

Blood Agar Plates and Hemolysis Protocols

||

Created Friday, 30 September 2005

Author Rebecca Buxton

Information

History

The history of blood agar, as we know it today, is uncertain. The inclusion of blood as a nutritive supplement in culture media may predate the use of agar. In their 1903 *Manual of Bacteriology*, Muir and Ritchie list its inclusion before they discuss “agar-agar” as a replacement for gelatin as a solidifying agent.

In the same discussion, however, they note that Robert Koch preferred plates poured by mixing bacterial inocula with melted gelatin rather than streaking material on the surface. Koch recommended media that were “firm, and where possible, ...transparent...” It appears that pour plates were the standard procedure for many years due largely to problems with surface contamination upon incubation. (It should be noted that, initially, agar “plates” were, indeed, sterilized flat glass plates, not Petrie dishes as we know today.)

An interesting method of adding blood to agar media is described in Bulloch’s 1938 *The History of Bacteriology*: Human blood or the blood of animals may be used. “Sloped tubes” of agar are employed. ...Purify a finger first with 1% mercuric iodine solution, dry, and then wash with absolute alcohol to remove the iodine. Allow the alcohol to evaporate. Prick with a needle sterilized by heat, and, catching a drop of blood in the loop of a sterile platinum wire, smear it on the surface of the agar. The excess of the blood runs down and leaves a film on the surface. Cover the tubes with India rubber caps, and incubate them for one to two days at 37°C before use, to make certain that they are sterile. Agar poured out in a thin layer in a Petri dish may be smeared with blood in the same way and used for culture. In investigating the diseases of races other than the white, it appears advisable to use the blood of the race under investigation. Any reader interested in the history of microbiology should explore Wolfgang Hesse’s biographical sketch of his grandparents, Walther and Angelina (“Lina”) (below). Walther was a protégé of Koch, and Angelina served as his assistant and illustrator. Sometime prior to the end of 1882, Walther was frustrated by the melting of his gelatin-coated culture tubes in the summer heat. He quizzed Lina about her jellies and puddings which maintained their solid consistency even at the warm temperatures.

imagined that Walther stirred blood into his cooled, melted agar in the same way that Lina did her fruit and meat juices!
(Rebecca Buxton with archival help from Jeff Karr)

ASM News article ["Walther and Angelina Hesse - Early Contributors to Bacteriology"](#) by Wolfgang Hesse and translated by Dieter H. M. Groschel (1992).

Purpose

Blood Agar is a general purpose enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties.

Theory

"Blood Agar" is not a consistently defined medium. The term "blood agar" generally refers to an enriched base medium to which defibrinated mammalian blood has been added.

In the

NaCl 5.0 g
 Agar 15.0 g
 Distilled water 1,000 ml

Combine the ingredients and adjust the pH to 7.3. Boil to dissolve the agar, and sterilize by autoclaving.

Blood Agar

To sterile Blood Agar Base which has been melted and cooled to 45 to 50°C, add 5% (vol/vol) sterile defibrinated blood that has been warmed to room temperature. Swirl the flask to mix thoroughly, avoiding the formation of bubbles, and dispense into sterile plates, continuing to avoid bubbles and froth on the surface. (NOTE: Cooling the agar and warming the blood are essential steps in this procedure. Hot agar can damage red blood cells, and cold blood can cause the agar to gel before pouring.)

PROTOCOL

Interpretation* of Hemolysis on Blood Agar Plates

(*) To read the hemolytic reaction on a blood agar plate, the plate must be held up to a light source and observed with the light coming from behind (transmitted light).

Beta hemolysis () is defined as complete or true lysis of red blood cells. A clear zone, approaching the color and transparency of the base medium, surrounds the colony. Many species of bacteria produce toxic by-products that are capable of destroying red blood cells.



FIG. 2

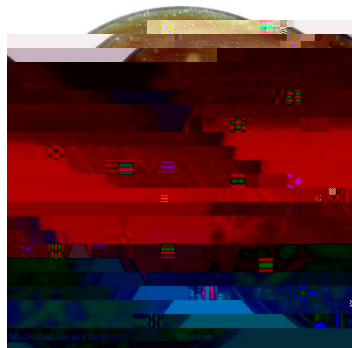


FIG. 3

FIG. 4

FIG. 2. Beta hemolytic *Streptococcus* species, *Streptococcus pyogenes* (transmitted light) (Lancefield group A).

FIG. 3. Normal Upper respiratory flora mixed with beta-hemolytic *Streptococcus* species. (The presence of beta-hemolytic

Streptococcus species with the presence of Lancefield group A - *Streptococcus pyogenes*)



FIG. 9

FIG. 7. *Streptococcus agalactiae* (Lancefield group B) viewed with incident light: No obvious hemolysis.

FIG. 8. *Streptococcus agalactiae* (Lancefield group B) viewed with transmitted light: Subtle hemolysis.

FIG. 9. *Listeria monocytogenes*, removing colonies to see the subtle pink hemolysis directly beneath the colonies

Alpha hemolysis (α) is the reduction of the red blood cell hemoglobin to methemoglobin in the medium surrounding the colony. This causes a green or brown discoloration in the medium. The color can be equated with "bruising" the cells. Microscopic inspection of alpha-hemolyzed red blood cells shows that the cell membrane is intact, so it is not, in fact, true lysis. Some text book authors refer to alpha as "partial hemolysis," which may be confusing to the student. It is most important to not confuse this "partial" or "incomplete" hemolysis with the "weak" or "subtle" lysis of *Streptococcus agalactiae* or *Listeria monocytogenes*, as seen above. Beta hemolysis will never include the brown or green discoloration of the cells in the surrounding medium. On prolonged incubation, many alpha hemolytic organisms will begin to appear more clear, but if the surrounding medium contains any shades of brown or green the "hemolysis" is still considered "alpha."



FIG. 10

hemolysis of some “gamma streptococci.”

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the [ASM Curriculum Recommendations: Introductory Course in Microbiology](#) and the [Guidelines for Biosafety in Teaching Laboratories](#).

COMMENTS AND TIPS

This section is to evolve as feedback on the protocol is discussed at ASMCUE. Please contact the project manager for further information.

REFERENCES

For Recipes and Protocol:

1. Gerhardt, Philipp, R. G. E. Murray, Willis A, Wood, Noel R. Krieg . 1994. *Methods for General and Molecular Bacteriology*. American Society for Microbiology, Washington, D. C. (p. 619, 642, 647).
2. Difco Manual. 1984. *Dehydrated culture media and reagents for microbiology*, 10th ed., Difco Laboratories, Detroit.

For History:

1. Muir, Robert and James Ritchie . 1903. *Manual of Bacteriology* . The MacMillan Company, London. (p. 226 -229)
2. Bulloch, William . 1938. *The History of Bacteriology*. Oxford University Press, London. (p. 42).
3. Hesse, Wolfgang . 1992. Walther and Angelina Hesse – Early Contributors to Bacteriology. *ASM News*. 58: 425 -428.

REVIEWERS

This resource was peer-reviewed at ASM Conference for Undergraduate Educators 2005.

Participating reviewers:

Samuel Fan
Bradley University, Peoria, IL

Ashalla Freeman
University of North Carolina, Chapel Hill, NC

Roxana Hughes
UNT Biological Sciences, Denton, TX

D. Sue Katz
Rogers State University, Claremore, OK

Lucy Kluckhohn Jones

Santa Monica College, Santa Monica, CA

Patricia Shields
University of Maryland, College Park, MD

Erica Suchman
Colorado State University, Ft. Collins, CO