

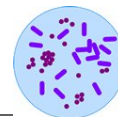
Unit 2: Working with Bacteria

In this unit we develop work tasks concerning isolated bacteria. Students will use prior knowledge learned in unit 1 and can be more autonomous when discussing issues.

Teaching notes 2.1 – Identification of Bacteria

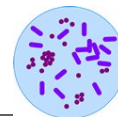
Timing: 3:00 h	Room: computer room
Teaching aims: <ul style="list-style-type: none"> ○ Strategies to identify bacteria ○ Preliminary identification ○ Haemolysis, catalase test and oxidase test 	
Prior knowledge: <ul style="list-style-type: none"> ○ Lessons 1.1, 1.3 and 1.4 	
Resources: <ul style="list-style-type: none"> ○ Internet access ○ Digital projector ○ Document “Preliminary tests” for activity 3 in “Supplementary materials” ○ Power point presentation with answer key Unit 2 in “Supplementary materials” 	

Activity 1 – Strategies for identification of bacteria	
Subject difficulty: ↑	Language difficulty: ↔
Activity 1.a	
Procedure: <ul style="list-style-type: none"> ○ Explain activity 1.a ○ Students read the text individually ○ They complete the chart working in pairs ○ Feedback in plenary 	
Language support: <ul style="list-style-type: none"> ○ Write difficult words on the blackboard and explain them ○ Language provided on the text 	
Resources: <ul style="list-style-type: none"> ○ Worksheet 2.1 	



Answer key:	
Steps that are always compulsory	Steps that depend on purpose
- Preliminary identification - Speciation	- Serological identification - Genetic identification
Activity 1.b	
Procedure:	
<ul style="list-style-type: none"> ○ Explain activity 1.b ○ Working in pairs, students complete the activity ○ Feedback in plenary 	
Language support:	
<ul style="list-style-type: none"> ○ Headings in the diagram 	
Resources:	
<ul style="list-style-type: none"> ○ Worksheet 2.1 	
Answer key:	

Activity 2 – Preliminary identification	
Subject difficulty: ↔	Language difficulty: ↓
Procedure:	
<ul style="list-style-type: none"> ○ Explain activity 2 ○ Students read the text individually ○ Students work in pairs to complete the activity ○ Feedback in plenary 	



Prior knowledge:		
<ul style="list-style-type: none"> ○ Lessons 1.1, 1.3 and 1.4 		
Language support:		
<ul style="list-style-type: none"> ○ Word bank with specific words ○ Descriptions of categories in the text 		
Resources:		
<ul style="list-style-type: none"> ○ Worksheet 2.1 ○ Worksheets 1.1, 1.3 and 1.4 		
Answer key:		
	Category	Examples
1	Media of growth	Selective media
		Differential media
2	Macroscopic characteristics of culture	Colonies shape
		Colonies size
		Haemolysis
3	Respiratory metabolism	Atmospheric conditions
4	Gram stain	Cells shape
		Cells arrangements
		Gram reaction
5	Basic Biochemical tests	Oxidase
		Catalase

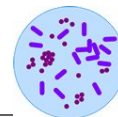
Activity 3 – Information exchange: haemolysis, catalase and oxidase

Subject difficulty: ⇄

Language difficulty: ↑

Procedure:

- Explain activity 3
- Give the three different texts on three different sheets to individual students. The texts will be in “Supplementary materials”. As colour is important, students’ worksheet may be printed in colour, or if not, the power point in “Supplementary materials” must be used.
- Students read their text individually and complete the section referring to it
- Check students know difficult words by giving clues if they should know them or explain if words are new.
- In groups of three, students exchange information to complete the other two sections. They explain their texts to the others by using their text frames.
- Feedback in plenary
- At the end of activity, make sure all the students have a copy of each text for reference
- Alternatively, to shorten the time, students do only one text and exchange information in plenary.



