#### Experiment 3: Gram Staining

Gram staining has been a major breakthrough in the identification and classification of microorganisms. Gram staining distinguishes microbes broadly in to Gram-positive and Gram-negative groups. These two groups can cause very different and important health consequences. Gram-positive bacteria have thick cell walls made up of many (up to 40) layers of peptidoglycan. This thick cell wall prevents microbes from losing the purple stain (upon de-staining). Gram-negative bacteria have relatively thin cell walls with only a few layers of peptidoglycan and an outer cell membrane. Gram-negative bacteria are visualized with a counterstain that creates a reddish-pink color on the bacteria.

### ৪ Materials

Gram slide from Experiment 1	Safranin
Crystal violet	Timer
Deionized water	Parafilm <sup>i</sup>
Gram Iodine	*Sink or disposable plastic container
2 <sup>nd</sup> half of bibulous paper sheet	
Decolorizer	*You must provide

#### Procedure

- 1. Over a sink or disposable plastic container, saturate the sample with crystal violet and wait one minute.
- 2. Rinse the slide with deionized water for 30 seconds.
- 3. Saturate the sample with the Gram iodine and wait one minute.
- 4. Rinse the slide with deionized water for 30 seconds.
- 5. Cover the sample with the decolorizer for five seconds. Do not let the decolorizer remain on the sample for more than five seconds as this will completely destain even a Gram-positive organism.
- 6. Rinse the slide with deionized water and wait 30 seconds.
- 7. Saturate the sample with the safranin stain for one minute.
- 8. Rinse the slide with deionized water for 30 seconds
- 9. Use the bibulous paper to blot the excess water from the slide. Take caution not to disturb the sample.
- 10. If you have a microscope available, observe the stained slide under 100X, 200X and 400X magnification and record what you see at each magnification in Table 3. If there is no microscope is available, refer to Figures 15 18.



## Structure and Microscopy

- 11. Place your slide in a disposable plastic container, and pour bleach over the surface until the sample is completely covered/saturated. Allow the bleach to soak for approximately 20 minutes and then rinse the bleach down the sink with running water.
- 12. Wrap the slide in Parafilm<sup>i</sup> and dispose of them in the trash.

Lab 5



Figure 13: Use caution when working with the decolorizing agent. Image courtesy of the CDC: Public Health Image Library.



Figure 14: Use the deionized water provided in your lab kit to rinse the slide. Image courtesy of the CDC: Public Health Image Library.



Figure 15: Unidentified Gram stained bacterial organism. Image courtesy of the CDC: Public Health Image Library.



Figure 16: The image shows the presence of hyphae in the fungal organism <u>Exophiala castellanii</u>. Image courtesy of the CDC: Public Health Image Library.



# Lab 5 Structure and Microscopy



Figure 17: Gram-negative bacteria sample. Cells take up the safranin stain. See the intracellular diplococci of this <u>coccobacilli</u> species. Image courtesy of the CDC: Public Health Image Library.



*Figure 18: Gram-negative <u>gonorrhoeae</u> bacteria sample. Cells are stained by the safranin stain. Image courtesy of the CDC: Public Health Image Library.* 

Table 3: Experiment 1 Staining Observations	
Stain Used:	
Magnification: 100X	
Magnification: 200X	
Magnification: 600X	

