Subject:

Clinical Microbiology

Topic:

Professional English for Clinical Microbiology

Student's worksheets

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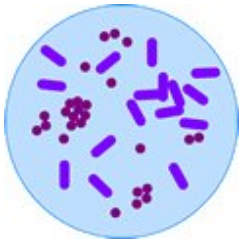
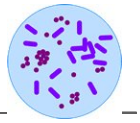
NILE 2011





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Unit 1: Laboratory Techniques

Introduction

In this lesson we shall learn about the basic techniques used in the Microbiology laboratory. Contents are ordered in a chronological work flow: when we receive a specimen, first of all we have a look on it to know what is inside and then we culture it to make bacteria grow.

Lesson 1.1 – Gram Stain

Activity 1 – Significance of Gram Stain

Read the text and fill the gaps. Use the word bank below to help you.

Gram stain is the main stain procedure in Microbiology. It enables us to know several things about bacteria in the smear:

- _____
- _____
- _____ of bacteria

Bacteria are divided into _____ large groups depending on their Gram-stain reaction: the so-called _____ (GP) that remains coloured after the **decolouration** procedure and the _____ (GN) that do not retain the dye and take the colour of the **counterstain**. Those differences rely on the basis of **cell wall** structures.

Considering together Gram reaction and shape we may set the _____ big groups of bacteria: **GP bacilli**, **GP cocci**, **GN bacilli**, and **GN cocci**.

arrangements	Gram-negative	Gram-stain reaction	
shape	two	four	Gram-positive

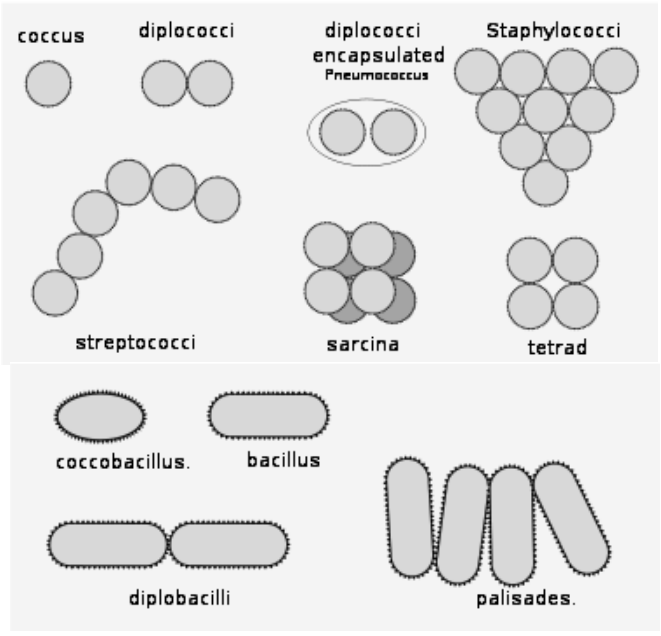
Activity 2 – Morphological classification of bacteria: shape

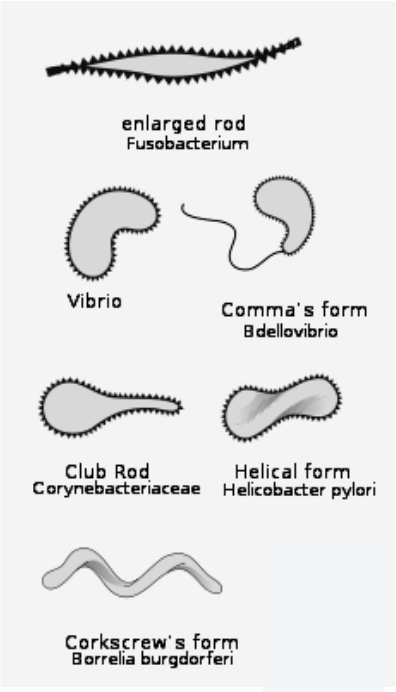
Complete the chart by using the diagram below and transferring the words in the word bank. But be careful: Latin is widely used in English.

Bacterial morphology chart	
Straight rods are called …	
Small straight rods are …	
Curved rods are called …	
Sphere shaped bacteria are …	
Spiral shaped bacteria are …	
Rods with tapered ends are …	

Word bank
Vibrio
Cocci
Fusobacteria
Bacilli
Coccobacilli
Spirilla

Latin rooted words	
Singular	Plural
One bacterium	Some bacteria
One bacillum	Some bacilli
One coccus	Some cocci





Activity 3 – Morphological classification of bacteria: arrangements

Sometimes we observe different patterns of arrangements. They suggest a particular genus or species. They cannot be used as a complete identification of bacteria, but may give some clue to the doctor while culture is awaited.

Match Arrangement column and Suggested bacteria column. Transfer the words from the word bank into the suggestion column. Work in pairs and search for help in: http://medical-dictionary.thefreedictionary.com/
http://archive.microbelibrary.org/asmonly/details.asp?id=2380

Word bank		
Neisseria gonorrhoeae	*Streptococcus pyogenes*	Cocci
Streptococcus pneumoniae	*Staphilococcus sp.*	Diphteroids

Bacterial arrangement chart	
Arrangement	***Suggestion***
GPC single-celled	
GPC in lanced-shaped pairs	
GNC in coffee-bean shaped pairs	
GPC in chains	
GPC in grape-like clusters	
GPB club-ended in palisade	

To pronounce Latin bacteria names properly, look at:
http://www.atsu.edu/faculty/chamberlain/Website/studio.htm

Activity 4 – Gram Stain procedure

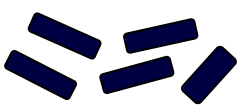
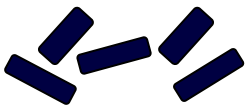
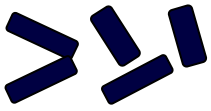
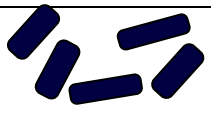
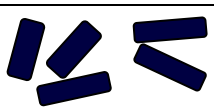
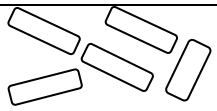
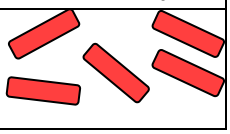
Fill the blanks on the text below by using the words in the word bank. Look at the pictures at the end of the activity to help you.