Option:

- In mixed skilled groups match two strong students with one weak

Language support:

- Text frames for each text
- Pictures
- Online dictionaries:
 - General English: <http://www.wordreference.com>
 - Medical English dictionary with sound: <http://medical-dictionary.thefreedictionary.com/>

Resources:

- Worksheet 2.1
- Internet access
- Supplementary materials: document “Preliminary tests” for exchange of information
- Tutorial on haemolysis: <http://www.wisc-online.com/Objects/ViewObject.aspx?ID=MBY4307>

Answer key:

3.a – Haemolysis

► Text frame:

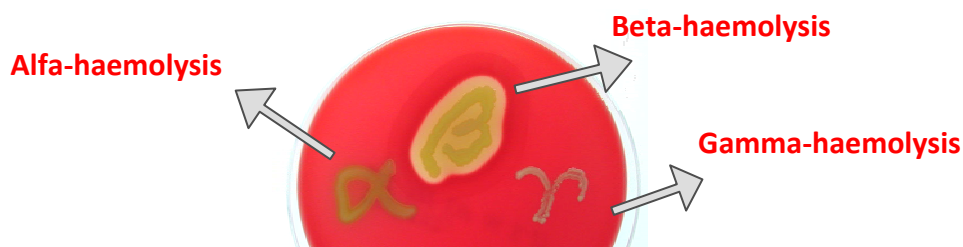
The name of the test is **Haemolysis**.

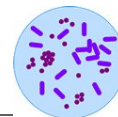
We have to use **blood agar** to observe this feature. When it appears, this means that bacteria **lyse** red blood cells in blood agar. Macroscopically, colonies may present three appearances:

1. **A yellow halo** around the colonies, which means lysis of RBC is complete and we call this **beta-haemolysis**.
2. **A green halo** around the colonies, which means lysis of RBC is incomplete and we call this **alfa-haemolysis**.
3. **No halo** around the colonies, which means there is no lysis of RBC and we call this **gamma-haemolysis**.

It is an important observation for **Streptococci**.

► Identify in the image below the three kind of haemolysis (see ppt for coloured version):





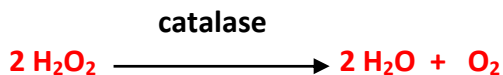
3.b - Catalase test

► Text frame:

The name of the test is **Catalase**.
This enzyme **breaks down** hydrogen peroxide into **water** and **oxygen**. We demonstrate this by **emulsifying** a colony in a drop of hydrogen peroxide. If there is the enzyme, **bubbles** will appear.
Catalase may exist in **aerobic bacteria** that produce **hydrogen peroxide** as a result of oxygen metabolism.
We may obtain false positives if we use **a metal wire loop** to pick up the colony or a colony from a **blood agar** plate.
The test is important to differentiate GPC: *Staphylococci* are **positive** and *Streptococci* are **negative**.

► Questions:

- (i) Complete the chemical reaction that involves catalase:



- (ii) How will we report the catalase test on the image and why?.

Catalase positive. Because we may see bubbles produced by gaseous oxygen released in the chemical reaction.

3.c - Oxidase test

► Text frame:

The name of the test is **Oxidase**.
This enzyme takes part in aerobic respiration helping **oxygen** to accept hydrogen and produce **water**. We demonstrate its presence by mixing a colony with a drop of **Kovac's oxidase reagent**. This is used by bacteria as hydrogen acceptor instead of **oxygen**. The reduced reagent develops a **blue-purple colour**.
Oxidase may exist in **aerobic bacteria**.
We may obtain false positives if we use **a metal wire loop** to pick up the colony or a colony from media containing **some dye**.
The test is important to differentiate GNB: *Pseudomonas* are **positive** and Enterobacteriae are **negative**.

► Questions:

- (i) Deduct how the oxidase test in image 2 was performed and the name of the method.

Kovac's reagent has been added directly to the plate. We may call this method: plate method.

- (ii) Do we have to take any precaution when performing this second method?

We cannot use the plate method with media that contain dyes because we may obtain a false positive because of the dye.

