** Introductory Medical Microbiology**

**Laboratory Notes**

**BIOL 2161L**

# GRAM STAINING

The information obtained from observation of microorganisms by simple staining is limited to morphology.  Other techniques must be employed to differentiate organisms within the same morphological category. The Gram stain is one of several **differential staining** procedures.  Use of the Gram stain technique will demonstrate differences between the cell wall composition of bacterial types.  Most bacteria have cell walls containing the chemical **peptidoglycan**.  The substance reinforces the cell wall and protects the cell against strong osmotic forces.

 Most bacteria can be categorized into one of two groups depending upon the relative amounts of peptidoglycan present.  One group has a dense peptidoglycan layer, which withstands **decolorization** by ethyl alcohol and retain the **primary stain** in the Gram stain procedure.  This group is designated as **Gram positive (G+)** The other group has a thin peptidoglycan layer surrounded by **lipopolysaccharide**, which can be stripped by ethyl alcohol rinse, releasing the primary stain. To be seen, these must be re-stained using a **secondary stain (counterstain)**. The bacteria are designated **Gram negative (G-)**.

|  |  |  |
| --- | --- | --- |
| **Gram Positive (+)** |  | **Gram Negative (-)** |
| **Purple** | ***Primary Stain***  **Crystal Violet** | **Purple** |
| **Purple** | ***Mordant Stain***  **Gram’s Iodine** | **Purple** |
| **Purple** | ***Decolorizer***  **Ethyl Alcohol (EtOH)** | **Colorless** |

***Counterstain***

**Saffranin**

**Red/Pink**

**Purple**

Read the following steps and explanations, then try the procedure with each of your cultures.  Remember:  **Do not** cross contaminate your cultures.  Use aseptic techniques to keep them pure.

|  |  |
| --- | --- |
| **Step** | **Explanation** |
| Using aseptic technique, make a thin  bacterial smear on a clean slide. Allow the smear to air dry or dry it on top of the Bacti-cinerator. Heat fix the smear using the technique from the previous staining lab. Allow the slide to cool. | If you can see substance on the loop or slide surface, your smear will be too thick.  The stains cannot penetrate or rinse out of thick samples. The slide must cool before the next step, so that the dye won't "cook". |
| Place **2-3 drops of crystal violet stain** on the slide balanced across the slide rack over the sink.  Time the preparation for **60 seconds.** | The crystal violet is the **primary stain**.  It will stain all bacteria present a dark purple color. |
| Rinse using the distilled water from the wash bottle and blot gently between the pages of bibulous paper. | Rinsing stops the dye action, blotting keeps  the next step from being diluted.  *Too much pressure in blotting will break the slide.* |
| Place several drops of Gram’s iodine on the smear and let it sit on the staining rack for **60 seconds**. | The iodine acts as a **mordant** (“sets” the stain).  It complexes with the crystal violet to form a larger molecule more resistant to rinsing out |
| Rinse and blot gently. | same as above |
| With the slide tilted against a light background, use a dropper to **drip 95% ethyl alcohol (ETOH) across the smear**.    Watch for purple dye to rinse off.  Continue as long as purple still rinses.  Stop when you see no more evidence of dye running off.  This should take 1 ½ droppers’ full and **18-20 seconds.**  This step is individualized.  You may have to adjust the time once you have seen your first results. | This is the most critical step and it is the hardest to perform correctly.  The ETOH is a **decolorizer**.  It is dissolving any lipid in the cell wall.  Since G+ cells have little lipid, their peptidoglycan cell wall retains the dye under the alcohol rinse and will remain purple.  G- cells, possessing much lipid, will develop weaknesses in the wall, and the dye will rinse out, leaving them transparent. |
| Rinse with distilled water and blot gently. (Be sure to do this immediately following decoloraization in order to stop the action of the alcohol) | If the alcohol acts too long, it will eventually decolorize even the G+ cells. |
| Place several drops of **saffranin** on the smear, leave for  **60 seconds** (across the rack). | This is the **secondary** or **counterstain**.  It will stain the transparent cells so that they may be seen.  The color will be pinkish-red, usually much paler than the purple cells |
| Rinse, blot dry and observe. | The G+ cells will be purple.   The G- cells will be pink *S. epi* is G+; *E. coli* is a G-short rod.   *B. sub* is a G+ spore-forming long rod.   If your results differ from this, let the instructor observe and make suggestions for adjusting the technique |

# Record the stain results for each of the following organisms below:

|  |  |  |
| --- | --- | --- |
| Organism | Color Observed | Gram Result (+/-) |
| *E. coli* |  |  |
| *B. subtilis* |  |  |
| *S. epidermidis* |  |  |
| *M. luteus* |  |  |
| *M. smegmatis* |  |  |
| *S. marcescens* |  |  |

# You should also note the morphology for each of the organisms observed:

|  |  |
| --- | --- |
| Organism | Morphologic Description/Drawing |
| *E. coli* |  |
| *B. subtilis* |  |
| *S. epidermidis* |  |
| *M. luteus* |  |
| *M. smegmatis* |  |
| *S. marcescens* |  |