

DAIRYGUARD PROTOCOL

Results from the DairyGuard platform will be presented in a format with 7 different levels for each organism.

- Negative - No DNA present
- Scant - An extremely slight signal that may or may not be significant
- + - A very low amount of DNA present
- ++
- +++ - A moderate amount of DNA present
- ++++
- +++++ - A very large amount of DNA present

Theoretically, the stronger the fluorescent signal received, the more of the DNA and subsequently, the more of that specific bacterial organism present in the sample. I would be very hesitant getting worked up over a scant signal, however, if it's on a client that has no history of that organism, or only intermittent positives. Until the scant signal is repeated through several weeks or samples, or until you start to see a stronger signal, I would treat it as a 'wake-up call', and only use it to increase your vigilance. Even with only a 1+ signal, until we get more history on our dairies and more experience with the increased sensitivity of Dairyguard, we should probably be cautious triggering a huge management response and herd cultures.

Following are very brief summaries of the organisms Dairyguard can detect and how to think about them and communicate about them with our clients.

Contagious organisms: Unchecked contagious mastitis organisms on a dairy that can be a concern not only because they can spread quickly but also because some of them cannot be treated effectively, and they can lead to an increased somatic cell count.

- *Staphylococcus aureus* - *S aureus* is one of the most serious organisms we are looking for in our milk quality lab. An unchecked or unmonitored *S aureus* outbreak on a dairy can be extremely costly, however many dairies do continue to function with a very low level of *S aureus* in the herd. There are literature reports of successful treatment for *S aureus*, but often the best response is careful quarantine or culling of the animal to prevent her being a source of infection for other animals. Streak canal contamination (low level positive milk samples in non-mastitic fresh animals, particularly heifers) does happen, and if the heifer does not have mastitis, resampling is probably warranted to ensure that she does not have an infection and does not continue to shed. See the other documents on the website regarding *S aureus*, and discuss the particulars of any dairy that is concerned about *S aureus* with any of the partners, all of whom have dealt with this difficult situation many times before. A positive *S aureus* result on DairyGuard should also have a positive *Staphylococcus* spp. result.

- *Mycoplasma bovis* - *M bovis* is considered one of the most pathogenic and infectious, as well as probably the most common *Mycoplasmas* in bovine milk. A positive *M bovis* result in DairyGuard should also have a positive *Mycoplasma spp* result. See the *Mycoplasma spp* summary for more information.
- *Mycoplasma bovigenitalium* - *M bovigenitalium* is the second most common isolated species of *Mycoplasma* in milk samples. A positive *M bovigenitalium* result in DairyGuard should also have a positive *Mycoplasma spp* result, and see the *M spp* summary for more information. *M bovigenitalium* may also be a normal (sometimes pathogenic) organism of the caudal reproductive tract of cattle, but animals that have mastitis and shed *M bovigenitalium* in the milk should be considered infected.
- *Mycoplasma californicum* - The third most common *Mycoplasma* detected in milk, often leads to very sudden outbreaks with high numbers of cows infected very quickly. Also one of the easiest *Mycoplasmas* to grow and culture. A positive *M californicum* result in DairyGuard should also have a positive *Mycoplasma spp* result, and see the *M spp* summary for more information.
- *Mycoplasma spp.* – A positive signal for these organisms will be triggered with any of the *Mycoplasmas* that commonly affect the mammary gland of cattle, including the three species above. *Mycoplasma* infections can occur in the inner ear, in joints, in the udder, or in the lungs and respiratory tract. Depending on the species and the site of infection, treatment with antibiotics may or may not be effective. There is no effective treatment for a cow shedding *Mycoplasma* in the milk...she should be separated from other cattle (quarantine or cull) to prevent her being a source of infection for them. Anecdotally, most *Mycoplasma* mastitis infections will show abnormal milk, and will find their way to the hospital. When a herd is having a *Mycoplasma* mastitis issue, addressing proper milking procedures in the pit, particularly while the hospital is being milked, is essential. See the handouts on the Valley Vets website for more information, and should you have a client or herd with *Mycoplasma*, or questions about *Mycoplasma*, contact one of the partners.
- *Prototheca spp.* - *Prototheca* is a unicellular algae present in wet environments that can cause mastitis and chronic increased SCC in dairy cattle. Treatment is ineffective, and the organism evades the cow's immune system. There are some indications that it is contagious, and culling or quarantining the animal may be worth considering.
- *Streptococcus agalactiae* - *S agalactiae* (*S ag*) is highly contagious. It is the one contagious mastitis organism that can be cured relatively easily by appropriate antibiotic therapy. Historically, it is much less prevalent than it once was, but the modern movement towards less antibiotic dry cow therapy has raised concerns that we may see *S ag* more frequently and on more and more herds.

