MVST BOD & NST PART IB — **Thurs 26th & Fri 27th Jan 2012**

Pathology Practical Class 18 — **Antibiotics and Toxins**

AIMS

The aims of this class are to provide varied insights into the impact and activity of antibacterial agents and toxins.

PLEASE TURN THE HOTPLATE ON BEFORE STARTING ANY PRACTICAL WORK

18.1 TITRATION OF ANTI-STREPTOLYSIN O BY TOXIN NEUTRALISATION

Streptococcus pyogenes (a so-called Group A β-haemolytic streptococcus) is commonly associated with throat infections, but it may also give rise to rheumatic fever and glomerulonephritis. Rheumatic fever, an immune disorder, was once common in Europe and the USA. Most patients (usually children aged 5-15 years old) have had a recent sore throat, typically caused by a group A β-haemolytic streptococcal infection.

A high proportion of patients infected with *Streptococcus pyogenes* produce an antibody which inhibits the lysis of erythrocytes by Streptolysin O. This inhibition will serve as a model toxin-antitoxin reaction and illustrates the application of this reaction as a means of detecting *Streptocooccus pyogenes* infections. The antibiotic treatment of such infections may prevent the onset of rheumatic fever or acute post-streptococcal glomerulonephritis

Examine the **photograph** on each bench.

In the performed experiment, serum from a patient was heated to inactivate complement, diluted and titrated against Streptolysin O prepared from a filtrate of a broth culture of *Streptococcus pyogenes* (a reducing agent was added to protect the toxin from inactivation by oxidation).

Dilutions of Patient's Serum							**Controls**	
Tube number	1	2	3	4	5	6	7	8
Buffer	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Patient's serum diluted 1:50.	0.5 ml, then 0.5ml serial dilutions discarding 0.5 ml from tube 6						0	0.5 ml
Streptolysin O	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0

After incubation for 15mins at 37°C a 5% suspension of horse red blood cells was added to each tube. After a further hour's incubation the end point was read.

Note that two control tubes were employed. The first contained the extract and the red blood cells, the second diluted serum and red blood cells.

Q1 Why were these controls included?

Catalogue No.	Small Image	Image Map	Large Image
M_BI_ST_25.jpg	Anti-streptolysin O titration		Anti-streptolysin O titration

18.2 ELEK PLATE: A TEST FOR TOXIN PRODUCTION BY *C. diphtheriae*

The gene encoding the diphtheria toxin of *Corynebacterium diphtheriae* is carried on a bacterial virus (the β bacteriophage), integrated into the bacterial chromosome. A non-toxigenic strain of *C. diphtheriae* can be converted into a toxigenic strain by becoming infected with β bacteriophage. Toxin formed can be detected immunologically using an ELEK plate

Toxigenic strains of *C. diphtheriae* may be distinguished from non-toxigenic strains by growing the organisms in thick streaks at right angles to a strip of filter paper that has been soaked in antitoxin. Lines of precipitate form after several days at the sites where toxin and antitoxin meet.

Procedure

Examine the photograph of the Elek plate provided, and make a diagram of it in your notes.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_CY_24.jpg	Elek plate		Elek plate

18.3 MORPHOLOGY OF CORYNEBACTERIUM DIPHTHERIAE

Corynebacteria characteristically contain polyphosphate granules, which may be detected using Albert's stain. These bacteria also have a tendency to form clumps which are said to look a bit like the characters used in written Chinese (kanji).

Procedure

Examine the photograph of *Corynebacteria* stained by Albert’s method and note the dark blue/green polyphosphate granules.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_CY_22.jpg	C. dip – Gram stain		C. dip – Gram stain
M_BI_CY_34.jpg	C. dip – polyphosphate granules		C. dip – polyphosphate granules

(B) Materials:

A blood agar plate inoculated with *C. diphtheriae.*

Procedure:

Examine the colonies on the blood agar plate, gram stain and note their appearance.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_CY_18.jpg	C. dip		C. dip

18.4 TRACHEA AND LARYNX: DIPHTHERIA

R64.375

This is an old but valuable specimen from a child.

Examine the specimen. Note the grey-yellow necrotic tissue and fibrin form a membrane on the mucosal surface of the hyperaemic trachea and larynx.

