BACTERIOTHERAPY FOR DOGS

Juan Angel Diaz Benitez

Thanks to my parents and friends who helped me in this project. Special thanks to Dr John Minarcik PhD, also known as the super pathologist, thank you for your excellent lessons in the pathology course, and Josiah Zayner PhD; your dynamism motivation and vision inspired me to write this book.

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Prologue

In this book will delve into bacteriotherapy, fecal and skin microbiome transplant in dogs, we will analyze the probiotics and we will analyze some of the treatments in which they use bacteria to treat diseases. The bacteriotherapy offers us a new vision of the disease, away of the classical idea that only they are of importance when they are pathological, but they offer possible new treatments for illnesses so diverse from bacterial infections to disorders of allergic or metabolic type. I hope this book help you to better understand the bacteriotherapy

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Bacteriotherapy for dogs

Introduction

In this book will delve into bacterioterapy, that is the use of beneficial bacteria to displace infectious agents or treat metabolic and nutritional illnesses of dogs, we will see from the probiotics commonly used to improve the intestinal health to the transplantation of intestinal and skin microbiome . We will analyze some of the treatments in which they use bacteria as well as the main characteristic of some of these bacteria.We will analyze the main characteristic of bacteria used as probiotic in skin and intestine health Besides we will share a couple of recipes to elaborate probiotics The probiotics have a lot of benefits for the organism. This because of various mechanisms as by means of the secretion of inhibitory substances of pathogenic agents,

preventing the colonization of infectious agents by kidnapping of nutrients and even regulating the expression of some genes going in with this last in the area of knowledge of of the epigenetics.

Probiotics

To be considered probiotic, the bacteria have to result innocuous for the dog, arrive alive to the intestine (that it is where exert his function), have antibacterial capacity and of modulation of the immunological response and influence positively in the metabolic activities. Besides, they have to be able to establish by himself same in the tissue where will exert his beneficial function.

The main profits of the probiótics are:

1. Fight intestinal illnesses like colitis and intestinal inflammation.
2. Combat illnesses like cancer, candidiasis, hemorrhoids and urinary infection.
3. Improve the digestion and combat the acidity
4. Combat the constipation and the diarrhea, regulating the intestinal traffic
5. Augment the absorption of nutrients, like vitamin B, calcium and iron;
6. Strengthen the immune system, for augmenting the production of cells of defense called macrophages;
7. Prevent the proliferation of bad bacteria in the intestine;
8. Help to digest the lactose, especially in individuals with intolerance to the lactose.
9. Warn problems like obesity, tall cholesterol and hypertension;
10. Warn allergies and alimentary intolerances.

Main probiótics

Lactobacillus acidophilus

This bacterium its present in a variety of foods, included the milk, the flesh, the fish and the cereals. And it's found in the intestines of the animals, the mouth and the vagina. The *L. acidophilus can* absorb the lactose and metabolize it, forming lactic acid. As any bacterium can be destroyed by some high temperatures, humidity, or the UV light.

His presence is important for maintain the balance in the diversity and protects harmful effect of other microorganisms. The destruction of nutrients that makes this microorganism produces lactic acid, acetic acid, peroxide of hydrogen and other by-products that create a half hostile for other undesirable organisms. This bacterium consumes the nutrients of other microorganisms controlling the development of these. Help in the digestion of the lactose, due to the fact that one of his properties is that they present

enzymatic activity lactasa, that is to say are able of

By what this probiotic could be use in patients intolerants to the lactose and unlike other treatments based in the previous consumption of enzymes , before the consumption of foods that contain lactose, the fact to generate his colony and integrate in the intestinal microbiome , does that with a daily dose was sufficient to be able to tolerate lactose during all day, by what result very convenient .

During the process of digestion, help in the production of niacin, folic acid, vitamin K and vitamin B6 (pyridoxine).Some studios show that the *L. acidophilus* Can help to the separation of the amino acids.

The use of this probiotic can cause a sufficient increase in the production and intestinal absorption of vitamin K what can diminish the effectiveness of the warfarin.

Lactobacillus casei

This probiotic is an anaerobic bacteria that is found in the intestine and mouth of humans and domestic animal . The L. casei, producer of lactic acid, employs in the dairy industry in the preparation of foods It uses especially in cases of diarrhea or after a prolonged treatment with antibiotics.

