PEER REVIEWED

Evaluation of the Azotest® Strip as Recommended for the Estimation of Milk Urea Nitrogen Concentrations in Individual Cow, Milk Line and Bulk Tank Samples

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Abstract

The objectives of this study were to determine inter-reader agreement and to describe the accuracy of the Azotest® strip for estimating urea nitrogen concentrations in milk samples from individual cows (n = 214), milk lines (n = 41) and bulk tanks (n = 41). Samples were split, with one portion used for milk urea nitrogen (MUN) analysis using the Azotest® strip, and the second portion submitted to an accredited diagnostic laboratory for MUN analysis using wet chemistry analysis as the gold standard test.

There was excellent inter-reader agreement (Kappa = 0.87) in this study for distinguishing between individual cow samples with either normal or high MUN values. However, the overall accuracy of the Azotest® strip was poor, with MUN results from the Azotest® strip being different than those from wet chemistry analysis ($P < 0.01$). There was poor agreement between the two test methods (Kappa = 0.12 – 0.14); average Azotest® results were approximately 5 mg/dl units higher than the wet chemistry analysis method for either individual cow, milk line or bulk tank samples. Forty-five percent of individual cow samples, and 45% of milk line and bulk tank samples were correctly categorized as low, normal or high.

The sensitivity and specificity of the Azotest® strip for detecting high MUN concentrations (vs normal or low) in individual cow samples was 98.7 and 33.1%, respectively, and in pooled milk line and bulk tank samples was 100 and 21.1%, respectively. For this data set, the predictive value of a positive test result when looking for high MUN measures (vs normal or low) was only 33.1 to 35.7%. Thus, approximately two-thirds of samples identified as high by the test strip actually had a normal MUN concentration.

The sensitivity and specificity of the Azotest® strip for detecting low MUN concentrations (vs normal or high) in individual cow samples was 0 and 100%, respectively. The test failed to correctly identify any truly low MUN samples as being low, and overestimated the MUN concentration in a high proportion of truly low and normal samples. Given this high degree of test inaccuracy in these data, we conclude that the Azotest® strip will not be beneficial to commercial dairy producers or dairy consultants. The authors recommend that producers wishing to monitor MUN submit milk samples to an accredited diagnostic laboratory for MUN analysis.

Résumé

Les objectifs de cette étude étaient d'une part de déterminer l'accord entre les utilisateurs du test et d'autre part d'examiner la fiabilité des mouillettes Azotest dans l'estimation des concentrations d'urée dans des échantillons de lait provenant de vaches (n = 214), de lignes à lait (n = 41) et de réservoirs (n = 41). Une partie des échantillons était utilisée pour le test de l'urée du lait avec les mouillettes Azotest et l'autre partie était soumise à un laboratoire diagnostic accrédité pour l'analyse de l'urée du lait. La méthodologie chimique était considérée comme l'étalon.

On a démontré une très grande concordance entre les utilisateurs (kappa = 0.87) dans cette étude lorsqu'il s'agissait de distinguer les échantillons de vaches avec des concentrations d'urée normales ou élevées. Toutefois,

la fiabilité des mouillettes Azotest était pauvre car la concentration de l'urée du lait déterminée avec les mouillettes était différente de celle obtenue avec le test chimique ($P < 0.01$). Il y avait une faible concordance entre les deux tests (kappa = 0.12-0.14). Les concentrations moyennes obtenues avec les mouillettes étaient approximativement plus élevées de 5 mg/dl que celles déterminées par le test chimique pour les trois types d'échantillons. Un total de 45% des trois types d'échantillons était bien classé dans les catégories faible, normale ou élevée.

La sensibilité et la spécificité des mouillettes Azotest pour distinguer les concentrations élevées d'urée par rapport aux concentrations plus faibles étaient égales respectivement à 98.7% et à 33.1% dans les échantillons de lait individuels et à 100% et à 21.1% dans les échantillons provenant des lignes à lait et des réservoirs. Dans cet ensemble de données, la valeur prédictive d'un test positif pour distinguer les concentrations élevées par rapport aux concentrations plus faibles variait entre 33.1% et 35.7%. Par conséquent, près des deux tiers des échantillons identifiés comme élevés par les mouillettes avaient en fait des concentrations normales d'urée.

