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Performance of a milk leukocyte differential test for decision-making in a selective dry cow therapy program

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Abstract

The objectives of this study were 1) to determine the operating characteristics of a commercial milk leukocyte differential (MLD) test to detect intramammary infections in quarters of late-lactation dairy cows as compared to bacteriological culture and 2) to evaluate the milk production and udder health parameters between cows treated following blanket vs selective dry cow therapy (DCT) using the MLD test results. In a first experiment, the MLD test was compared to the bacteriological culture results (gold standard) of 363 quarters from 94 cows. The sensitivity, specificity, and predictive values for the identification of infection using the MLD test were determined. Sensitivity ranged from 44% to 77%, and specificity from 54% to 92%. In the second experiment blanket DCT was compared to selective DCT based on the results of MLD test, and treating only positive quarters; a total of 328 cows were randomly assigned to 1 of the 2 treatment groups. The proportion of quarters positive to bacteriological culture, and the incidence rate of moderate and severe cases of clinical mastitis events, did not differ between treatment groups. Results of these experiments provide information to support decision-making in a selective DCT program in low-SCC herds using the MLD test.

Key words: dairy cow, milk, leukocyte differential, selective dry-cow therapy, udder health

Résumé

Les objectifs de cette étude étaient de déterminer les caractéristiques d'opération d'un test commercial pour le comptage leucocytaire différentiel dans le lait (CLL) pour détecter les infections intramammaires dans les quartiers de vaches laitières en fin de lactation par rapport à la culture bactériologique et aussi d'évaluer la production de lait et la santé du pis chez des vaches traitées suivant la thérapie systématique ou sélective pour vaches taries sur la base des résultats du test CLL. Dans la première expérience, le test CLL a été comparé aux résultats de la culture bactériologique (l'étalon) dans 363 quartiers de 94 vaches. La sensibilité, la spécificité et les valeurs prédictives pour l'identification de l'infection avec le test CLL ont été calculées. La sensibilité variait de 44% à 77% alors que la spécificité variait de 54% à 92%. La seconde expérience avait pour objectif de comparer la thérapie systématique et la thérapie sélective pour vaches taries sur la base des résultats du test NLL en ne traitant que les quartiers positifs. Des vaches (n = 328) ont été allouées aléatoirement à l'un des deux groupes de traitement. La proportion de quartiers positifs à la culture bactériologique de même que l'incidence de cas avec mammite clinique modérée ou sévère n'étaient pas différentes entre les deux groupes de traitement. La production de lait et le comptage de cellules somatiques dans la lactation subséquente variaient d'un jour de test à l'autre mais n'étaient pas différents dans les groupes avec thérapie systématique ou sélective pour vaches taries.

Introduction

Udder health is of great importance to the dairy industry because it affects the welfare and production of dairy cows.[21,32] Blanket dry cow therapy (**DCT**), which consists of infusing all quarters of all cows with a long-acting intramammary antimicrobial product, is used to eliminate ongoing infection at dry-off and prevent new infections.[7,34] This approach is used for mastitis control in over 80% of dairy herds in the United States and Canada.[45,52] In Europe, some countries have implemented new regulations which have mandated a decrease in the use of blanket DCT.[23,38] However, many countries still have over 80% of their herds using blanket DCT.[33,49] Concerns regarding antimicrobial resistance, as well as high costs associated with blanket DCT, are motivating the use of alternative approaches. Selective DCT consists either of treating all quarters of cows with at least 1 quarter infected,

or of treating only infected quarters.[7,37] To achieve success with selective DCT, intramammary infection (**IMI**) must be accurately identified at dry-off.[10,43,51]

Bacteriological culture is a specific diagnostic tool which identifies and characterizes the presence of pathogens. However, it is a time-intensive and costly procedure. Furthermore, milk culture requires aseptic collection of samples, which can prove difficult in many situations. As an alternative, somatic cell count (SCC) in milk is widely available and is used as a proxy for udder health and IMI.[47] Unfortunately, the availability of Dairy Herd Improvement SCC data that is in close time proximity to the day of dry-off occurs randomly, and is not common. A recent study, however, reported that combining the results of the last 3 test-days before dry-off accurately identified non-infected cows (specificity = 79-88%), but less accurately identified infected cows (sensitivity = 28-38%).[30] Recent studies suggest that the distribution of the leukocyte population can increase accuracy in identification of IMI.[39,48] In this regard, Advanced Animal Diagnostics[a] has developed an automated milk leukocyte differential[b] test to diagnose IMI at the quarter-level in dairy cows.

Differential cell count profiles were shown to be different in quarters with and without IMI.[13,39,48] A recent study evaluated the ability of the QScout MLD[b] test to identify IMI in early- and late-lactation dairy cows.[18] Multiple indexes are available when using the automated test. Overall, the sensitivity and specificity of the dry-off indexes QScout MLD test were fair to good in late-lactation cows, depending on threshold setting. This study was conducted in commercial Midwest dairy herds with high bulk-tank SCC. As such, it is of interest if the test would yield similar accuracy in a herd with low bulk-tank SCC. Moreover, it is unclear how the accuracy of the MLD test relative to bacteriology would influence its performance as a tool to identify cows in a selective DCT program. In this regard, describing which quarters and cows are wrongly identified as uninfected (false negative) and infected (false positive) would help to understand the best ways to use the test by producers and veterinarians.