Streptococcus *thermophilus*

This bacterium is also known as *Streptococcus salivarius* its *a species of* bacterium Gram-positive anaerobic facultative. It is a probiotic (survives in the stomach) and generally its used in the production of yoghurt. Among his profits for the health this the improve the cases of intolerance to the lactose and improve the acute diarrheas by excess of antibiotic.

Bifidobacterium animalis

Bifidobacterium animalis it's a gender of bacteria gram-positive, anaerobic, no mobile, the bacteria reside in the colon. They help in the digestion, and are associated with a minor incidence epidemiologic of allergies and also warn some forms of growth of tumors

Some bifid bacteria use like probiotics recently has proposed that this type of bacteria play an important paper in the beneficial effect that exerts the chocolate in the organism.According to this proposal, east and another type of bacteria convert the chocolate in the stomach in powerful agents anti-inflammatory, with special profit for the heart.

Lactobacillus jhonsonii

Lactobacillus johnsonii is a species of the gender Lactobacillus. His type of strain is ATCC 33200. It is part of the vaginal microbiome and has identified that has charitable properties for the organism like improving the absorption of some nutrients and coadyuvant in the treatment of intestinal infections.

Staphylococcus epidermidis

It is a normal habitant of the skin of human, dogs and some mammals . It is coagulase-negative, term nuclease-negative although sometimes varied, and presents frequently in the skin of humans and of animals and in mouth. It is sensitive to the antibiotic novobiocin; distinguishing it of other common organisms of coagulase negative like *S. saprophyticus*.

It can have an regulatory activity in the growth of pathogenic bacteria that causes illnesses like skin pioderma.

Pseudomonas fluorescens

Although it is not a commonly known bacterium its beneficall in fish when regulating the activity of pathogenic bacteria of the generate Vibrio, in addition to segregates substances that limit the growth of pathogens like the staphylococcous aureus and some funguses that can colonize the skin is a bacillus that can be find in soil and water.

It does not have capacity to form spores and grows aerobically. The optimum temperature for his operation is of 25 to 30 °C, although it can grow from the 5 until the 42 °C roughly. It does not grow under sour conditions (pH ≤ 4.5) and needs preferably neutral pH. His fluorescent pigment (pioverdine) does it react in front of the light ultraviolet, although no always releases it by what in occasions does not shine of visible way.

The *Pseudomona fluorescens* can grow in a half mineral with ions of ammonium or nitrate and an alone organic compound that works like only source of carbon and energy. The energetic gain is obtained by aerobic breath, no by fermentation and his growth is fast.

They find in the surface of the roots, since they are versatile in his metabolism and can use several sustrates produced by the same, but do not establish a symbiotic relation with the plant. It is important to do note that when doing contact the skin of animal when these walk and rest in the soil could colonize it givin profits to these.

We can stand out a complementary property of the *Pseudomonas fluorescens* that is the one to produce some substances -antibiotics - that act limiting the growth and development of fungic pathogens so it can act as skin probiotic bacteria.

Administration of the probiotic

The principal form of administration of the probiótics is oral .By this way they can colonize the gastrointestinal tract

Probiotic drink recipe for dogs

This is a selective probiotic recipe very effective to fight intestinal pathogens as well as correct other digestive issues .Its easy an can be made at home.

Ingredients

- 1 liter of whole milk
- 50gr of dairy product fermented with Lactobacillus casei or yogurt

- 1 Container, clean and disinfected
- 1 Cloth to cover them
- 1 big elastic band

PROCEDURE

1. Emty the milk in the container
2. Heats until it begin to boil (this destroy big part of the present bacteria in the milk)
3. Wait until the milk this to temperature acclimatize
4. Warm the probiotics to use
5. Add them to the milk of slow way
6. Cover the container to avert his contamination with paper or plastic
7. Leave ferment of 1 to 5 days days in a dry and dark place for grow the bacteria

Note: Depending the type of product used will be the final characteristics and the benefits of the probiotics that you will get.

Microbiome intestinal transplant

The transplantation of intestinal microbiome is effective in determinate illnesses. It bases in the use of feces of a healthy donor that they have to be homogenized in a salt solution and administered to the patient for restore his altered intestinal. It is a matter of microbial ecology. If we enter billions of bacteria of a healthy person in a patient, will achieve to colonize and restore his intestine with benefic bacteria, in addition to displacing to the pathogens that had colonized the intestine of the patient. It is a simple process but that is not effective for all type of illnesses and that of course requires of some regulation and sanitary controls (among them, check that the donor is healthy for real), by what never has to practise out of the sanitary surroundings.

