

**National Mastitis Council
Research Committee Report**

**Interpreting Bacteriological Culture Results to Diagnose
Bovine Intramammary Infections**

Monitoring bovine udder health ultimately involves judging the presence of bacterial species-specific intramammary infection (IMI) by means of bacteriological culture of mammary milk samples. There has been much discussion about the culture result criteria that justify concluding that a mammary quarter was infected at the time of sampling while minimizing the risk of misinterpreting bacteria that were merely present temporarily in the teat cistern, for example. Many different decision criteria have been discussed; they typically vary by bacterial species, purity of sample, and density of bacteria cultured from a standardized quantity of milk sample. It has been difficult to achieve uniformity and comparability of IMI definitions because of differing needs to minimize the risks of “false-negative” conclusions on one hand versus “false-positive” conclusions on the other hand. There are also varied opinions about what would constitute an IMI if a perfect detection method (gold standard) existed. Consequently, it is challenging for users of routine mammary bacteriological data to determine the appropriate decision criteria. Decision making is even more complex and accompanied by risk of error when interpreting the bacteriology results of milk obtained from all four quarters combined into a single composite sample.

This factsheet summarizes recent research to provide users of bovine mammary quarter milk sample bacteriology with a uniform approach to interpreting bacteriological culture results as applied to *Staphylococcus aureus*, *Streptococcus* species, *Escherichia coli*, and coagulase-negative *Staphylococcus* species (CNS).

How does mastitis differ from intramammary infection?

Over the years, the terms ‘mastitis’ and ‘IMI’ have been used interchangeably, but they represent different entities, as shown in Table 1. These terms should not be used interchangeably.

Table 1: Definitions and features of intramammary infection and mastitis.

	Intramammary Infection	Mastitis
International Dairy Federation definition	An infection occurring in the secretory tissue and/or the ducts and tubules of the mammary gland.	Inflammation of one or more quarters of the mammary gland, almost always caused by infecting microorganisms.
Diagnosis mainly by:	Bacteriological culture of milk samples obtained aseptically.	<u>Subclinical</u> : Measure of indicators of inflammation in milk samples such as somatic cell count (SCC) or California Mastitis Test (CMT). <u>Clinical</u> : Visual observation of milk and/or physical examination of the udder.

How do we determine if a quarter is infected with a microorganism?

The ability of a single quarter milk sample culture result to classify a quarter as infected or not is reflected in its sensitivity (Se) and specificity (Sp). The Se is the ability to detect an IMI, while the Sp is the ability to correctly identify non-infected quarters. These are usually determined by comparing test results to a gold standard (definitive result). While no perfect gold standard exists, a consensus was recently reported to consider a quarter positive if 2 out of 3 consecutive samples were positive for the same organism or if a single sample had ≥ 10 CFU/10 μ L (see references listed below for details). Table 2 shows the Se and Sp for several organisms, based on two different culture thresholds (1 or 10 CFU/10 μ L) for bacteriological results from a single quarter milk sample. For example, using the

threshold ≥ 1 colony of *S. aureus* as a criterion, 90.4% of quarters infected with *S. aureus* would be detected, and 99.8% of non-infected quarters would be correctly classified (the other 0.2% would have a false positive test result).

Table 2: Sensitivities and specificities for diagnosing an intramammary infection based on culture of a single milk sample. Data are categorized by pathogen type and two different detection thresholds.

Threshold for detection (CFU/10 μ L)	Sensitivity (%) / Specificity (%)			
	CNS*	<i>Staphylococcus aureus</i>	<i>Streptococcus</i> spp.**	<i>Escherichia coli</i>
≥ 1	61.2 / 84.3	90.4 / 99.8	29.1 / 94.8	76.5 / 99.9
≥ 10	26.0 / 98.1	72.0 / 100.0	6.9 / 99.3	47.1 / 100.0

*CNS = coagulase negative staphylococci. ** Primarily *S. uberis*

While acceptable Se and Sp may be obtained when diagnosing an IMI from a single quarter milk sample, culturing duplicate milk samples (collected at the same milking or close together in time) can help maximize either Se or Sp, but not both. If a sample is considered positive when either of the 2 samples is positive, then Se will be enhanced (but Sp will decrease). If both samples need to be positive before the quarter is classified as positive, then the Sp will be enhanced but the Se will decrease. There is limited improvement (primarily in Sp) when interpreting triplicate milk samples. In a similar manner, composite samples are sometimes used for diagnosing IMI at the cow level (see additional readings below).

Is an “intramammary infection” the same as a “new intramammary infection”?

There is no definite agreement on what constitutes a ‘New IMI’. Although this would require consecutive sampling of quarters, factors that should be taken into consideration are the infection characteristics of the pathogens, expected outcome and frequency of sampling, among others.

Conclusions

The criteria chosen for diagnosing IMI must take into consideration the objectives in view. In some situations criteria which maximize Se will be chosen, while in others it is more important to have high Sp. However, based on the organisms evaluated to date, using a criterion of ≥ 1 CFU/10 μ L from a single milk sample achieves a reasonable balance of Se and Sp.

References/Additional Reading

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