

AIMS

The aims of this practical class are to introduce you to some basic techniques in bacteriology.

16.1 INTRODUCTION TO COURSE SAFETY AND ASEPTIC TECHNIQUES

The safety procedures that must be observed during these classes together with a description of aseptic techniques will be shown in a brief video.

16.2 TYPES OF BACTERIAL GROWTH MEDIA

Familiarise yourself with the appearance of these media

1. Sterile Nutrient broth

Nutrient broth is a general purpose medium, based upon a soluble extract of enzymatically digested meat.

2. Overnight broth culture of *Escherichia coli*.

When bacteria grow in nutrient broth, the broth becomes turbid - a sample of such a broth is provided (there are about 5×10^8 bacteria per ml).

3. Nutrient agar

Nutrient broth is solidified with **agar** (a polysaccharide extracted from seaweed) to form **nutrient agar**.

4. Blood agar

Many pathogenic bacteria are fastidious in their growth requirements and will only grow on media enriched with blood. **Blood agar** is made by enriching the nutrient agar with horse blood.

A large number of different bacterial growth media have been devised and are in common use. In these classes most of the bacteria you see will be grown either on nutrient agar or on blood agar.

16.3 RECOGNITION OF BACTERIAL COLONIES ON SOLID MEDIA

The macroscopic features of the colonies of most bacterial species can be used as an aid to bacterial identification. However, you need to be aware that *environmental* factors can affect these features e.g. crowded colonies are smaller, owing to competition for nutrients or the formation of inhibitory substances. To find a typical colony of any species, always look for one that is well separated from its neighbours. *Genetic* variation can also affect the features of a colony e.g. how smooth or rough it is. Since bacteria cannot be identified solely by the appearance of their colonies there is a need to apply supporting tests; these tests will be described later.

Materials:

Nutrient agar plate cultures of:

1. *Staphylococcus aureus*
2. *Staphylococcus epidermidis*
3. *Pseudomonas aeruginosa*
4. *Escherichia coli*

Catalogue Number	Small Image	Image Map	Large Image
M_BI_SP_10.jpg	Staphylococcus aureus		Staphylococcus aureus

M_BI_SP_06.jpg	<i>Staphylococcus epidermidis</i>		<i>Staphylococcus epidermidis</i>
M_BI_PS_11.jpg	<i>Pseudomonas aeruginosa</i>		<i>Pseudomonas aeruginosa</i>
M_BI_ES_15.jpg	<i>Escherichia coli</i>		<i>Escherichia coli</i>

Procedure:

Examine the colonies carefully, using a hand lens. Make notes of the colonial appearance under the headings size, shape, elevation, surface appearance, type of margin, colour, opacity, presence or absence of changes in the surrounding medium. Your Demonstrator will help you with these descriptions.

16.4 BLOOD AGAR: HAEMOLYSIS

Pathogenic strains of many bacterial species produce toxins or enzymes which may lyse host cells such as erythrocytes i.e. they are haemolytic. You will be shown other organisms that produce haemolysins in later practical classes.

Materials:

Plate cultures of:

1. *Streptococcus pyogenes* showing complete haemolysis(β -haemolysis)
2. *viridans Streptococci* showing incomplete haemolysis(α -haemolysis)
3. *Staphylococcus aureus*

Catalogue Number	Small Image	Image Map	Large Image
M_BI_ST_23.jpg	<i>St. pyogenes</i> – top and back lit		<i>St. pyogenes</i> – top and back lit
M_BI_ST_24.jpg	<i>viridans St</i> – top and back lit		<i>viridans St</i> – top and back lit
M_BI_SP_15.jpg	<i>S. aureus</i> – top and back lit		<i>S. aureus</i> – top and back lit

Procedure:

Familiarise yourself with the appearances of these colonies. Examine the colonies carefully. Make notes of the colonial appearance.

