DAIRY VETERINARY NEWSLETTER

November 2016

Genomic Testing of Bovines - What is the Best Sample and How Should it be Collected?

During the last two to three years, commercial bovine genomic testing options have greatly and rapidly increased in the dairy industry. One thing that one does not always consider is how frustrating and costly it can be if the sample(s) collected for genomic testing do not yield enough volume of DNA of sufficient quality for the testing. We experienced this problem a few years ago, until we got some new expert advice on collection of DNA samples in contrast to some older recommendations. Dairy veterinarians will certainly continue to have more clients testing animal genomics in some form in the future, and advising them on, or being directly involved in sample collection, such as at time of calfhood vaccination will be of increasing importance.

In an article in the January 2016 issue of Progressive Dairyman by S. Bauck, a nice summary of DNA testing sample collection was presented. Dr. Bauck is a veterinarian and the manager of GeneSeek, the genomics division of Neogen. As always, the purpose of this newsletter article is not to endorse any particular brand; identification of trade names is only for clarity and to maximize practicality.

Some major points in the article referenced above:

- Samples for genomic testing can include tissue, hair, blood or semen (note: nasal swabs have also been used)
- Tissue collection with a device such as an Allflex Tissue Sampling Unit (TSU) has the following advantages:
- Dairymen are familiar with the applicator devices used to take the sample
- The device takes an appropriate sample for genomic testing
- The 2D barcode on the tissue sample collector is useful for simple sample identification recording

Genomic DNA sample collection that I have had the best success with used ear notches, just like those used for BVD PI animal identification for example. Ear notches or other tissue samples should be stored in a DNA protectant solution right away. (We tried hair samples many times, and DNA isolation was never satisfactory; I don't recommend hair samples anymore.) If using the Allflex device, the TSU is actually the individual-use tissue punch holder with DNA protectant reagent and fits into an applicator similar to an ear tagger, like several other tissue sampling devices. A punch tissue sample is collected from the outer third of the ear. It is important to avoid tattoo ink in ear punch samples.

Bauck states, "If you wish to re-test the animal at a later point in life, the tissue sampling unit is not necessarily the best device, since the only way to retain the sample is to save the extracted DNA."

Image of the TSU (Tissue Sampling Unit) applicator and the TSU itself

Storage of the DNA in liquid reagent for possible later use is the only sample storage method I have had experience with. However, the article mentions that hair and blood sampling can be done using a paper card, " - usually supplied by the genomics testing lab - that is inexpensive and easy to use, but somewhat less automated". The cards have bar codes which can link to the animal ID. Others report the cards used for nasal swab DNA also.

"The cost of the card is about 50 cents and one distinct advantage to this sampling method is the ability to return to that sample later in the animal's life and re-extract the DNA for another test (something which is done frequently on the part of the A.I. industry, for example)." I am interested in this because extracting DNA later from stored samples, especially hair, has been a problem in our experience. (Our post-extraction DNA stored in liquid has never been retested so far.) Not having to keep DNA in liquid after extraction and being able to go back to something like a bar coded card to get more DNA later would be very useful I think. However, the cards would have to be stored in a clean and organized way of course, and storage of large amounts of paper records is often a challenge on many farms, including some of the largest farms. The cards are reported to store DNA at room temperature for years.

An article describing use of paper DNA cards was written by M. McClure et al. in BNC Research Notes, 2009.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2704227/pdf/1756-0500-2-107.pdf

Returning to the subject of tissue collection using the TSU, it can also link to an animal ID; devices exist which can read an electronic ID such as an ear tag and link that with the bar code on the individual TSU. Bar code reading and linking can be difficult and confusing, though. It is also possible to write an animal's ID in a box within a pre-printed list of TSU ID's which include visible numbers such as 02060 according to this TSU Tissue Collection website:
https://genetics.zoetis.com/canada/_locale-assets/documents/tissue-sampling-en.pdf

I can envision that this writing of animal ID's on the pre-printed lists of 10 TSU ID's (they come in boxes of 10) might be easier to keep straight when working and sampling animals.

Proper ID of the animal linked to its DNA sample - essential

One of the most commonly unappreciated sources of error in any sample collection from animals or people is possible misidentification of who the sample came from. I agree with Bauck when he states how important proper sample ID is: "More than half of the focus for development of efficient sample collection systems is around the process of associating the sample barcode with the animal's permanent I.D. to ensure 'chain of custody' - like a crime scene investigation. The sample barcode is the vital piece of information from the lab perspective, but in the end, the information must be related to the animal I.D. to be useful for the dairyman. Sample identification and animal I.D. recording can be done by hand, however, this method can be time-consuming and lead to transcription errors."

