Microbiology at Home: A Short Non-Laboratory Manual for Enthusiasts and BioArtists

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Disclaimer: Whilst every effort has been made to highlight the safety issues regarding the cultivation of bacteria and other microorganisms outside of a laboratory, the author will not accept any liability for use of the methods and media described here.

STERILIZATION

Microbes live and survive in all habitable, and even in some seemingly inhabitable environments. As a consequence of this many will be naturally present on culture vessels, the ingredients for microbiological agar, and in water used to prepare this. If these microbes are not removed, then they will at the very least grow and contaminate your precious media so that you will end up with cultures that resemble rotten food. This makes it impossible to gain any meaningful results. In the worst case scenario, some of these organisms may be disease causing, and failure to eliminate these, may lead to their inadvertent culture and generate a serious infection hazard.

Waste materials produced from microbiology experiments will contain living microorganisms and these can represent a very real contamination hazard. As a consequence, all cultures and materials containing these lifeforms **must** be inactivated before disposal. To minimize risk, it is important to get rid of the materials that you no longer need as quickly as possible.

In microbiological terms, sterilization refers to the complete elimination of all living organisms in or on the materials being sterilized. There are no degrees of sterilization and items are either sterile or not. For the above reasons, sterilization procedures are a vital aspect of microbiology, and in the home, the most effective and available methods all involve the use of heat to destroy microbes.

Autoclaving

In microbiology laboratories the most widely-used method for heat sterilization is autoclaving. The autoclave is a large and complex machine that uses pressurized steam, heated to 121 °C, to destroy microbes. The high pressure (typically 15 psi) allows the temperature to exceed 100 °C, the normal boiling point of water at atmospheric pressure. To achieve sterility, a holding time of 15-20 minutes is required and such a treatment, if carried out properly, will inactivate all fungi, bacteria, viruses and also bacterial spores. The autoclave is effectively a giant pressure cooker, and so conveniently for the home microbiologist, a conventional pressure cooker can serve as a very effective autoclave for the home. The Prestige Hi Dome Aluminium Pressure Cooker (6 Litre) (£40-60) is a simple and robust appliance, which when used with the highest weight setting (15lb), will reach the elevated temperatures needed for sterilisation. It makes an ideal home autoclave and in fact, if you’re serious about microbiology at home, this or a similar pressure cooker is essential, as it will allow you to make sterile media and to safely deactivate any microbiological waste that you produce.

Please follow the instructions below to use your pressure cooker as an autoclave and note that these differ slightly from the instructions for its use in pressure cooking for reasons that are explained.

1. Pour tap water into the bottom of the pressure cooker. This can be tap water but if you live in a hard water area using bottled spring water will prevent scaling of the device.

2. To prevent items being immersed in the water and becoming soaked, the metal trivet should be inverted and used as a rack and any containers or packets placed on top of this.

3. For liquid media, robust glass vessels are needed during sterilization. Containers must have tops that can be fitted loose, and therefore vented, and should be capable of holding at least 20% more than the intended volume of medium, to allow for expansion during sterilization. If screw cap jars are used, the cap must be loosened prior to sterilization. Kilner Screw Top Glass Preserving Jars (500ml) are ideal. Solid items can be wrapped in aluminium kitchen foil but will need to be dried afterwards (2-3 days in an airing cupboard) without opening the packet to maintain sterility.

4. Put on the lid and close the pressure cooker but do not yet put the weight on top on the steam valve.

5. Place the cooker on the heat source and turn to a high setting

6. Once the water in the cooker starts to boil, steam will come out of the open valve. Allow steam to pass out of the valve for about 5 minutes, and then put the heaviest weight (15lb) weight on top of the valve. This step is important because it will ensure that all of the trapped air is removed. Air is a poor conductor of heat and too much air will decrease the efficiency of the sterilization process.

7. The steam will build up in the inside of the pressure cooker until it reaches the correct pressure. At this point, the steam will lift the weight and start to escape. As soon as the steam starts to escape, start timing the sterilization and turn down the heat so that the steam is only just escaping and not rushing out. Aim to maintain a gentle hissing.

