Fundamental microbiological working methods

Biology | Microbiology & genetics | Basics of microbiology

Applied Science | Medicine | Histology & Medical Microbiology

Difficulty level	Group size	Preparation time	Execution time
hard	2	30 minutes	45+ minutes

This content can also be found online at:

http://localhost:1337/c/5f0ed4cfb6127b0003044958

PHYWE
Robert-Bosch-Breite 10
37079 Göttingen
Tel.: 0551 604 - 0
Fax: 0551 604 - 107
info@phywe.de
www.phywe.de

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Teacher information

Application

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Experimental setup

The history of microorganisms starts with the invention of the microscope, at the dawn of the 17th century. After that Louis Pasteur established the first important methods when working with bacteria. Robert Koch elevated the microbiology for medical usage. His methods are the basis of microbiological work today, for example the use of agar.

This experiment is to acquire the basic knowledge for working with microorganisms.

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Robert-Bosch-Breite 10
37079 Göttingen

Tel.: 0551 604 - 0
Fax: 0551 604 - 107

info@phywe.de
www.phywe.de

Other teacher information (1/2)

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Prior knowledge

In order to prevent nutrient media and cultures from being contaminated with microorganisms that adhere to the working equipment, the equipment, nutrient media, and nutrient solutions must be sterilised.

Scientific principle

The nutrient medium is poured into the dishes either from test tubes, with one test tube holding the required quantity of ready-made medium for one Petri dish, or from Erlenmeyer flasks if a large number of plates needs to be prepared at the same time.

Other teacher information (2/2)

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Learning objective

The students are to acquire the knowledge of basic work techniques specific for microbiological work.

The students are to practise the following working techniques:

Tasks

1. Sterilisation of equipment
2. Preparation of standard nutrient agar for bacteria
3. Preparation of standard nutrient agar for moulds and yeasts
4. Preparation of a standard nutrient solution for bacteria
5. Preparation of slant agar tubes
6. Inoculation of microorganisms

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37079 Göttingen

Tel.: 0551 604 - 0
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info@phywe.de
www.phywe.de

Safety instructions

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For this experiment the general instructions for safe experimentation in science lessons apply.

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Student information

PHYWE

Robert-Bosch-Breite 10
37079 Göttingen

Tel.: 0551 604 - 0
Fax: 0551 604 - 107

info@phywe.de
www.phywe.de

Motivation

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The history of microorganisms starts with the invention of the microscope, at the dawn of the 17th century. After that Louis Pasteur established the first important methods when working with bacteria. Robert Koch elevated the microbiology for medical usage. His methods are the basis of microbiological work today, for example the use of agar.

The purpose of this experiment is to acquire the basic knowledge for working with microorganisms.

Tasks

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Experiment setup

Practise the following working techniques:

1. Sterilisation of equipment

2. Preparation of standard nutrient agar for bacteria

3. Preparation of standard nutrient agar for moulds and yeasts

4. Preparation of a standard nutrient solution for bacteria

5. Preparation of slant agar tubes

6. Inoculation of microorganisms

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Equipment

Position	Material	Item No.	Quantity
1	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
2	Beaker, Borosilicate, tall form, 600 ml	46029-00	1
3	Graduated cylinder, Borosilicate, 100 ml	36629-00	1
4	Tripod,ring d=140 mm, h=240 mm	33302-00	1
5	Microscopic slides, 50 pcs	64691-00	1
6	Graduated pipette 10 ml	36600-00	2
7	Test tube rack for 12 tubes, holes d= 22 mm, wood	37686-10	1
8	Erlenmeyer flask, Duran®, narrow neck, 500 ml	36121-00	2
9	Test tube, 160 x 16 mm, 100 pcs	37656-10	1
10	Meat extract 10 g	31521-03	1
11	Peptone,dry,from meat 50 g	31708-05	1
12	Sterile stoppers f. id 15mm, 250	39266-00	1
13	Sterile stoppers f. id 29mm, 100	39267-00	1
14	Pipettor	36592-00	1
15	Spatula, double blade, 150 mm	33460-00	1
16	Glass rod,boro 3.3,l=300mm, d=7mm	40485-05	1
17	Wire gauze with ceramic, 160 x 160 mm	33287-01	1
18	pH test sticks 6.5-10, 100 sticks	30301-04	1
19	Bunsen burner, natural gas,w.cock	32167-05	1
20	Safety gas tubing, DVGW, sold by metre	39281-10	1
21	Sodium hydroxide, pellets, 500 g	30157-50	1
22	Water, distilled 5 l	31246-81	1
23	Agar-agar, powdered 100 g	31083-10	1
24	Autoclave with insert	04431-93	1
25	Watch glass, dia.60 mm	34570-00	1
26	Hydrochloric acid,approx.5% 250ml	30315-25	1
27	Tweezers, l = 130 mm, straight, blunt	64610-00	1
28	Universal oven, 32 liters, 230 V	49559-93	1
29	Ethyl alcohol, absolute 500 ml	30008-50	1
30	Petri dish, d 100 mm	64705-00	1
31	pH test sticks 4.0-7.0,100 sticks	30301-03	1
32	Heating + cooking hotplate,230V	04025-93	1
33	Wire loop, streaking	64936-00	1