In part of my job at USU as the epidemiologist at the Utah Veterinary Diagnostic Laboratory, and before that when I worked in mastitis and udder health for many years, I have seen that many producers and others involved in sample handling take proper ID of each sample for granted. However, when handling hundreds or thousands of samples at one time, it is vitally important and not always simple to make sure that every sample container is properly linked back to the animal it came from.

Bauck continues, "Required data includes an acceptable and unique animal identification (registration number or electronic I.D. tag number), breed designation, birthdate, sex, sire and dam, and whether or not the animal is a twin or a singleton.

In most instances, this data can be routinely retrieved from commonly used record systems and uploaded to the genomic services provider where your samples are sent. If that isn't possible, a simple electronic spreadsheet can be sent via email." When I have done genomic testing, we never were asked and never considered whether a cow was a twin (or triplet) or not. As animals age or if they were purchased, the information above can be difficult to impossible to obtain.

Age or times to sample DNA

It is often logical to sample calves early in life; decisions can be made whether to keep or sell them based on genetic potential. However, the article makes this point: " - - depending on calf mortality at your farm, it [may] be financially beneficial to wait until you can feel confident in that calf's survival. In other instances, you may want to screen for diseases, such as animals persistently infected with bovine viral diarrhea (PI), before investing in the cost of genomic testing. One strategy that has been proposed is to sample a heifer at or shortly after birth, screen the sample immediately for BVD-PI and hold the sample for several months while the heifer gets through the critical health period in the first few months of life."

What about ID tagging an animal and sampling DNA at the same time? There are "matched pair" sets available for this purpose. I have seen them but never used them; I have only ear notched adult cows for DNA genomic testing as part of field research. The "pair" sets actually have 3 parts, visual tag, RFID tag, and the TSU:

Image of matched pair of visual tag, RFID tag, and TSU

The best video I have found of using the matched sets is a U.S. Jersey Association video:
https://www.youtube.com/watch?v=KTMfr4O3R6o

Tissue samples can be stored at room temperature, refrigerated or frozen. The major question is what does the DNA genomic testing lab where they will be sent require for sample storage and shipping conditions?

Consult with your DNA testing laboratory regarding sample types, shipping and handling

Generally, despite the long-term storage of paper cards, most DNA testing labs recommend shipping cards within 48 hours of collection. This suggests to me that if one wants to store card DNA on the farm for possible future testing, you would need to send one card to the lab and collect another blood or nasal swab sample on another card to keep. The cards close like a matchbook and can be shipped at room temperature (hair root samples can be as well, but again we have not had satisfactory DNA extraction results with hair samples).

I have commented in this newsletter several times in the past what it is like to try to reach someone to get an answer from the FDA. I discovered that that process was paradise compared to trying to get an answer from several bovine DNA testing laboratories regarding the question of what the storage and shipping conditions are for sending tissue punch samples to them. I talked to several people who had no idea, and multiple interminable phone menus that

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eventually concluded by hanging up. We have all experienced that many phone menus today seem designed never to have to actually answer the phone; these succeeded. However, one company indeed had a person who answered who could tell me that for their lab, as I suspected, any tissue punch sample in DNA protectant can be sent at ambient temperature with no special requirements, and by any courier service. This has also been our experience in handling our ear notches in DNA protectant; no special temperature requirements. However, there are many conflicting sources of internet information that recommend freezing DNA tissue samples, or say never to freeze, etc. Therefore your clients will need to find out from the specific lab where they are sending DNA what the shipping and handling requirements are. Obviously this needs to be clearly understood before sample collection.

One thing is clear; tissue punch samples in DNA protectant are becoming a common specimen for bovine genomic testing. Sampling at the same time as putting in an ID tag is also a common practice. Waiting until calves are at least a few weeks old to do testing for the reasons described earlier makes some sense, and this is easier with protected DNA samples than those on the paper cards. As always, I hope any readers with additional experiences or insights regarding bovine genomic testing sample collection will share them.

Please let us know your comments and also suggestions for future topics. I can be reached at (435) 760-3731 (Cell), (435) 797-1899 M-Tues, (435) 797-7120 W-F or **David.Wilson@usu.edu**.

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