The limitations of bacteriological culture as a gold standard to diagnose IMI are well described in the literature.[2,16] While it is useful to understand the operating characteristics (sensitivity and specificity) of the MLD test compared to an acceptable gold standard, validating its use in a selective DCT approach can also be done by comparison to blanket DCT in a randomized controlled trial. For example, selective DCT using on-farm culture or SCC in the 3 months prior to dry-off had no negative impact on udder health, milk production, and milk quality as compared to blanket DCT.[10,11,25] To our knowledge, MLD for selective DCT has not been compared to blanket DCT in a randomized trial.

The objectives of the present study were 1) to determine the operating characteristics of different thresholds of QScout MLD test to detect IMI in late-lactation dairy cows compared to bacteriological culture of milk, and 2) to compare the risk of developing clinical mastitis between cows treated at dry-off based on QScout MLD test results (quarter-level selective DCT) and cows treated based on a blanket DCT program.

Materials and Methods

Experiment 1 – Cross-sectional Study

Animals and housing. This cross-sectional study was conducted in November 2016 on a single 1,030-cow Holstein (75%) and Jersey (25%) dairy herd in North Carolina. Cows were housed in a freestall barn on fresh and recycled sand, and were milked 3x daily in a DeLaval double 16 parallel parlor. In 2016, the average DIM, milk yield, and bulk tank SCC was 160 d, 75 lb (34 kg) per cow/d, and 182,000 cells/mL, respectively. The minimum sample size of 245 quarters was calculated to identify a sensitivity of 70% (H_0 = 50%) and a specificity of 70% (H_0 = 60%) of IMI at the quarter-level, assuming a prevalence of IMI of 20%, power of 80% and confidence of 95%.[9] On the sampling day, all cows that were over 280 DIM and had no visible changes to the composition of their milk were enrolled in this study. For all enrolled cows, parity, DIM, and linear score at their last milk test were extracted from PCDart.[c]

Milk sampling and analyses. Standard pre-milking preparation, including pre-dipping, and drying the teat ends with a laundered cloth, was done by farm personnel. After the teat preparation, milk samples for the study were collected. Three to 5 streams of milk were removed from each quarter, followed by collection of 2 to 4 mL of milk into the respective quarter's individual well of the MLD Q4 collection device[b] provided by the manufacturer. The corresponding plug was inserted into the device to seal the milk sample, and the sample was set aside for processing upon arrival at the laboratory. Then, 1 of 2 trained technicians swabbed each of the 4 teat ends with cotton balls soaked in 70% methanol, and 2 milk samples were aseptically collected from each quarter. After collection, samples were immediately placed on ice and transported approximately 60 miles (100 km) to the AAD laboratory.

Samples collected for bacteriology were frozen by adding dry ice to coolers, then shipped overnight to a milk quality laboratory[d] for bacteriological analysis. Milk bacterial culture procedures followed National Mastitis Council guidelines.[36] Milk samples with 3 or more unique colony types were classified as contaminated. Intramammary infection was defined as a concentration of a given organism ≥ 100 cfu/mL of milk, except for CNS organisms, for which ≥ 200 cfu/mL of milk was required to be called an IMI.[16] As recently described,[18] quarters were classified as infected using 3 different approaches with the 2 samples per quarter collected: a single milk sample, both samples in parallel, or both samples in series. When using the single milk sample, the quarter was classified as infected according to the first analyzed sample.[15] If the first sample was contaminated, then the second sample was used.[3,18] When using both samples

interpreted in parallel, the quarter was classified as infected if 1 or both samples were positive for IMI.[41] When using both samples in series, the quarter was classified as infected if both samples were positive for IMI with the same pathogen.[2] In all 3 approaches, cows were classified as infected if at least 1 quarter was classified as infected.

Milk samples in the Q4 collection devices were processed within 12 hours of initial collection, as recommended in the product user manual. Milk from each quarter sample was used to measure the quarter-level SCC (cells/mL) with an optical cell counter.[e] Each sampling device was shaken across a 0.3 m span 20 times to thoroughly mix the milk sample. The plug insert was removed from the collection device and samples were analyzed with the MLD test. A lid with MLD test attached was placed on the base of the Q4 collection device, and the device was flipped to allow milk to load into each quadrant of the MLD test. The MLD test slide was then placed into the QScout DairyLab[f] reader for analysis. Lymphocytes, macrophages, neutrophils, and non-cellular debris were identified and recorded by the software. Samples were processed in Research Mode setting, and simulation in SmartResult was performed to allow a diagnosis to be generated at each possible threshold. The different thresholds were previously determined using models for prediction of high-risk quarters (unpublished data), and were used as indexes 1 to 12 in the present study.

Statistical analysis. Analyses were performed using SAS Studio 3.6.[g] Both the quarter and the cow were used as the unit of interest in this study. Prevalence of IMI with 95% confidence interval (CI) was calculated at the quarter- and the cow-level. Sensitivity, specificity, and predictive values to identify infected quarters and cows with the MLD test were determined for each of the 12 indexes (PROC SQL).[54] Characteristics of quarters (SCC, percentage of lymphocytes, neutrophils, and macrophages) correctly identified as infected using the MLD test (true positive) and incorrectly identified as non-infected (false negative) were compared using logistic regression models (PROC GLM). Quarters correctly identified as non-infected (true negative) and incorrectly identified as infected (false positive) were also compared.