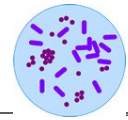


Teaching notes 2.2 – Biochemical Tests

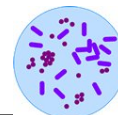
Timing: 2:00 h	Room: computer room
Teaching aims: <ul style="list-style-type: none"> ○ Fundamentals of biochemical tests ○ Examples of biochemical tests ○ Commercial kits 	
Prior knowledge: <ul style="list-style-type: none"> ○ Lesson 2.1 	
Resources: <ul style="list-style-type: none"> ○ Internet access ○ Digital projector ○ Power point presentation with answer key Unit 2 in “Supplementary materials” ○ Document “Commercial kits” for activity 3 in “Supplementary materials” ○ Alternative worksheet for activity 3 in “Supplementary materials” 	

Activity 1 – Fundamentals: what is in a biochemical test?

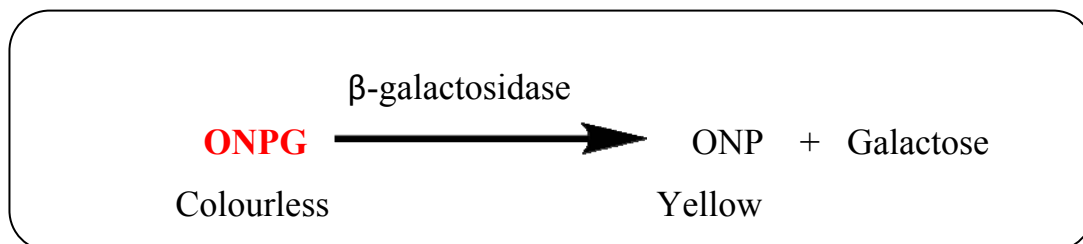
Subject difficulty: ⇔	Language difficulty: ⇔
Procedure: <ul style="list-style-type: none"> ○ Explain activity 1 ○ Students read the text individually ○ They complete the chart working in pairs ○ Feedback in plenary 	
Language support: <ul style="list-style-type: none"> ○ Write difficult words on the blackboard and explain them ○ Students are expected to know these basic concepts already 	
Resources: <ul style="list-style-type: none"> ○ Worksheet 2.2 	
Answer key: <div style="text-align: center; border: 1px solid black; border-radius: 15px; padding: 20px; margin: 10px auto; width: 80%;"> <p style="text-align: center;"> SUBSTRAT Colour A $\xrightarrow{\text{ENZYME}}$ FINAL PRODUCT Colour B </p> <p style="text-align: center;">INDICATOR</p> </div>	



Activity 2 – Taking biochemical test to pieces	
Subject difficulty: ⇄	Language difficulty: ⇄
Activity 2.a	
Procedure:	
<ul style="list-style-type: none"> ○ Explain activity 2.a ○ Students read the text individually ○ Students work in pairs to complete the activity ○ Feedback in plenary 	
Prior knowledge:	
<ul style="list-style-type: none"> ○ Activity 1 	
Language support:	
<ul style="list-style-type: none"> ○ Word bank with specific words ○ Labels in the diagram 	
Resources:	
<ul style="list-style-type: none"> ○ Worksheet 2.2 	
Answer key:	
A) Lysine Decarboxilase Test	
<p style="text-align: center;"> $\text{Lysine} \xrightarrow{\text{Lysine Decarboxilase}} \text{Cadaverine} + \text{CO}_2$ Yellow Red </p>	
B) Urease Test	
<p style="text-align: center;"> $\text{Urea} \xrightarrow{\text{Urease}} \text{NH}_3 + \text{CO}_2$ Yellow Red </p>	
C) Indol Test	
<p style="text-align: center;"> $\text{L-Tryptophan} \xrightarrow{\text{Tryptophanase}} \text{Indol} + \text{Pyruvic acid} + \text{NH}_3$ Yellow Violet </p>	



D) ONPG Test



Activity 2.b

Procedure:

- Explain activity 2.b
- Students work in pairs to complete the activity
- Students check answers with another pair
- Feedback in plenary

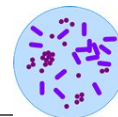
Resources:

- Worksheet 2.2

Answer key:

1	Which one is an example of a chromogenic reaction? The ONPG test is
2	Which one is an example of a test that has to be revealed? The indole test is
3	Which are the different strategies followed to name the tests? The test may have the name of the enzyme or the name of the substrate or the name of the product
4	Which is the strategy for indicators used in the reactions above and why is it useful? Indicator may be a pH indicator that changes colour when the product of reaction is acid or basic. This is the case of Phenol Red.

What will happen if there is no trap for H₂S in the medium?
If there is no trap ... **for H₂S, then it would be released into the atmosphere because it is a gas and we will not be aware of the reaction.**



Activity 3 – Commercial kits

Subject difficulty: ↔

Language difficulty: ↑

Procedure:

- Explain activity 3
- Divide the class into groups of 3
- Give each student one text about how to inoculate a different commercial kit. The texts are in “Supplementary materials” (document “Commercial kits”).
- Students look at the table and read one of the texts individually
- Students underline the sentences in their text that are relevant to the table. They have to fit the sentences into the table.
- Then, they exchange information within their group to complete the other two sections.
- Feedback in plenary
- At the end of activity, make sure all the students have a copy of each text for reference
- Alternatively, to shorten the time, students do only one text and exchange information in plenary.

Option:

- In mixed skilled groups match two strong students with one weak

Language support:

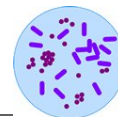
- Some cells completed in the chart
- Online dictionaries:
 - General English: <http://www.wordreference.com>
 - Medical English dictionary with sound: <http://medical-dictionary.thefreedictionary.com/>

Resources:

- Worksheet 2.2
- Document “Commercial kits” for activity 3 in “Supplementary materials”
- Alternative worksheet for activity 3 in “Supplementary materials”
- Internet access
- Instructions for use with ENTEROTUBE II:
<http://www.bd.com/europe/regulatory/Assets/IFU/HB/CE/ETUT/IA-273176.pdf>
- Instructions for use with API 10 S:
<http://www.microbelibrary.org/images/shoeb/api20.html>
- Instructions for use with DIATABS:
<http://www.rosco.dk/default.asp?mainmenu=20&submenu=23&webmanage=Instruction%20for%20use%20for%20Diatabs>

Answer key:

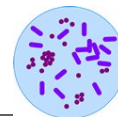
	Enterotube II	Api 10 S	Diatabs
Description of the kit	Sealed tube with 12 compartments containing media, a foil wall, an inner wire and two caps	Strip with wells containing media and an incubation box made of tray with cells and lid	Glass tubes, caps for the tubes and tablets



How to prepare inoculum	Pick up 1 colony with the inoculating wire. Take a visible amount of inoculum	Emulsify one colony into 5 ml sterile water or saline	Prepare a suspension 4 McF in 0'25 ml sterile saline
How to inoculate the tests	Withdraw the wire through all the 12 compartments	Distribute bacterial suspension into the wells	Each tests goes in a different tube
Assuring aerobic conditions	Punch holes through the foil wall on the aerobic compartments	Nothing special. All the aerobic wells are open	Nothing special. Tubes are used for aerobic incubation
Assuring anaerobic conditions	When you reinsert the wire it closes the anaerobic compartments	Seal the anaerobic wells with sterile paraffin oil	Seal the anaerobic tubes with sterile paraffin oil

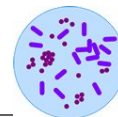
Teaching notes 2.3 – Variability of Bacteria

