

the pathogens most commonly isolated (22% of all cases), followed by coagulase-negative staphylococci (CNS) (12%), coliforms (7%), and others (5%). The quarter risk to receive primary IMM antibiotic therapy because of study assignment was only 28.2% for CB (vs 100% for PC) ( $P < 0.01$ ). When accounting for cases not treated initially that ended up being treated in the CB group, the proportion of quarters treated was 35.9% ( $P < 0.01$ ). Overall, the average number of IMM antibiotic tubes per CM quarter case was 1.13 and 3.29 for CB and PC, respectively (DiffPC (95% CI) = -2.16 [-2.49 to -1.83];  $P < 0.01$ ). Consequently, there was a reduction of 1 day in days out of the tank for cases assigned to CB (DiffPC (95% CI) = -1.02 [-1.44 to -0.56];  $P < 0.01$ ). It was 5.72 and 6.73 days for CB and PC, respectively. Days to clinical cure were not different between treatment programs (DiffPC (95% CI) = 0.32 [-0.17 to 0.82],  $P = 0.21$ ). Including in the calculation cases that did not receive IMM therapy, the mean days to clinical cure were 3.45 and 3.12 days for cases assigned to CB and PC, respectively. Also, the proportion of quarters with bacteriological cure was not different (ORPC (95% CI) = 1.0 (0.49 to 2.06);  $P = 0.98$ ). It was 76.9% and 77.1% for cases assigned to the CB and PC, respectively. Similarly, there was no difference on new intramammary infection risk between groups (ORPC (95% CI) = 0.78 (0.41 to 1.48);  $P = 0.74$ ) at 20.2% and 24.6% for cases assigned to the CB and PC, re-

spectively. Interestingly, CM recurrence in the same quarter between 14 and 60 days after the first case in cases assigned to CB happened half as much as that for cases assigned to PC (ORPC (95% CI) = 0.44 (0.23 to 0.84);  $P < 0.01$ ). The risk of CM recurrence was 12.8% and 25.2% for cases assigned to CB and PC, respectively. Similarly, risk of removal from herd was half for cases assigned to CB, although this was only a trend and not statistically significant (ORPC (95% CI) = 0.48 (0.18 to 1.14),  $P = 0.09$ ). The risk of removal from herd within 21 days after CM was 7.1% and 14.4% for cases assigned to CB and PC, respectively.

### Significance

The selective treatment of only CM cases from which environmental streptococci were isolated by a professional laboratory resulted in an approximate two-thirds reduction both in the number of cases treated and the number of IMM tubes used, as well as a reduction of 1 day out of the tank. Furthermore, the withholding of antibiotic treatment did not increase the time for milk to return visibly normal or affect bacteriological cure or new infection risk. Interestingly, CM recurrence was significantly lower and removal from herd tended to be lower when only environmental streptococci were treated with IMM antibiotics.

## Evaluation of an automated milk leukocyte differential test for detecting intramammary infection in early- and late-lactation quarters and cows

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### Introduction

The dairy industry must develop mastitis control practices that can use antimicrobials judiciously while maintaining or improving udder health. One opportunity is the adoption of selective dry cow therapy (SDCT) programs that treat only infected quarters or cows. Another approach may be to identify and apply selective treatment to infected quarters soon after calving. However, for such programs to be successful, we require on-farm tests with sufficient accuracy and utility to differentiate infected from uninfected quarters or cows. The objective of this study was to describe the diagnostic test characteristics of an automated milk leukocyte differential (MLD) test to identify intramammary infection (IMI) in early-lactation (EL) and late-lactation (LL) quarters and cows.

### Materials and Methods

Eighty-six EL (7 to 15 DIM) and 90 LL (277 to 721 DIM) cows were sampled from 3 midwest herds with bulk tank SCC values between 333,000 to 390,000 cells/mL. On the day of sampling, aseptic duplicate quarter samples were collected for bacterial culture, and quarter milk samples were collected into the MLD test sample collection device. MLD testing of quarter milk samples was completed within 3 to 8 hr of sample collection as per the manufacturer's directions using the "Research Mode" setting. MLD results were stored on a hard drive, and later transferred to the manufacturer (AAD, Durham, NC). Here they were interpreted according to a proprietary algorithm, and then quarter level results (positive/negative) reported back to the primary investigator as though the test had been set in the "3 minute Smart Mode",

as would be used on a farm, and at each possible threshold setting (1 to 18 for EL; 1 to 12 for LL). A quarter was infected if culture of a single milk sample showed bacterial growth of 1 or 2 species. At the cow level, a cow was classified as infected if bacterial culture indicated that 1 or more quarters were infected, and a cow was defined as MLD-positive if 1 or more quarters yielded a positive MLD test result. For both EL and LL quarters and cows, 2x2 tables were then created to compare bacterial culture results (referent test: infected/not infected) against MLD test results (pos/neg), for all threshold settings, in order to calculate the MLD test characteristics including sensitivity (Se), specificity (Sp), accuracy (Ac), predictive values, and agreement (Kappa).

### Results

The quarter-level prevalence of IMI was 25.2% and 25.8% in EL and LL quarters, respectively. The cow-level prevalence of IMI was 71.2% and 64.4% of EL and LL cows, respectively. At the quarter level, Se of the MLD test to identify IMI was low ( $\leq 32.5\%$ ) for EL quarters and low-to-fair (46.6 to 69.9%) for LL quarters, depending on the setting used. Specificity was fair-to-very good (60.0 to 95.6%), depending on the lactation stage and setting. Accuracy was only fair over-

all (62.5% to 74.8%), and agreement between quarter-level MLD test and milk culture results was slight ( $< 0.20$ ) to fair ( $< 0.26$ ) for EL and LL quarters, respectively. At the cow level for EL cows, Se of the MLD test to identify infected cows was low (25.0% to 53.8%) and Sp was fair to very good (66.7% to 95.2%), with low-to-fair Ac (45.2% to 58.9%), depending on the setting. At the cow level for LL cows, Se was fair to good (59.6% to 80.9%) but specificity was low (34.6% to 50.0%), with only fair Ac (56.2% to 64.4%), depending on the setting. Agreement between MLD test results and bacterial culture, at the cow level, was only slight to fair (0.08 to 0.24) for both EL and LL cows.

### Significance

The MLD test performed better in LL quarters and cows than in EL quarters and cows, but had limitations in both populations. The MLD test may have limitations for adoption in SDCT or fresh-cow screening programs in high SCC herds, since many truly infected quarters or cows would go undetected and untreated, while many non-infected quarters or cows would be treated needlessly. Future studies should reevaluate the diagnostic test characteristics of the MLD test in low and moderate SCC herds.