1. Place a drop of sterile saline or water on the slide. Transfer a small portion of a colony with a _____ and gently mix to emulsify.

2. Let air-dry.
3. _____ the cells to the slide by heat by passing it (cell side up) through a flame. Do not let the glass become hot to the touch.
4. Place your slide on a slide holder.
5. Cover the entire slide completely with crystal violet and leave it for 1 minute. Pour it off and _____ briefly with running tap water. Drain the slide.
6. Cover the slide with iodine solution. Let it stand for 1 minute as well. Pour off and rinse with tap water. _____ the slide.
7. Add the _____ drop by drop until the runoff remains clear. Wash off briefly with tap water and drain the slide.
8. _____ with safranin for one minute and wash off briefly with tap water to remove excess dye.
9. Drain slide and let it air-dry in an _____ position.

drain	wire loop	rinse	decolouriser	fix	counterstain	upright

The drawing below shows the sequence of colouring events based on the Gram-reaction of bacteria:

	Crystal Violet	Gram´s Iodine	Decolourizer	Safranin
GP				
GN				

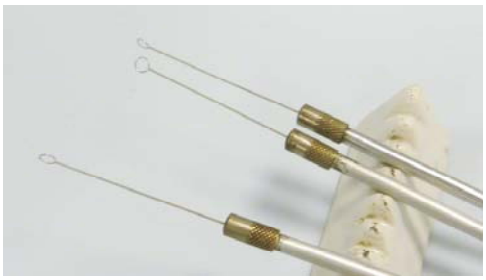


Image: wire loops

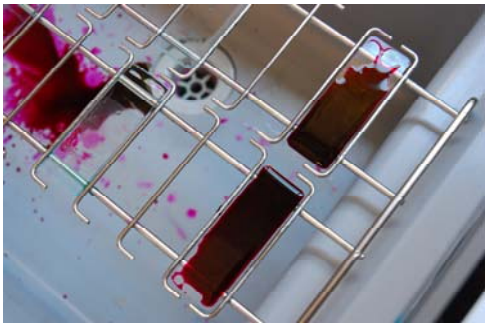


Image: counterstain

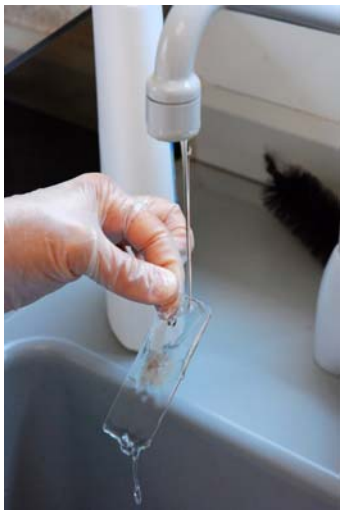


Image: running tap water

Activity 5 – Revision: sequencing the Gram Stain

Explain the Gram Stain of a smear filling the gaps on the text below:

First, you cover the smear with _____. Next, you wash and cover it with _____. Then you wash and decolourise it and finally you _____ the smear with _____. As a result, Gram-positive bacteria stain _____. And Gram-negative bacteria stain _____.

Lesson 1.2 – The Microscope

The microscope is one of the most frequently used and fragile pieces of equipment in the laboratory. It is extremely important to follow protocols strictly for carrying, operating and cleaning it, in order to avoid any damage and to obtain high quality images.

Activity 1 – History of the light microscope

Read the text and complete the time line with the facts related to the microscope.

The fact that a piece of transparent crystal thicker in the middle than at the edges made things look larger was already known from Roman times. These objects were called "lenses" because of their shape like the seeds of a lentil. From then, the history of microscope began in 16th century.

About 1595, two Dutch lens makers, Zaccharias Janssen and his son Hans, joined several lenses in a tube and discovered that objects appeared more enlarged than using only one lens. Magnification obtained was less than 10 diameters that is 10 times the actual size. These instruments were called "flea glasses" because they were used to observe fleas and other insects. This was the first compound microscope.

Robert Hook, British physicist, improved the compound microscope to obtain up to 30 diameters magnification. He discovered the "cell" while looking at a sliver of cork (1665). He believed the cells had served as containers for the "noble juices" of the once-living cork tree. He thought these cells existed only in plants, since they hadn´t been observed in animals. He wrote "Micrographia", the first book describing observations made through what they called "magnifying glasses and where the word "cell" was used for the first time.

Image: Micrographia. Flea.