Timing: 2:00 h	Room: ordinary classroom
Teaching aims: <ul style="list-style-type: none"> ○ Concept of variability ○ Limits for variability ○ Effective use of biochemical profiles of bacteria 	
Prior knowledge: <ul style="list-style-type: none"> ○ Lesson 2.2 	
Resources: <ul style="list-style-type: none"> ○ Worksheet 2.3 ○ Power point presentation with answer key Unit 2 in “Supplementary materials” ○ Alternative worksheet for activity 1 in “Supplementary materials” ○ Digital projector for feedback 	



Activity 1 – Limits for variability	
Subject difficulty: ↑	Language difficulty: ↑
Procedure: <ul style="list-style-type: none"> ○ Explain activity 1 ○ Working in pairs, students complete the activity ○ They check the answers with another pair ○ Feedback in plenary 	
Language support: <ul style="list-style-type: none"> ○ Word bank 	
Resources: <ul style="list-style-type: none"> ○ Worksheet 2.3 ○ Alternative worksheet for activity 1 containing scaffolding in “Supplementary materials” 	
Answer key: <p>1.- When percentages are 98 or 99, that is over 85% and it means that almost all the isolated cultures will give the same result and we may consider the species is positive to the test, but even so, we will find some strain will be negative. These tests have high significance because statistically they have quite sure results.</p> <p>2.- On the other hand we have some percentages that are not so clear. Percentages around 85% may be considered as positive reactions of the species, but accepting this means that nearly 15% are negative strains. The one that has only 65% will be considered indeterminate for the referred test and that means that the test in this case has no significance.</p> <p>3.- The most puzzling situation here is that of <i>E. coli</i> for Indol test: 50% means that half the isolated strains will be positive and half negative. The most indeterminate result we could ever have. For percentages under 50% we may apply the same reasoning as before for percentages about 65%.</p>	

Activity 2 – Making it clear	
Subject difficulty: ↔	Language difficulty: ↔
Activity 2.a	
Procedure: <ul style="list-style-type: none"> ○ Explain activity 2.a ○ Students read the text in activity 1 individually ○ Working in pairs, students match the columns in the substitution table ○ They check the answers with another pair ○ Feedback in plenary 	
Language support: <ul style="list-style-type: none"> ○ Substitution table 	



Resources:

- Worksheet 2.3

Answer key:

1	Test results with a low percentage probability have low significance
2	Variability of species is the cause for indeterminate results for tests
3	Negative results with high percentage probability have high significance
4	Variability of species is the cause for unexpected results
5	Test results with a low percentage probability may lead us to misclassify bacteria

Activity 2.b

Procedure:

- Explain activity 2.b
- Students read the text in activity 1 individually
- Students work individually to complete the mind map
- They check the answers with their partners
- Feedback in plenary

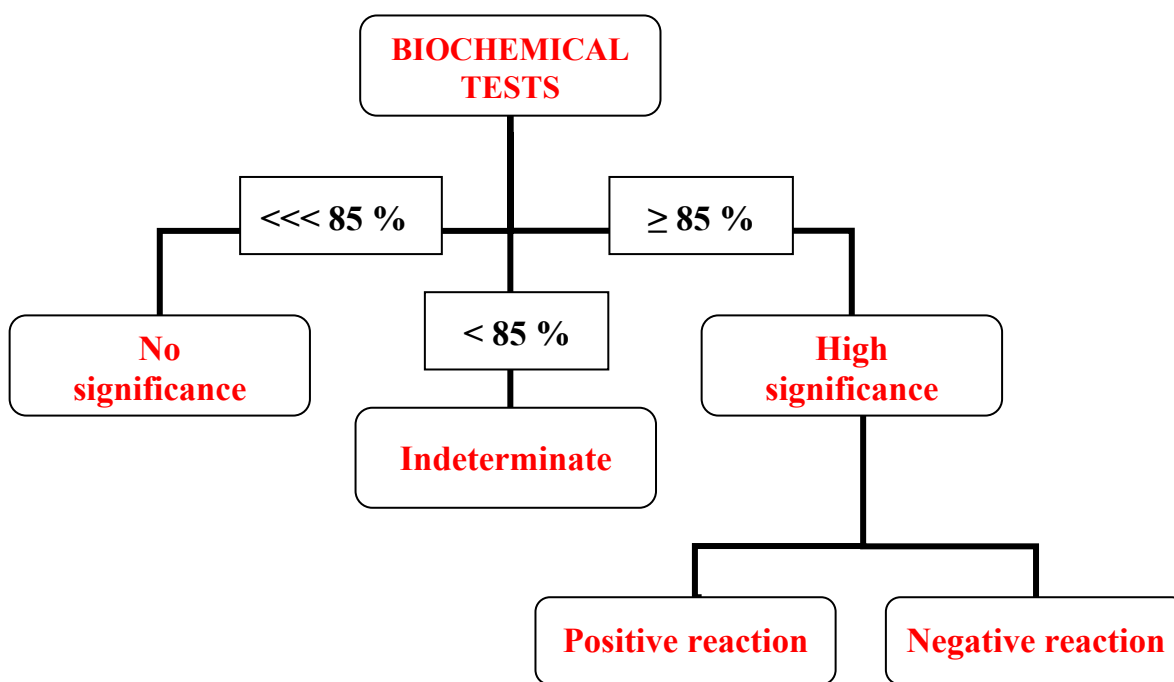
Language support:

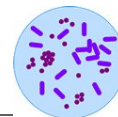
- Word bank

Resources:

- Worksheet 2.3

Answer key:

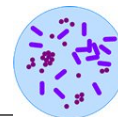


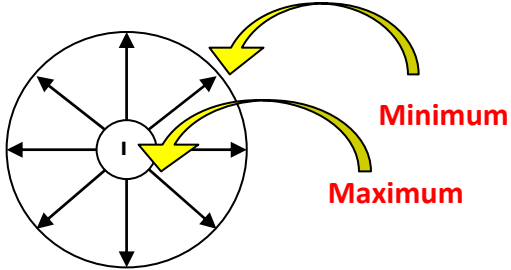
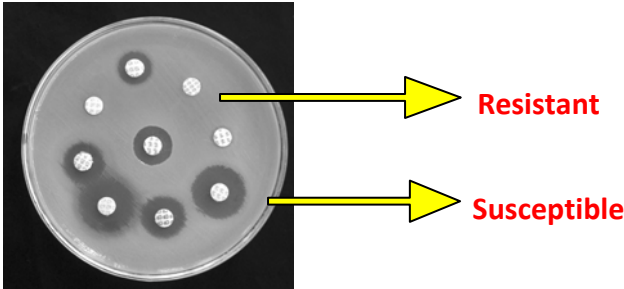


Activity 3 – Functional matrix																																									
Subject difficulty: ⇔			Language difficulty: ⇔																																						
Procedure: <ul style="list-style-type: none"> ○ Explain activity 3 ○ Students read the text individually ○ Students work individually to complete the matrix ○ They check the answers with their partners ○ Feedback in plenary 																																									
Resources: <ul style="list-style-type: none"> ○ Worksheet 2.3 																																									
Answer key: <table border="1" style="width: 100%; text-align: center;"> <thead> <tr> <th>SPECIES</th> <th>ODC</th> <th>H₂S</th> <th>URE</th> <th>TDA</th> <th>IND</th> </tr> </thead> <tbody> <tr> <td><i>Citrobacter freundii</i></td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> </tr> <tr> <td><i>Escherichia coli</i></td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> </tr> <tr> <td><i>Enterobacter aerogenes</i></td> <td>+</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> </tr> <tr> <td><i>Proteus mirabilis</i></td> <td>+</td> <td>-</td> <td>+</td> <td>+</td> <td>-</td> </tr> <tr> <td><i>Aeromonas hydrophila</i></td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>+</td> </tr> </tbody> </table>						SPECIES	ODC	H ₂ S	URE	TDA	IND	<i>Citrobacter freundii</i>	-	-	-	-	-	<i>Escherichia coli</i>	-	-	-	-	-	<i>Enterobacter aerogenes</i>	+	-	-	-	-	<i>Proteus mirabilis</i>	+	-	+	+	-	<i>Aeromonas hydrophila</i>	-	-	-	-	+
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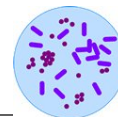
Teaching notes 2.4 – Susceptibility Tests