8 After 20 minutes turn off the heat and leave to cool.

9 Wait until pressure is completely reduced then lift the weight off the valve allowing any remaining steam to escape. **Never open the pressure cooker until the steam valve has been opened to release the pressure.** Open the pressure cooker carefully using the metal lid to shield yourself. The contents will still be very hot so allow them to cool for 10-15 minutes before handling. During sterilization the molten agar will tend to sink to the bottom of the containers so to ensure **a** uniform distribution before pouring, the media should be gently shaken after sterilization,

while it cools. Don’t take too much time over this as agar will set to form a firm gel in the bottle at about 32 C to 45 C.

Dry-Heat Sterilization

The dry-heat sterilization process works via conduction whereby heat is absorbed by the exterior surfaces of an item and then gradually passes throughout it so that eventually the entire object reaches the proper temperature required to achieve sterilization. Air is a poor conductor of heat and consequently, the times needed for sterilization here are much longer than for autoclaving. Effective time and temperature and temperature combinations for dry-heat sterilization are 160°C (320°F) for 2 hours or 170°C (340°F) for 1 hour and consequently, a conventional household oven is ideal for this process. This method is usually used for materials which can withstand the high temperatures (not media), such as metal and glass, and for those which could either be corroded by steam or must remain dry. Again materials can be wrapped in foil to maintain sterility after treatment.

Flaming (Red Heat)

As the name suggests, flaming involves placing an object in a flame. This might be from a Bunsen burner, an alcohol lamp, or at home from a gas cooker or portable gas burner. The item is left in the flame until it glows red and the intense heat ensures that any microbe is rapidly inactivated. This method is commonly used for small metal (loops, wires and blades) because it only takes a matter of seconds to sterilize these but it is obviously not suitable for larger, flammable, or heat sensitive items. The heated item must also be cooled before it can be used to transfer microbes as the residual heat would also kill these, and this can be achieved conveniently by pressing the item into sterile agar-based media until it stops fizzing.

A variation on flaming is to dip the object in 100% ethanol (at home methylated spirits is an ideal substitute for this) and then to merely touch the object briefly to the flame, but not hold it there. The ethanol will ignite and burn off in a few seconds. This process will kill most but not all microbes.

ASCEPTIC TECHNIQUE

Microbiological media will not only support the growth of those microorganisms that you have chosen to grow but will also readily grow undesirable contaminants from your body (skin and hair) or any other non-sterile surface that the media comes into contact with. Microbes cannot fly but are readily carried on the air, and on microscopic dust particles, and can readily make their way onto your sterile media if it is carelessly handled.

Simple tips

• Microorganisms, food and eating don't mix safely because there is a risk of accidental ingestion, so whilst sterile media may be prepared in the kitchen, once microbes have grown on it, it would be better to handle it in some other room.

• Keep all surfaces clean and dry and treat with household disinfectants prior to and after use.

• **Avoid** clutter and get rid of the materials that you no longer need as quickly as possible.

• Make sure that all media and culture vessels have been sterilized prior to use.

• Keep culture dishes covered. When it is necessary to open a dish, keep the lid close to the dish and open it only as far and as long as is necessary to accomplish the procedure.

• If you have long hair, make sure it does not hang into your culture media as hair is a major source of contaminating microorganisms.

• Never reach over sterile media as you will contaminate it.

MEDIA AND STAINS

Bacteria display a huge range of nutritional and physical requirements for growth and accordingly, there is no single medium or environment that will allow the cultivation of all bacteria. Some bacteria will grow in little more than water whilst others (in fact most of the bacteria that you are likely to study here) are a little more demanding and require certain complex nutrients. In the lab these needs are most often met by including extracts or enzymatic digests of meat, milk, plants or yeast. Fortunately, many of these ingredients have counterparts on supermarket shelves and simple microbiological media can be easily made at home without having to resort to commercial media suppliers, who would most likely not release the materials to the general public in any case. More esoteric ingredients however, such as brain-heart infusion or defibrinated horse blood, are not likely to be as readily available to the home microbiologist.