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info@phywe.de
www.phywe.de

Setup (1/9) Sterilisation of equipment


Abb. a

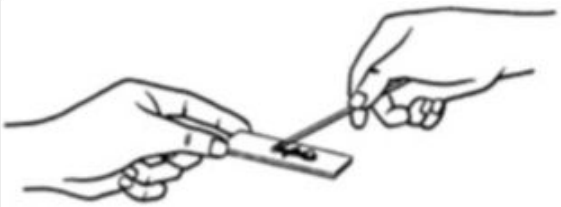
The equipment must be sterile in order to avoid contamination. Metal equipment is sterilised with the flame of a gas burner. Before and after their use, inoculation loops and needles are heated in the flame of a gas burner until they are red hot and then they are allowed to cool. The holder is flamed if it can come into contact with the cultures or any sterile equipment. Small glass objects, such as microscope slides and coverslips, are also sterilised by flaming. Prior to flaming, they can be immersed in 96% ethyl alcohol. During this process, the microscope slides and cover slips must be held with a pair of tweezers. Pipettes are sterilised by filling them twice or three times with 96% ethyl alcohol, by letting the alcohol flow out, by blowing the residual ethyl alcohol into the flame of a gas burner, and by passing the pipette repeatedly through the flame afterwards. All other equipment parts made of glass are heated up to 160 °C for 1 to 1½ hours in a hot-air steriliser. The risk of shattering is lower, since they can cool more slowly. This is why glass equipment is always placed in a cold steriliser where it is also left to cool after the sterilisation.

Setup (2/9) Standard nutrient agar for bacteria

Nutrient medium is constituted to meet the requirements for the target organisms, in this case bacteria. The gelling agent that is used is agar-agar or short agar. Unlike gelatine, agar can be decomposed by few types of bacteria. Weigh the required quantities of all of the ingredients, fill them into a beaker, and boil them until the agar has completely dissolved. The beaker must be large enough to hold four times the quantity of the prepared nutrient medium, because agar boils over rather easily. As soon as the solution boils, reduce the flame and stirr the solution repeatedly. Weigh the meat extract. To do so, unroll the glass rod on the microscope slide (Image on the next slide) and flush the meat extract into the beaker. The microscope slide can also be boiled together with the solution and removed afterwards.

PHYWE
Robert-Bosch-Breite 10
37079 Göttingen
Tel.: 0551 604 - 0
Fax: 0551 604 - 107
info@phywe.de
www.phywe.de

Setup (3/9) Standard nutrient agar for bacteria



Weighing the meat extract

The nutrient medium should include: **Liebig's meat extract 0.3%; Peptone 0.5%; Agar 2.0%;** Distilled water is used as the solvent.

When all of the ingredients have completely dissolved, the nutrient medium is brought to a **pH value of 7.4 to 7.6** by adding drops of a 1% sodium hydroxide solution. While doing so, check the pH value with pH test sticks (pH 6.5 to 10.0). Bacteria grow best in a neutral to weakly alkaline environment, i.e. at a pH value of approximately 7.0 to 7.2. Since the concentration of hydrogen ions will decrease slightly during the sterilisation of the nutrient media, it is adjusted right from the onset to a higher level than the actually desired level. The prepared nutrient medium is then filled into test tubes; approximately 12 to 15 ml (approximately half full) for plate pouring.