Catalogue Number	Small Image	Image Map	Large Image
A_IN_BI_TC_02.jpg	Trachea		Trachea

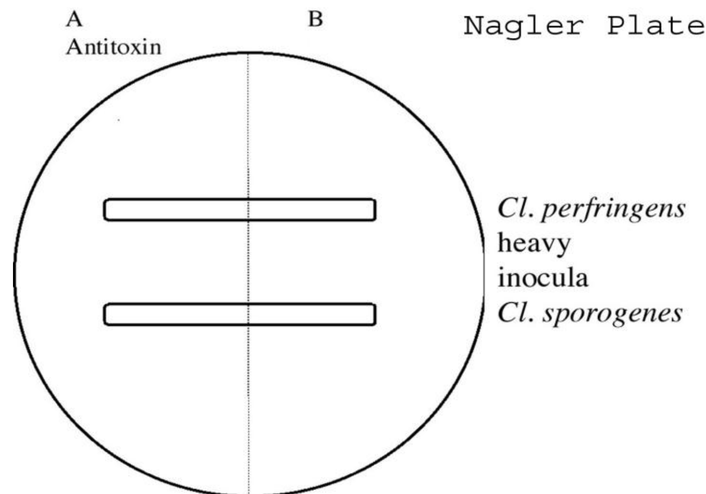
18.5 ACTION OF *CLOSTRIDIUM PERFRINGENS* α-TOXIN

At least 12 different toxins have been identified in strains of *Cl. perfringens*. One of these is the α-toxin, a lecithinase that hydrolyses the phospholipid lecithin (a component of cell membranes) to a diglyceride and phosphorylcholine. The activity of the α-toxin can be demonstrated by growth on agar containing egg yolk (as a source of lecithin): an opaque zone representing insoluble diglyceride, becomes evident around colonies of *Clostridium perfringens* (the Nagler reaction). The activity of α-toxin is inhibited by anti-α-toxin antibody (generated by vaccination with α-toxoid).

The photograph provided (**19.2**),shows a nutrient agar plate enriched with egg yolk. Anti α-toxin was spread over half of the plate and a heavy inocula of *Cl. perfringens* and *Cl. sporogenes* were streaked across the plate at right angles to the anti α-toxin boundary.

Examine the photo and interpret the result.

Q1 Do both bacterial species produce α toxin?



Catalogue Number	Small Image	Image Map	Large Image
M_BI_CL_27.jpg	Nagler reaction		Nagler reaction

18.6 ANTIBIOTIC SENSITIVITY OF COMMON PATHOGENS

Demonstration plates (labelled SS, Standard Strains) and photographs are provided which show the spectra of inherent antibiotic sensitivity of selected standard strains from the American Type Culture Collection, (ATCC) of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.*

Catalogue Number	Small Image	Image Map	Large Image
M_BI_AB_25.jpg	S. aureus - ATCC		S. aureus - ATCC
M_BI_AB_25.jpg	E. coli - ATCC		E. coli - ATCC
M_BI_AB_25.jpg	Ps. aeruginosa - ATCC		Ps. aeruginosa - ATCC

Q2 How do you account for the inherent differences between the antibiotic sensitivities of the standard strains of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* ?

N.B. Clinical isolates of any bacterial species can vary widely in their patterns of antibiotic sensitivity and resistance.

18.6 ENZYMATIC DESTRUCTION OF ANTIBIOTIC

The soluble β-lactamases produced by penicillin-resistant *Staphylococcus aureus* or *Neisseria gonorrhoeae* or by ampicillin-resistant *Haemophilus influenzae* convert penicillin into penicilloic acid, resulting in a pH change. In the case of *Haemophilus influenzae,* if bacteria are spotted out onto test paper strips containing penicillin and an appropriate pH indicator, the presence of β-lactamase is indicated by acid production. Clinical awareness of the presence of antibiotic resistant bacteria can be life saving.

Materials:

1. Paper strips containing indicator (bromothymol blue) and penicillin.
2. Cultures of known β-lactamase-positive and β-lactamase-negative *Haemophilus influenzae.*
3. Phosphate-buffered saline (PBS).
4. Test cultures.

Procedure:

1. Place a paper indicator strip on a glass slide.
2. Damp the paper with just sufficient PBS to hold it to the slide. Do not flood.
3 Apply a heavy loop of bacteria from the test culture and from the control culture to the appropriate printed points indicated on the test paper. Leave for a few minutes. Then look to see if pH changes have occurred at the points at which the bacteria were applied to the paper i.e. whether penicilloic acid has been formed.

18.7 EXAMINATION OF ORGANISMS CULTURED FROM A BURN

In this exercise, you are provided with an agar plate labelled **D** that has been inoculated with fluid taken from a burn in the inguinal (groin) region, and you are asked to identify the bacteria present in the wound.

Examine Plate D.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_MX_23.jpg	Plate D		Plate D

Q3. How many colony types are present? Gram-stain the organisms.
Q4. Can you now identify them?

Follow the notes from class 17.

PLEASE NOTE:

Cover the microscope and put it away.`Turn off the hotplate.
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.