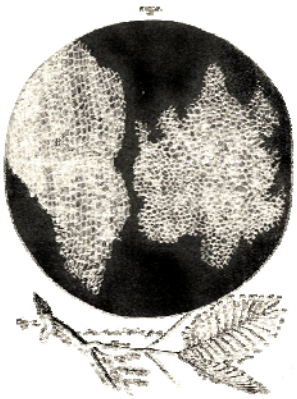
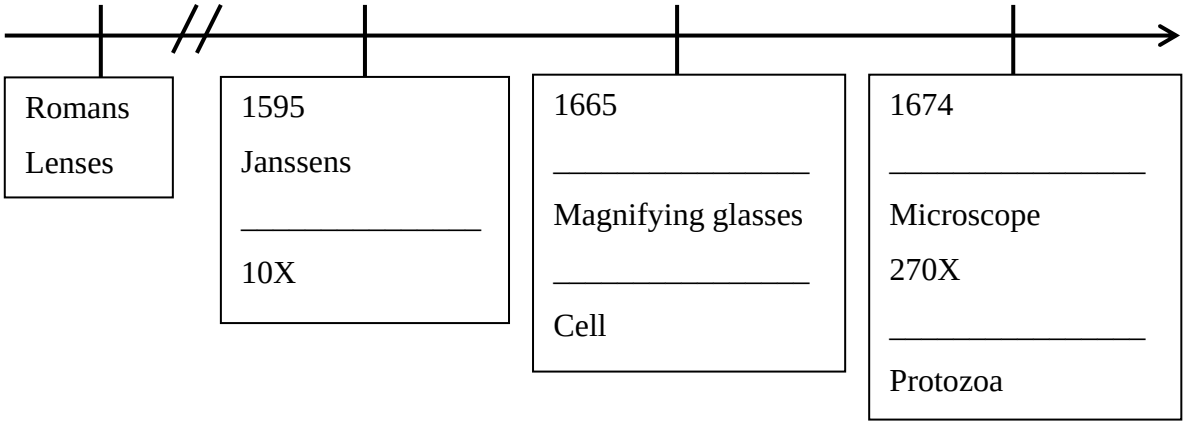


Image: Micrographia. Suber cells.

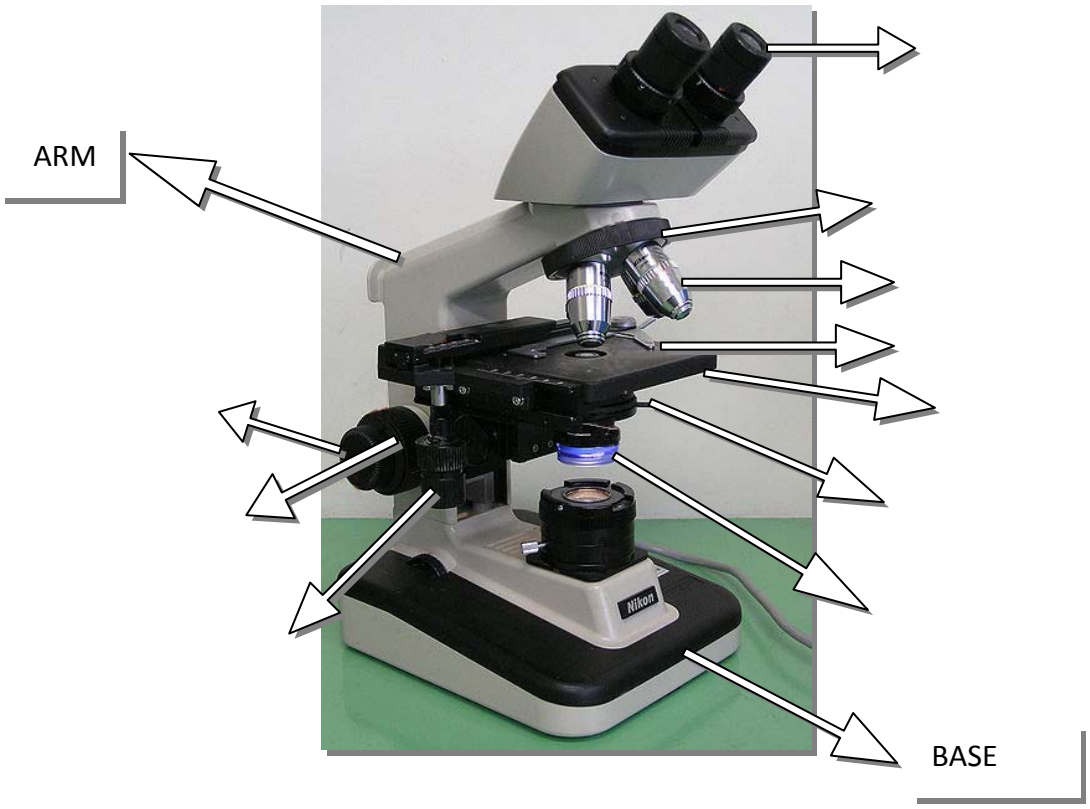
Anton van Leeuwenhoek, Dutch as well, tried new methods for polishing tiny lenses of great curvature which gave magnifications up to 270 diameters. These led to the building of his "microscopes" and the discoveries for which he is famous. He was the first to see and describe bacteria and protozoa (1674).



Activity 2 – Parts of the bright-field microscope

2.a - Label the parts of the microscope. Choose the names from the definitions that appear in the table below. Use your common sense and be prepared to justify your choice.

▶ The **stage** is the platform where you put the slide
▶ The **objective** contains lenses and you see things under them
▶ The **fine focus knob** is used to obtain a sharp image
▶ The **condenser diaphragm lever** controls how much light enters through the lens
▶ The **coarse focus knob** is used to approach the image until you can recognize what you are seeing
▶ The **condenser** focuses the light on the slide you are looking at
▶ The **stage clips** hold the slides in place
▶ The **nosepiece** connects all the objectives on a microscope
▶ The **eyepieces** contains lenses and you look into them to see your specimen
▶ The **stage knobs** are used to move the stage and change the field of view



2.b - Describe the position of the arm, the base and the light source on the image. Use the prepositions of place that are in the box below:

I - The **arm** is _____

ii - The **base** is _____

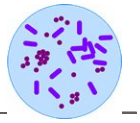
iii - The **light source** is _____

Prepositions of place:

At the top

In the top left-hand corner		In the top right-hand corner
On the left	In the middle	On the right
In the bottom left-hand corner		In the top right-hand corner

At the bottom



Activity 3 – Standard operating procedure to look under the microscope

The flow chart on next page is the standard operating procedure to examine a microbiology stain.