Microorganisms may be grown in liquid, solid or semisolid media. The media included in this manual, however, are all for solid media because these allow the formation of colonies and consequently, are the most useful for assessing the diversity of bacteria from different environments, for the isolation of pure cultures and for the short-term maintenance of microbes. To make solid medium 1 to 2% agar is simply added to a solution of appropriate nutrients in order to convert it into a solid gel.

Agar is a natural compound that is extracted from certain types of red-purple marine algae and it has a number of unique properties that make it the gelling agent of choice for microbiology. It melts at approximately 85°C, a different temperature from that at which it solidifies, 32-40°C, allowing it to form a strong gel at room temperature but which remains firm at temperatures up to 65°C. In addition, most, but not all bacteria, are unable to degrade agar meaning that your media will be stable in the presence of these organisms. Compare this gelling agent to gelatin, which can also be used to make solid culture media if agar is not available. Many bacteria possess the ability to degrade gelatin and consequently what starts off as an ideal solid media some becomes a liquid as the gelling compound is degraded by the very bacteria growing on it. This process is not without artistic merit and in certain circumstances may be actually desirable. Both agar and gelatin are commonly used as gelling agents in cooking so head for the home baking section of your local supermarket and you should be able to find all you need to solidify your microbiological agar.

Solid microbiological media was traditionally prepared in reusable glass Petri dishes with lids and today, clear plastic disposable Petri dishes, typically 95 or 100 mm in diameter are used. Should these prove difficult to acquire, then many containers, provided they can be sterilised, will serve as replacement Petri dishes, such as metal

jam jar lids, and metal tins used for baking cupcakes/muffins. These can all be covered with foil to maintain sterility during incubation.

What follows are descriptions and instructions for a variety of specially developed microbiological agars, suited for isolating particular groups of bacteria, and all of which can be made in the home or kitchen, with readily accessible ingredients.

1. General Kitchen Agar (GKA)

Background and Intended Use

General Kitchen Agar is a novel modification of Plate Count Agar. It can be used for obtaining microbial counts from any environment, and is specifically designed for use in facilities where the availability of chemicals may be restricted by Health and Safety issues or by problems with supply. Unlike any other microbiological agar, it is edible, but consumption is not recommended once it has been inoculated.

Principles of the Medium

The casein present in dried skimmed milk powder provides amino acids and other complex nitrogenous substances that are necessary to support bacterial growth. Marmite, is a form of yeast extract, and primarily supplies the B-complex vitamins need as co-factors. Honey is a natural source of carbohydrates (fructose and glucose) and provides the energy source for growth. The medium can be supplemented with various natural chromogenic compounds that will change colour depending on microbial activity (extract of red cabbage and turmeric are recommended).

Directions for Preparation

Formula Per Litre (1 litre makes approximately 40 agar plates)

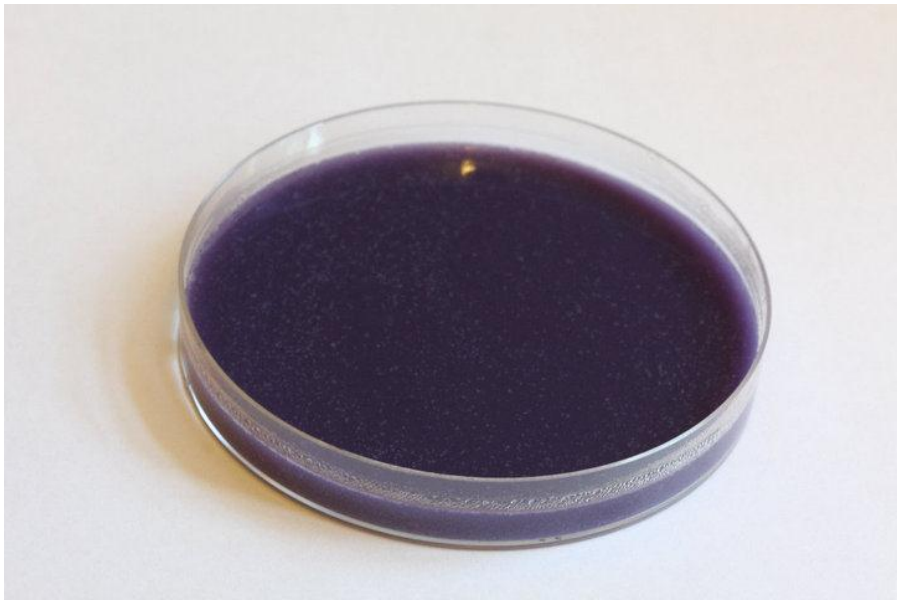
Dried Skimmed Milk Powder (Marvel, or Supermarket Own Brands).....5.0 g
Marmite (or Vegimite or Bovril).. .....2.5 g
Honey..... .....1.0 g
Agar (Clearspring but many brands are available).....15.0g
Natural chromogenic supplement (optional)