16.5 IDENTIFICATION OF BACTERIA ON INDICATOR MEDIA

There are a number of different media in common use for the study of enteric (intestinal) organisms. Note these examples which you will see again in later classes. See also demonstration boards. MacConkey medium contains bile salts, which inhibit the growth of non-enteric organisms, lactose, and a pH indicator, neutral red which is red or purple at low pH and colourless at neutral or high pH. Lactose-fermenting bacteria (e.g. *E. coli*) grow as red/purple colonies, as the acid they produce from lactose fermentation produces a localized pH drop that changes the colour of the pH indicator. Non-lactose-fermenting colonies (e.g. *Salmonella* species) are pale or colourless.

Cysteine Lactose Electrolyte Deficient Agar (CLED) is used clinically in the analysis of urine samples, and as with MacConkey's medium, lactose and non-lactose fermenters are differentiated. The indicator is bromothymol blue which is yellow at acid pH. In the demonstration plates provided, colonies of *E. coli* and *Ent. faecalis* are yellow, those of *Salmonella* are pale green/blue. The electrolyte deficiency prevents the swarming of *Proteus mirabilis*, an organism that is not discussed in this course, but which after *E. coli*, is one of the more common causes of urinary tract infections. Swarming gives rise to a thin film of bacteria which may cover the entire surface of an agar plate, thus obscuring other bacterial colonies.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_ES_19.jpg	E. coli – blood agar		E. coli – blood agar
M_BI_ES_13.jpg	E. coli - Mac		E. coli - Mac
M_BI_ES_22.jpg	E. coli - CLED		E. coli - CLED
M_BI_SL_13.jpg	Salmonella – blood agar		Salmonella – blood agar
M_BI_SL_07.jpg	Salmonella - Mac		Salmonella – Mac
M_BI_SL_16.jpg	Salmonella - CLED		Salmonella – CLED
M_BI_ET_04.jpg	Ent. faecalis – blood agar		Ent. faecalis – blood agar
M_BI_ET_05.jpg	Ent. faecalis - Mac		Ent. faecalis - Mac
M_BI_ET_08.jpg	Ent. faecalis - CLED		Ent. faecalis - CLED

Materials:

Pure cultures of *E. coli*, *Salmonella sp* and *Ent. faecalis*, on Blood agar, MacConkey's medium, & CLED.

Procedure:

1. Examine the colonies.
2. Make notes of their appearances on the different media.

16.6 SEROLOGICAL IDENTIFICATION OF SALMONELLAE

Members of the genus *Salmonella* are ubiquitous human and animal pathogens that cause gastroenteritis, enteric fever and septicaemia. The genus contains more than 1800 serotypes and surveillance of *Salmonella* serotypes is a major component in monitoring outbreaks of salmonellosis. The serotypes are based on three major antigens: H, or flagellar antigen, O or somatic antigen, and Vi antigen, two of which are demonstrated here.

In the photograph provided suspensions of two *Salmonella* serotypes were mixed on a glass tile with antisera specific for particular O and H antigen serotypes. The clumping of the bacteria indicates a positive reading.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_SL_15.jpg	Salmonella antigen serotyping		Salmonella antigen serotyping

16.7 THROAT SWABS

Work in pairs.

Materials:

1. Sterile swab in plastic cover.
2. A willing partner.
3. Blood agar plate.

Procedure:

Label the blood agar plate with your partner's name or bench number, then gently swab your partner's throat/tonsils using the demonstrated technique. Inoculate a small area of the labelled plate with material from the swab, and then dilute the bacteria across the plate, using a sterile loop.

The plate will be incubated at 37°C.

16.8 ANALYTICAL PROFILE INDEX (API)

The Analytical Profile Index strip provided, API 10 S, is a standardised identification system for Enterobacteriaceae and other non-fastidious Gram-negative rods. Alternative strips are available for other groups of bacteria.