Setup (4/9) Standard nutrient agar for moulds and yeasts

Slime mold

Moulds and yeasts grow mainly on slightly acid substrates, which must be taken into consideration when preparing the nutrient media. The nutrient medium should include: **syrup (beet juice) 5.0%; agar 2.0%**; distilled water is used as the solvent. Fill the required quantities of all of the ingredients into a beaker and proceed analog to bacteria medium. When all of the ingredients have dissolved, test the **pH value** of the nutrient medium with a test stick. It should be approximately **5.0-6.0**. If the pH value differs from this reference value, add drops of a 1% sodium hydroxide solution or of 1% hydrochloric acid. The prepared nutrient medium is then filled into test tubes; approximately 12 to 15 ml (approximately half full) for plate pouring and 5 to 6 ml (approximately one quarter full) for the preparation of slant agar tubes. Larger quantities that can be used in one go for pouring plates are filled into Erlenmeyer flasks. Seal the test tubes and Erlenmeyer flasks with sterile stoppers and sterilise them in the autoclave. Most yeasts and moulds will grow well on this type of nutrient medium. Proceed in the same manner in order to prepare nutrient media with a different composition for yeasts and moulds.

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Robert-Bosch-Breite 10
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Setup (5/9) Sterilisation of nutrient media and solutions

Benchtop autoclave

The ingredients of the nutrient media and solutions as well as the tools and equipment that are used for their preparation are contaminated with a wide range of microorganisms that would quickly grow and contaminate the prepared nutrient media. To remove contamination, they are placed in an autoclave and sterilised in flowing steam. Hot-air sterilisers are not suitable for nutrient media and solutions because their water content would be reduced, thereby increasing the nutrient concentration.

Autoclaves are steam pots that can be sealed in an air-tight manner. They enable the sterilisation under overpressure and at temperatures above 100 °C, thereby also killing bacteria spores. However, the killing time strongly depends on steam saturation in the autoclave.

Setup (6/9) Sterilisation of nutrient media and solutions

This is why it must be ensured that, after the water in the autoclave has started to boil, as much air as possible can escape in order to achieve a high level of steam saturation. The autoclave must be filled with the necessary quantity of water - in general just below the insert - and then the objects to be sterilised are added. Several test tubes are placed in a container as a group. Set the sterilisation conditions to **1.4 - 2,7 bar, approx. 125 - 140 °C;**After the water in the autoclave has started to boil, which is indicated by steam escaping from the steam release valve, keep the steam release valve open for another 3 minutes until the autoclave has been vented and then close it. The operating pressure will now be reached because of the pressure increase, and steam will escape from the sterilisation valve. This means that a little steam should escape the entire time during the sterilisation. After a high pressure of 1.4 bar has been reached, the sterilisation duration is 20 minutes or 15 minutes at 2.7 bar. After the sterilisation, leave the items in the autoclave in order to allow them to cool. Wait until the high pressure has subsided before opening the autoclav to ensure that no sterilisation product boils out of the vessel. Then, remove the lid.

PHYWE
Robert-Bosch-Breite 10
37079 Göttingen
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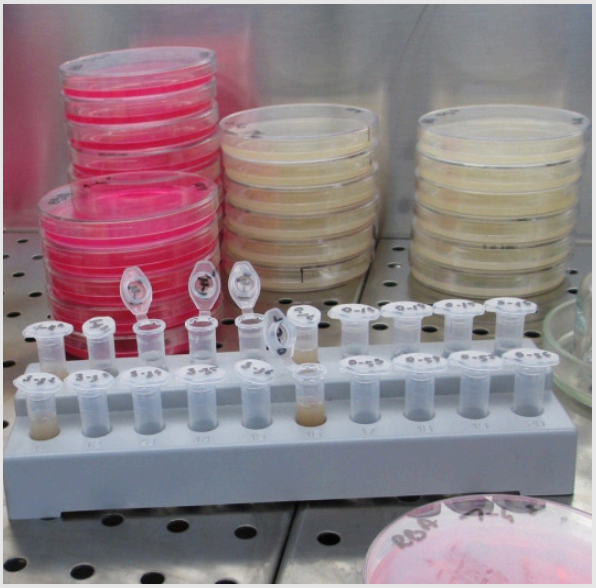
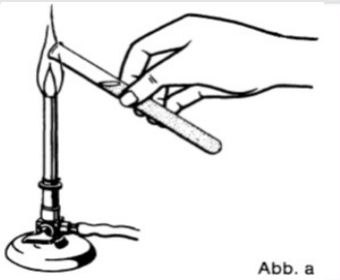
info@phywe.de
www.phywe.de

