Canary-AG™ accurately predicts onset of respiratory infections in beef feedlot cattle via breath

The beef cattle feedlot market holds 30 million head with an estimated market size of $65 billion annually in the United States. A leading cause of economic loss for beef producers is bovine respiratory infection (BRI), estimated at $1 billion dollar annual due to loss of production, medication costs, and increased labor for care. Calves entering feedlots are the most vulnerable cattle population for developing infections and tend to be the most expensive to treat. BRI accounts for >75% of illnesses and 70% of cattle deaths in feedlots. Early and appropriate intervention in illnesses is vital to improved health outcomes. Moreover, unnecessary prophylactic treatment is undesirable because of the risk of antibiotic-resistant bacterial strains, higher treatment costs, USDA mandates and consumer market pressure.

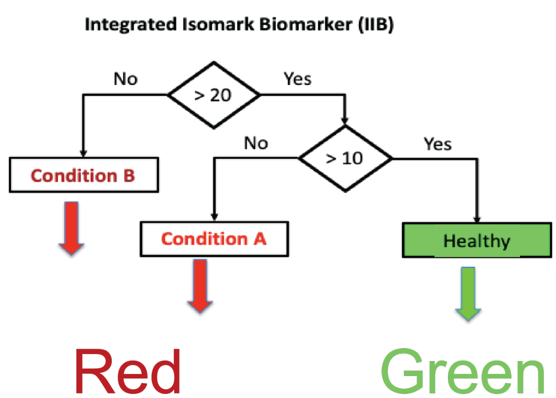
The current methods for detecting infections rely heavily on visible signs such as lack of appetite or activity and depend on the skill of the rancher or veterinarian's skill in observation. Furthermore, visible signs appear after the infection has progressed to a point where it is having a significant systemic impact on animal health. This subjective approach is not data driven and often detects infections too late for effective interventions.

Isomark has developed a pre-symptomatic breath biomarker for early detection of infection. Isomark's device called the Canary-AG™, monitors patented biomarkers in exhaled breath and predicts onset as within 4-6 hours of infection onset, and 24-48 before visible outward signs. Isomark's non-invasive and rapid detection technology is focused on monitoring changes in natural carbon isotopes ratios in exhaled breath CO_2. The changes in breath markers are reported as red or green lights indicating animals' health status, infected vs. healthy. The biomarker can be thought of as the "temperature of the immune system," providing a pre-symptomatic biomarker for infection at the earliest times, giving ranchers and veterinarians up to two days' notice before conventional methods. Early detection provides the ability to begin treating infection at their earliest point resulting in improved treatment success, reduced antibiotic use/costs, and lower morbidity and mortality losses due to disease. ***New intervention strategies, based on Isomark's noninvasive monitoring technology, will change the current reactive paradigm to a more proactive approach and will improve health and reduce costs.***

Isomark Health. Inc, 5930 Seminole Centre Ct., Suite F, Fitchburg, WI 53711
Email: info@isomark.com Ph. (608) 571-2609

The science behind technology:

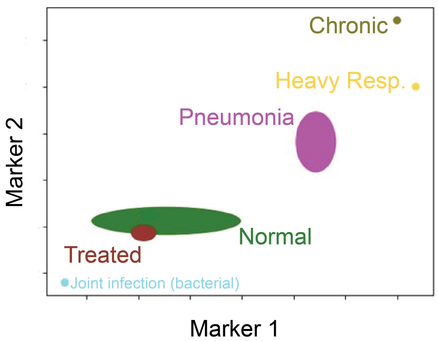
Isomark's technology is based on 25 years of research at University of WI-Madison. The patented biomarkers rely on measuring the $^{13}CO_2/^{12}CO_2$ ratio called the BDV. BVD is used as a reliable and highly sensitive indicator for the onset to progression of infection. During the early onset of the acute phase response the BDV is inversely related to the severity of a developing infection. Tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1) induce changes in secondary metabolism. Amino acids are rapidly released from skeletal muscle where they can be used to make acute phase proteins or be metabolized for fuel [1]. Amino acids released during the acute phase fractionate based on the molecular weight of the amino acid [2]. This fractionation follows the principles of the kinetic isotope effect [3]. Approximately 1% of the world's stable carbon has the atomic weight of 13 (^{13}C) and most of the remaining carbon is ^{12}C. Amino acids and products with higher amounts of ^{13}C are less likely to be fully metabolized to CO_2 than those amino acids that have a majority of ^{12}C, the lighter isotope. The "heavier" carbon amino acids remain as products of the acute phase (i.e., acute phase proteins) while the "lighter" carbon amino acids are more likely to be completely oxidized to CO_2. The result of this phenomenon is that the ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath decreases during the infection onset [2, 4]. However, as the infection progresses, metabolism shifts and becomes more anaerobic, and macronutrient metabolism changes from primarily a mixture of carbohydrates and lipids to primarily carbohydrate and body proteins. Due to isotopic discrimination against ^{13}C during several steps in synthesis, lipids are 3-5‰ lighter than carbohydrates or proteins. Thus, a shift in macronutrient oxidation during the progression of infection causes a rapid increase in the $^{13}CO_2$:$^{12}CO_2$ ratio. Because of the competing isotopic mechanisms during an untreated infection, the BDV has a higher variance than normal. In practical terms, a shift of greater than 1‰ above or below the baseline level are indications of the presence of infection. The information from the BVD is then combined with other proprietary breath biomarkers with a machine learning approach to predict health status with the Integrated Isomark Biomarker indicated in the user interface by a green light for healthy and a red light for sick.

