



Wisconsin Veterinary
Diagnostic Laboratory
UNIVERSITY OF WISCONSIN-MADISON

Mastitis: Milk Culture Interpretation Guidelines

Individual Cow Samples

- **Contagious Pathogens**-Infection with contagious pathogens occurs from the milk of other infected animals. These infections can be prevented with proper milking hygiene including post-milking teat disinfection, milking infected animals last and effective management of clinical cases. For more information on individual pathogens, see our website.
 - **Gram-positive**
 - Streptococcus agalactiae*
 - Streptococcus uberis*
 - **Gram-positive, Coagulase-positive**
 - Staphylococcus aureus*
 - **Other**
 - Mycoplasma sp.*
 - Prototheca sp.*

- **Environmental Pathogens**-Infection with environmental pathogens occurs from bacteria entering the teat end from dirt, manure, bedding, milking machines that have been contaminated with these pathogens. These infections can be prevented with proper hygiene, milk machine maintenance and pre-milking teat disinfection. For more information on individual pathogens, see our website.
 - **Streptococcus (Gram-positive cocci)**

<i>Aerococcus sp.</i>	<i>Enterococcus hirae</i>
<i>Aerococcus viridans</i>	<i>Enterococcus saccharolyticus</i>
<i>Enterococcus sp.</i>	<i>Lactococcus gravieae</i>
<i>Enterococcus casseliflavus</i>	<i>Lactococcus lactis</i>
<i>Enterococcus faecalis</i>	<i>Micrococcus sp.</i>

Streptococcus sp.
Streptococcus bovis
Streptococcus dysgalactiae
Streptococcus equi

Streptococcus uberis
Streptococcus vestibularis

○ **Other Gram-Positive**

Trueperella pyogenes
Bacillus sp.

Corynebacterium sp.
Listeria monocytogenes

○ **Staphylococci (Gram-positive, Coagulase-negative cocci)**

Staphylococcus sp.
Staphylococcus chromogenes
Staphylococcus saprophyticus

Staphylococcus simulans
Staphylococcus xylosum

○ **Gram negative**

Acinetobacter sp.
Acinetobacter baumannii
Aeromonas sp.
Citrobacter sp.
Enterobacter sp.
Enterobacter amnigenus
Escherichia coli
Flavimonas sp.
Hafnia sp.
Klebsiella sp.
Klebsiella oxytoca

Klebsiella pneumonia
Pantoea sp.
Plesimonas shigelloides
Proteus sp.
Pseudomonas sp.
Pseudomonas fulva
Salmonella sp.
Serratia asp.
Serratia marcescens
Stenotrophomonas sp.
Yersinia sp.

○ **Others**

Yeast
Nocardia sp.

Prototheca sp.

▪ **Semi-quantitation of results**

Pathogens are reported semi-quantitatively to assist veterinarians in understanding the levels at which a pathogen was detected in the milk sample. The scheme uses:

- +1- very few

- +2- few
- +3- moderate
- +4- numerous

It is important to note that milk stored improperly, such as at room temperature for extended amounts of time will allow for the growth of pathogens that will change the semi-quantification of that pathogen. Contaminants or mixed flora can also be reported semi-quantitatively. *Prototheca* are reported quantitatively rather than semi-quantitatively.

▪ **“No Growth” Samples**

When a milk sample yields no colonies the results will be reported as no growth.

Reasons for no growth

- The infection was cleared by the cows immune system (Gram-negative infections)
- Culture was taken after antimicrobial therapy was initiated.
- Improper handling/transport caused overgrowth of non-pathogenic organisms or reduced the number of viable pathogenic organisms.
- Organisms are below detectable levels.
 - Avoid taking composite udder samples and instead sample each quarter individually.
- Improper test selected for organism of interest
 - *Mycoplasma* is not included in routine mastitis culture and will not grow on routine mastitis culture media. Please request *Mycoplasma* culture.
- Cow was sampled after milking.
- The cow is not shedding pathogen or is not infected.

▪ **“No Significant Growth” Samples**

When a milk sample yields three or more dissimilar colony types in low levels, and no pathogen is obvious, the milk samples is likely contaminated with a small amount of contaminating microorganisms. It is near impossible to collect a milk sample without some amount of contamination. This growth does not indicate a problem with sampling the milk, but rather this samples was collected correctly and can be interpreted as ‘no growth’.

- When a milk sample with ‘no significant growth’ is improperly stored, the microorganisms multiply and the sample will then have moderate to numerous levels of contaminants and will be reported as such.

▪ **“Contaminated” Samples**

When a milk sample yields three or more dissimilar colony types in moderate to numerous levels and there is no predominant organism and no obvious pathogen, the milk sample is likely contaminated upon sampling and will be reported as so. If a pathogen has been identified in addition to three or more dissimilar colony types, the reported results will include the pathogen in addition to “mixed flora” or “contaminants”. Organisms considered ‘contaminants’ include common skin and environmental bacteria such as: environmental *Streptococcus*, *Enterococcus*, coliforms, diphtheroids, *Micrococcus*, *Bacillus*, and coagulase-negative *Staphylococcus* species.

Reasons for contamination

- Dirty teat ends
- Milk touching fingers before entering the sample collection vial
- Non-sterile tubes used for sample collection
- Environmental bacteria contaminated the sample during collection.
- Improper handling/transport caused overgrowth of organisms.
- Sample was not sent to the laboratory promptly, was not refrigerated promptly, or was not sent with cool packs that maintained a cool temperature to limit the growth of contaminating microorganisms.

▪ ***Prototheca* isolated**

The laboratory identifies *Prototheca* species by growth on *Prototheca* Isolation media, which is a highly selective media for *Prototheca* species. *Prototheca* also grows well on blood agar and Eosin Methylene Blue (EMB) agar. If *Prototheca* are identified, the laboratory will report out those results quantitatively. However, if *Prototheca* are not identified, this is not necessarily due to a lack of *Prototheca* as the WVDL has observed a slight decrease in *Prototheca* recovery when milk is frozen. Therefore, please send cold milk samples to the laboratory and avoid freezing if *Prototheca* mastitis is suspected.

Bulk Tank Samples

See the document titled “Interpretation of Bulk Tank Milk Results” on our website.

Resources

<http://www.mndhia.org/uploads/5/0/3/6/50366013/interpretingcultureresults.pdf>

<https://extension.tennessee.edu/publications/Documents/W181.pdf>