Experiment 2 – Randomized Controlled Clinical Trial

Animals and housing. The enrollment for this randomized controlled trial was done in November and December 2013 on a single 7,416-cow Holstein dairy herd in Idaho. Cows were housed on dry lots and were milked 3x in either a double 36 parallel milking parlor or a double 24 parallel parlor. In 2013, the average DIM, milk yield, and bulk tank SCC was 182 d, 83 lb (37.6 kg)/cow/d, and 132,000 cells/mL, respectively. The hypothesis of this study was that the use of a selective DCT program, based on determination of IMI status using the MLD test results, would not result in an increased risk of clinical mastitis of more than 10% compared to a blanket DCT program. A sample size of 326 cows (163 per treatment) was calculated to identify a difference in clinical mastitis of 10% (assumed proportion of 25% and 21% based on previous literature[35]), with 97.5% confidence (1-sided test), 80% power, and a 12% loss to follow-up (WinPEPI[1]). Cows were enrolled weekly on the day prior to dry-off and were randomly assigned within lactation and number of positive quarters to blanket or selective DCT, using a random number generator.

Milk sampling and analyses. At enrollment, 24 h before dry-off, and at d 7 to 17 postpartum, milk samples from each quarter were collected for the MLD test. Samples from each quarter were also aseptically collected at enrollment for bacteriological culture. After collection, samples for MLD were processed within 12 hours, as recommended in the product user manual and analyzed by the DairyLab software.[h] Lymphocytes, macrophages, neutrophils, and non-cellular debris were identified and recorded by the software. Samples were processed with the SmartResult mode using index 6 for the threshold, which classified quarters as positive, borderline, or negative. Quarters classified as positive and borderline were considered positive.

Samples collected for bacteriology were sent to a commercial milk testing laboratory[i] for bacteriological culture. After 24 h, samples were classified as not infected (no growth), or infected (growth of major or other pathogens).

Treatment. All quarters of all cows in the blanket DCT group were treated with intramammary cloxacillin benzathine[j] and external teat sealant.[k] Cows in the selective DCT group were treated with intramammary cloxacillin benzathine and external teat sealant in quarters positive to MLD test, and with only external teat sealant in quarters negative to MLD test. The external teat sealant was applied once at dry-off.

Udder health and production. A clinical mastitis event was defined as an alteration of the milk appearance, udder swelling, or both, that necessitated a treatment with an IMM antimicrobial product, with or without systemic signs. These events were recorded for the whole lactation. Milk production and SCC (log transformed) data were obtained from DHI reports, approximately bimonthly for the 6 tests following parturition. Mastitis events, culling dates, and 305 d predicted milk production were obtained from DHI-Plus.

Statistical analysis. Analyses were performed using R version 3.4.3.[42] The number of clinical mastitis events per cow-day at risk was assessed using a quasi-Poisson regression model which includes a dispersion parameter to correct for over-dispersion (glm function).[53] The use of antimicrobial in the first 100 days postpartum was also compared using a logistic regression model (glm function). Monthly milk production and SCC (log transformed) at DHI test for 6 tests following parturition were compared between the blanket DCT and selective DCT groups using mixed linear regression models with a repeated measures structure (unstructured covariance structure; lmer function). Treatment group was included in all models. Parity, milk yield (in lb) before dry-off, length of the dry period (short: ≤ 56 d; normal: > 56 d),

and quarter MLD and IMI status at dry-off were offered to the models and kept if their unconditional association had a $P<0.30$. The final models were built using a backward stepwise approach, keeping confounders with a $P<0.20$.[31] Two-way interactions between treatment and confounders were kept in the model if significant ($P<0.05$). The fit of the quasi-Poisson model was assessed using the deviance and Pearson's chi-square-tests. For the mixed linear regression models, normality and homoscedasticity of the residuals was assessed graphically using standardized residuals, and their fit was evaluated using outliers (residuals and student residuals), extreme (leverage), and influential data (Cook's distance and DFFITS). To ensure removal of cows from the herd did not differ between groups, time to culling and the characteristics of the culled cows were compared between treatment using a Cox proportional hazard model (coxph function), and using logistic and linear regression models, respectively.

Results

Experiment 1 – Cross-sectional Study

A total of 94 cows were enrolled in the study from first to seventh lactation (median = 2, mean = 2.02, SD = 1.24), and between 284 and 786 DIM (median = 342, mean = 356.25, SD = 74.53). The median SCC at the quarter-level was 67,500 cells/mL (mean = 176,633; SD = 340,341. On average, the proportion of lymphocytes was 19.7% (median = 18.1, range = 0 to 50.0), the proportion of macrophages was 30.4% (median = 29.5, range = 0 to 64.0), and the proportion of neutrophils was 49.9% (median = 50.0, range = 13.9 to 84.4). Of the 373 quarters sampled for milk bacteriology (3 blind quarters), 2 were omitted for analysis due to contamination of both samples (0.5%). Eight (2.2%) quarters had disagreeing results (4 had 1 of the 2 samples contaminated, and 4 had discordant pathogen results). As shown in Table 1, the discordance between samples was small. Consequently, the difference between the 3 approaches used to classify quarters was minimal. The bacteriology result from the single milk sample was used as a gold standard for analyses, both at the quarter-level and the cow-level. The true prevalence of IMI was 12.1 (9.0 - 15.9) % in quarters, and 31.9 (19.9 - 39.0) % in cows.