Suspend the above ingredients in 1 Litre of purified water (any bottled still mineral water should be suitable) in a saucepan. Bring to the boil and continue to stir and simmer until all of the particles of agar have dissolved. This step may take some time but it is crucial to ensure that all of the agar has dissolved. If the medium is to be used without complete sterilization it may be cooled at this point to 55 ^{o}C and poured into Petri dishes. At this point the medium can also be sterilized by heating it in a household pressure cooker at maximum pressure for 20 minutes. Cool and pour as described above.

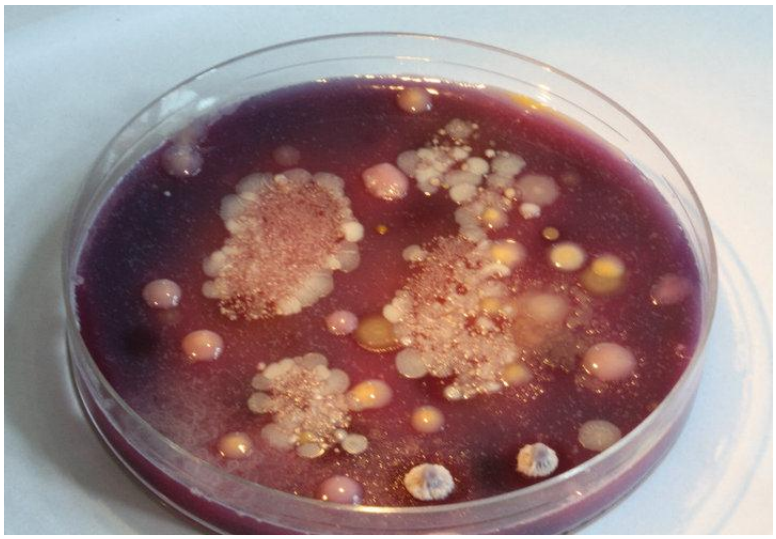
Notes

This is a highly nutritious medium that will support the growth of many bacteria including pathogens. It must therefore only be handled and used in accordance with good microbiological practice. It should only be disposed of via a validated method that ensures the complete inactivation of any microorganism which grows on it. The medium is intended for only the non-specific isolation of bacteria from the environment and to reduce the risk of the growth of pathogenic bacteria the plates should be incubated at 25 ^{o}C.

General Kitchen Agar (GKA) Images



Uninoculated GKA. This version of the agar is purple because it contains an extract of red cabbage. This is a natural pH indicator and turns green/yellow if the bacteria generate alkaline conditions or red if they produce acid. Purple indicates neutral conditions.



Bacterial colonies that grew on GKA after it had been inoculated with soil. A wide variety of bacteria grew. The powdery nature of the colony in the close-up suggests that it might be an actinomycete.