La sensibilité et la spécificité des mouillettes Azotest pour distinguer les concentrations faibles d'urée par rapport aux concentrations plus élevées étaient égales à 0% et à 100% respectivement dans les échantillons de lait individuels. Le test ne permis pas d'identifier correctement comme faible aucun des échantillons effectivement faibles et surestimait la concentration d'urée dans une forte proportion des échantillons vraiment faibles ou normaux. Compte tenu de la pauvre fiabilité du test avec ces données, nous concluons que l'utilisation des mouillettes Azotest ne sera pas bénéfique ni aux producteurs de lait ni aux consultants laitier. Les auteurs recommandent aux producteurs qui désirent surveiller l'urée du lait de soumettre leurs échantillons à un laboratoire de diagnostic accrédité.

Introduction

Over the past several years, there has been a great deal of interest in monitoring urea nitrogen concentrations as a measure of efficiency of protein utilization in dairy herds. Research has established that serum urea and milk urea nitrogen (SUN, MUN) concentrations are sensitive to concentrations of dietary crude protein (CP), rumen degradable protein (RDP), rumen undegradable protein (RUP) and protein-to-energy ratios.[3,4,6,8,9,16,18,20,22] Potential benefits of monitoring MUN concentrations include more efficient use of expensive dietary protein (*i.e.*, reduced feed costs), improved health, productivity, and reproductive performance in the animal, and reduced excretion of excess dietary nitrogen into the environment.

There are several approaches to sampling and testing MUN concentrations. The most common sampling strategy is to collect a metered milk sample from individual cows on Dairy Herd Improvement Association (DHIA) test day, and then submit those samples for MUN testing along with routine milk component testing. The two most common MUN test methods used in North American DHIA laboratories are either a wet chemistry analysis or near infrared (NIR) analysis. Independent evaluation has demonstrated that DHIA samples analyzed using the NIR method of analysis are suitable for MUN concentrations, provided that data are interpreted at the group level.[11] The cost of testing through DHIA or other milk testing laboratories ranges from $0.10 to $1.00 per sample, depending on the number of samples submitted, method of analysis, and the laboratory used. Additionally, the producer must wait two to three days for test results. Because of large cow-to-cow variability in MUN levels, MUN test results from individual cows should be interpreted at the group level.[5,7,19,21,24]

One disadvantage of testing using individual cow DHIA samples is that producers may have to wait for their next DHIA test day in order to monitor or detect a herd or group response to a recent change in nutritional management. Additionally, producers would like to save on the expense of testing all milking animals. As such, it would be very useful to producers if they had an on-farm test that could be used on a subset of individual cows or on milk line or bulk tank samples collected from a group of cows on the same ration.

An alternative test available for use is the Azotest® strip,[a] an on-farm dipstick test marketed to dairy producers and consultants.[1] This is a urease-based test coupled to a pH indicator dye. Higher concentrations of urea in the milk sample cause darker green color changes on the reagent strip (Figure 1). The Azotest® strip costs approximately $2.00 (US) per test strip. The manufacturer recommends that, due to individual cow variation, the test be performed for at least ten cows, with a minimum of five from the same feeding group. The test must be performed using a metered milk sample representative of the entire milking, and not a pre-milking or post-milking stripping sample. Finally, the manufacturer recommends interpreting test strip results as follows: "...perform the test on several cows to obtain an estimate of the average value as well as the proportion of cows outside the desirable range. If 40% or more (4 of 10 or 2 of 5) samples are outside the desirable range, it is advised to review the feeding strategy to correct the problem".[1]

The Azotest® strip has potential convenience, time and cost advantages over laboratory testing. However,