The results from 363 quarters were available from the MLD test, as 3 quarters did not have enough milk to fill the MLD test, 4 quarters (1 cow) were omitted, and 1 sample failed to be read. None of the 8 missing quarters was positive for IMI. The MLD test characteristics in quarters compared to laboratory culture of milk samples when using a single milk sample to identify IMI are presented in Table 2. Sensitivity ranged from 44.4% (setting 12) to 73.3% (settings 1 and 2), and specificity from 75.5% (setting 1) to 91.5% (setting 12). Positive predictive value ranged from 29.7% (setting 1) to 42.6% (setting 12), and negative predictive value from 92.1% (setting 12) to 95.4% (setting 2).

The MLD test characteristics in cows compared to laboratory culture of milk samples when using a single milk sample to identify IMI are presented in Table 3. Sensitivity ranged from 50.0% (setting 12) to 76.7% (settings 1 and 2), and specificity from 54.0% (settings 1 and 2) to 81.0% (setting 12). Positive predictive value ranged from 44.2% (setting 1) to 55.6% (setting 12), and negative predictive value ranged from 77.3% (setting 12) to 82.9% (settings 1 and 2).

False-negative quarters with the MLD test at setting 1 (highest sensitivity) were identified with CNS (n = 7; plate count = 250 to 2,300 cfu/mL), *Corynebacterium* spp (n = 3; plate count = 180 to 450 cfu/mL), and mixed infection (n = 2; plate count = 690 to 1,590 cfu/mL). These quarters had lower SCC (76,666 ± 169,773 cells/mL) than true-positive quarters (654,484 ± 102,377 cells/mL; $P=0.01$), and higher lymphocyte percentage (20.6 ± 1.7 %) than true-positive quarters (16.7 ± 1.0 %; $P=0.05$). False-negative quarters with the MLD test at setting 12 (lowest sensitivity) were identified with CNS (n = 15; plate count = 250 to 2,300 cfu/mL), *Corynebacterium* spp (n = 3; plate count = 180 to 450 cfu/mL), *Enterococcus faecium* (n = 3; plate count = 180 to 1,190 cfu/mL), and mixed infection (n = 4; plate count = 560 to 1,590 cfu/mL). These quarters had lower SCC (148,120 ± 100,371

Table 1. Prevalence and etiology of intramammary infection in late-lactation quarters as determined by culture of quarter milk samples (reference test) when using 3 different definitions of IMI.

Quarter status	Single milk sample (%)	Duplicates, in parallel (%)	Duplicates, in series (%)
Total quarters (n)	371	371	363
No growth	326 (87.9)	323 (87.1)	318 (87.6)
Infected	45 (12.1)	48 (12.9)	45 (12.4)
Staphylococcus aureus	1 (0.3)	1 (0.3)	1 (0.3)
Enterobacter cloacae	1 (0.3)	1 (0.3)	1 (0.3)
Lactococcus lactis	2 (0.5)	2 (0.5)	2 (0.6)
Streptococcus equinus	1 (0.3)	1 (0.3)	1 (0.3)
Streptococcus gallolyticus	3 (0.8)	3 (0.8)	3 (0.8)
Enterococcus facium	6 (1.6)	7 (1.9)	6 (1.7)
Yeast	1 (0.3)	1 (0.3)	1 (0.3)
CNS	31 (8.4)	33 (8.9)	31 (8.5)
Corynebacterium spp	4 (1.1)	4 (1.1)	4 (1.1)

Table 2. Test characteristics of the milk leukocyte differential (MLD) test* to diagnose IMI in late-lactation quarters as compared with laboratory culture of milk samples when using a single milk sample to identify IMI (true prevalence = 12.1%) in 363 quarters from 93 Holstein cows (estimate [95% confidence interval]).

MLD setting	Number of positive quarters	Apparent prevalence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
MLD 1†	111	30.6 (25.9-35.6)	73.3 (58.1-85.4)	75.5 (70.4-80.1)	29.7 (21.4-39.2)	95.2 (91.8-97.5)
MLD 12‡,§	47	13.0 (9.7-16.8)	44.4 (29.6-60.0)	91.5 (87.9-94.3)	42.6 (28.3-57.8)	92.1 (88.5-94.8)
MLD 6¶	79	21.8 (17.6-26.4)	60.0 (44.3-74.3)	83.7 (79.1-87.5)	34.2 (23.9-45.7)	93.7 (90.2-96.2)

* QScout MLD, Advanced Animal Diagnostics, Inc, Morrisville, NC
† Setting with maximum sensitivity.
‡ Setting with maximum specificity.
§ Setting with maximum accuracy.
¶ Factory setting of MLD test recommended by manufacturer for use on farms.

Table 3. Test characteristics of the milk leukocyte differential (MLD) test* to diagnose IMI in late-lactation cows as compared with laboratory culture of milk samples when using a single milk sample per quarter to identify IMI (true prevalence = 31.9%) in 93 Holstein cows (estimate [95% confidence interval]).