3. a - Pay attention to the teacher´s reading and performance of part (A). In the box below you have the headings of each part A-E of the procedure. Choose the heading for part (A) and write it on the procedure´s chart.

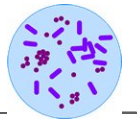
LIGHT ADJUSTMENT		**MOVE UP IN MAGNIFICATION**
FIRST FOCUS	**STARTING**	**SCANNING**

3.b - Pay attention to the teacher´s reading and performance of part (B). Choose the heading and write it as before. Answer the questions below on points 4 and 5.

What happens if …
… you start focusing with the high magnification objective instead of the medium-power one?
… you start focusing already looking down the microscope and rotate the coarse focus knob moving the objective to the slide?

3.c - Pay attention to the teacher´s reading and performance of part (C). Choose the heading and write it as before. Complete the chart below with the information in points 9, 10 and 11.

	40X objective
Condenser must be …	
Diaphragm lever must be …	



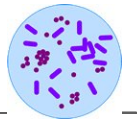
3.d - Pay attention to the teacher´s reading and performance of part (D). Choose the heading and write it as before. Answer the question below on point 12.

What happens if …
… you do not have a set scan pattern?

3.e - Pay attention to the teacher´s reading and performance of part (E). Choose the heading and write it as before. Complete the chart with the information in point 17 and answer the questions below on points 16 and 17.

	100X oil objective
Condenser must be …	
Diaphragm lever must be …	

What happens if …
… you use the coarse focus knob when changing from one objective to another?
… you do not adjust the condenser again when changing from one objective to another?



(A)

1. Place your slide on the stage and hold it in place with the metal clips
2. Turn on the microscope by rotating the power switch
3. Move the stage knobs until the specimen is over the light beam coming down up

(B)

4. Place the 40X* objective pointing down at the stage
5. Before you look down the microscope, rotate the coarse focus knob until the objective lens is as close to the slide as possible
6. Looking down into the microscope, adjust the eyepieces to your interpupillary distance by pushing the eyepieces together or apart until you see one uniform field of view
7. Start focusing by slowly turning the coarse focus knob so that the lens moves away from the slide
8. Then turn the fine focus knob to improve the image

*We read 40X as "40 times"

(C)

9. Lower the condenser by turning the condenser focusing knob
10. Open the condenser diaphragm lever until you obtain a sharp image
11. Keep doing little adjustments of condenser knob and diaphragm lever until you achieve the most detailed image.

(D)

12. Scan the smear by moving the slide continuously and at a slow pace, according to a set scan pattern. Select a good microscopic field to look under higher magnification.

(E)

13. Rotate the nosepiece just half the way to put the high-power objective into position
14. As it is a 100X immersion objective, put a little drop of immersion oil on the smear
15. Finish turning it into position
16. Adjust focus by turning the fine focus knob
17. Adjust light by raising the condenser and closing its diaphragm lever
18. During use of the microscope, one hand should remain on the fine focus as constant readjustment will be needed, and the other hand on stage knobs to change fields of view

Activity 4 – Revision

Match beginnings and endings of the sentences and you will find the reasons for some actions in the procedure. Transfer the words into the table of beginnings:

Turning the focus knobs you obtain …
Adjusting the condenser you obtain ...
You change lens to a higher power objective for ...
We start under medium-power objective to …

1	... enhanced contrast	3	... higher resolution
2	... a good quality image	4	… do a quick scan of the smear

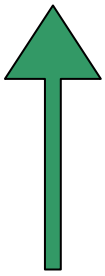
Useful definitions for the activities:

- **Resolution** – It is the ability of a lens to distinguish small objects that are close together. This is a technical property of lenses that differs from one kind to another.
- **Contrast** – It is the difference between the object and the background. Easiest ways to enhance contrast is to use a dye or to manipulate light.

Activity 5 – Reporting results in direct specimens: case studies

When doing microscopic examinations of a direct specimen, we have to score bacteria and all the different elements we identify in the smear. Find below the key phrases used to indicate quantity.

Grading expressions in Microscopy



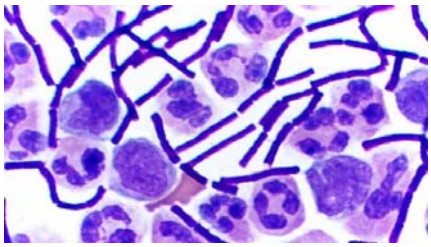
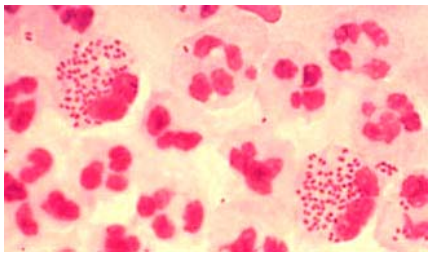
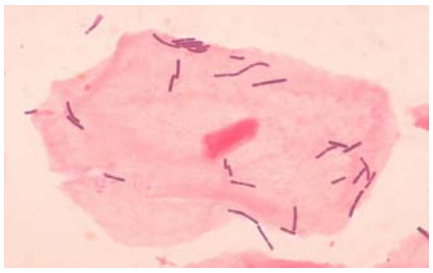
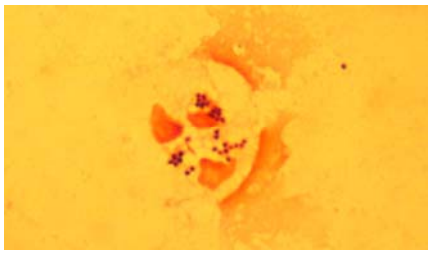
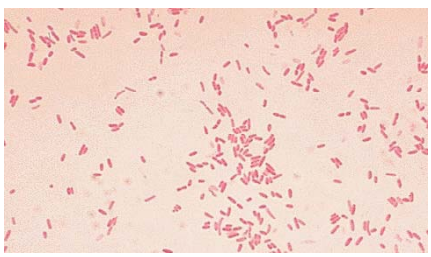
Numerous …

A moderate number of …

Scanty …

No … revealed

In the chart below, you have several stains of different specimens and some reported microscopy results. Match the images and their results.