Principle:

The API strip consists of ten micro-tubes containing dehydrated media and substrates. When a bacterial suspension is added, the contents of each tube are rehydrated. During subsequent incubation the metabolic activity of different bacterial species have differing effects upon the various substrates. These different effects are detected, after overnight incubation, by colour changes that occur either spontaneously or after the addition of other reagents.

Procedure

Each bench is provided with four previously inoculated and incubated strips, W, X, Y & Z, and in this experiment, you are asked to use the API method to identify the bacteria in each strip.

To start the procedure, mark the colour changes in tubes ONPG, GLU, ARA, LDC, ODC, CIT, H₂S, URE as positive and negative according to the results table (Figure 1) below. The results + or - are then marked on the chart (Figure 2) below in the space above each label.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_AP_01.jpg	API – before reagents		API – before reagents

To read the remaining results proceed as follows:

- 1) To tube TDA add one drop of TDA reagent. Read and record result.
- 2) To tube IND add one drop of JAMES reagent. Read and record result.
- 3) To tube ONPG add one drop of oxidase reagent. Read result immediately (a purple colour developing after a minute is not positive). Record result in OX on chart.
- 4) To tube GLU add one drop of NIT1 and one drop of NIT2. Record result after two minutes in NO₂ on the chart.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_AP_02.jpg	API – after reagents		API – after reagents

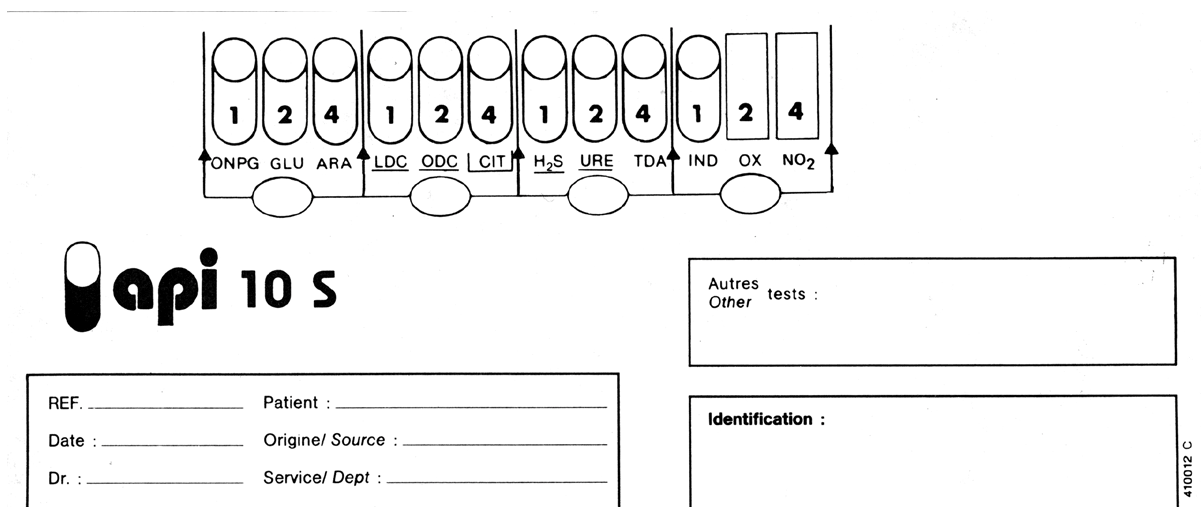
Figure 1

Test	Substrate	Reaction/Enzyme	Results
------	-----------	-----------------	---------