MLD setting	Number of positive cows	Apparent prevalence (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
MLD 1†	52	55.9 (45.2-66.2)	76.7 (57.7-90.1)	54.0 (40.9-66.6)	44.2 (30.5-58.7)	82.9 (67.9-92.9)
MLD 12‡	27	29.0 (20.1-39.4)	50.0 (31.3-68.7)	81.0 (69.1-89.8)	55.6 (35.3-74.5)	77.3 (65.3-86.7)
MLD 11§	31	33.3 (23.9-43.9)	56.7 (37.4-74.5)	77.8 (65.5-87.3)	54.8 (36.0-72.7)	79.0 (66.8-88.3)
MLD 6¶	41	44.1 (33.8-54.8)	63.3 (43.9-80.1)	65.1 (52.0-76.7)	46.3 (30.7-62.6)	78.9 (65.3-88.9)

* QScout MLD, Advanced Animal Diagnostics, Inc, Morrisville, NC
† Setting with maximum sensitivity.
‡ Setting with maximum specificity.
§ Setting with maximum accuracy.
¶ Factory setting of MLD test recommended by manufacturer for use on farms.

cells/mL) than true-positive quarters (940,750 ± 112,219 cells/ml; $P<0.01$), higher macrophage percentage (27.2 ± 2.3 %) than true-positive quarters (18.5 ± 2.6 %; $P=0.02$), and lower neutrophil percentage (55.2 ± 2.0 %) than true-positive quarters (63.6 ± 2.2 %; $P=0.01$).

False-positive quarters with the MLD test at setting 1 (lowest specificity) had higher SCC (358,487 ± 23,072 cells/mL) than true-negative quarters (54,697 ± 13,126 cells/mL; $P<0.01$), lower lymphocyte percentage (18.1 ± 0.9%) than true-negative quarters (20.6 ± 0.5%; $P=0.02$), and higher neutrophil percentage (52.6 ± 1.1%) than true-negative quarters (47.4 ± 0.6%; $P<0.01$). False-positive quarters with the MLD test at setting 12 (highest specificity) had higher SCC (604,222 ± 37,347 cells/mL) than true-negative quarters (85,034 ± 11,357 cells/mL; $P<0.01$), lower macrophage percentage (20.8 ± 2.4 %) than true-negative quarters (32.4 ± 0.7 %; $P<0.01$), and higher neutrophil percentage (59.6 ± 1.8 %) than true-negative quarters (47.6 ± 0.6 %; $P<0.01$).

Experiment 2 – Randomized Controlled Clinical Trial

A total of 328 cows (1,312 quarters) were enrolled in the study, from first (n = 196; 60%) and second (n = 132; 40%) lactation. At dry-off, cows were between 271 and 551 DIM (median = 318, mean = 331.5, SD = 52.4). Their median SCC at the latest milk test was 31,000 cells/mL (mean = 71,433; SD = 86,448), and their median milk production at the latest milk test was 61 lb (27.7 kg) (mean = 27.8 lb [12.6 kg]; SD = 8.1 lb [3.7 kg]). The proportion of quarters positive to MLD at dry-off test did not differ between blanket (41.7 ± 1.9%) and selective DCT groups (40.0 ± 1.9%; OR = 1.1 ± 0.1; $P = 0.56$). The proportion of quarters with SCC > 100,000 cells/mL was lower, but did not differ between blanket (41.7 ± 1.9%) and selective DCT groups (40.0 ± 1.9%; OR = 1.1 ± 0.1; $P=0.56$). Also, the proportion of quarters positive to milk bacteriological culture did not differ between groups (blanket DCT: 16.2 ± 1.4%; selective DCT: 19.3 ± 1.5%; OR = 0.9 ± 0.1; $P=0.17$). Pathogens identified by the bacteriological culture are presented in Table 4. Major IMI (*Staphylococcus aureus*, *Streptococcus uberis*, and *Escherichia coli*) were identified in 4.4% of the bacteriological samples. Only 1 quarter positive to a major pathogen (*E. coli*) was left untreated in the selective DCT group.

Six cows were excluded during the dry-off period due to death (n = 5) or culling (n = 1), resulting in 322 cows used for analyses. According to the farm records, the causes of death and culling were suspicion of cancer (n = 2) and metritis (n = 1), pneumonia (n = 2), and lameness (n = 1). Of the excluded animals, 2 were in the blanket DCT group and 4

Table 4. Prevalence and etiology of intramammary infection 24 h before dry-off of 328 cows enrolled in a randomized controlled trial to compare blanket to selective dry-cow therapy (DCT).