2. Cyanobacterial Kitchen Agar (CKA)

Background and Intended Use

Cyanobacteria are a type of bacteria that have formed the oldest fossils known, from more than 3.5 billion years ago, and yet they are still very common and are one of the largest and most important groups of bacteria on earth. Today, they can be found in almost every conceivable environment on earth, from oceans to fresh water, from bare rock to soil and even in the most extreme niches such as hot springs and in Antarctic ice. Their story is also intricately linked to the evolution of more complex life on earth as they were the first organisms to evolve to become photosynthetic, that is, to be able convert the simple gas carbon dioxide into more complex carbon compounds (particularly sugars) using light as an energy source. As oxygen is produced as a by-product of this process, as a consequence of this cyanobacteria also created the oxygen rich conditions in our planet's early atmosphere that were necessary for the evolution of all oxygen-dependent life including us. The other great contribution made by cyanobacteria is the origin of plants. The chloroplast, a small body within all plant cells which carries out the vital process of photosynthesis is actually derived from a cyanobacterium which some time during the late Proterozoic, or in the early Cambrian periods took residence in a larger type of cell and began making food for its host cell via photosynthesis return for accommodation.

Principles of the Medium

As mentioned above cyanobacteria are photosynthetic and will grow on little more than water, air (carbon dioxide) and light. As a consequence of this this media provides the cyanobacteria with just essential minerals and they are able to grow on this media by using light to manufacture their own food. The soluble plant food and the chalk in the media are an ideal source of minerals for the These must be sterilized separately from the agar and then added to the agar when cool to prevent the components reacting together at high temperature. Once the medium is made and inoculated with cyanobacteria, it must be incubated in daylight to allow the bacteria to carry out photosynthesis.

Directions for Preparation

2x Mineral Stock:
Miracle-Gro All Purpose All Purpose Soluble Plant Food……...2 x 1.25ml measures
Chalk (finely ground from a natural source)…… 1g
Natural Bottled Spring Water……... 500ml

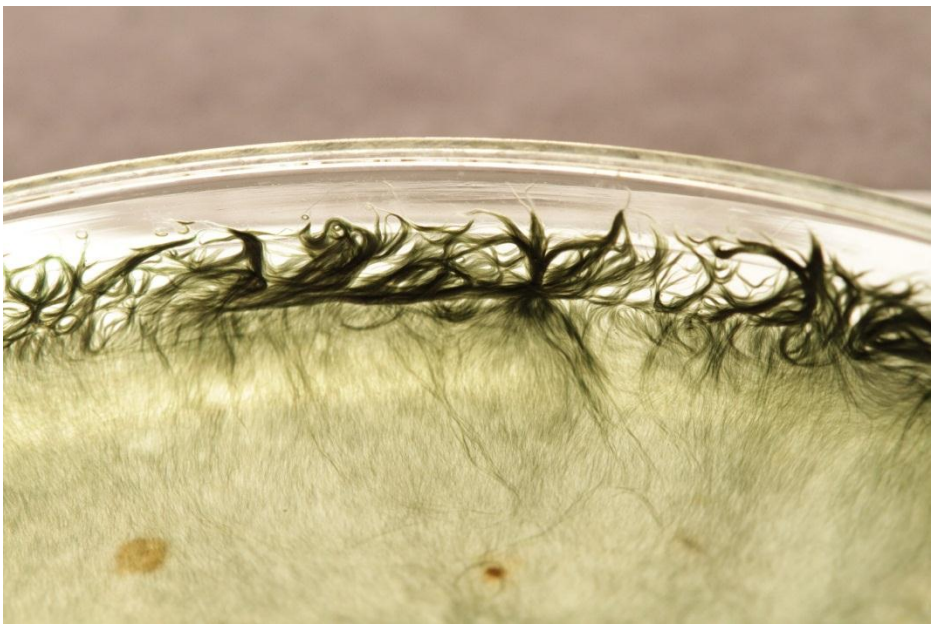
Agar Solution:
Agar (Clearspring but many brands are available).....3.0g
Natural Bottled Spring Water……100ml

Make up the two separate stock solutions as outlined above. Sterilize these by heating them in a household pressure cooker at maximum pressure for 20 minutes. When the two solutions are cool enough to handle safely, combine them and make sure that they are mixed thoroughly. Pour into suitable containers and allow to set before using. Please note that the calcium carbonate present in the chalk is insoluble and will not dissolve and to mix it into the media the container should be shaken gently just before pouring.

Notes
Cyanobacteria only grow in water or extremely damp places so lakes and ponds are a good source of these bacteria. Cyanobacteria can also grow to very high levels in waters polluted with agricultural fertilisers and form the extensive and visible blooms that appear as a blue-green paint or scum and these would be a good source. Be careful handling bacteria and water from such blooms as some cyanobacteria produce powerful toxins.

