Lesson 2.1 – Identification of Bacteria
Activity 3 - Information exchange: preliminary tests (3 texts)

WS Lesson 2.1 – Identification of Bacteria
Activity 3.c – Oxidase test

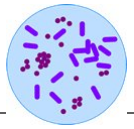
The test detects oxidase production in bacteria. This is an enzyme involved in oxygen metabolism. Its aim is to facilitate the role of oxygen as a final hydrogen acceptor in aerobic respiration, giving water as a final product. Oxidase is present only in some aerobic bacteria.

We use a colourless reagent, **Kovac´s oxidase reagent** that is used for oxidase instead of oxygen: we deceive bacteria to change their usual substrate by our substrate. As a result, oxidized reagent takes a purple-blue colour.

This tests is most useful for differentiating non-fermenters Gram-negative bacilli (i.e. *Pseudomonas* ...) that are oxidase positive from fermenters Gram-negative bacilli (Enterobacteriae) that are negative.

We may use the **swab method**: we dip the swab into the reagent and then touch the colony with it. **Positive** reactions will develop a **blue-purple colour** in less than ten seconds. If there is no oxidase, the reagent will remain **colourless** and the test is reported as **negative**.

Metal loops and media containing dyes may give false positives. Use plastic or wood sticks to pick up colonies and be careful not to take out agar with the colony.



WS Lesson 2.1 – Identification of Bacteria
Activity 3.a – Haemolysis

Some bacteria produce haemolysis on blood agar. This means they cause lysis of red blood cells contained in the culture media. There are three different kinds of haemolysis:

- **α haemolysis** – It is a partial lysis of red blood cells and appears as a green halo surrounding the colonies.
- **β haemolysis** – It is a complete lysis of red blood cells and appears as a clear, yellowish halo around the colonies.
- **γ haemolysis** – It is the expression for non-haemolitic bacteria when there is no halo around the colonies.

This is a taxonomic feature for some species such as those of genus *Streptoccocci*, which are classified according to the kind of haemolysis they produce.

WS Lesson 2.1 – Identification of Bacteria
Activity 3.b - Catalase test

The test detects catalase production in bacteria. This is an enzyme involved in oxygen metabolism. Its aim is to destroy hydrogen peroxide produced in aerobic respiration by breaking it down into water and oxygen. Anaerobes do not have it because they do not need it, and aerobes may have some other different enzyme.

The presence of catalase can be checked by the **slide method**: we put a drop of 3% **hydrogen peroxide** on a slide and emulsify bacteria in the drop. If there is catalase, **bubbles** of oxygen will appear in a few seconds and the result is reported as **positive**. If there is no catalase, nothing will happen and the result will be reported as **negative**.

Metal loops and blood agar may give false positives. Use plastic or wood sticks to pick up colonies.

This test is most useful for differentiating between *Staphilococci* and *Streptococci,* both Gram-positive cocci and facultative aerobes, but one contains catalase and the other does not.

Lesson 2.2 – Biochemical Tests
Activity 3 – Information exchange: commercial kits (3 texts)

Instructions for use with API 10 S (TM Biomerieux)

Preparation of the strip

1. Prepare an incubation box (tray and lid) and distribute about 3 ml of distilled water into the cells at the bottom of the tray to create a humid atmosphere.
2. Remove the strip from its packaging.
3. Place the strip in the incubation box.

Preparation of the inoculum

1. Use any tube containing 5 ml of sterile saline or sterile distilled water, without additives.
2. Using a pipette remove a single isolated colony.
3. Carefully emulsify to achieve a homogeneous bacterial suspension. This suspension must be used immediately after preparation.

Inoculation of the strip

1. With the same pipette, distribute the bacterial suspension into the wells of the strip. For the CITRATE test, fill the well completely. For the other tests, fill only up to the edge. For the tests LDC, ODC, H_2S and UREASE, create anaerobic conditions by overlaying with sterile paraffin oil.
2. Close the incubation box.
3. Incubate at 36°C ± 2°C for 18-24 hours.

Instructions for use with DIATABS (TM Rosco Diagnostica)

Inoculum preparation

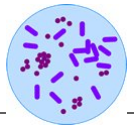
1. Prepare a heavy suspension (at least McFarland 4 standard) of the test organism in 0.25 ml saline in a sterile tube.
2. A battery of Diatabs tablets may be inoculated with a single inoculum, each one in a different tube. Each Diatabs contains one different dehydrated media.

Inoculation

1. Add each Diatabs to the corresponding tube. Some Diatabs additionally require 3 drops of sterile paraffin oil added to the tube to create anaerobic conditions.

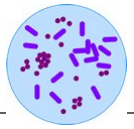
Incubation and reading of tubes

1. Close the tube and incubate at 35-37 °C for 4 hours. Negative results must continue incubation overnight.



Instructions for use with ENTEROTUBE II (TM BBL)

1. Take one Enterotube tube and remove both caps. The tip of the inoculating wire is under the white cap. Do not flame the wire.
2. Pick one well isolated colony directly with the tip of the inoculating wire. A visible amount of inoculum should be seen at the tip and the side of the wire. Avoid touching agar with the wire.
3. Inoculate the Enterotube by first twisting the wire, then withdrawing the wire through all twelve compartments applying a turning motion. The withdrawing tip has a handle to pull the wire and a mark below it.
4. Reinsert the wire (without sterilizing) into Enterotube, using a turning motion through all 12 compartments, until the mark on the wire is aligned with the opening of the tube. Break the wire at the notch by bending. The portion of the wire remaining in the tube maintains anaerobic conditions necessary for some reactions.
5. With the broken off part of the wire, punch holes through the foil wall of the tube on the last eight compartments (adonitol, lactose, arabinose, sorbitol, Voges-Proskauer, dulcitol/PA, urea and citrate) in order to support aerobic growth in these compartments. Replace both caps.
6. Incubate the tube at 35 to 37° C for 18 to 24 hours in an upright position.



Lesson 2.2 – Biochemical Tests
Activity 3 – Alternative worksheet: commercial kits

You are going to read a text about how to inoculate a commercial kit. Look at the chart first and then read the text. Underline what is relevant to the chart. Finally, fit them to the word frame in the boxes to complete the sentences. Check meaning and pronunciation of any words you need in the online dictionaries you know.

There are three different texts for three different kits. Form a group of three with students that have read the other texts. Explain your text to each other and exchange information to fill in your charts.

	Enterotube II	**Api 10 S**	**Diatabs**
Description of the kit	Sealed tube with 12 compartments containing media, a foil wall , an inner w_____ and two caps	Strip with wells containing media and an incubation box made of a t_____ with cells and its l_____	G_____ tubes, caps for the tubes and tablets
How to prepare inoculum	Pick up 1 colony with the inoculating wire. Take a visible amount of i_____	E_____ one colony into 5 ml sterile water or saline	Prepare a suspension 4 McF in 0'25 ml sterile s_____
How to inoculate the tests	Withdraw the wire through all the 12 c_____	Distribute bacterial suspension into the w_____	Each tests goes in a different t_____
Assuring aerobic conditions	Punch holes through the f_____ 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 c_____	Seal the anaerobic wells with sterile p_____ oil	Seal the anaerobic tubes with sterile p_____ oil

Lesson 2.3 – Variability
Activity 1 – alternative worksheet: limits for variability

The text in the worksheet reflects the meaning of variability. Fill the gaps of the missing words approximating numbers. Use the word bank in the worksheet and the scaffolding below to approximate numbers. There may be some word left over and some repeated.