Image 1			*A - Vaginal discharge:* *Gram stain shows scanty epithelial cells and a moderate number of Gram-positive bacilli.*
Image 2			*B - Cerebrospinal fluid:* *There are numerous Gram-positive bacilli and numerous polymorphonuclear cells.*
Image 3			*C - Urethral discharge:* *Gram stain shows numerous polymorphonuclear neutrophils and moderate number of intracellular and extracellular coffee-bean shaped diplococci.*
Image 4			*D - Urine:* *The specimen includes numerous Gram negative bacilli. No cells revealed.*
Imatge 5			*E - Sputum:* *Gram stain shows scanty polymorphonuclear cells and clusters of Gram positive cocci.*

Lesson 1.3 – Preparing Culture Media

Bacterial culture is a method of studying bacteria by growing them on media containing nutrients under laboratory conditions. Different bacteria have different nutritional needs and because of this there exists such a wide range of media.

Preparation has three stages:

1. Prepare solution
2. Sterilise
3. Pour into plates

Word bank	
One culture medium (sg)	Several culture media (pl)

Activity 1 – Previous knowledge

1.a - Choose the correct word for the written mathematical operations:

times	minus	plus	divided by

+	**Addition**	2 + 2 = 4	Two _____ two are four
-	**Subtraction**	5 – 3 = 2	Five _____ three are two
x	**Multiplication**	4 x 2 = 8	Four _____ two is eight
÷	**Division**	6 / 2 = 3	Six _____ two is three

1.b - Measures of volume and mass in the International System of Units are in the box. Write the symbol for each one:

Measures of VOLUME		**Measures of MASS**		**Measures of LENGTH**	
Units	**Symbols**	**Units**	**Symbols**	**Units**	**Symbols**
Litre Millilitre		Gram Milligram		Metre Millimetre	

Activity 2 – Preparing culture media: preparing solution

Dehydrated media are commercially available and should be reconstituted following the manufacturers' recommendation. This appears on the label of media containers.

2.a - Some facts about the media:

1. The powder is **hygroscopic** which that means it absorbs moisture and then clumps and sticks to surfaces.

2. Solid media contain **agar**. This is a gelling polymer obtained from seaweed and its gelling properties depend on temperature.

Underline the correct word looking at the words in the box:

- Agar *melts / solidifies* when heated above 85ºC
- Agar *melts / solidifies* when cooled from 42ºC and below
- Agar must be *molten / solidified* to be handled

solidify (pp solidified)
Solid agar ⇆ Liquid agar
melt (pp molten)

2.b - In the chart below, you have the **manufacturer's instructions** for the Chapman Agar used in our laboratory. Calculate how much powder and how much water you need to prepare 200 ml and read your calculations using the vocabulary in activity 1. Read decimals as explained below.

Mannitol Salt Agar (Chapman Agar) **Technical data sheet**

Directions
Suspend 111 g of powder in 1 L of distilled water and bring to the boil. Dispense in tubes or flasks and sterilize by autoclaving at 121°C for 15 minutes.

Reading decimals: 2.548 is read "two point five four eight"

2.c - Once you have done calculations, you have to put them into practice. Fill in the blanks and choose the materials from the box:

For weighing substances, you have to use a _____to put the powder on the _____, and a _____ to measure the water.

 Scales	 Graduated cylinders
 Weighing dish	

2.d - After this, you have to mix the **solvent** and the **solute** in a flask. Complete the sentences 1, 3 and 6 about the procedure. Use information from earlier in the lesson, your previous knowledge and your common sense to help you.

1	The vessel must be about twice the final volume of the medium because …
2	First of all, you have to put the powder inside the flask and secondly the water.
3	As you pour the water, use it to wash the weighing dish because …
4	Pour this water used for cleaning into the flask

5	You will obtain a cloudy liquid.
6	Heat the mix to boil for 1 minute to …
7	When it is completed, the mixture becomes clear.

Activity 3 – Prepare solid culture media: sterilizing

Sterilisation destroys all microorganisms. In the laboratory, we sterilise by applying moist heat in **autoclaves**. They are closed vessels where water boils under high pressure.

2.a - Label the diagram about changes in the state of water by using the words in the box:

condense	solidify	evaporate	melt

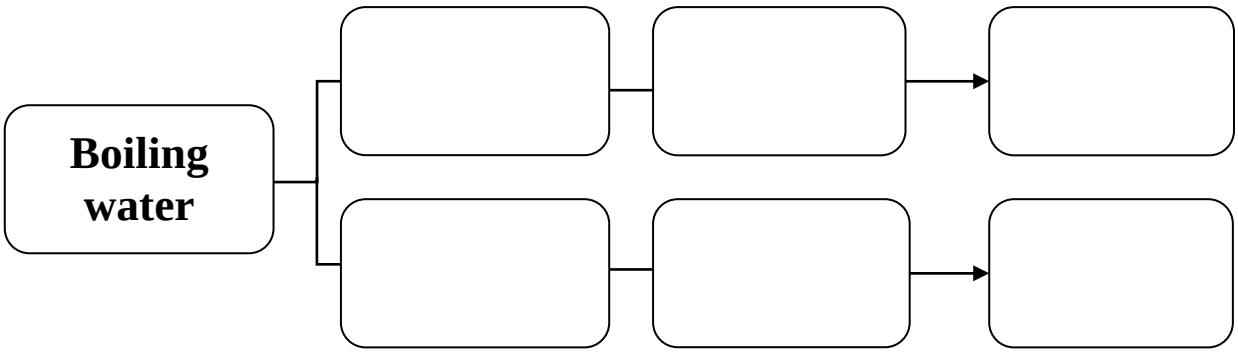
ice ⇆ water ⇆ vapour /steam

2.b - Read the text and complete the tree-diagram below about sterilizing conditions explained before. Use the expressions in the box:

At atmospheric pressure, water boils at 100ºC (not enough to sterilise). When water is boiled at increased pressure, it boils at a higher temperature. When steam comes into contact with an item, it condenses on its surface and transfers the heat to it.