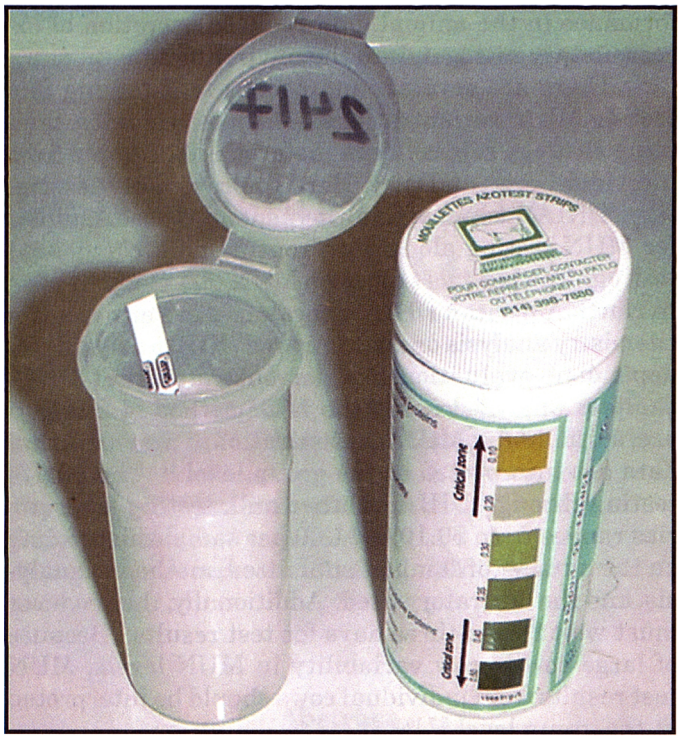

Figure 1. Milk sample being tested for milk urea nitrogen concentration using the Azotest® strip.

such a test needs to be accurate to be useful to the producer. To our knowledge, there are no published studies evaluating the accuracy of the Azotest® strip for measuring urea concentrations in milk samples. The first objective of this study was to describe the accuracy and agreement of the Azotest® strip in estimating urea nitrogen concentrations as compared to wet chemistry analysis. The second objective was to describe the inter-reader agreement using the Azotest® strip on milk samples from individual cows.

Materials and Methods

Milk Sample Collection and MUN Analysis

A total of 214 metered milk samples were collected from individual cows at three separate milking events in two different dairy herds; twice at the dairy barn on campus at the University of Minnesota (one morning and one evening milking, three months apart) and once at a commercial dairy farm in Minnesota (one morning milking). These two dairies were selected for sampling out of convenience and because it was known, from previous MUN testing through MN DHIA,[b] that they should produce milk samples representing a wide range of MUN concentrations. Milk was collected from the milk meter of each cow at the time of regular milking, chilled, and transported directly to the College of Veterinary Medicine, University of Minnesota.

A total of 41 paired milk line and bulk tank samples (total of 82 samples) were collected from 22 Minnesota dairy herds at different milking events. This was done as part of a different study that resulted in the validation of using milk line samples to monitor milk component and udder health data.[12,13] The reader can refer to these papers for details on the installation and use of the QMI Safe Septum Sani-elbow.[c] Milk line and bulk tank samples were cooled immediately upon collection and transported directly to the College of Veterinary Medicine, University of Minnesota.

Upon receiving individual cow, milk line and bulk tank milk samples, each sample was well agitated and then split into two subsamples. The first subsample from each pair was submitted to the Dairy Quality Control Institute (DQCI) Services Laboratory[d] for analysis of MUN with a wet chemistry reference test method.[e]

The second subsample was immediately tested for MUN at the University of Minnesota using the Azotest® strip following the manufacturer's directions.[1] Milk samples were tested at room temperature. After agitation of each sample, the Azotest® strip was set in the milk sample vial for five minutes. The strips were then quickly rinsed under a light stream of cold tap water for three seconds, and then immediately read by comparing the color change on the test strip against a color chart provided by the manufacturer on the test product bottle. The color change readings were categorized into 6 different semi-quantitative levels of MU: 0.1, 0.2, 0.3, 0.35, 0.4 and 0.5 g/L (Figure 1). Corresponding levels after converting units to MUN are 4.7, 9.3, 13.9, 16.3, 18.6 and 23.2 mg/dl, respectively. Readers should note that in the United States, urea is most frequently reported as mg/dl of MUN, while the rest of the world often reports mmol/L of MU. One gram of urea nitrogen is equivalent to 2.146 grams or 0.0357 moles of urea. The conversion factor to estimate MUN mg/dl = MU g/L x 46.45, since nitrogen represents 46.45% of urea by weight.

For 43 of the individual cow samples, Azotest® strip measurements were analyzed twice. Tests were run and read independently in the same room by two individuals within 15 minutes of each other. Both individuals were masked to each other's results.