In the 17th century and in the veterinary field was contemplated the possibility to use feces of healthy animals like therapy to treat the ailing animals, therapy designated *transfaunación* by the Italian doctor Fabrizi

D´Acquapendente. In that period, neither the microbiology neither the concept of bacterium existed like such and considered to these small beings animáculos. Later, during the Second World War the German army employed this practical pairto treat the dysentery.

In humans in this days, and especially during the last five years, the use of transplantation of fecal microbiome has been popularized like the most effective method for the elimination of the intestinal pathogen *Clostridium difficile*, when the antibiotics are not effective. This pathogen is characteristic by his resistance to antibiotics and for generating recurrent infections in the patients, diminishing his quality of life drastically and even causing the death in 30% of the cases. When the treatments with antibiotics are not effective resorts to the transplantation of fecal microbiome, employing the microbiome intestinal healthy of a person that administers oral road or by means of colonoscopy to the patient, achieving eradicate the pathogen until in 95% of the cases . At present they are carry studies to see if the transplantation of fecal

microbiome can be effective in isease like the illness of Crohn or colitis.To make a fecal transplant in dogs we have been used the next procedure with very good results :1-Selection of the donor , it has to be a young healty dog, dewormed and tested for parvovirus and hepatitis.

2-Collection of the stool, 20 grams its enough

3-Blend it with 100ml of sterile fisiologial saline solution

4-Filtered

5-Collect the sample with 20 or 10ml syringue

6-Administered slowly in the rectum

Previous to the transplant in some cases we administered ampiciline (20mg/Kg PO) twice a day to make room for the new bacteria.Sometimes when the dogs its healty we also used to give them pills with feces inside but we don’t recommend to do this if the dogs is immunosupressed or weak because the bacteria can cause proliferate and cause disease.

Skin microbiome transplant

Following the same base of the transplantation of fecal microbiome, there have been studies that analyze the modification of the microbiome in the skin, due to the fact that a lot of cutaneous illnesses are due to proliferation of pathogenic bacteria in the skin, at present have done investigations about the use of bacteria that could act regulating to other pathogenic microorganisms.

The cutaneous microbiome is particularly complex with variations. It is formed by a set of microorganisms (bacteria, yeasts, virus and parasites) and at least 19 big families of bacteria have been identified; the main are Actinobacteria (Corynebacterium, etc.), Firmicutes (Staphylococcus, Streptococcus, etc.) and Proteobacteria (Acinetobacter, etc.). The composition of the microbiome varies, on the other hand, according to the cutaneous zones and goes from 100 microorganisms by cm^2 in the loin to 106 in the stop or the abdomen.

This microbiome constitutes from the birth by the contact with the vaginal flora after a birth or by the contact with the abdominal flora in case of cesárea. Also it depends of the sex and of the state of health and lifestyle of the animal, immune state, hygiene, antibiotics, products applied, The cutanemous microbiome has, a fundamental paper of defense against the infectious agents it Produces, for example, antimicrobial peptides and *Propionibacterium acnes* contributes to the acidity of the skin. These two strains are also involved in the local immunity. Besides, the skin microbiome could modulate the immune system as equal as does it the intestinal microbiome.

The interactions among these microorganisms are innumerable and the alterations of this balance associate to cutaneous problems like the acne, the illnesses of type psoriasis or dermatitis, by what have developed even creams with bacteria like probiotics to treat the acne and other illness.

This is a clinicall case of skin microbiome transplant for a patient who has pioderma.

Patient: Canine

Breed:Pitbull

Age: 10 years

Clinical sign

The patient presents dermatitis, purulent secretion with presence of pustules in the dorsal part of the body.It also presents crusts and alopecia.

Citology

We made a Gram stain and observed numerous Staphylococcus in violet colour

Diagnostic

Because of the results of the cytology and to the clinical signs we diagnosed a dermal infection of staphilococcus a bacterium that proliferates in conditions of bad hygiene.

Treatment

Due to the fact that the bacterium is resistant to the penicillin in this case we madee a transplantation or bioaugmentacion of the probiotic bacterium Pseudomona fluorescens to the skin of the animal, for this use a strain obtained of commercial way. To make this, first desinfect the area of the injuries with clorhexidine and give inoculate the previously prepared bacterium . We made several inoculations along a week improving quickly the appearance of the injuries, the hot spots and finally the area covered of hair. To check that the bacterium is found in skin we made analysis of the skin with Wood lamp, observing the fluorescense characteristic of the bacteria (geen fluorescent). Later we took samples of the skin and make a culture in Muller Hinton agar observing the colonies with the characteristic fluorescence.