Cyanobacterial Kitchen Agar (CKA) Images

The ingredients for CKA, CKA plates and a natural isolate of the cyanobacterium *Nostoc* waiting to be culture



The growth of *Nostoc* CKA. This cyanobacterium can grow in a filamentous form as seen here. The filaments are called trichomes.

3. Bioluminescent Kitchen Agar (BKA)

Background and Intended Use

Where there is no sunlight, living organisms can create their own form of biological light called bioluminescence. In fact, it is estimated that 90% of deep-sea marine life produce this form of light and they use it to attract prey or mates, to distract or repel predators, and for communication. Some, but not all of the organisms that use bioluminescence cannot produce light themselves and have to rely on bioluminescent bacteria to do this for them. In these situations, the animal and bacterium form a complex relationship whereby, the bacterium gains a home and is fed, and the host gains the ability to make light and to put this light to its own uses. Certain types of bioluminescent bacteria live in seawater on marine animals and, the latter make a good source for these interesting bacteria.

Principles of the Medium

As mentioned above many bioluminescent bacteria are marine in origin so the media used to grow them has to mimic some of the properties of seawater and consequently the media contains a high concentration of salt. The source of the salt is important here and it is better to use natural sea salt as will also contain natural minerals and not additives like refined salt. These bacteria also require amino acids and small fragments of protein for growth and the hydrolysed vegetable in the Marigold Swiss Vegetable Boullion Powder provides an excellent source of these. Marmite, is a form of yeast extract, and primarily supplies the B-complex vitamins the bacteria need as co-factors. Glycerine (also known as glycerol) is available from most pharmacies and is source of carbon and also provides the energy source for growth and light production. As the bacteria grow they produce acids which unless neutralised will kill them. Chalk (essentially calcium carbonate) is included in the media because it reacts with the acids to neutralise them.

Directions for Preparation

Marigold Swiss Vegetable Boullion Powder (8g gives slightly better bioluminescence but 2g gives slightly less light but results in larger colony formation)