Statistical Analysis

Three methods of analysis were used to compare results between the Azotest® strip and the reference method. First, a non-parametric test of differences between groups was required, instead of a conventional ANOVA, because the results of the Azotest® strip are reported as ordinal type data. The MUN results from the wet chemistry analysis were also categorized as low (<10.0 mg/dl), normal (10.0 to 16.3 mg/dl), or high (> 16.3 mg/dl). These categories were created both to match MUN cut points for categories as indicated on the

Azotest® strip interpretation chart (low = 4.7 or 9.3 mg/dl; normal = 13.9 or 16.3 mg/dl; high = 18.6 or 23.2 mg/dl) as well as to match with current recommended ranges reported in the scientific literature.[16] The PROC NPAR1WAY procedure (SAS version 8.0), using the Wilcoxon statistic, was used to test for differences between the MUN results of the two test methods (Azotest® vs reference method).[23] This method produces a chi-square approximation for the significance of the Kruskal-Wallis test when the WILCOXON option is used.[10] The Kruskal-Wallis test was performed once on data containing the 214 individual cow samples, and then again using data from the 41 milk line and 41 bulk tank samples. The latter two sample types were combined in the analysis because they are both representative of a pooled sample type that producers might frequently elect to use on a dairy.

The second test performed on data for both individual cow samples and pooled (milk line and bulk tank) samples was a 3 x 3 Kappa, or test of agreement, between categorized (low, normal, high) results from the Azotest® strip as compared to the reference method. Finally, this categorized data was also used to calculate the sensitivity, specificity, and predictive values of a positive and negative the Azotest® strip result, both to accurately identify a sample with a high MUN concentration (vs normal or low) and to accurately identify a sample with a low MUN concentration (vs normal or high). This was done separately for the individual cow samples and the pooled samples. A 2x2 Kappa value was also used to measure inter-reader agreement for the 43 individual cow samples that were read by two individuals.

Results and Discussion

Descriptive statistics are presented in Table 1 showing MUN results for the 214 individual cow samples, 41 milk line samples and 41 bulk tank samples for both the Azotest® strip and the reference test method. In these descriptive data the Azotest® strip appeared to overestimate the wet chemistry analysis mean MUN by approximately 5 mg/dl, regardless of the type of sample (individual cow, milk line or bulk tank). A graphic comparison of Azotest® strip and wet chemistry analysis MUN results for the 214 individual cow samples is also presented in Figure 2. This scatter plot shows not only the strong tendency for the Azotest® strip to produce a higher MUN estimation than wet chemistry analysis, but also shows a great deal of variation in wet chemistry analysis results (continuous results reported) for any one level of MUN produced by the Azotest® strip (semi-quantitative results reported).

After categorization of results into low, medium and high MUN levels, a significant difference was detected between the Azotest® results and the reference method. This was true both for the individual cow samples ($P < 0.0001$) and for the pooled samples (milk line and bulk tank samples; $P < 0.0001$).

The level of agreement between the two tests for estimating low, normal or high categories of MUN results for individual cow samples was poor (K = 0.12; Table 2). The level of agreement for estimating normal or high categories of MUN results between the two test types for pooled samples (milk line and bulk tank samples) was also poor (K = 0.14; Table 3). The latter analysis had no low MUN values for either milk line or bulk tank samples, so only a 2 x 2 Kappa could be estimated.

When considering the ability of the test to differentiate high MUN values, the sensitivity and specificity of the Azotest® test strip for identifying an individual cow sample with a high MUN concentration (vs normal or low) was 97.8 and 45.8%, respectively (Table 2). Using the prevalence of truly high values (21.5%) in this data set, the corresponding predictive values of a positive (high) and negative (normal or low) test result were 33.1 and 98.7%, respectively. Thus, if the Azotest® strip yielded a high result in the current data set, a producer could only be 33.1% confident that the MUN value was truly high. Put another way, if the Azotest® strip yielded a high test result for three cows, likely only one of those cows would be truly high, while the other two would be false positives, with their true MUN value falling in the normal or low range. Thus, when faced with interpreting high test results, the inaccuracies of the test could lead a producer to the false conclusion that MUN val-

Table 1. Azotest® strip and wet chemistry analysis milk urea nitrogen concentration (mg/dl) test results for individual cow, milk line and bulk tank samples.