Quarter status	Blanket DCT (%)	Selective DCT* (%)
Total quarters (n)	632	652
No growth	530 (83.9)	527 (80.8)
Infected	102 (16.1)	125 (19.2)
Staphylococcus aureus	1 (0.2)	2 (0.3)
Escherichia coli	1 (0.2)	2 (0.3)
Streptococcus uberis	1 (0.2)	3 (0.5)
Environmental streptocci	77 (12.2)	82 (12.6)
Streptococcus spp	1 (0.2)	0
Staphylocuccus spp	33 (5.2)	50 (7.7)
Pseudomonas spp	1 (0.2)	1 (0.2)
Prototheca spp	3 (0.5)	2 (0.3)
Bacillus spp	1 (0.2)	2 (0.3)
Yeast	0	1 (0.2)

* QScout MLD, Advanced Animal Diagnostics, Inc, Morrisville, NC

in the selective DCT group. The 133 cows culled after calving were included in analyses for postpartum IMI, and in analyses for clinical mastitis, milk production, and SCC until they were culled. The time to culling did not differ between groups (hazard ratio blanket vs selective DCT = 1.1; *P*=0.28), and the culled cows did not differ between groups for their lactation group (*P*=0.96), their odds of mastitis in the lactation (blanket DCT = 24 ± 5%, selective DCT = 29 ± 6%; *P*=0.46), and their projected milk production (mature equivalent 305; blanket DCT = 32,965 ± 4,947 lb [14,953 ± 2,244 kg], selective DCT = 31,716 ± 5,098 lb [14,386 ± 2,312 kg]; *P*=0.16).

The length of the dry period was on average 55.9 days (SD = 6.3) and ranged from 27 to 56 days in the short dry period category (mean = 51.7 ± 4.1), and from 57 to 79 days in the normal dry period category (mean = 60.4 ± 3.4). After parturition, the proportion of quarters positive to MLD test did not differ between blanket (19.3 ± 1.6%) and selective DCT (22.9 ± 1.6%; OR = 0.9 ± 0.1; *P*=0.13). The number of moderate and severe cases of clinical mastitis events per cow-day at risk did not differ between blanket and selective DCT groups (*P*=0.89; Table 5). The incidence rate of clinical mastitis event for blanket and selective DCT was 6.8 and 7.8 clinical mastitis events per 10,000 cow-days at risk, respectively, and the odds of antimicrobial use for mastitis treatment in the first 100 days postpartum did not differ between blanket and selective DCT groups (blanket DCT = 6.6 ± 2.1%; selective DCT = 6.8 ± 2.1%; OR = 1.0; *P*=0.95). As shown in Figure 1, milk production varied between DHI test-days (*P*<0.01), but not between blanket and selective DCT groups (*P*=0.90; group by test interaction: *P*=0.58). Similarly, SCC (log transformed) varied between DHI test-days (*P*<0.01), but not between blanket and selective DCT groups (*P*=0.50; group by test interaction: *P*=0.40).

Discussion

This study provides information concerning use of the MLD test for decision-making in a selective DCT approach. The goals of DCT are to eliminate IMI present at dry-off and to prevent new IMI in the subsequent lactation.[7] Even though the sensitivity of MLD test was below 70% at the quarter level (Experiment 1), there was no difference in IMI at calving and CM in the lactation between cows treated with selective and blanket DCT (Experiment 2).

Experiment 1 – Cross-sectional Study

The sensitivities found in Experiment 1 were similar to that previously reported,[18] and the specificities were higher. In the present study, duplicate bacteriological results were very consistent and contamination was infrequent, and only the single milk sample results were used. As the MLD test is using the leukocyte differential for identifying IMI, the ac-

Table 5. Quasi-Poisson model presenting the association between dry-cow therapy (DCT; blanket or selective), and the number of clinical mastitis events per cow-day at risk in the subsequent lactation, in 322 Holstein dairy cows, adjusted for parity as confounder. Cows in the blanket DCT were all treated with intramammary cloxacillin benzathine and external teat sealant in all quarters. Cows in the selective DCT were treated according to their results from MLD test* in SmartResult mode (index 6): positive and borderline quarters were treated with intramammary cloxacillin benzathine and external teat sealant, and negative quarters were treated with external teat sealant only.

Predictor	n	Coefficient	SE†	Incidence rate ratio (95% CI)	*P* value
Intercept		-7.17	0.27		< 0.01
Treatment					
Blanket DCT	160	Referent			
Selective DCT	162	0.14	0.27	1.15 (0.68-1.95)	0.61
Dry period length					
Normal (> 56 days)	154	Referent			
Short (≤ 56 days)	168	-0.85	0.28	0.43 (0.25-0.43)	< 0.01
MLD status at dry-off					
All negative quarters	122	Referent			
At least 1 positive quarter	200	0.59	0.29	1.80 (1.02-3.18)	0.04
Dispersion		3.03			

* QScout MLD, Advanced Animal Diagnostics, Inc, Morrisville, NC

† The covariance matrix was multiplied by a factor of Pearson's chi-square divided by the degrees of freedom.