Timing: 2:00 h	Room: computer room
Teaching aims: <ul style="list-style-type: none"> ○ Fundamentals of Disk Diffusion Tests ○ Kirby-Bauer Method ○ MIC concept ○ Breakpoint tables 	
Resources: <ul style="list-style-type: none"> ○ Worksheet 2.4 ○ Power point presentation with answer key Unit 2 in “Supplementary materials” ○ Digital projector for feedback 	



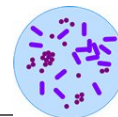
Activity 1 – Fundamentals of Disc Diffusion Test	
Subject difficulty: ⇔	Language difficulty: ↓
Procedure: <ul style="list-style-type: none"> ○ Explain activity 1 ○ Working in pairs, students complete the activity ○ They check the answers with another pair ○ Feedback in plenary 	
Resources: <ul style="list-style-type: none"> ○ Worksheet 2.4 	
Answer key: <div style="border: 1px solid black; padding: 10px;"> <p>1.a</p>  <p>1.b</p>  </div>	

Activity 2 – Kirby-Bauer Method	
Subject difficulty: ⇔	Language difficulty: ⇔
Procedure: <ul style="list-style-type: none"> ○ Explain activity 2 ○ Students work individually ○ Students read the text to complete after seeing the video tutorial ○ Play the video-tutorial: http://student.ccbcmd.edu/courses/bio141/labmanua/lab21/images/kirby%20bauer.htm ○ Students watch the video and fill the gaps on the text with the words that appear in the video. The text to fill and the text that appears in the video are a bit different. ○ Alternatively, explain the procedure instead of projecting the video ○ Before watching the video-tutorial, make sure that the students know all the words. Give clues if they know the words or explain them if they are new ○ They check the answers with their partners ○ Feedback in plenary 	



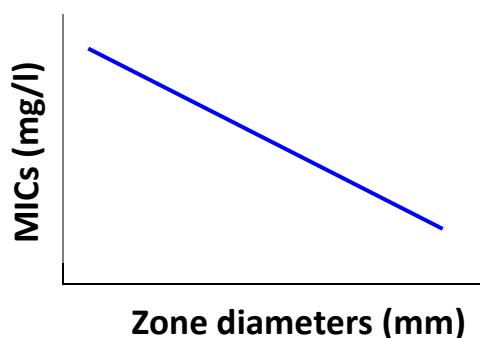
<p>Language support:</p> <ul style="list-style-type: none"> ○ Text written in the video-tutorial ○ Alternatively, difficult words written on the blackboard
<p>Resources:</p> <ul style="list-style-type: none"> ○ Worksheet 2.4
<p>Answer key:</p> <ol style="list-style-type: none"> 1. Preparation of inoculum: <i>Prepare a direct suspension with 3 to 5 well isolated colonies in distilled water. Adjust turbidity to 0.5 MacFarland standard.</i> 2. Inoculation of plates: <ol style="list-style-type: none"> a) <i>Dip a swab in the suspension and remove excess liquid by pressing it against the inner side of the tube.</i> b) <i>Spread the inoculum evenly over the entire surface of the plate by streaking in three directions. Finally swab the rim of the agar. Take care not to leave gaps between streaks. Allow the plate to dry.</i> 3. Application of disks: <i>Apply the disks to the surface of the plate using tweezers to pick them from the container. Flame the tweezers before taking each disk and press over the disk on the plate to ensure full contact. Put no more than 6 equidistant disks per plate of 9 cm. Do not relocate disks once they have touched the agar surface.</i> 4. Incubation: <i>Invert the plate and incubate overnight at 37°C.</i> 5. Measure of zones: <i>Measure the diameters of zones of complete inhibition around each disk from the back of the plate.</i> 6. Interpretation of susceptibility: <i>Interpret diameters into categories of susceptibility according to published tables.</i>