Sample type	Test method	n	Mean	Std. Dev.	Min.	Max.
Individual cow	Wet chemistry	214	13.7	3.4	2.7	21.7
	Azotest® strip	214	18.7	2.8	13.9	23.2
Milk line	Wet chemistry	41	15.2	2.1	11.1	21.6
	Azotest® strip	41	20.4	2.8	16.3	23.2
Bulk tank	Wet chemistry	41	15.2	2.1	10.8	22.0
	Azotest® strip	41	20.4	2.8	16.3	23.2

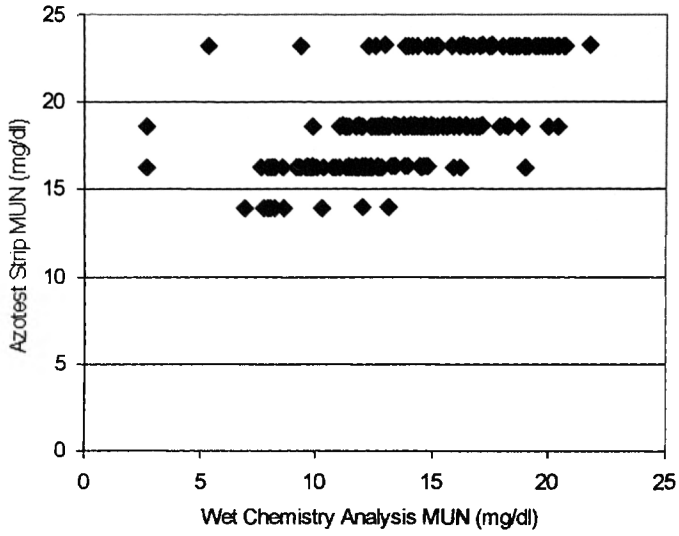

Figure 2. Comparison of Azotest® strip and wet chemistry analysis milk urea nitrogen results for 214 individual cow samples.

Table 2. Milk urea nitrogen classification results comparing the Azotest® strip and wet chemistry analysis test method for individual cow milk samples.

	Wet chemistry analysis MUN (mg/dl)			
Azotest® strip MUN (mg/dl)	Low (< 10.0)	Normal (10.0-16.3)	High (>16.3)	Total
Low (4.7 or 9.3)	0	0	0	0
Normal (13.9 or 16.3)	26	51	1	78
High (18.6 or 23.2)	4	87	45	136
Total	30	138	46	214

Table 3. Milk urea nitrogen classification results comparing the Azotest® strip and wet chemistry analysis test method for milk line and bulk tank samples.

	Wet chemistry analysis MUN (mg/dl)		
Azotest® strip MUN (mg/dl)	Normal (10.0-16.3)	High (>16.3)	Total
Normal (13.9 or 16.3)	12	0	12
High (18.6 or 23.2)	45	25	70
Total	57	25	82

ues in the group are higher than they truly are. However, when looking to detect high MUN measures, if the test yielded a negative test result (normal or low), a producer could be 98.7% confident that the MUN value truly was not high.

Results were similar when using pooled samples (milk line and bulk tank samples) to identify high MUN concentrations (Table 3). In pooled samples, the sensitivity and specificity of the Azotest® test strip to identify a sample with a high MUN concentration (vs normal) were 100 and 21.0%, respectively. Of 25 samples with truly high MUN concentrations, 100% (25 of 25) were identified as high by the test strip. However, for 57 samples with truly normal concentrations, only 21% (12 of 57) were identified as normal using the test strip. The remaining 79% (45 of 57) of normal samples were identified as high. The overall accuracy was 45.1%. Using the prevalence of high MUN values (30.5%) in this data set, the corresponding predictive values of a positive (high) and negative (normal) test result were 35.7 and 100%, respectively. Thus, if a producer received a high reading on an Azotest® strip from an individual milk line or bulk tank sample, he could only be 35.7% confident that the true MUN result was high (a 64.3% chance the true value was normal). However if a normal test result was received using the Azotest® strip, the producer could be 100% confident that the true MUN value was not high.

When considering the ability of the test to differentiate low MUN values and using individual cow samples, the sensitivity and specificity of the Azotest® strip to identify individual cow samples with a low MUN concentration (vs normal or high) were 0 and 100%, respectively (Table 2). Of 30 samples with truly low MUN values, none were identified as low with the test strip. Based on the prevalence of low values (14.0%) in this data set, the corresponding predictive value of a negative (normal or high) test result would be 86.0%. Thus, when attempting to detect low measures with this data set, if the test yielded a negative test result (normal or high), a producer could be 86.0% confident that the MUN value truly was not low. This predictive value of a negative test would be much poorer in a dataset that contained a higher prevalence of samples with low MUN concentrations. The predictive value of a positive (low) test result could not technically be calculated for this study because one cannot divide zero by zero. However, the authors speculate that if the Azotest® strip did report a low test result (not observed in this study), there would be a very high probability that there was truly a low MUN concentration in the sample. Similar analysis could not be performed for the milk line and bulk tank sample set because that data set did not contain any pooled samples representing a truly low MUN value.