			Negative	Positive
ONPG	ortho-nitro-phenyl-galactoside	beta-galactosidase	colourless	yellow
GLU	glucose	fermentation/oxidation(1)	blue/blue green	yellow
ARA	arabinose	fermentation/oxidation(1)	blue/blue green	yellow
LDC	lysine	lysine decarboxylase	yellow	orange
ODC	ornithine	ornithine decarboxylase	yellow	red/orange
CIT	sodium citrate	citrate utilisation	pale green/yellow	Blue green/blue
H ₂ S	sodium thiosulphate	H ₂ S production	colourless/greyish	Black deposit/ thin line
URE	urea	urease	yellow	red/orange
TDA	tryptophane	tryptophane desaminase	yellow	dark brown
IND	tryptophane	indole production	pale green/yellow	pink
OX		cytochrome oxidase	colourless	violet
NO ₂		NO ₂ production	yellow	red

(1) Fermentation begins in the lower portion of the tubes; oxidation begins in the cupule

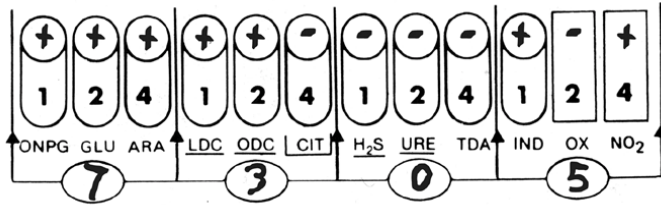
Figure 2



A four-figure code is found by addition of positive results as shown in the example (Figure3), and then used to identify the bacterial species using the directory provided (Analytical Profile Index directory)

Deduce the identity of the bacteria you have analysed (W, X, Y or Z). Compare your results with the other groups on your bench.

Figure 3



api 10 S

Autres tests :
Other tests :

REF. _____ Patient : **Example**
Date : _____ Origine/ Source : _____
Dr. : _____ Service/ Dept : _____

Identification : **E. coli**

410012 C

IDENTIFICATION DIRECTORY (reference only)

