The Practitioner's Mastitis Laboratory

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The most informative publication that is available to you on this subject is obtainable from the National Mastitis Council, Inc., 910 17th St., N.W., Washington, D.C. 20006. The title of the publication is *Microbiological Procedures for the Diagnosis of Bovine Mastitis.* It will be necessary for you to include a check for $2 with your request for this booklet. There are excellent photographs of the common mastitis pathogens on plain blood agar.

Milk samples are collected in accordance with specifications laid out by the National Mastitis Council. Basically, this means collection at or close to a normal milking time, the udder being thoroughly washed with an acceptable sanitizer and dried with paper towels. The next step is thorough scrubbing of the external teat sphincter with 70% isopropyl alcohol. The tube is then held in such a position that environmental contamination would be kept at a minimum.

Samples should be plated as soon as possible after taking; however, they may be refrigerated for a period of time prior to plating. If samples are refrigerated for a 12- to 18-hour period, many times a preincubation of samples is advisable for an hour or two prior to plating. I personally find that this procedure is unnecessary many times. I have included a handwritten sheet indicating the method I follow in inoculating the culture plate.

I will concentrate primarily on reading and interpreting the results of inoculating this particular plate. There are three types of media: the bright red media is blood agar and naturally permits the growth of almost all mastitis pathogens. The purple-red media is a special strep media, permitting the growth of only streptococci. The restrictive or inhibitory nature of this medium is due to the inclusion of thallium and crystal violet in media. The clear purple-blue-colored media is a media that permits only the growth of coliform organisms. Thus, if plates are inoculated in the manner prescribed and read at 12 to 24 hours of incubation, you will have your staphylococci as well as other organisms growing on blood agar; your streptococci on the strep media; and your coliforms on the coliform media. This permits accurate clinical diagnosis of 99% of all mastitis pathogens.

Since we are using the blood agar for identification of staphylococci, I will describe colony morphology briefly. Staph on blood agar after 12 to 24 hours of incubation appear as opaque white to yellow porcelain-like colonies with varying types of hemolysis. These colonies are usually rather large and very easy to identify.

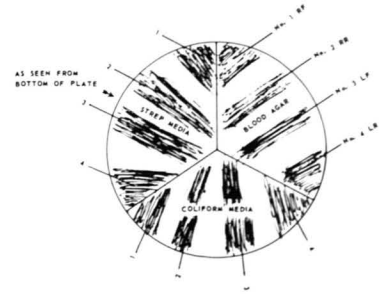
Colony morphology on the strep media is as follows: strep agalactae appears as a clear colony with a rather large zone of clear hemolysis surrounding it. This is the characteristic CAMP reaction. The media has red blood cells in it as well as a hemolysin from a beta hemolytic staphylocci. This gives the characteristic CAMP reaction. Streptococci, not *strep agalactae,* appear as small, dark colonies with a dark zone, or small clear colonies without a dark zone. The dark zone indicates fermentation of esculin. These streptococci may be of the *strep Uberus* group. Please see National Mastitis Council publication for this interpretation.

The clear purple-blue-colored media permits primarily the growth of coliforms. These coliforms produce rather large, soft colonies with dark-colored centers. *E. Coli* produces a colony with a characteristic metallic green sheen. Klebsiella species and aerobacter species do not produce this sheen, however, they do produce colonies with dark-colored centers. The klebsiella group produces a colony that has a homogenous pink color.

If you should desire to have an autogenous staph vaccine or an autogenous coliform vaccine made, they can be made from the colonies on these triplates, provided they are shipped to me in a satisfactory condition.

Plates have a much longer shelf-life when stored in the refrigerator; and, in addition, many of the problems of contamination of the blood agar section of the plate are avoided. Shipment of the plain blood agar long distances does present a problem.

METHOD OF PLATE INOCULATION



1. Insert swab into tube with milk, move swab up and down several times and then touch swab to side of tube prior to removing from tube. This permits excess milk to drain off the swab.
2. Inoculate blood agar first; rotate swab 1/3 turn and inoculate strep media; rotate swab 1/3 turn and inoculate coliform media.
3. Avoid excessive quantities of milk on plate or streaks will run together.
4. Incubate plates upside down in incubator 37°. Read in 12, 18, to 24 hours.

NOTE: Store plates upside down in the refrigerator as this will prevent excessive moisture accumulation on surface of the agar.