Activity 3 – Interpreting diameters	
Subject difficulty: ↑	Language difficulty: ↓
<p>Procedure:</p> <ul style="list-style-type: none"> ○ Explain activity 3 ○ Students read the text individually ○ Students work individually to complete the activities ○ They check the answers with their partners ○ Feedback in plenary 	
<p>Resources:</p> <ul style="list-style-type: none"> ○ Worksheet 2.4 	
<p>Language support:</p> <ul style="list-style-type: none"> ○ Alternative words to choose ○ Word bank 	



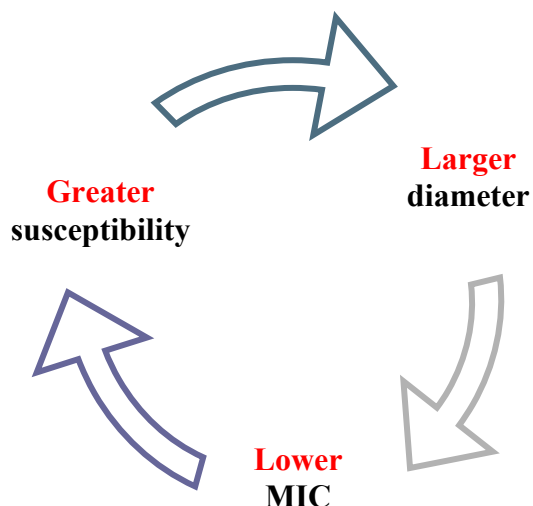
Answer key:

3.a



This *bar graph* / *line graph* shows that MICs and zone diameters are *directly* / *inversely* proportional. That is, as diameter *increases* / *decreases*, MIC *increases* / *decreases*.

3.b



Activity 4 – Using breakpoint tables

Subject difficulty: ⇄

Language difficulty: ⇄

Procedure:

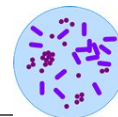
- Explain activity 4
- Students match the columns
- They check the answers with their partners
- Feedback in plenary

Language support:

- Language provided on the text

Resources:

- Worksheet 2.4



Answer key:

Susceptible		That means bacteria are not affected by this antibiotic at all or maybe they are but at too high concentration
Intermediate		That means bacteria are susceptible to low concentrations of it
Resistant		That means bacteria are susceptible to higher concentrations than that in usual dosages

Activity 5 – Life test

Subject difficulty: ⇔

Language difficulty: ↓

Procedure:

- Explain activity 5
- Students interpret the diameters with the breakpoints in the text
- They check the answers with their partners
- Feedback in plenary

Language support:

- Breakpoint table in text

Resources:

- Worksheet 2.4
- Complete breakpoint tables in “Supplementary materials”

Answer key:

Antibiotic	Diameter (mm)	Result
Piperacillin	22	S
Aztreonam	24	I
Imipenem	16	R
Ceftazidime	16	S
Gentamicin	0	R
Ciprofloxacin	20	R

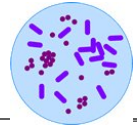
Assessment

Procedure:

- At the end of the unit, students in pairs do a power point to explain a specific biochemical test from a list.
- They prepare the activity by their own and give the presentation in plenary
- Assessment criteria as shown below

Resources:

- Assessment 2 in “Supplementary materials”
- Tick box template for assessment



Tick box template – Assessment criteria UNIT 2

		Students																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Criteria																					
1	Does the student include 10 slides?																				
2	Does the student develop all the suggested issues?																				
3	Can the student explain concepts properly?																				
4	Can the student link the contents presented?																				
5	Can the student present clear visuals?																				
6	Can the student use technical words correctly?																				
7	Does the student show self-confidence?																				
8	Can the student talk fluently?																				
9																					