To autoclave media and not spoil them, we use a standard autoclaving cycle that is "121 º C, 15 minutes". That means keeping the media at 121ºC for 15 minutes. For other items, we may use higher temperatures.

121ºC or more	At high pressure	Not sterilise
At atmospheric pressure	100ºC	Sterilise

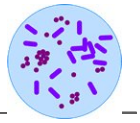


Activity 4 – Prepare solid culture media: pouring into plates

After the removal of media flasks from the autoclave, we have to pour their contents into **Petri dishes**. To do this we have to perform a sequence of different actions.

The 11 sentences below explain the procedure to pour the prepared medium into the plates. Order and transfer them to the box to produce a complete text. Look at the picture about how pouring agar into plates to help you.

▶ And with your left hand open the cover of the plate just wide enough to pour the media
▶ Leave the plates undisturbed to cool and solidify
▶ Cool the flask to 50ºC in a water-bath for half an hour
▶ Lay Petri dishes on the bench top
▶ Hold the flask with your right hand
▶ That avoids burns and keeps the medium still liquid
▶ This is enough to set an even agar layer of 0.5 mm thick over the bottom of the plate
▶ Once the agar has set, invert the plates to store them



▶ Wrap a paper towel around the flask to handle it
▶ Condensation falling from the lids into the agar surface will cause problems when inoculating the plate
▶ Pour about 20 ml into each plate

Use connectors to link the sentences and add punctuation. Choose them from the box below. You do not need them all and there will be some left.

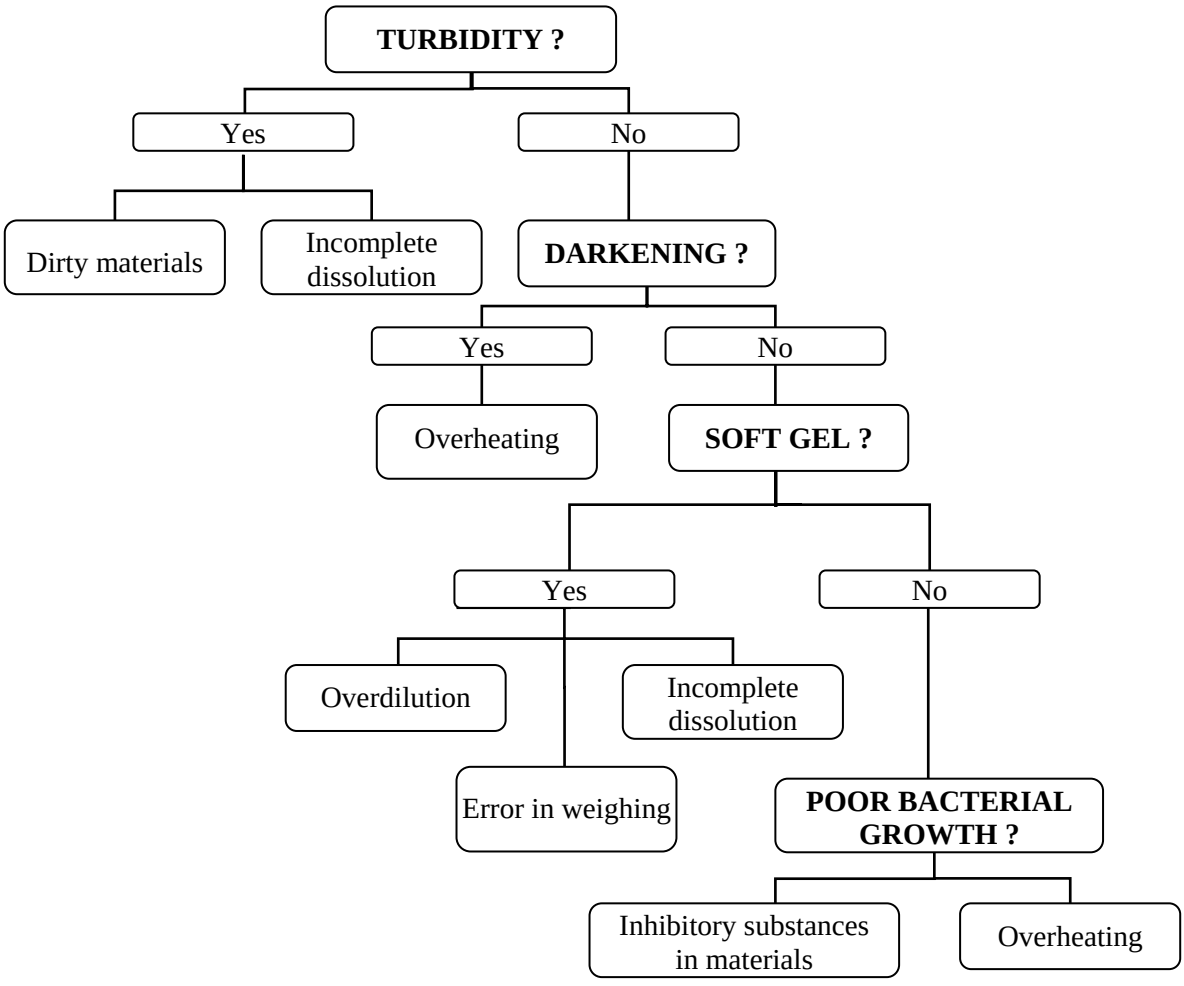
Sequencing	**Contrasting**	**Cause and effect**
first of all meanwhile then	otherwise alternatively	therefore because

Activity 5 – Revision

At the bottom of the page, you have a dichotomous key to analyse faults in self-made media and find out possible causes for them.

