Isolation of Bacteria using Gram Staining and Selective Media Using *Unknown #12*

Lee, Ha Jung
BIO 3302L D075
Professor Taddeo
March 14, 2018

Introduction:

Gram staining, named after the founder Hans Gram, is a differential stain that is the most important and frequently used staining technique in microbiology. It is the first step in identifying an isolated unknown organism. Bacteria are distinguished by color into gram-positive (purple) and gram-negative (pink/red) bacteria. The response to color is due to the chemical composition of the cell wall where gram-positive cells have a thick layer of peptidoglycan and gram-negative cells consist of a thin peptidoglycan and an outer membrane of lipopolysaccharide (Cowan, 2015). In gram-positive cells the porosity of the cells is decreased whereas gram-negative cells increase the porosity of the cells by dissolving the lipid in the outer layer.

The Gram Stain technique involves the use of a primary stain (imparts color to the cells), a mordant (fixes the color or intensifies the color), decolorization (removes primary stain), and counterstain (absorbed by the cell). It is best to use young cultures that are less than 24 hours for best results, due to the possibility of gram-positive bacteria acting as a gram-negative. Also, it is important to note that the decolorization step is most important step and the right amount is needed to gain optimal results (Obenauf, 2015).

In order for bacteria and other microbes to successfully grow, there needs to be a suitable environment. Culture medium is the term used for a combination of ingredients that will support the cultivation of microorganisms by providing the essential nutrients. Blood Agar (BA) plate is an enriched media with highly nutritious materials (sheep blood) for the purpose of cultivating the bacteria *Streptococcus.* It demonstrates the hemolytic (process of breaking down RBC) properties of microorganisms and this is seen in the change of agar. Alpha hemolysis is a partial hemolysis and the agar surrounding the colonies darkens in color. Beta hemolysis is complete hemolysis and agar is cleared around the colonies. Gamma hemolysis is when no hemolysis occurs. MacConkey (MAC) Agar is a selective media that prevents the growth of Gram-positive organism, allowing the isolation of Gram-negative bacteria. It is also a differential media where it differentiates enteric bacteria on the basis of their ability to ferment lactose, where the colonies of bacteria will turn red (Obenauf, 2015). Phenyl Ethyl Alcohol (PEA) agar is a selective medium used to cultivate Gram-positive organisms and inhibits the growth of gram-negative organisms by interfering with DNA synthesis. For this experiment, only selective media was used for the MAC and PEA plates.

Methods and Materials:

Gram Stain:

Materials:

Clean slide, staining tray, slide holder, Bunsen burner, striker, loop, distilled water

Primary Stain-Crystal Violet (2 min)

Mordant- Gram's Iodine (1 min)

Decolorizer- Alcohol

Counterstain- Safranin (2 min)

Bacteria Used:

Unknown #12

BA Plate:

Materials:

Blood (sheep blood) agar plate, black sharpie, inoculating loop, Bunsen burner

Bacteria Used:

Unknown #12

MAC Plate:

Materials:

MacConkey agar plate, black sharpie, inoculating loop, Bunsen burner

Composition of MAC plate:

Crystal violet, bile salts, carbohydrate lactose, neutral red

Bacteria Used:

Unknown #12

PEA Plate:

Materials:

Phenyl ethyl alcohol plate, black sharpie, inoculating loop, Bunsen burner

Bacteria Used:

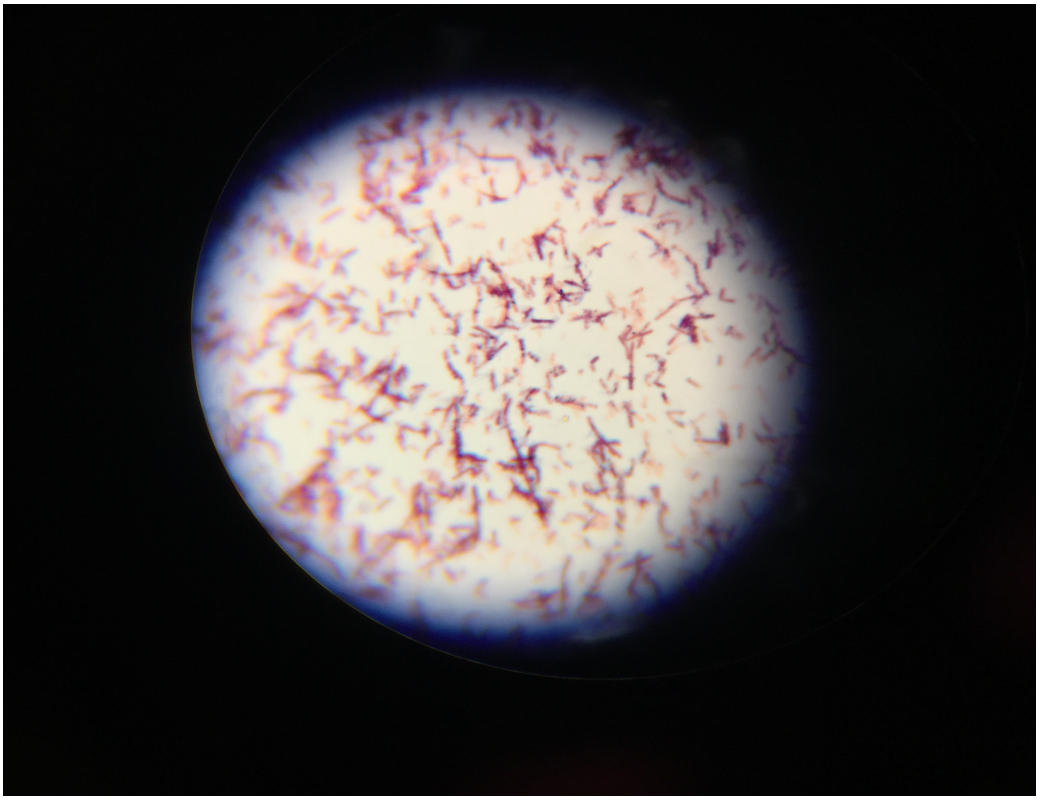
Unknown #12

Results:

Gram Stain:

Bacteria	*Color*
Gram-negative	Pink/Red
Gram-positive	Purple

Unknown microorganism #12 showed a purple pigment under the light microscope, confirming that it was a gram-positive bacteria.

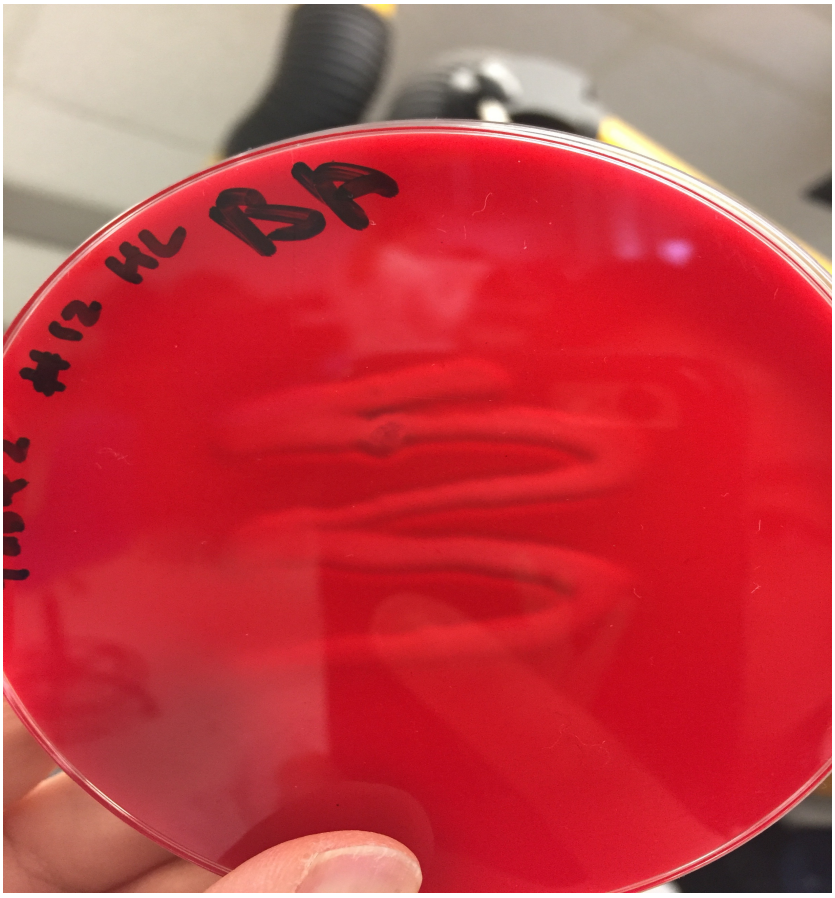


**Unknown #12* microorganism under light microscope at 100X (oil immersion).

BA Plate:

Hemolysis Activity	Agar Change
Alphia-hemolysis (partial lysis)	Darkening greenish “halo” of the BA around colony
Beta-hemolysis (complete lysis)	Colorless clearing of the BA
Gamma-hemolysis (no lysis)	No change at all

Unknown microorganism #12 showed an alpha hemolysis with a darkening halo around the bacteria.



*_Unknown #12_ microorganism observed one week later after being incubated for 24 hours at 35 degrees Celsius.

