D2Dx™ Immunity Test for Cattle Health and Reproduction Management

-----Better Immunity, Better Health

A balanced immune system is important for the health and productivity of farm animals. Studies by scientists have shown that animals with good immune functions are less prone to diseases, healthier and more productive. When an animal is infected by bacteria, viruses or other pathogens, the immune system of the animal is activated. The detection of an active immune response is a sign of potential infection in the animal. During pregnancy, the immune system of cows and heifers has to make certain changes to accommodate the growth of the fetus. However, these changes also make pregnant and transition cows and heifers more vulnerable to infectious diseases. Closely monitoring the immune status of cattle herd can help farm owners identify sick animals earlier, identify cows and heifers with reproductive problems, avoid excessive antibiotics treatment on healthy animals, and make more informative management decisions.

What is the D2Dx™ Immunity Test?

In both humans and animals, two types of immune responses co-exist in the immune system: Th1 (T helper 1) and Th2 (T helper 2) responses. At any given time, there is an inverse relation between Th1 and Th2 immunity: when one is stronger, the other is weaker. Maintaining a balanced Th1/Th2 immunity ratio is important for cattle health and reproduction.

D2Dx™ is a blood test that measures the Th2 immune response. D2Dx™ test uses a gold nanoparticle reagent as a universal pathogen to probe the Th2 immune response from human or animal blood (Figure 1). Upon mixing the gold nanoparticle (AuNP) reagent with a blood serum, proteins from the humoral immune system including IgG, IgM, and complement proteins, will interact with the gold nanoparticles in a way similar to what happens *in vivo*. This interaction is measured and quantified by monitoring the average nanoparticle size change of the assay solution using dynamic light scattering (DLS). Stronger Th2 immune response gives higher D2Dx™ test score.

While D2Dx™ test only detects the Th2 immunity, because of the inverse relation between Th1 and Th2 immunity, D2Dx™ test result is essentially a measure of the Th1/Th2 immunity balance.

When D2Dx™ test score is either too high or too low, it suggests an unbalanced Th1/Th2 immune activity and underlying health problems in the animal. D2Dx™ is not a diagnostic test; however, together with other tests and evaluations, D2Dx™ immunity test can potentially bring the following benefits to cattle owners:

- Identify animals that have contracted an infectious disease for isolation, further diagnosis and timely treatment;
- Aid in treatment decisions and in controlling the spread of infectious diseases;
- Identify animals that are more disease resistant and thereby reduce the potential use of unnecessary treatments and use of antibiotics in cattle;
- Monitor the health of cows and heifers during pregnancy and transition period, discover underlying health and reproduction problems earlier for timely treatment and care;
- Predict the productivity potential of young calves, provide owners with additional information to make better management decisions.

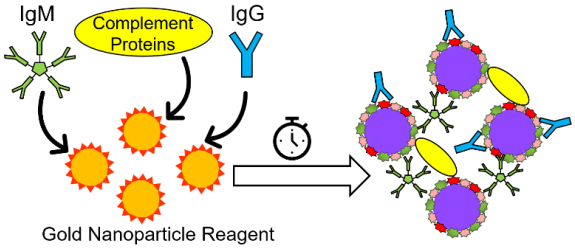


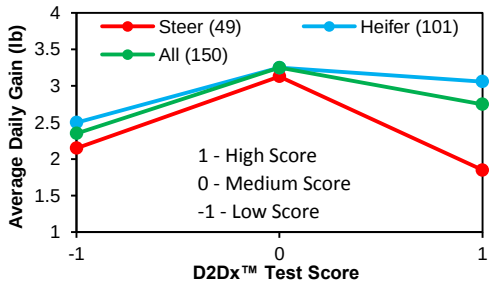
Figure 1. The principle of D2Dx™ immunity test. Gold nanoparticle reagents interact with the blood serum proteins including IgG, IgM and complement proteins from the humoral immune system to form nanoparticle aggregates.

Case Study 1 – Better Immune Health, Higher Average Daily Gain of Calves

A study was conducted at a feedlot on 150 calves at the age of 6-8 months old. The calves were tested upon arrival at the feedlot using the D2Dx™ immunity test and identified as having high, medium, or low test scores. 50 days later, the calves were weighed, and the average daily gain (ADG in lb) was calculated. The study found:

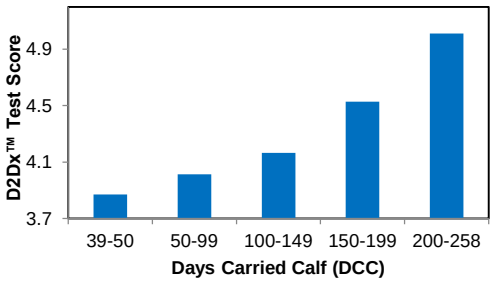
- Calves with medium scores have the highest ADG;
- For steers, the difference in ADG between medium and high score group is about 1.2 lb;

- When steers and heifers are combined together, the difference in ADG between medium test score group and other two groups is 0.8 lb.



