Techniques of On-Farm Culturing

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With the increasing concern about the prudent use of antibiotics, a more evaluative approach is needed to target the use and estimated success when using antibiotics to treat cows with mastitis. Production losses due to subclinical mastitis were recently estimated to cost the dairy industry $1 billion dollars annually. Knowing which cows have treatable mastitis and identifying which do not within 24 hours can help the farmer make a better treatment decision with more judicious use of antibiotic therapy and reduce the cost of discarded milk. One simple and effective way to cut cost, while also cutting antibiotic usage is to use on-farm culturing of milk from cows with clinical mastitis signs or elevated somatic cell count (SCC). On-farm culturing can reduce antibiotic usage by 50%. Getting started utilizing on-farm culturing can be simple and quick. Here are some tips.

- **Location for your laboratory**: Correctly locating the on-farm laboratory is critical. Find a place that is dry, well lit, clean, and away from human food preparation. Setting up in the cleanest spot possible increases your chances on getting good results and not letting the samples get contaminated. Some simple appliances are needed for on-farm culture, a simple incubator to grow the cultures, and a refrigerator to keep the plates and collected milk samples cold.
- **Learn how to interpret results on plates**: A simple mistake made by famers is not understanding the results from the cultured plates.
    - Biplates (plates containing 2 different growth media) are the simplest plates used for on-farm culturing. The red media is usually where gram-positive bacteria grow, and the other media is a pink color and grows gram negative bacteria. Some simple rules are: (1) if growth occurs on the gram positive side you should consider treating the cow especially if she is clinical, (2) if growth occurs in the gram negative side you should not treat because the mastitis cannot be cured with antibiotics, and (3) no treatment when no growth occurs. Realize that 30 to 50% of cultured samples for mastitis will result in no growth being detected since the cow's immune system may have cleared the infection or the bacteria is currently not being shed into the milk.
    - Triplates include both medias from the biplate as well as a darker red media which is selective for streptococci mastitis. This plate allows the separation of Staph. and Strept. species. To differentiate between *Staph aureus* and other Staph species, i.e CNS, additional analysis is needed.
    - Quadplates include all the medias from the triplate, as well as a blood agar section. If no growth occurs in the gram positive and negative as well as the streptococci section but occurs in the blood agar section, a sample should be sent to a lab for further examination.

- **Sterile milk samples needed**: Getting a sterile, fresh sample is critical for culturing the bacteria causing mastitis. This process should include: (1) the sampler should always

be wearing gloves, (2) the normal process for prepping a teat should be done beforehand including cleaning the teat, using the pre-dip, dried and fore-striped, (3) after the teat prep is done, an alcohol pad should be used on the teat end to clean the teat end and make it aseptic. This insures all the bacteria is removed before the milk sample is taken, and (4) using an unopened, sterile milk tube , collect your milk sample, and then label the tube and refrigerate until it's time to plate the milk.

- **Plating milk samples**: Plating the milk sample is just as important as any other step in this process. Since the media grows any bacteria, getting just a tiny contaminating particle in the sample or plate could mess up the entire process and result in no usable results.
    - Make sure the station where you will be plating the milk is clean.
    - Use sterile gloves and sterile swabs when applying the milk to the media.
    - When plating the sample, go in a zig zag pattern to insure the bacteria is spread out evenly throughout the plate. Remember to streak the media on plates in the correct order for the best results.
    - Once the milk has been plated, place the left-over milk in the freezer in case you need to send it to a lab for further analysis.
- **Place plated samples in incubator media side up**: Following this practice increases your chances of success in growing the bacteria causing clinical mastitis.
    - After the milk has been streaked onto the plates, place the plates in the incubator upside down, the media should be on top. This prevents condensation from forming and dropping on the media.
    - Set the incubator to body temperature, which is 98.6° F or 37° C.
    - Leave the sample in the incubator for a minimum 24 hours which gives the bacteria plenty of time to grow.
- **Reading plates**: Reading the plate and determining what the growth means.
    - If colonies are in big clusters or following the streak, it is a good sample.
    - If colonies show growth in all the sections, it is a good chance that the plate is contaminated. Never use a contaminated plate to make a diagnosis.

A farmer who is using these tips can more accurately diagnosis the type of mastitis and treat it properly.