Association Council on Scientific Affairs developed evidence-based clinical guidelines for professional topical application of fluorides that have endorsed the use of in-office fluoride gels and fluoride varnishes.As with chlorhexidine varnish, the use of fluoride varnish for caries management is considered off-label, as it is cleared for marketing by the FDA for the treatment of dentin hypersensitivity associated with the exposure of root surfaces. The use of 5,000 ppm prescription fluoride toothpaste and home-use fluoride rinses has also been recommended. Fluoride varnish is a concentrated topical fluoride designed to stay in close contact with the tooth surface for hours, enhancing fluoride uptake during the early stages of demineralization. Because of the large amount of fluoride that can be deposited in the demineralized enamel, varnishes are effective when used on early white spot lesions. The caries preventive efficacy of fluoride varnish is well-studied, and has been found in a systematic review to be more effective than traditional topical fluoride gels. Its ease of use and relative safety make it suitable for prevention in community-based dental programs. Most fluoride varnishes in the United States are 5% sodium fluoride (22,600 ppm fluoride ions), and several products offer single-unit-dose application, keeping the delivery cost-effective. Recently, manufacturers have added amorphous calcium phosphate or tricalcium phosphate to enhance remineralization and fluoride uptake . Another effective fluoride varnish contains 0.9% difluorosilane in a polyurethane base with ethyl acetate and isoamylpropionate solvents (Fluor Protector, Ivoclar Vivadent) and is equivalent to 0.1%, or 1,000 ppm in solution. As the solvents evaporate, the concentration of the fluoride at the tooth surface will rise, resulting in effective fluoride binding and uptake. In addition, the viscosity of this varnish allows it to flow easily on the tooth surface. The ADA's clinical guidelines suggest that applications of fluoride varnish two to four times per year are effective in reducing carious lesions in children and adolescents who are at high risk for caries, and the CAMBRA clinical guidelines recommend a frequency of application of fluoride varnish as indicated by the patient's caries risk.

Effective Lifestyle Habits

While the use of fluoride has decreased the need for strict dietary control of sucrose, dental caries disease does not occur in the absence of dietary fermentable carbohydrates. Reducing the amount and frequency of sugar consumption, including the "hidden sugars" in many processed foods, continues to be important for patients at high risk for caries. Consuming foods or snacks that do not promote carious lesion formation or progression would be ideal for patients at risk for dental caries. Hard cheese has been shown to coat teeth with a lipid layer, protecting surfaces from acid attack.Emerging science suggests increasing arginine-rich proteins in the diet, as it has been shown that consumption of these foods can rapidly increase plaque pH. Arginine rich proteins include a variety of nuts (peanuts, almonds, walnuts, cashews, pistachios), seeds (sunflower, pumpkin, squash), kidney beans, soybeans, watermelon and tuna. Ammonia production from arginine and urea metabolism has been identified as the mechanism by which oral bacteria are protected against acid killing, and it maintains a relatively neutral

environmental pH that may suppress the emergence of a more cariogenic microflora. Dental products that can assist in neutralizing acid and encourage a non-acidic environment include sodium bicarbonate products that can be found in commercially available toothpastes and rinses. The use of baking soda rinses has been suggested to neutralize an acidic oral environment. Chewing gum, especially high-dose xylitol gum, can raise plaque pH and reduce MS at the same time. Calcium phosphate products have also been shown to raise plaque pH in addition to delivering bioavailable calcium and phosphate to the tooth surface to enhance remineralization.A variety of calcium phosphate technologies are currently available, including amorphous calcium phosphate (ACP), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), calcium sodium phosphosilicate and tricalcium phosphate (TCP). The use of most calcium phosphate products is considered off-label because most of these products are accepted by the FDA as tooth-polishing or desensitizing ingredients only rather than as agents of remineralization. Sugarfree chewing gum with CPP-ACP has been shown to increase remineralization by approximately 20% compared with plain, sugar-free gum. Calcium phosphate therapy supports fluoride therapy and is not designed to replace the use of fluoride. For patients who have salivary hypofunction, including low or no flow, low pH, and poor buffering capacity, the use of these agents may be beneficial. CAMBRA clinical guidelines (>6 years old) suggest the use of calcium phosphate for patients with excessive root exposure or sensitivity and is recommended for use several times daily for patients classified as being at extreme risk. For pediatric patients (0-6 years old), CAMBRA clinical guidelines suggest alternating brushing between toothpaste and calcium phosphate, leaving the latter on at bedtime for patients classified as noncompliant and at moderate to extreme risk.

For those patients with high or extreme risk, a power toothbrush may be beneficial. While most research concerning power toothbrushes focuses on the ability of the brush to remove plaque biofilm, recent research has shown that power toothbrushes may be helpful in the delivery and retention of fluoride. Recent research has shown that one sonic toothbrush enhances fluoride effects on the plaque biofilm, causing increased fluoride delivery and retention at the tooth surface. In addition, for patients at extreme risk (demonstrating hyposalivation, or reduced salivary flow), the sonic power toothbrush has been shown to increase salivary flow and decrease the numbers of incipient and frank root caries, as compared to a manual toothbrush. Patient adherence to the recommendations made by the dental professional is critical to successful implementation of these caries protective factors. It is well-understood among dental professionals that adherence and motivation are issues for many patients, and lack of adherence or noncompliance affects outcomes across all dental disciplines. The ability of the clinician to motivate the patient to make positive behavior change is crucial. One technique gaining popularity among patient-centered clinicians is motivational interviewing. The main focus of motivational interviewing is to help the patient overcome ambivalence to behavior change. This is achieved through focusing on what the patient feels, wants and thinks, and involves the patient speaking and the clinician listening. The strategies involved in motivational interviewing are more persuasive and supportive than coercive and argumentative and are designed to tap into the patient's intrinsic motivation rather than being imposed extrinsically. Motivational interviewing with parents of pediatric patients has been shown to be more effective in reducing the number of carious lesions and has more of a protective effect compared to traditional educational counseling methods. Conclusion Multiple factors, such as the interaction of bacteria, diet and host response, influence dental caries initiation, progression and treatment. Time has proven that this disease cannot be controlled by restoration alone. Assessment of the caries risk of the individual patient is a critical component in determining an appropriate and successful management strategy. CAMBRA supports clinicians in making decisions based on research, clinical expertise, and the patient's preferences and needs. Motivating patients to adhere to recommendations from their dental professional is also an important aspect in achieving successful outcomes in caries management. Along with fluoride, new products are available to assist clinicians with noninvasive management strategies. While research exists for these newer preventive intervention and clinical guidelines, more in vivo clinical trials are needed to establish their true clinical relevance. This does not mean that clinicians should not consider these products, strategies and guidelines but rather that they should carefully weigh the benefits and risks of recommending these products for their patients. Best practices are an evolving approach to exceptional patient care, and CAMBRA offers clinicians the ability to apply the most relevant, research-based and helpful interventions to real-life practice.

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Highlights of the coming issue


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