Diseases wich bacteriotherapy could help

PARVOVIROSIS CANINE

It’s an infetious disease very lethal in puppies, the principal clinicall signs are:

- Vomits of dense consistency with green whitish foam or another type of tonality initially that become aqueous and abundant with blood.
- Fault of appetite and dehydration to fast rhythm because of the diarrhea and vomit.
- In grave cases, problems in the heart especially in puppies less to 6 months
- Diarrhea with or without blood (usually with blood) and very stinky.
- A big waned sadness.
- Weakness generalized, respiratory problems, dry eyes.

- Tall fever (this is one of the keys to detect the illness regarding others with the same symptoms)

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Morbility

- Very lethal in puppies of 1 to 6 months.
- It causes death by septicemia and dehydration.
- It needs urgent veterinary attention.
- Of bad prognosis.

The period of incubation approximated is of 5 days. The source of contamination is the faecal matter of the animals that have contracted the infection. It can have big quantity of virus in the faecal feces of the animals that suffer the illness. The virus is resistant under extreme climatic conditions and can survive during long periods.

.[1]

Treatment

There are not specific treatments: dehydration, replacing the liquids. In the case of the

parvovirus is one of the illnesses in where but can help the fecal transplant since to administer to early stages not only warns bacterial infections and improves the immune reaction but there is hypothesis that it can have bacteriophages that help to improve the dog condition.

Malabsortion syndrome

The main function of the small intestine is the digestion and the absorption of nutrients. By his part, the pancreas is the manager of segregate the main part of the digestive enzymes. Thus, problems in both organs relate with the syndrome of malabsorción.

Signs

Very likely that suffers the syndrome of malabsocion:

- Under weight, what indicates that the malabsorcion is already severe or chronic.
- Malnutrition, decrease of the muscular mass.

- Enormous appetite, what receives the name of polifagy, but also can have inapetence and anorexia.
- Coprophagy and pike, to try achieve nutrients.
- Diarrhea with three or four daily depositions.
- Foods without digesting in the excrements.
- The peel that it surrounds to the anus can appear greasy.
- Flatulence.
- Excessive intestinal noises.
- Chronic vomits, that are used to to be of yellowish white color.
- Abdominal pain.
- Dehydration.
- Anemia.

Any treatment for the malabsorcion has to base in the identification of the illness that is causing it, that is what treats. In any case, bases in the diet and in the medication,

according to the symptomatology that present. The administration of probiotics is useful because they may help in the absorption of nutrients because of enzymes that produces. Also probiotics restore the intestinal villi.

DISTEMPER

It transmits easily by the direct contact with dogs infected. The virus is present in the secretions of the nose and the tears. The dogs infected can disseminate the virus during several months. Although the virus is relatively unstable out of the infected dog, can travel some distance in the clothes and affect to other unprotected dogs. The contagion is very fast among the dogs of a group, especially in the kennels and in the canine exhibitions, from here the obligation to show that it has made a suitable program of vaccination in these situations. To the

Slight form

A lot of dogs possess a partial immunity provided by the mother or residual of past vaccines to which did not follow the doses

of pertinent memory, and can manifest only a light unrest, with tears, mucus and something of cough.

Respiratory form: course with a picture of tiring breath, nasal secretion and cough. It is possible that exist an secondary bacterial infection.

Digestive form: it consists in a gastroenteritis, with vomits and diarrhea.

Cutaneous form: Principally dermatitis

Nervous form: the animal begins to present muscular spasms, that end in the paralysis of the extremities . This symptom can be accompanied of a peculiar cough (sibilant “cough”, with whistles) product of the nervous injuries**.** In this form, the initial illness is used to to spend unobserved.

Ocular form: In her they appear signs of conjunctivitis.

Treatment

The virus does not have treatment. The bacterial infections secondary can treat , being also necessary the treatment of support.

In this case probiotics may increase the immune response when administered oral or intranasal, especially lactobacillus casei.