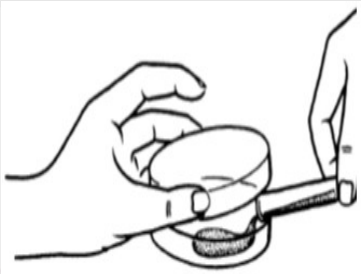
Setup (7/9) Pouring of plates

Petri dishes filled with a solidified nutrient medium are called plates. The nutrient medium is poured into the dishes either from test tubes, with one test tube holding the required quantity of ready-made medium for one Petri dish. Test tubes with nutrient medium are simply called tubes by microbiologists. In order to be able to pour plates at any time, it is useful to keep tubes with the ready-made nutrient media in stock. Tubes with nutrient agar are boiled in a water bath until their content liquefies. Tubes with nutrient gelatine are heated in a waterbath until the gelatine has become liquid. After the nutrient medium has liquefied, take one tube out of the water bath and remove the cotton plug. Then, flame the mouth of the tube briefly in the flame of a gas burner in order to remove any contamination (upper image on the next slide).



Setup (8/9) Sterilisation of nutrient media and solutions





Open the cover of a sterile Petri dish with a diameter of 100 mm to such an extent that the mouth of the tube fits between the upper and lower part without touching them. Then, pour the nutrient medium from the tube into the Petri dish (lower image) and re-attach the cover immediately. During pouring, the upper part of the Petri dish should cover the lower part as completely as possible in order to prevent any microorganisms in the air from falling into the dish. Perform a circular movement with the closed Petri dish on the tabletop in order to distribute the nutrient medium evenly over the entire surface of the dish. Then, let the plate rest until the nutrient medium has solidified.

Proceed in the same manner when pouring plates from Erlenmeyer flasks. Plates that are not used immediately can be stored in a refrigerator for some time with the cover facing downwards, i.e. upside down.

PHYWE
Robert-Bosch-Breite 10
37079 Göttingen
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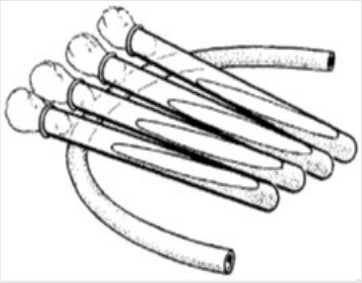
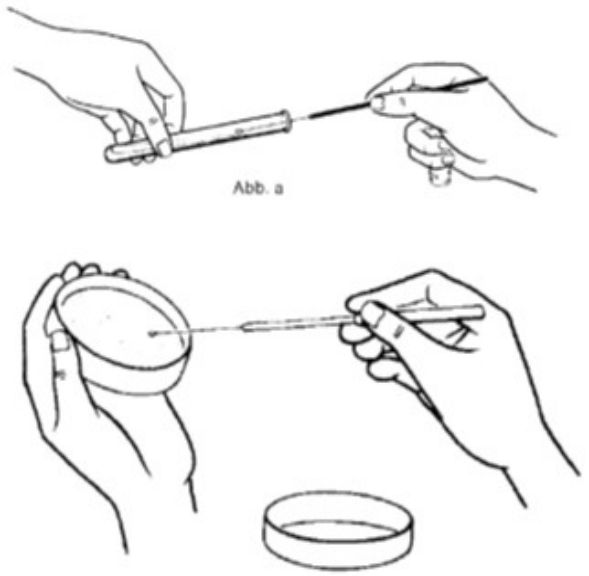
Setup (9/9) Preparation of slant agar tubes



Slant agar tubes are test tubes in which the nutrient media has solidified with a slant surface. As a result, a greater amount of the nutrient surface is available for inoculation. Since they are rather space-saving, they are often used instead of plates in test series. In addition, pure cultures of microorganisms are kept on slant agar tubes for collection purposes, provided that the culture conditions allow for it. Prepare the desired nutrient medium, fill 5-6 ml of it into some test tubes (approximately one quarter full), seal them with sterile stoppers, and sterilise them. Immediately afterwards, as long as the nutrient medium is still liquid, place the upper end of the test tubes on a strip of wood that is approximately 12-15 mm high. The tube of a gas burner is also ideally suitable for this purpose since it has the perfect diameter (upper left image). As a result, the nutrient medium will solidify in the test tubes and form a long, slant surface. The nutrient medium should reach approximately the beginning of the upper third of the tube.