0016 <i>Pseudomonas putrefaciens</i>	2654 <i>Proteus mirabilis</i>	6426 <i>Pseudomonas aeruginosa</i>
0075 <i>Proteus vulgaris</i>	2664 <i>Proteus mirabilis</i>	6445 <i>Providencia</i>
0206 <i>Pseudomonas putrefaciens</i>	2665 <i>Proteus</i>	6475 <i>Proteus vulgaris</i>
0216 <i>Pseudomonas putrefaciens</i>	2674 <i>Proteus mirabilis</i>	6514 <i>Salmonella</i>
0221 <i>Proteus morgani</i>	2675 <i>Proteus mirabilis</i>	6524 <i>Klebsiella / Serratia</i>
0225 <i>Proteus morgani</i>	2707 <i>Vibrio</i>	6604 <i>Enterobacter cloacae</i>
0261 <i>Proteus morgani</i>	2714 <i>Salmonella</i>	6614 <i>Citrobacter freundii / Salmonella</i>
0265 <i>Proteus morgani</i>	2724 <i>Serratia</i>	6674 <i>Proteus mirabilis</i>
0274 <i>Proteus mirabilis</i>	2764 <i>Proteus mirabilis</i>	6704 <i>Salmonella</i>
0412 <i>Pseudomonas putrefaciens</i>	2774 <i>Proteus mirabilis</i>	6707 <i>Vibrio</i>
0416 <i>Pseudomonas putrefaciens</i>		6714 <i>Salmonella</i>
0422 <i>Pseudomonas aeruginosa</i>	3004 <i>Shigella / Klebsiella</i>	6715 <i>Salmonella</i>
0475 <i>Proteus vulgaris</i>	3005 <i>Escherichia coli / Shigella</i>	
0500 <i>Pseudomonas maltophilia</i>	3007 <i>Aeromonas hydrophila</i>	7000 <i>Enterobacter agglomerans</i>
0504 <i>Pseudomonas maltophilia</i>	3014 <i>Citrobacter freundii</i>	7001 <i>Enterobacter agglomerans</i>
0616 <i>Pseudomonas putrefaciens</i>	3024 <i>Yersinia pseudotuberculosis</i>	7005 <i>Escherichia coli</i>
0664 <i>Proteus mirabilis</i>	3105 <i>Escherichia coli</i>	7006 <i>Aeromonas hydrophila</i>
0674 <i>Proteus mirabilis</i>	3107 <i>Aeromonas hydrophila</i>	7007 <i>Aeromonas hydrophila</i>
	3204 <i>Shigella</i>	7014 <i>Citrobacter freundii</i>
1500 <i>Pseudomonas maltophilia</i>	3205 <i>Escherichia coli</i>	7025 <i>Yersinia enterocolitica</i>
1504 <i>Pseudomonas maltophilia</i>	3224 <i>Yersinia enterocolitica</i>	7034 <i>Citrobacter freundii</i>
	3225 <i>Yersinia enterocolitica</i>	7040 <i>Enterobacter agglomerans</i>
2004 <i>Shigella / Klebsiella</i>	3305 <i>Escherichia coli</i>	7041 <i>Enterobacter agglomerans</i>
2005 <i>Shigella</i>	3307 <i>Aeromonas shigelloides / Vibrio</i>	7044 <i>Enterobacter agglomerans</i>
2035 <i>Proteus vulgaris</i>	3402 <i>Pseudomonas cepacia</i>	7045 <i>Enterobacter agglomerans</i>
2045 <i>Providencia</i>	3404 <i>Klebsiella / Serratia</i>	7105 <i>Escherichia coli</i>
2055 <i>Proteus vulgaris</i>	3406 <i>Pseudomonas cepacia</i>	7107 <i>Aeromonas hydrophila</i>
2064 <i>Proteus</i>	3407 <i>Aeromonas hydrophila</i>	7115 <i>Escherichia coli</i>
2065 <i>Proteus</i>	3465 <i>Proteus rettgeri</i>	7124 <i>Klebsiella / Serratia</i>
2074 <i>Proteus</i>	3502 <i>Pseudomonas cepacia</i>	7125 <i>Klebsiella oxytoca</i>
2075 <i>Proteus vulgaris</i>	3507 <i>Aeromonas hydrophila</i>	7204 <i>Shigella / Enterobacter</i>
2104 <i>Salmonella typhi</i>	3524 <i>Klebsiella / Serratia</i>	7205 <i>Escherichia coli</i>
2105 <i>Escherichia coli</i>	3614 <i>Citrobacter freundii</i>	7214 <i>Citrobacter freundii</i>
2114 <i>Salmonella</i>	3674 <i>Proteus mirabilis</i>	7224 <i>Yersinia enterocolitica</i>
2221 <i>Proteus morgani</i>	3704 <i>Serratia marcescens</i>	7225 <i>Yersinia enterocolitica</i>
2224 <i>Yersinia enterocolitica</i>	3707 <i>Vibrio</i>	7234 <i>Citrobacter freundii</i>
2234 <i>Proteus mirabilis</i>	3724 <i>Serratia marcescens</i>	7305 <i>Escherichia coli</i>
2241 <i>Proteus morgani</i>		7314 <i>Arizona</i>