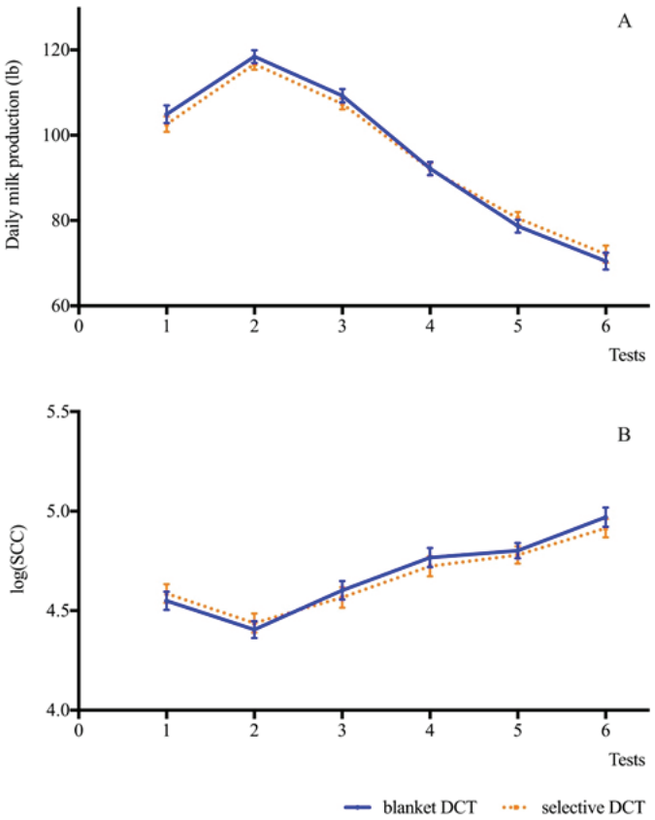

Figure 1. Average (± SE) daily milk production (A) and SCC (log transformed; B) for 322 Holstein dairy cows enrolled in a randomized controlled trial comparing blanket and selective dry-cow therapy (DCT). There was no difference between treatment groups over time for daily milk production (P = 0.23) and SCC (P = 0.14). Cows in the blanket DCT were all treated with intramammary cephapirin benzathine and teat sealant. Cows in the selective DCT were treated according to their results from MLD test* in SmartResult mode (index 6): positive and borderline quarters were treated with intramammary cloxacillin benzathine and external teat sealant, and negative quarters were treated with external teat sealant only.
*QScout MLD, Advanced Animal Diagnostics, Inc, Morrisville, NC

curacy of the test could be different between studies if the pathogens, or the inflammatory response to these pathogens, is different within the populations used in these studies.

It has been shown that quarters with IMI and low SCC (≤ 100,000 cells/mL) had higher lymphocyte and lower neutrophil percentages than cows with IMI and high SCC.[40] There is, to our knowledge, no indication that lower SCC and different leukocyte differential are due to a different stage of infection.[27] Pilla et al[40] reported that quarters with high SCC (> 100,000 cells/mL), but without bacterial growth, had lower lymphocyte and higher neutrophil percentages than normal quarters. It is not clear in these cases if the bacteriological culture produced false-negative results,[16] or if the MLD test produced false-positive results. The high SCC in the false-positive group suggest an inflammatory process[47] that is identified by the MLD test.

At the quarter level, the negative predictive value was over 90%, regardless of the setting used. This characteristic is desirable in a selective DCT approach, as it is less likely to leave infected quarters untreated. Predictive values are influenced by the prevalence of the condition, which means that quarters identified with MLD test as negative in a herd with low IMI prevalence at dry-off are very likely to be negative. On the other hand, higher IMI prevalence at dry-off will result in lower negative predictive values, which implies that negative MLD tests need to be interpreted according to the IMI prevalence on each farm.[18] In the false-negative samples, environmental and contagious pathogens have been identified with a large range of cfu/mL. Since the present study was conducted in a low bulk-tank SCC herd, only 1 sample was identified with a major pathogen among the false-negative samples. Major pathogens elicit stronger cellular reaction,[2,27] and it is possible that a population with more IMI with a major pathogen would have generated better sensitivity and specificity results. This hypothesis, however, needs to be verified. The study design of the present study does not allow for description of the dynamic process of the IMI. It is unclear if these pathogens were just beginning to colonize the udder, were chronically present, or were about to be eliminated. In other words, it is unknown if these IMI would have persisted through the dry-off period if left untreated.

The positive predictive values also need to be interpreted according to the IMI prevalence of the farm. In the present study, the positive predictive values of the MLD test were below 60%, both at the quarter- and cow-level. This indicates that the test, while accurately identifying non-infected quarters, classifies quarters with no growth as positive. The false-positive samples had higher SCC and neutrophil proportion, but the study design does not allow for evaluation of the dynamic nature of the IMI. It is also possible that the milk bacteriology missed a proportion of the IMI.[48]

Experiment 2 – Randomized Controlled Clinical Trial

Comparing the accuracy of the MLD test to bacteriological culture does not assess the impact of quarter misclassification on the results of a selective DCT program. The direct comparison between selective and blanket DCT in Experiment 2 resulted in no difference in moderate to severe clinical mastitis rate, milk production, and SCC in the subsequent lactation. Similar to our findings, previous studies have found no differences in clinical mastitis events, milk production, and SCC for cows treated with either blanket DCT or selective DCT, using laboratory and on-farm bacteriological culture for program decision-making.[10,11,24,25] When the selective DCT program was based on decision-making using SCC and clinical mastitis history of the lactation just being completed, untreated (low SCC) cows were more likely to have high SCC in the subsequent lactation than treated cows with low SCC.[43] However, these results are not directly comparable to the present study, since their study design did not compare selective versus blanket DCT. Yet, using MLD

tests for decision-making in the selective DCT program in the present study resulted in no difference in udder health parameters, including SCC, when compared to blanket DCT in the subsequent lactation.