Procedure (1/2)

When inoculating the microorganisms it is important to prevent the nutrient media or cultures from being contaminated with foreign microorganisms. Do not walk around the room during the inoculation to avoid any unnecessary air movement. Flame the inoculation loop in the flame of a gas burner until it is red hot in order to clean and sterilise it. Then, let it cool. When withdrawing microorganisms from a tube, remove the cotton plug from the tube carefully with the hand that holds the inoculation loop. Hold the stopper in this position (upper image on the next slide) until it is reinserted into the tube. Flame the open mouth of the tube briefly in the flame of a gas burner. Insert the inoculation loop carefully into the tube, withdraw a little from the culture, flame the mouth of the tube once more, and reinsert the cotton plug. Then, transfer the inoculation material onto or into the new nutrient medium.



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Robert-Bosch-Breite 10
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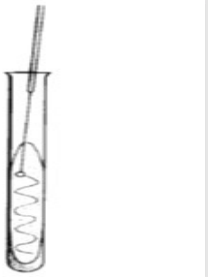
Procedure (2/2)



If a slant agar tube is to be inoculated, proceed in the same manner when opening the tube as during the withdrawal of the inoculation material. During the transfer into nutrient solutions, the mass of microorganisms is spread out carefully on the glass wall just below the liquid surface so that it distributes as evenly as possible in the nutrient solution. If plates are to be inoculated, they must be placed down with the cover facing downwards, i.e. upside down. Then, lift the lower part, turn it around, spread the inoculation material out, and place the lower part back onto the cover. Proceed in the same manner when withdrawing material from a plate on which microorganisms have grown.

On all types of nutrient media, the inoculation material is usually spread out in a zigzag pattern (upper image) in order to make optimum use of the available nutrient surface. After the inoculation, flame the inoculation loop immediately in the flame of a gas burner until it is red hot in order to clean and sterilise it. Flame the grip as far as it came into contact with the inoculation material.

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Protocol

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Robert-Bosch-Breite 10
37079 Göttingen

Tel.: 0551 604 - 0
Fax: 0551 604 - 107

info@phywe.de
www.phywe.de

Task 1

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What kind of living creature are moulds and yeasts?

- Bacteria
- Plantae
- Archaea
- Fungi

Task 2

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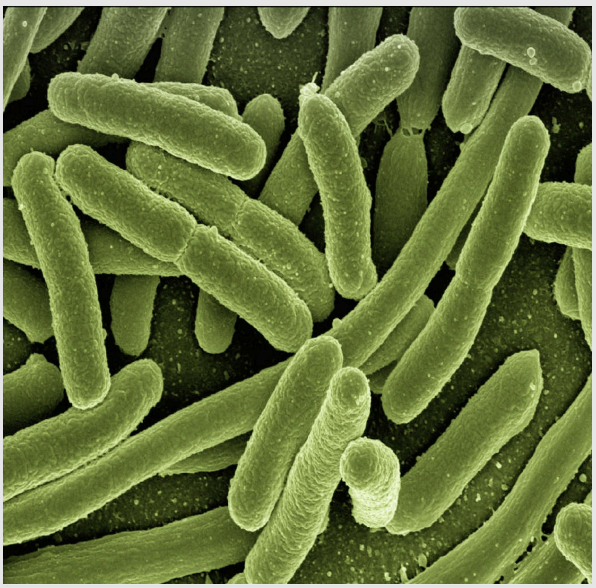
What part of the standard eukaryote cells do bacteria lack?

- [ ] Cellular membrane
- [ ] Cytoplasm
- [ ] Mitochondria
- [ ] Nucleus

Check



PHYWE

Robert-Bosch-Breite 10
37079 Göttingen

Tel.: 0551 604 - 0
Fax: 0551 604 - 107

info@phywe.de
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Task 3

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Why is agar used, instead of gelatine?

- Gelatine degrades when heated and does not solidify afterwards.
- Agar is porous and thus microorganisms can move through it.
- Unlike agar, gelatine can be processed by many microorganisms.
- Agar is derived from algae and is thus vegan.

Slide	Score / Total
Slide 22: Moulds and Yeasts	0/1
Slide 23: Bacteria	0/2
Slide 24: Agar-agar	0/1

Total Score 0 / 4

Show solutions Retry

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