The two participating Azotest® strip readers achieved a very high level of agreement when differentiating between normal and high MUN categories (K = 0.87; Table 4).

While individual cow samples represented a wide range (L, M, H) of MUN values, one weakness in this study was that the milk line and bulk tank sample set represented only normal and high true MUN concentrations. This was also true of the sample set used to measure inter-reader agreement. Ideally the evaluation of inter-reader agreement should be repeated, if a future opportunity arises, using a sample set representing a wider range of true MUN values. However the authors believe that the current study provides enough information about the Azotest® strip to reach a conclusion about its utility when used according to the manufacturer's recommendations.

Milk urea nitrogen testing has demonstrated itself to be a valuable diagnostic tool for monitoring the efficiency of dietary nitrogen utilization on dairy farms.[2,12,15] Advantages of Azotest® strip, compared to laboratory testing, include potential for reduced testing costs and the ability to test in a timely fashion relative to feeding management changes. An additional benefit suggested from this study is that there appears to be excellent inter-reader agreement in differentiating between samples with normal vs high MUN values. However the final and most important factor to consider when evaluating a test's utility is the accuracy of results. In this study the test was not accurate when used on single pooled samples such as milk line or bulk tank samples. A similar conclusion was reached when the test was used for individual cow samples and interpreted, as per manufacturer's recommendations, by examining both the group mean value and the proportion of animals with an abnormal test result. Based on our data, there is a high probability that herds with truly low or normal MUN concentrations could be falsely classified, when using the Azotest® strip, as having either normal or high values, respectively.

Table 4. Comparison of inter-reader results when using the Azotest® strip on individual cow milk samples with normal or high milk urea nitrogen concentrations. (Kappa = 0.87)

	Reader 1		
Reader 2	Normal MUN (13.9 or 16.3 mg/dl)	High MUN (18.6 or 23.2 mg/dl)	Total
Normal MUN (13.9 or 16.3 mg/dl)	32	1	33
High MUN (18.6 or 23.2 mg/dl)	1	9	10
Total	33	10	43

Conclusions

The Azotest® strip has several potential advantages over laboratory testing for MUN, including cost, convenience, timeliness of testing results and excellent inter-reader agreement. However, the overall accuracy of the Azotest® strip was generally poor when used as recommended. Azotest® strip results differed significantly from true MUN concentrations as measured using wet chemistry analysis. Only 45% of all individual cow samples (low, normal or high) and 45% of all pooled milk line and bulk tank samples (normal or high) were correctly identified. The test failed to correctly identify any truly low MUN samples as being low, and overestimated the MUN concentration in a high proportion of truly low and normal samples. With this data set the predictive value of a positive test result when looking for high MUN measures (vs normal or low) was only 33.1 to 35.7%. Thus, approximately two-thirds of the samples identified as high by the test strip truly have a normal or low MUN concentration. Given this high degree of test inaccuracy in these data, we conclude that the Azotest® strip will not be beneficial to commercial dairy producers or dairy consultants. The authors recommend that producers wishing to monitor MUN, instead, submit milk samples to an accredited diagnostic laboratory for MUN analysis.

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Footnotes

[a] Azotest® strip, P.A.T.L.Q., Ste-Anne-De-Bellvue, Quebec, Canada.
[b] Minnesota Dairy Herd Improvement Association, 307 Brighton Ave. South, Buffalo, MN 55313.
[c] Quality Management Inc., St. Paul, MN 55128.
[d] Dairy Quality Control Institute. Mounds View, MN 55112.
[e] Bentley Instruments, Chaska, MN 55318.

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*Roth MA, Carter DP. Comparison of Bovine Herpesvirus 1 Vaccines for Rapid Induction of Immunity. Veterinary Therapeutics. 2000; 1, 4: 220-228. Additional data on file. ®RELIANT and the CATTLE HEAD LOGO are registered trademarks of Merial. ™DUAL IBR is a trademark of Merial.

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