MAC Plate:

Selective media (promote growth of gram negative)

Bacteria	*Growth?*
Gram-negative	YES
Gram-positive	NO

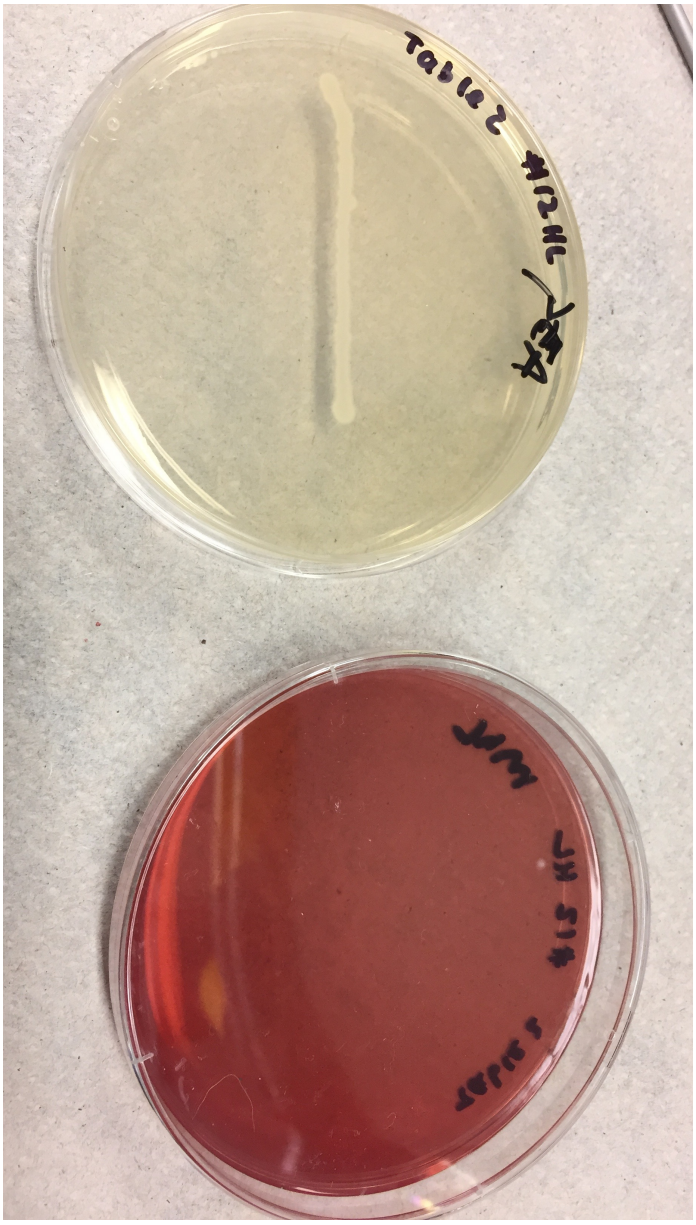
Unknown microorganism #12 proved to be a gram-positive because it did NOT show growth when observed one week later on the MacConkey Agar Plate.

PEA Plate:

Selective media (promote growth of gram positive)

Bacteria	*Growth?*
Gram-negative	NO
Gram-positive	YES

Unknown microorganism #12 proved to be a gram-positive because it showed growth when observed one week later on the Phenyl ethyl alcohol plate.



*_Unknown #12_ showed growth on the PEA plate (left) but not on the MAC plate (right) when observed one week later after being incubated for 24 hours at 35 degrees Celsius.

Conclusion and Discussion:

In the Gram Staining technique, the decolorizer (acetone-alcohol) acts as a solvent and creates large holes in the gram-negative cell's outer membrane. This porous characteristic of the gram-negative allows the iodine-crystal violet complex to be washed out and the

counterstain (Safranin) to stain the cells resulting in a reddish/pink pigment. This is not the case for gram-positive cells (*Unknown #12*). In gram-positive cells, the porosity is decreased and the thick peptidoglycan (cell wall) layer retains the iodine-crystal violet complex. Thus, even after the decolorizing step the gram-positive cells retain the purple pigment from the primary stain (Crystal Violet) and the counterstain has no affect. This is shown in *Unknown #12* where the cells display a purple pigment.

In the BA plate, the test was to see if the bacteria could make toxins called hemolysins that can break down red blood cells. For *Unknown #12,* the results of the agar change showed possible characteristics of both alpha-hemolysis and beta-hemolysis but the alpha-hemolysis (partial lysis) characteristic was stronger. The incubated plate shows a dark green halo around the colonies, caused by the oxidation of iron in hemoglobin molecules within red blood cells (Cowan, 2015).

The MAC plate and the PEA plates are selective mediums, which test for the inhibition/cultivation of either gram-negative or gram-positive cells. The crystal violet and bile salts composed in MacConkey Agar inhibit the growth of gram-positive organisms, allowing the isolation of gram-negative bacteria. Therefore, *Unknown #12* did not grow on the MAC plate. However, the PEA plate cultivates gram-positive cells and therefore *Unknown #12* was evident on the plate. It reduces growth of gram-negative microorganisms by interfering with DNA synthesis, interrupting the production of gene sequences in cells (Cowan, 2015).

References:

Cowan, M.K., *Microbiology Fundamentals: A Clinical Approach 2nd edition.* McGraw-Hill Education, 2015. Print.

Obenauf, Steven. *Laboratory Manual, Microbiology Fundamentals: A Clinical Approach 2nd edition*. McGraw Hill Education, 2015. Print.