Marmite Yeast Extract 0.7 g

Saxa Sea Salt 15.0g

Glycerine 5g

Ground Chalk 2.5g

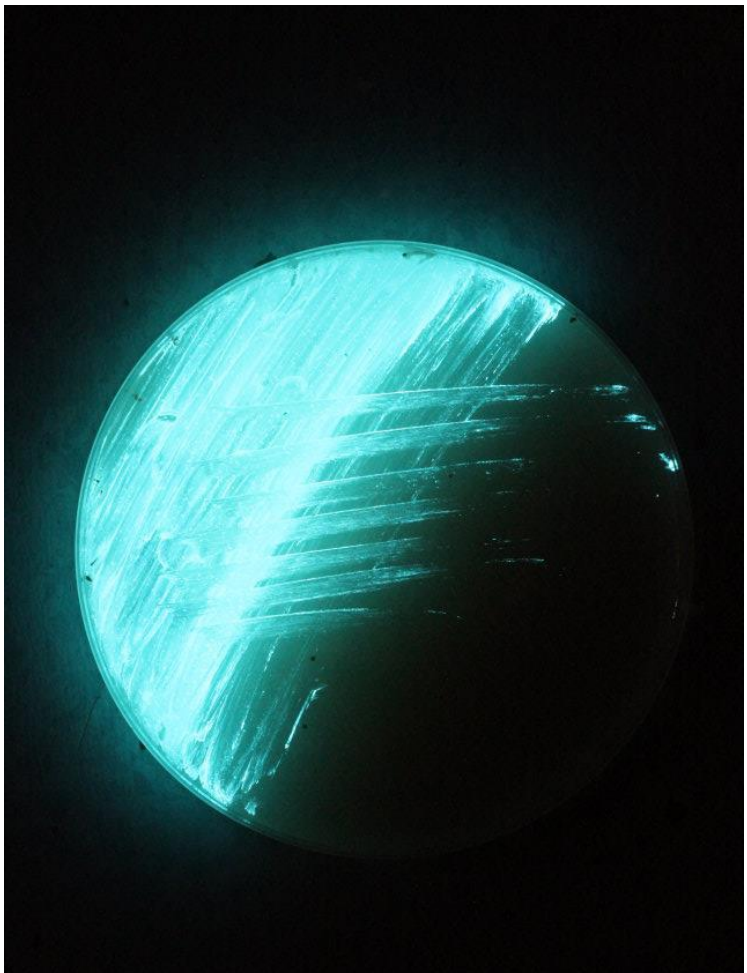
Agar 7.5g

Suspend the above ingredients in 500ml of purified water (any bottled still mineral water should be suitable) in a saucepan. Bring to the boil and continue to stir and simmer until all of the particles of agar have dissolved. This step may take some time but it is crucial to ensure that all of the agar has dissolved. If the medium is to be used without complete sterilization it may be cooled at this point to 55 oC and poured into Petri dishes. At this point the medium can also be sterilized by heating it in a household pressure cooker at maximum pressure for 20 minutes. Please note that the calcium carbonate present in the chalk is insoluble and will not dissolve and to mix it into the media the container should be shaken gently just before pouring.

Notes

This media can be made as a liquid broth if the agar is omitted. If you don't have a laboratory source of bioluminescent bacteria, you can immerse fresh (and not previously frozen seafood, like fish, squid, and prawns) into the broth. If you leave this for a few days in a cool room, this will enrich for bioluminescent bacteria and you can then culture and isolate these on BKA.

Bioluminescent Kitchen Agar (BKA) Images



The growth of *Photobacterium phosphoreum* on PKA. Imaged in darkness and strikingly bioluminescent.

4. Ehrlich Staining

Background and Intended Use

Bacterial and other microbial cells are usually colourless because the cytoplasm is mostly transparent, and so even when viewed a microscope, they are not generally visible. As a consequence of this bacteria and some other microorganisms need to be stained to make them visible.

Paul Ehrlich was a German physician who made countless contributions to science, in fields as diverse as histology, haematology, immunology, oncology, microbiology and pharmacology. In the course of his investigations Ehrlich came across the dye methylene blue, which he regarded as particularly suitable dye for staining bacteria. The process here is inspired by Ehrlich's early studies on staining bacteria and is a simple off-the-shelf/ DIYBio-staining procedure for bacteria, other microbes, human cells and DNA. It is based on methylene blue which is readily available as a "fish medicine". The brand I used here is King British Methylene Blue. It works very well as it comes in the bottle, and without the need for any messy preparation.

Methylene blue is unable to penetrate living cells and will thus leave them unstained. However dead cells are unable to keep the methylene blue from penetrating them and will stain blue. It will also specifically stain DNA blue.

Clean a glass microscope slide (or some other form of glass) and spread the sample to be observed over the slide

Directions

1. Clean a glass slide (or other form of glass) and place the sample to be examined on the slide. If the sample is liquid then it should be spread over the slide to form a thin a film. If the sample is solid then it will need to be mixed with a small volume of water before spreading.

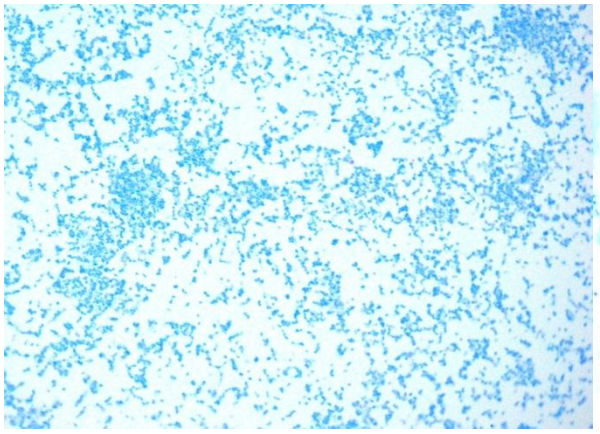
2. Allow the spread sample (called the smear) to air dry. It's best not to heat the sample nor blow on it to quicken drying because this could force bacteria into the air leading to contamination and possible infection.