Classify the information as “cause” or “its effects” and transfer it to the T-chart below example below. There is a worked example for you to see.

Cause	Effect (due to the cause)
Dirty materials	



Lesson 1.4 – Bacterial Culture

Bacterial culture is used to determine the cause of an infectious disease. Bacteria multiply by **binary fission**, that is when a mother cell divides into two daughter cells. They do this repeatedly and in a short time. As a result, they grow on solid media as colonies. A **colony** is a visible mass of millions of cells derived from a single mother cell.

Activity 1 – Previous knowledge: mathematics

Choose the correct word for the written mathematical expressions from the word bank below the chart:

to minus two	squared	to the four	to the twelve	cubed
two thirds	a quarter	five eighths	one hundredth	a half
nought (before the point)		oh (after the point)		

Fractions		Decimals	
½		3.14	Three point one four
¼		0.5	_____ point five
⅔		0.07	_____ point _____seven
⅝		6.003	Six point _____ _____ three
1/100		0.1×10^{-3}	_____ point one times ten to minus three

Powers		
X^y	2^4	Two _____
	5^2	Five _____
	2^3	Two _____

Potentials of ten (scientific notation)		
10^x	10^2	Ten _____
	4.7×10^{12}	Four point seven times ten _____
	0.5×10^{-2}	Nought point five times ten _____

Activity 2 – Selection of media

Media used to cultivate a clinical specimen depends on the bacteria that may be found in this kind of specimen. Each location in the body offers different environmental conditions to bacteria and only those that feel good in that environment settle there.

Because of this, routine protocols for culturing clinical specimens specify various media and atmospheric conditions for incubation.

There are several types of culture media:

1. **Enriched media** – They may include blood as a source of nutrients. They are design for **fastidious bacteria**, that is bacteria with complex nutritive requirements.

2. **Selective media** – They are media that include **specific inhibitors** for competitors of the target bacteria, and so their growth is made easier.

3. **Differential media** – They are designed on purpose for specific bacteria. They include **specific inhibitors** and a **detection system** that highlights a particular metabolic activity giving colonies different colours. This provides differentiation of the target bacteria from others that might grow on the medium.

Look for examples of media that you may use with Enterobacteriae, Staphilococcus, Streptococcus, Neisseria, Haemophilus or Vibrio. Search in media manufactures´ websites: BD (http://www.bd.com/products) and Oxoid (http://www.oxoid.com/uk/blue/index.asp).

Use the online dictionaries you know for help with difficult words.

Media name	Category	Utility
	Enriched	
	Enriched	
	Selective	

	Selective	
	Differential	
	Differential	

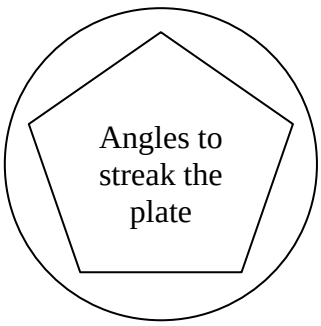
Activity 3 – Streak plate method

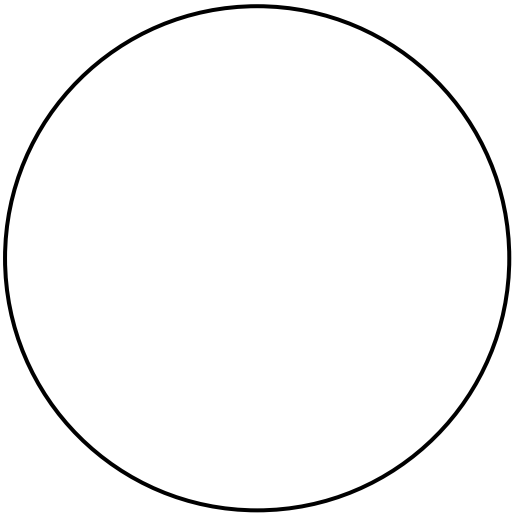
To do a culture, we must transfer a small quantity of the specimen to a prepared culture plate. We have to spread it over the plate and incubate it to give bacteria enough time to multiply. We must obtain isolated colonies after incubation. The most usual method for inoculating a plate is the streak plate.

Read the procedure and draw the layout of the spread in the plate sketch. You may look at an online dictionary for help with difficult words: http://wordreference.com or http://www.thefreedictionary/medicaldictionary

.

1. *Touch the specimen with the sterile loop and place the **inoculum** near the periphery at the top of the plate.*
2. ***Spread** it with the loop doing up to seven close and parallel **strokes**, go and return, over the plate surface. Do not lift the loop and cover one third of the total area of the plate.*
3. ***Flame** the loop in the Bunsen burner until it is red hot. Allow it to cool.*
4. *Turn the plate at less than 90º (look at the diagram below to see the angles).*
5. *Streak the plate as before, but now overlap the end of what was previously spread. Cover a similar area as before.*
6. *Turn the plate again and repeat now overlapping the end of the second spread.*
7. *Turn the plate the last time and repeat, but be careful not to close the ring of spreads.*
8. *Use the centre of the plate to make a last wavy line. It is "the signature".*





Activity 4 – Atmospheric conditions

To culture bacteria we need to fulfil their requirements, otherwise they will not grow. They have both nutritional and respiratory requirements. So, the inoculated plate must be incubated in the appropriate atmosphere. Usually, incubation takes 24 hours at 37ºC in an incubator.