2245 <i>Proteus morgani</i>	4402 <i>Pseudomonas fluorescent group</i>	7315 <i>Escherichia coli</i>
2254 <i>Proteus mirabilis</i>	4406 <i>Pseudomonas fluorescent group</i>	7400 <i>Enterobacter agglomerans</i>
2261 <i>Proteus morgani</i>	4422 <i>Pseudomonas aeruginosa</i>	7401 <i>Enterobacter agglomerans</i>
2264 <i>Proteus mirabilis</i>	4426 <i>Pseudomonas aeruginosa</i>	7405 <i>Enterobacter agglomerans</i>
2265 <i>Proteus morgani</i>		7407 <i>Aeromonas hydrophila</i>
2274 <i>Proteus mirabilis</i>	6000 <i>Acinetobacter calcoaceticus</i>	7414 <i>Citrobacter freundii</i>
2305 <i>Edwardsiella tarda</i>	6004 <i>Shigella</i>	7415 <i>Citrobacter freundii</i>
2307 <i>Vibrio</i>	6005 <i>Shigella / Escherichia coli</i>	7425 <i>Klebsiella pneumoniae var oxytoca</i>
2314 <i>Salmonella</i>	6045 <i>Providencia</i>	7434 <i>Citrobacter freundii</i>
2315 <i>Edwardsiella tarda</i>	6065 <i>Proteus</i>	7440 <i>Enterobacter agglomerans</i>
2324 <i>Hafnia alvei</i>	6104 <i>Salmonella</i>	7441 <i>Enterobacter agglomerans</i>
2365 <i>Proteus morgani</i>	6105 <i>Salmonella / Escherichia coli</i>	7444 <i>Enterobacter agglomerans</i>
2374 <i>Proteus mirabilis</i>	6114 <i>Salmonella</i>	7445 <i>Enterobacter agglomerans</i>
2402 <i>Pseudomonas fluorescent group</i>	6204 <i>Salmonella / Shigella</i>	7504 <i>Klebsiella / Serratia</i>
2404 <i>Klebsiella ozaenae</i>	6205 <i>Escherichia coli</i>	7505 <i>Klebsiella pneumoniae var oxytoca</i>
2405 <i>Providencia</i>	6214 <i>Citrobacter / Salmonella</i>	7507 <i>Aeromonas hydrophila</i>
2422 <i>Pseudomonas aeruginosa</i>	6224 <i>Yersinia enterocolitica</i>	7524 <i>Klebsiella pneumoniae</i>
2425 <i>Proteus rettgeri</i>	6225 <i>Yersinia enterocolitica</i>	7525 <i>Klebsiella pneumoniae var oxytoca</i>
2426 <i>Pseudomonas aeruginosa</i>	6261 <i>Proteus morgani</i>	7604 <i>Enterobacter cloacae / Serratia</i>
2444 <i>Providencia</i>	6265 <i>Proteus morgani</i>	7605 <i>Citrobacter</i>
2445 <i>Providencia</i>	6274 <i>Proteus mirabilis</i>	7614 <i>Citrobacter freundii</i>
2464 <i>Proteus</i>	6304 <i>Hafnia alvei / Salmonella</i>	7615 <i>Citrobacter freundii</i>
2465 <i>Proteus rettgeri</i>	6305 <i>Escherichia coli</i>	7624 <i>Enterobacter cloacae</i>
2474 <i>Proteus</i>	6307 <i>Vibrio</i>	7714 <i>Arizona</i>
2475 <i>Proteus</i>	6314 <i>Salmonella</i>	7702 <i>Pseudomonas cepacia</i>
2545 <i>Providencia</i>	6400 <i>Acinetobacter calcoaceticus</i>	7706 <i>Pseudomonas cepacia</i>
2624 <i>Proteus mirabilis</i>	6402 <i>Pseudomonas fluorescent group</i>	7724 <i>Enterobacter / Serratia</i>
2634 <i>Proteus mirabilis</i>	6406 <i>Pseudomonas fluorescent group</i>	7725 <i>Klebsiella oxytoca</i>
2644 <i>Proteus mirabilis</i>	6414 <i>Citrobacter freundii / Salmonella</i>	6422 <i>Pseudomonas aeruginosa</i>

DEMONSTRATIONS

- A. Demonstration boards of various bacteria: bacilli (rods), cocci (spheres or ovoids) and spirochaetes.
- B. Electron micrographs illustrating bacterial fine structure.

All the plates which need to be incubated must be placed in the aluminium racks at the end of your bench. Make sure that you have labelled your plates with your seat number &/or name and remember which rack you put them in for next practical session.

Wipe your bench top with disinfectant before you leave.

On the way out, wash your hands, turning the taps on and off with your elbows.

Thank You!