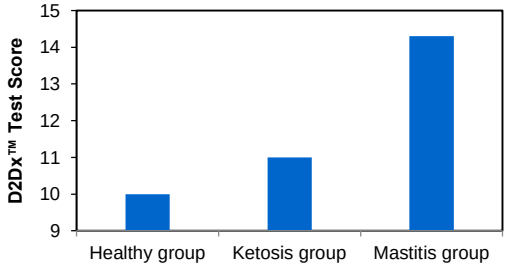
Case Study 2 – Th1/Th2 Immune Balance Shift During Pregnancy

A study was conducted on 79 pregnant cows in a dairy farm. Data shows that the D2Dx™ test score increases with the DCC (Days Carried Calf) of the pregnant cows. This increase reflects the immunity shift of pregnant cows from a Th1- to a Th2-dominant response.



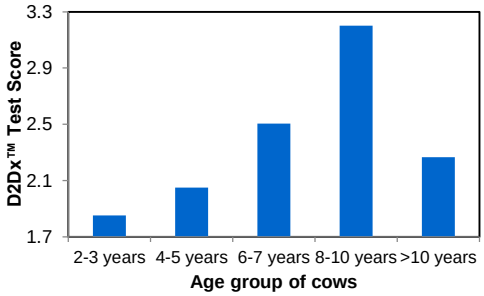
Case Study 3 – Unbalanced Immunity Increases Health Risk of Transition Cows

In another study of 20 pregnant dairy cows during peri-parturition period, blood samples were collected and tested 30 days before calving. Among the 20 cows, 8 were healthy, 8 developed ketosis and 4 developed mastitis after calving. The average test score of cows with ketosis and mastitis is significantly higher than the healthy group. This study suggests that if the Th2 immunity of pregnant cows is too strong, the cows will more likely have health problems after calving.



Case Study 4 – Aging Cows with Declined Immune Health and Productivity

A study was conducted at a beef cattle ranch on 165 cows with different ages. The study shows that the D2Dx™ test score increases over age. After peaking at an average age of 8-10 years, the test score decreases sharply. This change signals a decline in the immune health of the animals. With a declined immune health, the reproductive ability of the cow will most likely decline as well. Individual cows start to show declined immune health at different ages. Together with other tests and evaluations, D2Dx™ immunity test may assist owners to identify cows with reduced reproductive potentials and make informative decisions accordingly.



Additional Reading

1. Zheng, T.; Crews, J.; McGill, J.L.; Dhume, K.; Finn, C.; Strutt, T.; McKinstry, K. K.; Huo Q. A single-step gold nanoparticle–blood serum interaction assay reveals humoral immunity development and immune status of animals from neonates to adults. ACS Infect. Dis. 2019, 5, 2, 228-238.
2. Zheng, T.; Bott, S.; Huo, Q. Techniques for accurate sizing of gold nanoparticles using dynamic light scattering with particular application to chemical and biological sensing based on aggregate formation. ACS Appl. Mater. Interfaces 2016, 8, 33, 21585-21594.
3. Reiner, S.L.; Locksley, R.M. The regulation of immunity to Leishmania Major. Annu. Rev. Immunol. 1995, 13, 151-177.
4. Kidd, P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and diseases. Altern. Med. Rev. 2003, 8, 223-246.
5. Spellberg, B.; Edwards, J.E., Jr. Type 1/Type 2 immunity in infectious diseases. Clin. Infect. Diseases 2001, 32, 76-102.
6. Sykes, L.; MacIntyre, D.A.; Yap, X.J.; Teoh, T.G.; Bennett, P.R. The Th1/Th2 dichotomy of pregnancy and preterm labor. Mediators Inflamm. 2012, article 967629.
7. Yang, L.; Wang, Y.; Ma, X.; Wang, S.; Zhang, L. Changes in expression of Th1 and Th2 cytokines in bovine peripheral blood mononuclear cells during early pregnancy. Indian J. Anim. Res. 2016, 50, 466-470.

D2Dx™ Test Related Patents

1. *US9,005,994,B2* “Methods for biomolecule and biomolecule complex (BMC) detection and analysis and the use of such for research and medical diagnosis”, Huo, Q. Issued on April 14, 2015.
2. *US10,191,041,B2*, “Detection of analytes using metal nanoparticle probe and dynamic light scattering”, Huo, Q.; Liu, X.; Dai, Q. Issued on January 29, 2019.
3. PCT patent application, “Detection of interaction between an assay substance and blood or blood components for immune status evaluation and immune-related diseases detection and diagnosis”, Huo, Q.; Zheng, T.; McKinstry, K. Filed on March 15, 2019.