**MCB2010L –Microbiology Lab**

**Exercise 15: Bacterial Identification of Enterobacteriaceae Using EnteroPluri System**

* + The EnteroPluri is a biochemical identification system for the identification of members of the Enterobacteriaceae family.
		- Enterobacteriaceae are gram negative, oxidase negative
		- They are facultative anaerobes – they ferment but are not killed in the presence of oxygen
		- Include *E.coli, Salmonella, Yersinia, Klebsiella, Pseudomonas, Shigella*
	+ The tube is a self-contained, compartmented plastic tube containing twelve different media that allow determination of 15 biochemical reactions.
		- For example: does the bacteria ferment glucose and produce gas?; does the bacteria ferment lactose?; does the bacteria use citrate?
		- Combination of answers to these questions can indicate what bacteria you are dealing with.
	+ When would you use the EnteroPluri tube?

|  |  |  |
| --- | --- | --- |
| Is the bacteria in question gram -? | YES | NO |
| Is the bacteria bacillus in shape? | YES | NO |
| Is the bacteria oxidase negative?* Cytochrome oxidase is what is used at end of electron transport chain
* Enterobacteriaceae don’t need this because they are anaerobic
 | YES | NO |
|  | If you have circled YES for all, then you can use the EnteroPluri tube to further characterize/identify your unknown |  |



* + Work in pairs
		- Obtain one EnteroPluri tube.
		- Inoculate the tube with unknown bacteria*.*
		- Incubate overnight and interpret all reactions in the next period- page 96-98.



**Exercise 16: Antibiotic Sensitivity Using the Kirby-Bauer Method**

* + Once the pathogen has been identified, the physician will select an appropriate drug for the effective treatment of the disease.
	+ The Kirby-Bauer is a highly standardize disc diffusion method that is approved for wide use in clinical laboratories.
	+ Work in pair; procedure – page 102
		- Obtain two Muller-Hinton agar plates.
			* Why Muller-Hinton? This agar grows a variety of microbes. Also detoxifies toxins that the bacterial release which might alter antibiotic results.
		- Inoculate one plate with *Pseudomonas aeruginosa* and the other plate with *Staphylococcus aureus.*
		- Place BBL discs using dispenser.
		- Incubate the plates overnight
		- Examine plates and measure zones of inhibition in mm and compare with the chart provided – page 104-105
			* Just because there is a zone of inhibition does not mean that the bacteria is sensitive to the antibiotic – it all depends on the species and the antibiotic (that’s why it’s important to measure the zone and compare to the chart)