CONJUNTIVITIS

Conjunctivitis has a number of causes and describes an infection of the ‘conjunctiva’ of the eye, which is a mucous membrane covering the eyeball and lines the eyelid, which normally acts as a barrier to infection and foreign objects. When it is infected and inflamed it is known as conjunctivitis, or ‘pink eye’. The symptoms of conjunctivitis may include discharge from the eye, which can be clear or green. The whites of the eyes are often red, and the surrounding eye area swollen. You may also notice your dog pawing at the affected eye due to the discomfort caused, as well as squinting and blinking more than normal.

Conjunctivitis often starts in one eye and can spread to the other through contamination, but if an allergy (a common cause) or virus is the cause of the infection both eyes can be affected from the outset.

There are a number of things that can cause conjunctivitis and your vet will need to investigate to establish which is to blame. Potential causes can include:

- Foreign bodies such as a grass seed or grit
- Allergy
- Injury
- Bites in the eye area
- Blocked or infected tear duct
- Dry eye (caused by a lack of lubrication)
- Eye diseases such as glaucoma
- Bacterial infection (normally causes green or yellow discharge)
- Viral infection such as canine herpes or canine distemper
- Parasites such as eye worm

- The treatment your vet will give for conjunctivitis will very much depend on the cause.
- During an initial examination, your vet will give your dog a full eye exam and try to establish whether or not a foreign body is to blame. If it is, and is not easily removed, your dog may need to have it taken out under sedation or anaesthetic. Surgery may also be needed for a blocked tear duct. A course of eye drops and/or antibiotics will normally follow any surgery.
- If an allergy is likely to be to blame, antihistamines may be prescribed.
- If your vet thinks a bacterial infection is the cause, eye drops and antibiotics will normally be given. If a more serious underlying cause is suspected, further tests may be done.
- During the recovery period, a buster collar may be needed to prevent your dog from scratching at their eyes and causing any further irritation.
- There are several studies that use lactobacillus acidophilus to treat bacterian conjunctivitis with very good results, in our

case be have been used in eye drops with very good results

Dermatofitosis

The dermatofitosis, commonly known as it tinea, is a cutaneous infection caused by funguses of the gender Microsporum, Trichophyton and Epidermophyton, being the main causal agent Microsporum canis in the infections of dermatofitosis in cats.

is more frequent in cats that in dogs. It is common to see it in animals that live in groups and with the end to avert the contagion is important to detect it and treat it the before possible.

The clinic of dye it in dogs is visible among 2 and 4 weeks after the contagion, being faster the propagation if the animal is immunosupressed. Like this then, we will visualize circular injuries of alopecia that can be located or generalized. It dye it in dogs, on the contrary that in the humans, does not produce itchy and therefore less injuries by

It is important to diagnose it in initial phases as less aggressive will owe to be the treatment. Sometimes it is sufficient with improving the immune system of the animal affected, averting the propagation of the fungus. Usually it makes a treatment fungicidal commonplace in shape of ointment, powders or lotion in the whole body of the animal affected. In cases more advanced of main severity uses a systemic fungicidal.

There have been studies that show fungicidal properties of Pseudomona fluorescens and we have done inoculation of the non pathogenic bacteria strains in dogs with very good results.

Scabies

Exist diverse types of scabies that affect to the dogs, caused by distinct types of acaruses and his treatment is pretty much easy depending of the origin and moment of the process in which we begin it and the cures. We have to have very present that the scabies is perfectly treatable, although very annoying and for the one who suffers it, but if it does not carry a suitable treatment can give infections and

produce secondary illnesses and, even, the death of the animal.

Symptoms of scabies in dogs

To detect the canine scabies have to fix us in several symptoms that present that, luckily, are signs quite specific of this type of cutaneous condition and immediately call our attention. The most common symptoms of the scabies in dogs are:

- Itching and ardor of the skin.
- Inflammation of the skin.
- Scratches.
- Rubbed against objects and the soil in search of relief.
- Leave to eat (anorexia).
- Big loss of weight.
- Loss of the hair with zones entirely without fur.
- Eruptions in the skin (red stains).
- Cutaneous flakes.
- Wounds and sores in the skin.
- Badly smell of the skin.
- Dry skin.

If we detect some of these symptoms in our pet have to act quickly to avert the propagation of the condition. Although we can relieve symptoms of the scabies in dogs with some remedies that will see more advance, is vital that attend to the veterinary and follow the suitable treatment for each type of scabies as it indicate us the specialist.