Gram positive environmental organisms

- *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus similans*
- *Staphylococcus hyicus*, *Staphylococcus chromogenes*
- *Micrococcus caseolyticus*
- *Staphylococcus xylosus*, *Staphylococcus equorum*
- *Staphylococcus haemolyticus*

- *Staphylococcus saprophyticus*
- *Staphylococcus sciuri*
- *Staphylococcus species*

The above species of *Staphylococcus* are opportunistic and environmental *Staph* organisms which can cause both clinical and subclinical mastitis. A positive DairyGuard result for these organisms should also have a positive *Staphylococcus spp* result. They are differentiated for several reasons. *Micrococcus* can encode and share genes for antibiotic resistance. *S hyicus* may be coag positive, and may occasionally be contagious (which complicates culture diagnosis, as that is one of our most common differential tests for *S aureus* through traditional culturing methods.) Several of these organisms also create a biofilm that can make treatment less effective.

- *Streptococcus dysgalactiae*
- *Streptococcus uberis*
- *Streptococcus species*

The above species of *Streptococcus* are primarily opportunistic and environmental *Strep* organisms which can cause both clinical and subclinical mastitis. A positive DairyGuard result for these organisms should also have a positive *Streptococcus spp* result. Both *S dysgalactiae* and *S uberis* may rarely cause contagious mastitis.

- *Bacillus cereus group*
- *Bacillus subtilis group*

Bacillus organisms may commonly be contaminants in the sample, but there are reported instances of *Bacillus* mastitis. *Bacillus* is heat resistant and may survive pasteurization, which may create a potential food safety issue.

- *Corynebacterium bovis* - *C bovis* infection causes only a slight increase in SCC and is most commonly found in herds that do not post-dip.
- *Trueperella pyogenes* - *T pyo* (summer mastitis) is commonly associated with flies, teat injuries, and summer calvings and leads to markedly high amounts of pus in the milk. Treatment is usually ineffective and often the best course of action is drying off that quarter.
- *Enterococcus species* - Antibiotic resistance is very common in *Enterococcus* species
- *Aerococcus viridans* - Environmental gram positive bacteria usually susceptible to most beta-lactam antibiotics
- *Lactococcus lactis* - Emerging gram positive bacteria that may be resistant to treatment.

Gram negative environmental organisms

- *Escherichia coli* - *E coli* (MPEC – Mammary pathogenic *E coli*) is one of the most common environmental infections of the bovine quarter. There is a high level of antibiotic resistance to most intramammary treatments (Spectramast LC may be the most effective), and effective treatment of the cow is often supportive to allow her to combat the infection. Vaccines may help prevent the severe endotoxemia that can occur and lead to severe disease and death. One

of the most common causes of a high incidence of E coli mastitis is milking wet udders/wet cows, if the milking crew does not give them enough time in the drip pen, or animals walking back to their pen through a flush or other wet environment while their teat sphincter is still open.

- Enterobacter/Klebsiella - Gram negative organisms common in the environment that can cause different levels of mastitis. *Klebsiella* can become deep-seated in the mammary tissue and lead to chronic mastitis and chronic high SCC. *Klebsiella* may also be associated with certain types of bedding (anything with wood/wood pulp/paper), so a herd that is experiencing lots of *Klebsiella* mastitis should examine their bedding and bedding choices.
- Pseudomonas aeruginosa – Environmental Gram negative organisms sometimes associated with contaminated water, hoses or equipment. This organism does not typically respond well to antibiotic therapy.
- Proteus spp. - Gram negative environmental organism similar to the others in this category
- Citrobacter - Will cause a severe acute mastitis
- Serratia marcescens - Of note with this organism is that chlorhexidine in normal concentrations is ineffective at killing this organism. It has been reported that it can survive in dip cups, which may be a potential source of new infections.
- Salmonella - This organism may be misclassified here, as if it is present in the milk it may present a severe public health threat. Most commonly associated with a fecal-oral route of exposure, it is unclear if it can be shed in the milk or is a post-milking contaminant.

Organisms typically associated with dirty milking equipment

- Acinetobacter spp.
- Enterobacteriaceae
- Pseudomonas spp.
- Stenotrophomonas maltophilia

The presence of these organisms in a milk sample are indicative of dirty or contaminated milking equipment, and the cleaning and washing of the system and the potential replacement of rubber parts should be examined.

Public Health/Food Quality bacteria

- *Acholeplasma* spp. – *Acholeplasma* is considered a contaminant in milk, and there is no concern it is pathogenic to either cattle or humans. It can cause confusion with *Mycoplasma* (see above) on culture
- *Clostridium* spp.
- *Listeria* spp.
- *Campylobacter jejuni*

These other organisms can cause human disease when consumed, particularly in a raw (unpasteurized) milk product.