Transfer the information in the text below onto the lateral key, comparing the effect of atmosphere on bacteria and how it influences their growth. Tick only when bacteria grow:

There are bacteria that:

- *Use oxygen in aerobic, normal atmosphere (**strict aerobes**)*
- *Do not use oxygen (**strict anaerobes**). They may be **obligate anaerobes** (are killed by oxygen) or **aerotolerant anaerobes** that respire anaerobically and survive in the presence of oxygen.*
- *Need a high concentration of carbon dioxide (**carboxyphilic organisms**) added to an aerobic atmosphere. Strict aerobes grow too because what they need is oxygen.*
- *Need a lower concentration of oxygen than that in the air (**microaerobic organisms**) and are killed by higher concentrations*
- *Switch between aerobic and anaerobic metabolism to adapt to different circumstances (**facultative aerobes**).*

Group	Aerobic atm	CO_2 - rich atmosphere	O_2 - low atmosphere	Anaerobic atm
STRICT AEROBES				
CARBOXYPHILIC ORG				
MICROAEROBIC ORG				
FACULTATIVES				
STRICT ANAEROBES				

To obtain special atmospheres, we use different sachets with reagents inside that produce the desired gases. We put the sachet and the plates inside a jar, close it tightly and incubate.

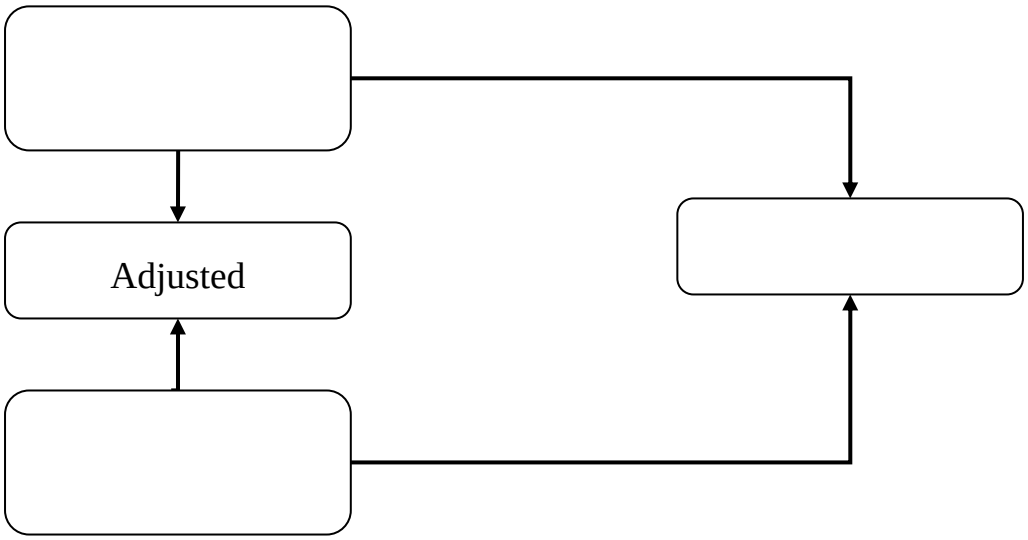
Activity 5 – Bacterial standards

They are useful in quality control assays and when subculturing a plate and testing requires a standard inoculum.

5.a - Complete the diagram below based on the information in the text:

McFarland Standards *are turbidity standards made from different concentrations of latex beads mixed in a buffer liquid and they are generally labelled 1 to 10. They are used to visually approximate the concentration of cells in a suspension.*

Bacteria in suspension produce turbidity too. Because of this, there is a correspondence between McFarland units and Bacterial concentration. That means that a bacterial suspension adjusted to the same turbidity of a McFarland Standard produces expected ***bacterial plate counts*** *when streaked on a plate.*



5.b - Describe the correspondences between McFarland units and bacterial counts using the word frame and the table below.

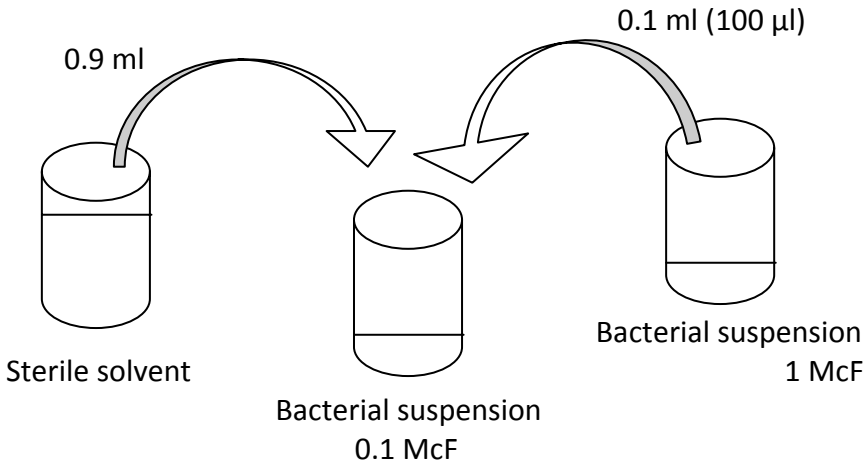
"The McFarland _____ Standard corresponds to a suspension of

_____ cells per ml".

McFarland Standard	Approximate Bacterial Density
1.0	3.0×10^8 cells/ml
2.0	6.0×10^8 cells/ml
3.0	9.0×10^8 cells/ml

5.c - Further dilutions of the adjusted suspensions give proportionally reduced counts. So, a 1/10 dilution of standard 1 McF gives a new standard 0.1 McF, and so on.

Explain the scheme below that shows how to prepare the standard 0.1 McF. You have the word-frame (write numbers in words)



1. For a final volume of _____ ml, first of all we dispense _____ ml of solvent into a sterile tube.
2. Then, we dispense _____ µl of the suspension 1McF.
3. Mix and use