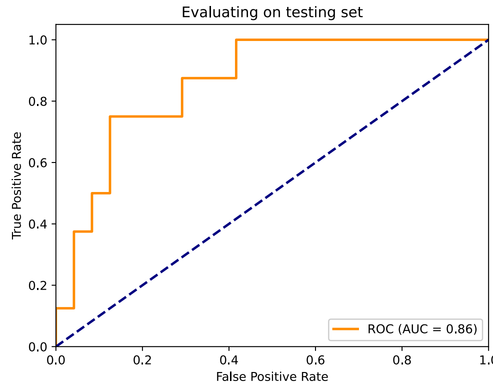


How it works:

The Canary-AG was deliberately engineered to measure the health status of cattle in feedlots and dairy applications. Key features of the Canary-AG are: 1) it is built for rugged environments, 2) it is user friendly and easy to operate, 3) it is available in stationary or portable options, 4) breath samples are easy to collect at chute side or from headlock for point-of-care applications, 5) and most importantly results are provided in 30 seconds of obtaining a breath sample. The Canary does not use any sample pre-processing or reagents thus it is considered a green technology with minimum requirement for disposables. The Canary-AG (cart option depicted) consists of a washdown compliant enclosure, zero air connections, internet connectivity and output data integration (wi-fi or cellular options available), electronic ID reader, and breath sampling handle and mask for breath collection. The user interface is intuitive and displays data in real-time as breath samples are collected and analyzed. Output data is stored locally on the Canary and reported automatically to users via email, mobile device, and/or health management software integration.

Isomark Health. Inc, 5930 Seminole Centre Ct., Suite F, Fitchburg, WI 53711
Email: info@isomark.com Ph. (608) 571-2609





Data collection and analysis:

Data were collected at a partner feedlot located in central Nebraska over the course of one year. Breath was sampled as cattle entered the feedlot during normal intake processing. Electronic identification (EID) numbers were individually assigned. The EID tag was then used to initiate the Canary sample collection procedure, and breath was captured using the handle/mask apparatus of the Canary. Once an adequate breath sample was obtained the Canary automatically analyzed the breath $^{13}CO_2/^{12}CO_2$ and proprietary breath markers and reported them to the feedlot manager and Isomark's central data server.

Health data was obtained from the feedlot's health management software. Focusing on the first 14 days of feedlot entry, health events were noted in 11.1% of animals measured, of which 49.3% were lung related infections. The algorithm was able to distinguish a variety of disease conditions including acute and chronic lung related conditions of bacterial or viral origins. To design an algorithm for predicting the health status of the animals, 80% of the healthy and 80% of the unhealthy animals were selected at random to construct a derivation dataset, and the remaining 20% of the data was considered as the validation dataset. A supervised machine learning algorithm was trained on the derivation dataset, and then the model was used to predict the health status of animals in the remaining validation dataset. The area under the curve (AUC) of the receiver operating characteristic curve of the model was 0.86 after 5-fold cross-validation. Health conditions can be represented in a two-dimensional visual representation of how diseases were segregated into types of conditions.

Conclusions:

Based on these data Isomark has produced an accurate predictive model, using advanced analytical methods, that can be used to screen beef cattle for infections including respiratory infections using novel breath biomarkers. The ability to screen beef cattle upon arrival to the feedlot in real time within 30 seconds, and predict which animals are most likely to become sick within the first two weeks on the feedlot enables ranchers to segregate these animals at the time of entry for follow-up care or other management decisions. The flexibility of either stationary or portable Canary options provides wide flexibility across feed yard applications. Segregating sick animals has the added benefit of preventing further exposure of potential disease to an otherwise healthy herd. Earlier diagnosis of diseases will also reduce cost of treatment, and/or provide a data driven approach to management decisions on an individual basis. Isomark's technology will significantly reduce the $1 billion dollar losses due to respiratory infections in beef cattle while improve the beef quality of healthy animals.

References:

1. Zamir, O., et al., *Tumour necrosis factor (TNF) and interleukin-1 (IL-1) induce muscle proteolysis through different mechanisms.* Mediators Inflamm, 1992. **1**(4): p. 247-50.
2. Butz, D.E., et al., *Changes in the natural abundance of 13CO2/12CO2in breath due to lipopolysacchride-induced acute phase response.* Rapid Commun. Mass Spectrom, 2009. **23**(23): p. 3729-3735.
3. Tieszen, L.L., et al., *Fractionation and Turnover of Stable Carbon Isotopes in Animal Tissues: Implications for δ 13C Analysis of Diet.* Oecologia, 1983. **57**(1/2): p. 32-37.
4. Boriosi, J.P., et al., *Changes in breath carbon isotope composition as a potential biomarker of inflammatory acute phase response in mechanically ventilated pediatric patients.* Journal of Analytical Atomic Spectrometry, 2014. **29**(4): p. 599 - 605.

Isomark Health. Inc, 5930 Seminole Centre Ct., Suite F, Fitchburg, WI 53711
Email: info@isomark.com Ph. (608) 571-2609