3. In order for the sample to be stained, and to stop the microorganisms simply washing off the slide during this process, it needs to be heat fixed. Holding the slide by one edge (forceps can be used here) pass it slowly through a Bunsen burner

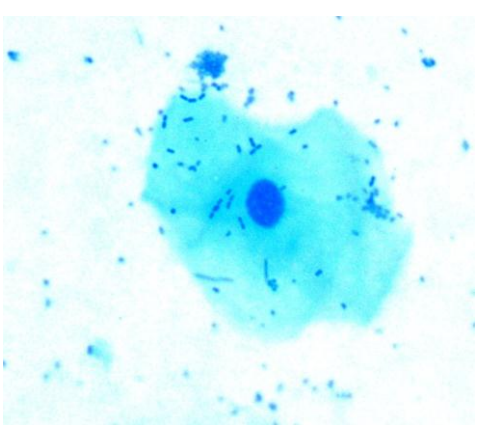
flame (other flames will also work). Do not move the slide so slowly that the edge of the slide that you're holding heats up to uncomfortable levels. This step leads to the denaturation of proteins causing the microbial cells to stick to the slide. It also kills any microorganisms making them safe for the following steps, and also renders them susceptible to the methylene blue staining

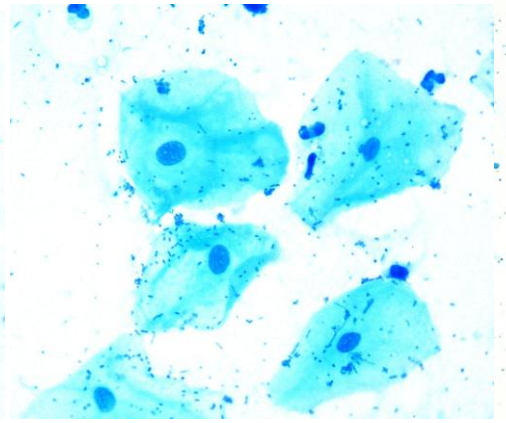
4. When the slide has cooled, cover the sample smear with the methylene blue stain solution (King British Methylene Blue). Allow the stain to work for 2 minutes. Remove the stain by rinsing with water from a tap and gently blot (do not rub) the stain dry using tissue paper. Air dry to ensure any residual water is removed and then observe with a microscope. You will need at least 400 x magnifications to see bacteria and other microbes, and preferably 1000x to see bacteria properly.

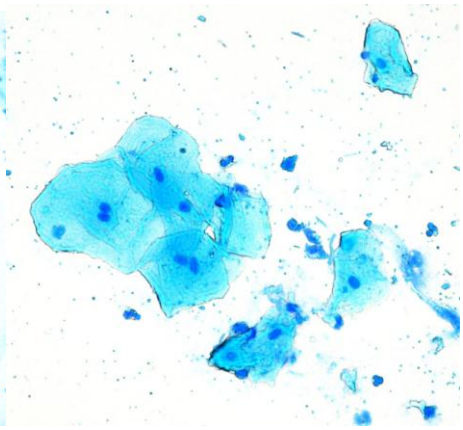
Ehrlich Staining Images



Bacteria from a bought probiotic yoghurt (left) and Baker's yeast (left), 1000x magnification







Here I have stained saliva from my own mouth and the stain has revealed my own cells, their nuclei, and the normal bacterial flora of my mouth. My personal buccal (cheek) epithelial cells are visible as large pale blue cells with a dark staining nucleus (the dye stains DNA) which contains my own genome. Numerous bacteria are visible either attached to the cells or in other parts of the stained sample (these are the small darkly stained shapes).

COMING SOON

Social Media, a media for the isolation and cultivation of Myxobacteria. Very social bacteria that build structures and hunt other bacteria in packs like wolves.

Kitchen *Staphylococcus* Agar (KSA), a medium for the selective isolation of *Staphylococcus* from the human body.

Natural Transformations, an off-the-shelf/DIYBio method that uses natural mineral waters to transform cells of bacteria (introduce plasmid DNA into them, a key step in genetic modification).