Considerable variation in the impact of selective DCT compared to blanket DCT on milk yield and SCC between herds has been reported.[43] As the data presented in the current study were obtained from only 1 farm, generalization of the findings should be done with caution. Moreover, the bacteriological cultures at enrollment in the present study showed a very small proportion of major IMI (*S. aureus*, *S. uberis*, and *E. coli*) at dry-off. It is unclear if a higher prevalence of major pathogens would have affected the performance of the MLD test for selective DCT. High self-cure rate has been reported in cows with low SCC and CNS at dry-off,[6,11] but this was not evaluated in the present experiment as there was no bacteriological culture done postpartum. It is also possible that the characteristics of the herd used in the current study could have lowered the risk of cross-quarter contamination and played an important role in the results, despite the interdependence of quarters for the acquisition of new IMI during the dry period.[5,8,44]

Features of this herd may also have played a role in the low incidence rate of clinical mastitis cases in the present study compared to previous reports.[4,26,46] Moreover, the definition for clinical mastitis used in the present study was constrained by the commercial setting of the farm and did not include mild cases, which also decrease the apparent incidence rate. It is possible that the comparison of udder health would have been different if the mild cases had been included, but the absence of difference in milk production and SCC between the groups suggests this difference did not have repercussions on milk production and quality.

The absence of difference observed in this experiment can also be due to the limited number of cows enrolled. The commercial setting in which it took place restrained the time and number of animals available for the study. Moreover, the sample size was calculated to identify a difference in mastitis cases, including mild cases (21 to 25%), but these were not recorded during the follow-up period. The results of this experiment are consequently lacking power, and future research should include more cows in order to support our findings.

The choice of the threshold in the present study was set following the manufacturer recommendations. Different thresholds have the potential to yield different results, and choosing a threshold that maximizes the NPV would have been ideal in a selective DCT context as it minimizes the untreated infected quarters. Following the results of Experiment 1, an index of 1 could have resulted in better performance and should be considered for future research.

The present study used external teat sealant for both treated and untreated cows. Considerable research has been published on the positive efficacy of internal teat sealant to prevent new IMI during the dry period.[6,17] However, internal teat sealant had a lesser impact on low-SCC cows.[6] In contrast, literature about external teat sealant is limited. Various herd management factors have been shown to affect the duration of adherence and overall efficacy of external teat sealants.[28,29] As such, it is unclear if the use of external teat sealant is beneficial in the implementation of a selective DCT program.

Interestingly, cows with at least 1 quarter positive on the MLD test at dry-off had higher clinical mastitis incidence rate in the subsequent lactation than cows with all MLD-negative quarters. While it is not clear what mechanism underlies this association, it could be linked to the decreased cure of IMI in quarters with high MLD. Increased SCC before dry-off has indeed been shown to be associated with increased odds of clinical mastitis in the first 30 d postpartum,[19] and high SCC during the lactation prior to dry-off has been associated with reduced odds of IMI cure.[22] The absence of association between the IMI status at dry-off, as determined by milk bacteriology, and the clinical mastitis incidence rate in the subsequent lactation is likely due to the low number of quarters infected with major pathogens, or because of the presence of bacteria that cannot be cultured but still have an impact on udder health.[50] There was, however, an association between the MLD status at dry-off and the clinical mastitis incidence rate. This suggests that the MLD test has a potential predictive power that is worth exploring in future research. Length of the dry period has not been associated with cure of IMI or new IMI, but longer previous lactation has been associated with increased odds of new IMI.[12,22] In the end, it is not possible to assess which mechanisms were involved in the present study as the postpartum IMI status was not evaluated.

In both experiments, the pathogens identified by bacteriological culture were not distinguished between major and minor pathogens, but a threshold of ≥ 200 cfu/mL was used for classifying the presence of CNS as an IMI.[16] The treatment of CNS at dry-off has been suggested to be inadequate, but remains controversial due to the multiple species grouped under CNS.[14,20,38] Future studies with higher prevalence of both major and minor pathogens could explore the accuracy of the MLD test to differentiate between the 2 groups.

While both experiments were conducted in single herds with low bulk-tank SCC and a low prevalence of major IMI pathogens, the results can be used to further the knowledge on MLD tests. The MLD test identifies accurately non-infected quarters of dairy cows in late lactation, and the characteristics of the misclassified samples suggest that the MLD test identifies inflammation accurately. Moreover, when the QScout MLD test was used for decision-making in a selective DCT program, the incidence rate of moderate and severe cases of mastitis events did not differ from a blanket DCT program. Experiments including a greater number of animals and farms with different characteristics (e.g., high bulk-tank SCC or higher rate of mastitis events) will be necessary to generalize these conclusions.

Endnotes

[a] Advanced Animal Diagnostics, Inc., Morrisville, NC
[b] QScout MLD, Advanced Animal Diagnostics, Inc., Morrisville, NC
[c] PC Dart, DRMS, Raleigh, NC
[d] University of Tennessee Milk Quality Laboratory, Knoxville, TN
[e] DeLaval DCC, Kansas City, MO
[f] QScout DairyLab reader, software Version 1.0.6.15013, Advanced Animal Diagnostics, Inc., Morrisville, NC
[g] SAS Institute Inc., Cary, NC
[h] DairyLab, software Version 1.0.6.62, Advanced Animal Diagnostics, Inc., Morrisville, NC
[i] Udder Health Systems Inc., Laboratory, Jerome, ID
[j] Dry-Clox®, Boehringer Ingelheim, Duluth, GA
[k] T-Hexx®, Hydromer Inc., Branchburg, NJ

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