Treatment

The treatment for the scabies in dogs that the especialist is generally with ivermectin, Besides, it will provide us analgesic, anti-inflammatory, some shampoo or powders for baths with products painkillers for the skin. Some study shown that bacteria like Pseudomona fluorescence have demonstrated to have acaricide effect by the secretion of chitinace that destroy the exosqueleton of mites.

Bacterioterapy and ethology

The relation between intestinal microbiome and behavior it’s not clear but some studies show evidence that indicates that the mammalian microbiome can affect behaviour, and several symbionts even produce neurotransmitters. One common explanation for these observations is that symbionts have evolved to manipulate host behaviour for their benefit, besides som e beahviour problems like coprophagy may improve when administered probiotics .Common causes of behavior problems

- Aggressiveness
- Fear
- Coprophagy
- Stress
- Anxiety by separation
- Jealousies
- Socialization

DUST BATH

This behavior is so innate that a lot of dogs have been seen to lie down and attempt to roll. It may be similar to scratching one's back. Rolling is usually put in the category of self-grooming or comfort behavior. It most certainly must have some adaptive function to have persisted for so long and among dogs kept in all types of environments. We believe dogs roll to scratch their backs, help shed winter coats and beneficall bacteria could colonize their skin providing them of multiple benefits.

Bioreactor

A bioreactor is a vessel in which a biological reaction or change takes place. The biological systems involved include enzymes, microorganisms, animal cells, plant cells, and tissues. The bioreactor is a place where an optimum external environment is provided to meet the needs of the biological reaction system so that a high yield of the bioprocess is achieved.

To multiply our probiotics bacteria we made a very simple bioreactor using a plastic bottle , draining bacteria from a small hole every time we need to administered.The procedure was as follows:

1-Disinfect a plastic bottle

2-Made a hole with a syringue

3-boil 100ml of milk (source of nutrients)

4-Add the milk to te plastic bottle

5-Wait 30 minutes

6-Add the probiotics

7-Make a hole in the bottle with a syringue (to extract the probiotics

8-desinfect the hole and place a tape

Test of methylene blue reduction

To measure the quantity of probiotics that be have in our cultures (milk, agar etc) we use the Test of reduction of the blue of blue methylene reduction test is based on the fact that the color imparted to milk by the addition of a dye such as methylene blue will disappear more or less quickly. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism causes the color to disappear. The agencies responsible for the oxygen consumption are the bacteria. Though certain species of bacteria have considerably more influence than others, it is generally assumed that the greater the number of

bacteria in milk, the quicker will the oxygen be consumed, and in turn the sooner will the color disappear. Thus, the time of reduction is taken as a measure of the number of organisms in milk although actually it is likely that it is more truly a measure of the total metabolic reactions proceeding at the cell surface of the bacteria.

The methylene blue reduction test has lost much of its popularity because of its low correlation with other bacterial procedures. This is true particularly in those samples which show extensive multiplication of the psychrotropic species

1. Sterilize all glassware and rubber stoppers either in an autoclave or in boiling water. Be sure all glassware is chemically clean.
2. Measure 1 ml of the methylene blue thiocyanate solution into a test tube.
3. Add 10 ml of milk and stopper.
4. Tubes may be placed in the water bath immediately or may be stored in the refrigerator at 0o to 4o C for a more convenient time of incubation. When ready to perform the test, the temperature of

the samples should be brought to 35o C within 10 minutes.

5. When temperature reaches 36o C, slowly invert tubes a few times to assure uniform creaming. Do not shake tubes. Record this time as the beginning of the incubation period. Cover to keep out light.
6. Check samples for decolorization after 30 minutes of incubation. Make subsequent readings at hourly intervals thereafter.
7. After each reading, remove decolorized tubes and then slowly make one complete inversion of remaining tubes.
8. Record reduction time in whole hours between last inversion and decolorization. For example, if the sample were still blue after L 5 hours but was decolorized (white) at the 2.5 hour reading, the methylene blue reduction time would be recorded as 2 hours. Decolorization is considered complete when four-fifths of the color has disappeared.

Factors Affecting the Test.–Many factors affect the methylene blue reduction test and

therefore the steps of operation should be uniform.

Since the oxygen content must be used up before the color disappears, any manipulation that increases the oxygen affects the test. Cold milk holds more oxygen than warm milk; pouring milk back and forth from one container to another increases the amount, and at milking time much oxygen may be absorbed.

Results

less than 30 minutes 20-30 millions

30 minutes -2 hours 4 to 20 millions

2 6 hours .5 to 4 millions

But of 6 hours less than 500000

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